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Faculty of Agriculture

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Research Article

Wheat Self-Sufficiency in Turkey: Production and Climate Change in Focus

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Abstract: Due to the fact that they can be preserved for extended periods of time and are utilized in virtually all cuisines, wheat and wheat products are the most popular grains to grow and produce. Beyond the current climate change impacts, closed national borders and growing limits for import-export products throughout the pandemic phase have caused governments to question their ability to produce enough food to meet their own needs in the short term. One of the objectives of this research is to determine what variables affect wheat self-sufficiency in Türkiye, which is one of the world's major wheat suppliers, and to develop recommendations for wheat production areas in the face of climate change's predicted impacts. With respect to Türkiye, wheat self-sufficiency data from the Turkish Statistical Institute (2000 to 2020) and regional climate change projections data from the General Directorate of Meteorology for the years 2050 and 2080 were used to identify the most significant variables, as well as the relationship between those variables and self-sufficiency. The findings indicate that wheat production is the most essential component in achieving wheat self-sufficiency and that climate change has a significant impact on wheat productivity and the areas where wheat is grown. Following this, the study concludes by detailing prospective wheat production regions in current great plain areas in the context of regional climate change projections, as well as critical policies for sustainable wheat cultivation.

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Footnote: This study is largely based on Zeynep Erdogan's ongoing PhD thesis and partly preliminary studies of Fatma Selçuk's ongoing MA thesis. Both theses are supervised by Aliye Ahu Akgün.

1. Introduction

The Covid-19 pandemic has affected not only health but also nutrition, food security, and food-related approaches all over the world (FAO, 2020). Many routines, such as dietary priorities, consumption patterns, and eating habits, have evolved with time (Chenarides et al., 2020; Grashuis et al., 2020; Laguna et al., 2020). Many individuals have made it a priority to have access to food as well as to use and store it for extended periods of time. Similarly, during the epidemic period, there has been a surge in food stockpiling and panic purchasing (Nicola et al., 2020). The current tendency has also had an impact on the overall efficiency of the food system (Nchanji and Lutomia, 2021). While food security and self-sufficiency have risen to the top of the priority list on a macro level, a rising trend in

demand for critical nutrients and long-life foods (flour, bread, pasta, and so on) has been observed at the micro level. According to a study conducted by Ipsos Turkey (2020), pasta, legume, and flour, all of which can be stored at home, are among the top three food products whose use has increased the most during the pandemic. The situation in which various export restrictions on staple foods such as rice and wheat have been implemented as a result of the pandemic (Laborde et al., 2020) underscores the necessity of agricultural production and self-sufficiency at the same time.

The European Food Behaviors Report (EIT Food, 2020) is a key source of information on food priorities and consumption behavior in the early pandemic period, revealing that flour saw the greatest increase in consumption (27 percent), followed by vegetables (27 percent), and fruits (32 percent). According to Google trends for 2020, 9 out of the top 10 most sought recipes are for various types of bread from around the world. Similarly, "ekmek" which means "bread" in Turkish, leads Türkiye's list, and the top ten recipes in Türkiye are all connected to wheat products, such as bread and pastries. These searches may provide as a measure of people's food choices and relationships with wheat during the pandemic lockdown period. A new conundrum has emerged: how to ensure that the most basic of nutritional commodities, such as wheat, are constantly available.

When examining wheat self-sufficiency, both production-based and productivity-based elements should be considered. Self-sufficiency factors are determined as endogenous variables in this context, while climate change variables are determined as exogenous variables. Climate change affects agricultural production, productivity, and self-sufficiency in both direct and indirect ways. From this vantage point, the study's initial goal is to establish what factors contribute to wheat self-sufficiency in Türkiye agriculture. The article then focuses on how climate change would affect self-sufficiency and how this change will affect wheat production patterns and places in Türkiye. Although the term "self-sufficiency" has multiple meanings in different settings, it is assumed in this study to mean a situation in which wheat output exceeds consumption. The study not only identifies the major determinants influencing wheat sufficiency, but it also establishes a framework for linking agricultural production and productivity. We focus on Türkiye because developing countries with fertile lands and agricultural potential, such as Türkiye, are critical to satisfying the world's growing population's food needs (FAO, 2020b). At the same time, the study is one of the first in current studies on agricultural production, food security, and pandemics to bring wheat self-sufficiency into question in the face of climatic change.

The study is structured as follows: it begins with a review of the literature on agricultural production and self-sufficiency from the perspective of food security, including productivity. In the next sections, we primarily focus on wheat self-sufficiency and production within Turkish agricultural production, as well as the influence of climate change on wheat production. The fifth section describes the study's methodologies and the materials employed. The sixth part, as a findings section, discusses the primary elements affecting wheat sufficiency in Türkiye, as well as wheat production scenarios under the impact of climate change in Türkiye for the years 2050 and 2080. In the following section, we address the consequences of factors that affect wheat sufficiency, the relationship between agricultural self-sufficiency and productivity, including wheat production and losses, and how wheat production patterns in Türkiye may alter as a result of climate change. Finally, the research finishes with strategies, evaluations, and recommendations for ensuring Türkiye's agricultural self-sufficiency in the future.

1.1.Macro perspective: Agricultural production and self-sufficiency

In Maslow's (1943) hierarchy of needs, physiological needs are at the top, and one of these is feeding. Throughout history, agriculture has been a major element in meeting the nutritional needs of human people. A shift from production to consumption through time has resulted in an increase in the area impacted by farming. The scarcity or surplus in the amount of agricultural production affects a wide range of social groups, from farmers' incomes to investments in agriculture-based industries, from raw material procurement to food safety (Trewin, 1999; Bayraç and Doğan, 2016).

As the world's population grows, so makes the demand for food, and this trend is only expected to continue (Kruse, 2010). By the year 2050, food production must increase by 60% to meet this need (FAO, 2015). Access to food is known as one of the most essential human rights (UN, 1948). This right can be connected to the concept of food security, which is "the ability of all people to have access to the fundamental foods they require physically and economically at all times" when supply and demand are in balance (FAO, 1983). There are four primary components to food security, according to the World

Health Organization (WHO) (2019) and the Food and Agriculture Organization of the United Nations (FAO) (2006). Covid-19 has had a direct impact on these three pillars, particularly food availability (Laborde et al., 2020). The first step in providing food accessibility is to ensure that there is enough food to go around, and this is largely based on the availability of sustainable agriculture.

Self-sufficiency can be defined in a variety of ways and measured in a variety of ways. Food self-sufficiency is defined by FAO (1999) as "the extent to which a country can meet its own domestic food demands from its own domestic production." Generally, self-sufficiency refers to a country's ability to meet its own needs in terms of production and consumption (Soltani et al., 2020). There are some analysts who define self-sufficiency as a country's ability to meet all of its food needs only through domestic production without being dependent on food imports (Clapp, 2017). We define wheat self-sufficiency in this study as a supply of wheat that is equal to domestic demand and also provides surplus production of each type of wheat in the event of a predictable condition like climatic change or an uncertain period like a pandemic.

The primary goal of agricultural production in most countries is to ensure that the population receives adequate and balanced nourishment. As part of a country's agricultural self-sufficiency, agricultural production is critical to preventing excessive foreign dependency (Acar and Aytüre, 2014; Bayar, 2018). There are no sustainable agricultural policies or policies that provide self-sufficiency in production when they are not based on production and resource productivity (Gülçubuk, 2020). Increased agricultural production is linked to agricultural self-sufficiency. Increasing cultivation areas is one of the most important methods for boosting agricultural production, and another is to enhance yield per unit area (Tuğay, 2012) while minimizing food losses. However, as arable land gets depleted, it is vital to find new ways to produce more goods with the help of already available agricultural inputs (Erçakar and Taşçı, 2011). As a result, the research focuses on preserving existing habitats and improving agricultural production and productivity.

Storage, marketing, and product price are just a few factors that can affect productivity. Other factors include the fragmentation of land, climatic conditions, land ownership, and organization, as well as agricultural inputs (seeds, fertilizers/pesticides) and irrigation (Mentz and Slater, 1999; Çelik, 2000). However, chemical inputs, seed advances, mechanization, expansion of arable land, and activation of irrigation potential might be represented as the most important variables in increasing agricultural production (Bayramoğlu, 2010). A second element that has an impact on production and productivity is the amount of food that is lost. In the food supply chain, food loss refers to a drop in quality and/or quantity losses. High food losses put the nation's food supply at risk (Keding et al., 2013). Due to food losses, there are more uncertainties and hazards. Food waste accounts for 14 percent of the world's food losses each year (FAO, 2020a). During the stages of food production, harvesting, and post-harvest activities (storage, processing, distribution, and consumption), food losses occur.

According to the level of development of the countries, food losses may vary (FAO, 2013). In middle- and high-income countries, the majority of food loss and waste occur in the distribution and consumption stage (FAO, 2011; Prusky, 2011; Permanandh, 2011). Food losses occur all the way up the food supply chain, despite the fact that food waste happens at the consumer and retail level (Cattaneo et al., 2020). The majority of the loss occurs during the production and post-harvest phases in low-income countries as well (FAO, 2011; Prusky, 2011; Permanandh, 2011).

1.2. Micro perspective: Agricultural production and self-sufficiency

In terms of total agricultural land and arable land amount, China and the United States are the two pioneer countries around the world. In addition to having an important agricultural land potential, Türkiye comes after its neighboring countries Russia and Ukraine (Figure 1.).

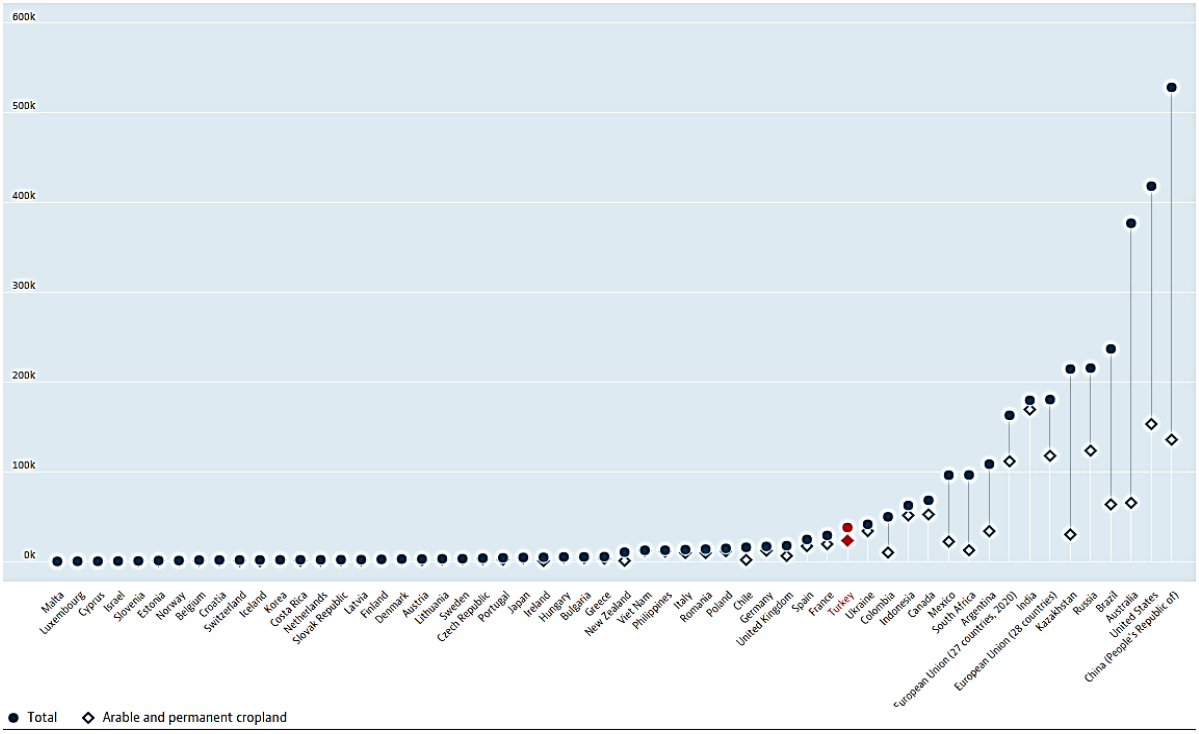


Figure 1. Agricultural Land- Total/Arable and Permanent Cropland, Thousand ha. (OECD, 2021).

To maximize output in Türkiye's agricultural sector, increasing the amount of arable land available for cultivation is a vital consideration. Because the amount of arable land in Türkiye is nearing its maximum, it is impossible to increase the boundaries of agricultural land, as is the case in many other countries (Bayar, 2018). Table 1. shows that since the 1980s, total agricultural land and cultivated land have decreased. On the map, wheat production areas and productivity values with the plain areas for 2020 are given (Figure 2.). The map shows that while Central Anatolia and Southeast Anatolia have a high-rate production in wheat, the west of Southeastern Anatolia, the east of the Mediterranean region, the west of the Aegean region, and the Marmara region have become prominent in terms of wheat productivity with the remarkable values.

Table 1. Total agricultural land and total arable/permanent cropland, Türkiye

Years	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**	**2020**
Total arable and permanent cropland (thousand ha)	25.324	27.339	28.182	27.856	26.379	24.394	23.145
	1990	**1995**	**2000**	**2005**	**2010**	**2015**	**2020**
Total agricultural land (thousand ha)	42.033	39.212	38.757	41.223	39.011	38.551	37.762

Source: TURKSTAT, 2021 (https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1)

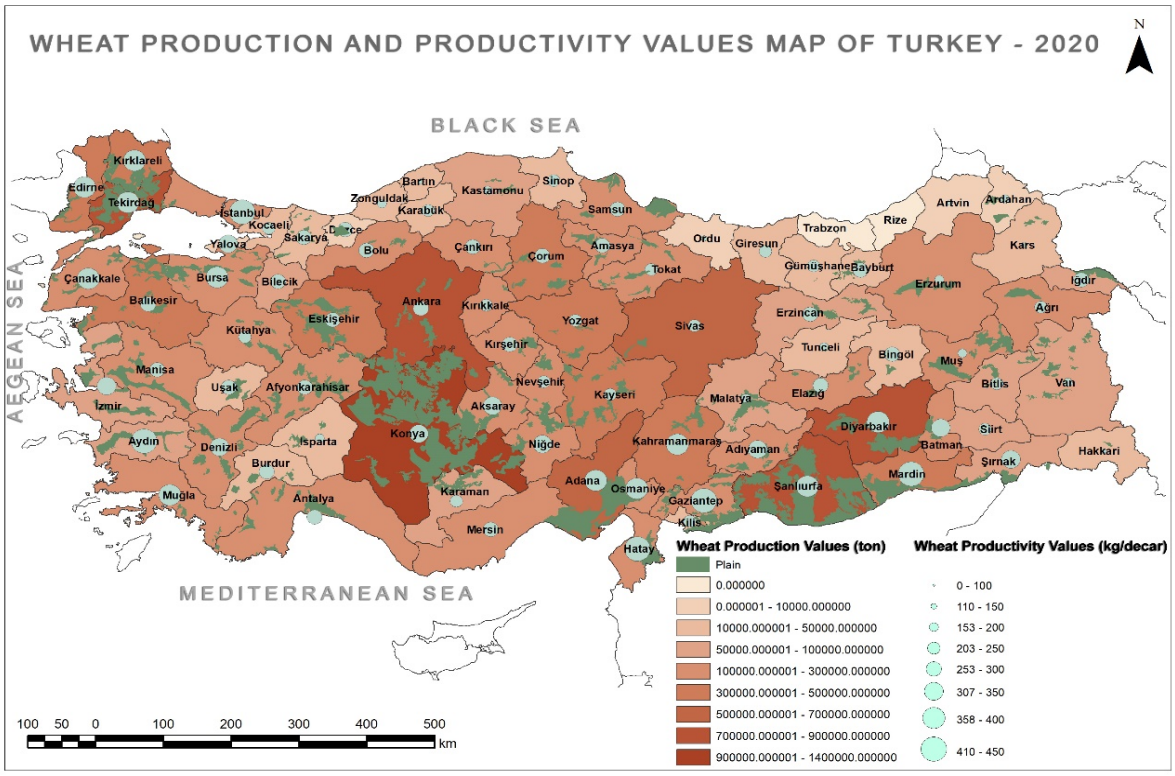


Figure 2. The map of wheat production and productivity values of Türkiye, 2020. *Source:* Based on the data derived from TURKSTAT (2020), map is prepared by the authors.

Besides the high input prices, Türkiye's agricultural land structure is exceedingly fragmented and small as a general concern (Demirdöğen et al., 2016). Although generalizations cannot be made for all agricultural products, irrigation and mechanization are not possible in small and fragmented areas. As a result, agricultural production and productivity are significantly impacted. In developing countries, 95% of food losses are due to "unintentional" mishaps that occur in the early stage of the supply chain (FAO, 2018). In Türkiye, most of the country's food losses occur during the production process. Fruits and vegetables suffer the greatest losses in plant/crop output, while grains suffer the least (Tatlıdil et al., 2013).

Wheat has been grown in Türkiye for more than a thousand years (Atalan-Helicke, 2019). After the financial crisis of 1929, official policies aimed primarily at wheat production in terms of agricultural policy in Türkiye. Wheat assistance purchases and price intervention were first implemented in 1932 under statute number 2056. Wheat was the target of price regulation and corrections under this statute (Silier, 1981). The second half of the 1930s saw the continuation of precautionary measures for wheat purchasing, such as the free charge of seeds, increased custom charges on wheat imports, and support for agricultural cooperation (Margulies and Yıldızoğu, 1990). At the end of the 1930s, wheat, which had been primarily an import, began to be exported (Özdinc, 2010). While wheat production grew in the 1950s, it relied on growing farmland, after the 1970s, it relied on a gain in productivity (Aysu, 2018). Since the 1980s, neoliberal policies and the free market have had a major impact on agricultural policies, and the state monopoly in the wheat market has shrunk as a result. Wheat production has not changed much in the recent three decades. However, imports of wheat have risen sharply in tandem with the country's growth as an exporter of flour (wheat) in the 2000s (FAOSTAT, 2021) (Figure 3.).

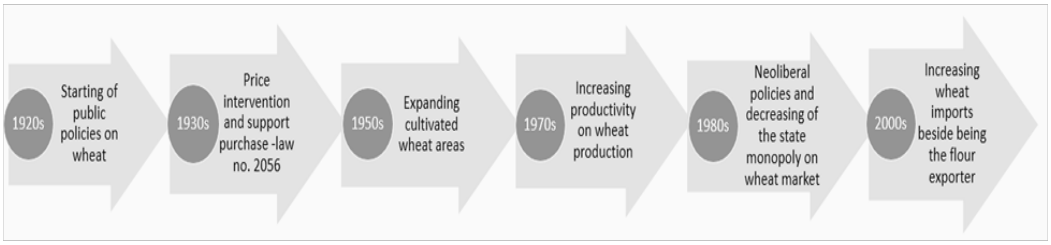


Figure 3. Timeline of wheat policies in Türkiye.

Türkiye is the 10th-largest agricultural exporter in the world, accounting for about 3% of global output (FAOSTAT, 2021; TMO, 2020). When it comes to grains, wheat is the most widely consumed and produced product (Demirbaş and Atis, 2005). Wheat accounts for 28 percent of the world's total cereal production, which totals 2.7 billion tonnes (FAO, 2021; USDA, 2021). About 66% of the world's wheat production is made by these five countries: China, India, the United States, and the Russian Federation (TEPGE, 2021, FAOSTAT, 2021). Türkiye accounts for around also 3% of global wheat production and ranks first in the world in terms of exports of processed wheat (wheat flour) (FAOSTAT, 2021).

Türkiye imports wheat primarily from Russia (62%) and Ukraine (15%) (TEPGE, 2021). Türkiye's wheat imports will be affected by any conflict between Russia and Ukraine that has an impact on wheat production in Russia and Ukraine. While 44 percent of Türkiye's cultivated grain fields are dedicated to wheat production (TURKSTAT, 2021), in terms of wheat exports, the country is a leader in both raw and processed wheat products (FAOSTAT, 2021). Türkiye's production has increased, even though the country's agricultural land has decreased during the past 15 years, as indicated in Figure 4. Because of the shrinkage of arable land, it is logical to assume that greater production is associated with higher productivity. Proof that production is greatly affected by productivity.

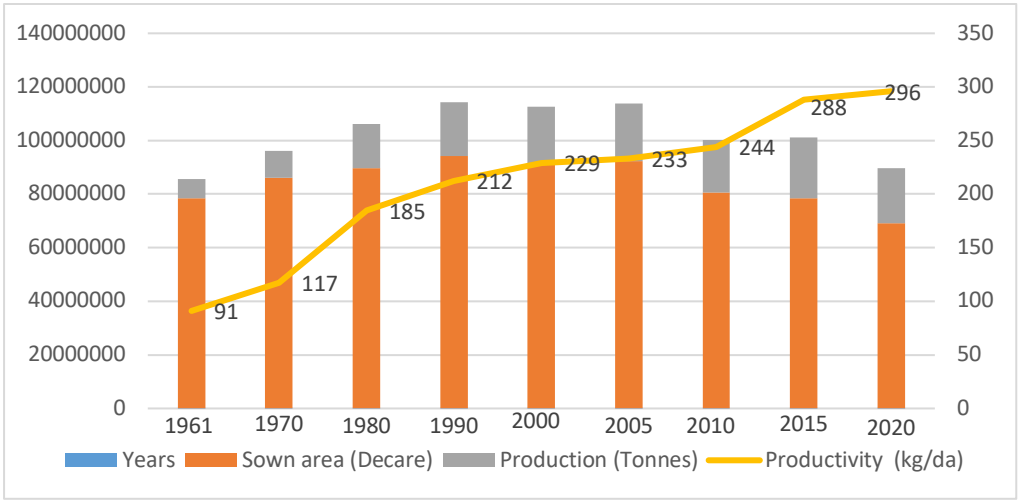


Figure 4. Wheat Sown Area, Production and Productivity, Türkiye. Source: FAOSTAT, 2021; TURKSTAT, 2021.

The last five years of data (2014-2019) demonstrate that wheat is among the top five items for international commerce with its many varieties, according to the Turkish Federation of Food and Drink Industry Associations (TGDF, 2020). Wheat flour and pasta are two of the top three exports, whereas durum wheat is the most common import. Even though the export value of wheat products is larger than the import value of durum wheat, wheat self-sufficiency should be studied in detail in the event of an emergency.

Wheat self-sufficiency is a concept that is met when looking into wheat production in depth. For example, how much of a product's demand in a certain location is met by local manufacturing. The TURKSTAT (2021a) report on self-sufficiency values notes that a percentage value of less than 100

indicates a shortage of supplies, whilst a percentage value of more than 100 indicates a surplus that may be stored or exported. The formula that is used by TURKSTAT is

[*Self-sufficiency = (Usable Production / Domestic Use)*100]*.

There have only been nine years in the last 20 years in which wheat self-sufficiency was between 95 and 100 percent on average in Türkiye, according to TURKSTAT (2021). (Figure 5). Despite Türkiye's apparent wheat self-sufficiency, the long-term viability of this position must also be considered.

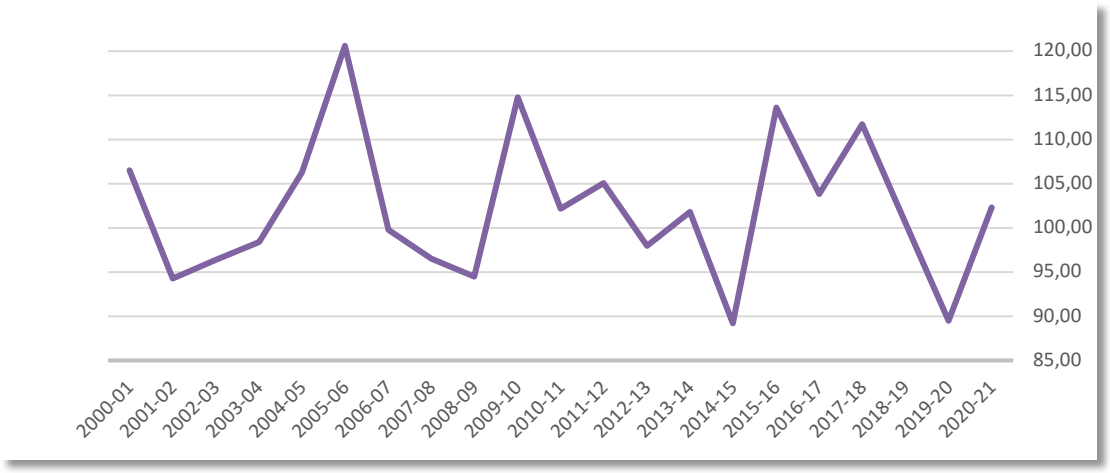
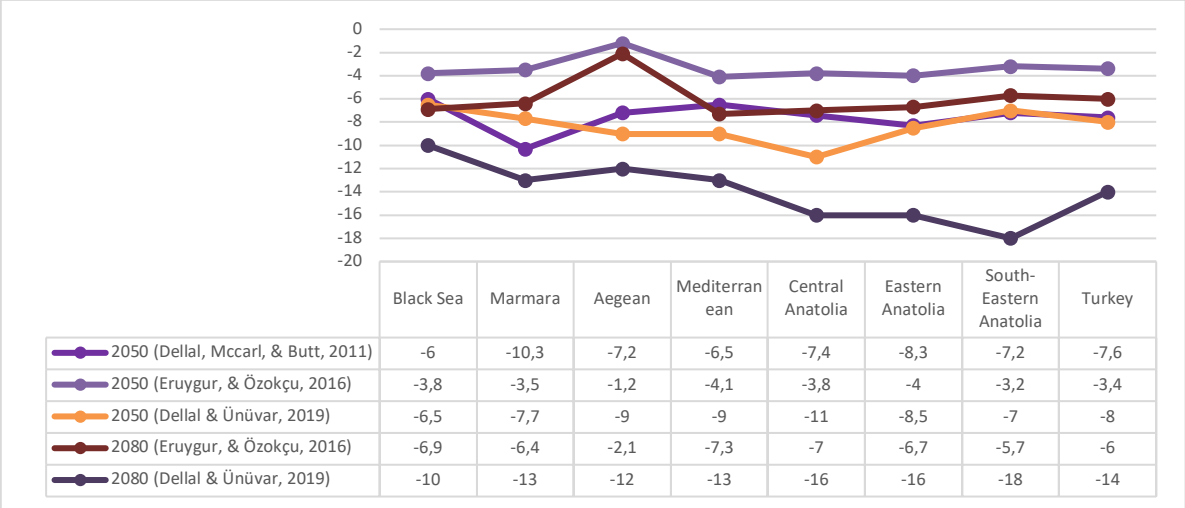


Figure 5. Degree of wheat self-sufficiency in Türkiye. Source: TURKSTAT, 2021.

Since the 1950s, increasing levels of greenhouse gas emissions have signaled the onset of climate change. Temperatures rise as a result of increased greenhouse gas and CO2 emissions, and precipitation patterns alter as well (IPCC, 2018). Climate change is affecting the environment, society, and economy, and agricultural food production is one of the most affected areas. Increases in high temperatures and decreased precipitation have led to an increase in drought, a drop in soil moisture, and a fall in crop yields (FAO, 2016).

Climate change has a significant impact on the world's primary grain products, including wheat. For each degree Celsius increase in temperature, global wheat yield is predicted to be lost by 6.0 ±2.9% (Zhao et al., 2017). Climate change may lead to a progressive decline in wheat planting areas due to an increase in average sunshine duration in wheat-producing regions (Karapınar et al., 2020). A wide range of climates is suitable for wheat. However, highly humid and hot climates are detrimental to the crop's growth. The ideal climate for growing wheat calls for temperatures ranging from 5 to 15 degrees Celsius and annual precipitation of 500-600 millimeters (mm) in order to achieve excellent yields (KTB, 2016).

Located in the semi-arid climate zone, Türkiye is one of the regions most affected by climate change. According to experts, most of Türkiye's wheat-producing regions, including Central Anatolia and Southeastern Anatolia, are likely to be faced with more severe and frequent droughts in the future decades (OECD, 2021). Climate change is anticipated to reduce wheat cultivation area, wheat production, and yield in seven regions of Türkiye in 2050 and 2080 (Dellal, McCarl & Butt, 2011; Eruygur and Özokçu, 2016; Dellal and Ünüvar, 2019). This is due to the increase in temperature and decrease in precipitation. Figure 6. below shows the estimated values for wheat by region based on existing studies in the literature.



	Black Sea	Marmara	Aegean	Mediterranean	Central Anatolia	Eastern Anatolia	South-Eastern Anatolia	Turkey
2050 (Dellal, Mccarl, & Butt, 2011)	-6	-10,3	-7,2	-6,5	-7,4	-8,3	-7,2	-7,6
2050 (Eruygur, & Özokçu, 2016)	-3,8	-3,5	-1,2	-4,1	-3,8	-4	-3,2	-3,4
2050 (Dellal & Ünüvar, 2019)	-6,5	-7,7	-9	-9	-11	-8,5	-7	-8
2080 (Eruygur, & Özokçu, 2016)	-6,9	-6,4	-2,1	-7,3	-7	-6,7	-5,7	-6
2080 (Dellal & Ünüvar, 2019)	-10	-13	-12	-13	-16	-16	-18	-14

Figure 6. The estimated values for wheat by regions for 2050 and 2080. Source: Based on studies of Dellal, McCarl & Butt, 2011; Eruygur & Ozokcu, 2016; Dellal&Ünüvar, 2019. Graph is prepared by the authors.

2. Material and Methods

The study is organized into two sections: statistical and spatial data analysis. As a first step, we looked at statistics on Türkiye's wheat values to determine its level of wheat self-sufficiency. The current variables on wheat self-sufficiency by TURKSTAT have been used to determine the primary factors of self-sufficiency. A table including sixteen variables (wheat production, area sown, harvest losses, Supply=Use, Usable production, Imports, Imports EU 27-28, Domestic use, Seed use, Animal Feed, Human consumption, Losses, Exports, Exports EU 27-28, Change in stocks, and Human consumption per capita) is used by TURKSTAT to explain the wheat self-sufficiency values. These TURKSTAT-determined variables are intimately linked to wheat production and consumption, as well as export and import. It is clear from the table content that every variable has some bearing on wheat production, even if they are not all included in the self-sufficiency calculation (TURKSTAT, 2021). In this study, the most recent TURKSTAT data on wheat self-sufficiency in the ten-year period from 2000-2001 to 2020-2021 was used.

The study examines how the variables listed in the wheat self-sufficiency table affect the concept's meaning. It is required to employ a method to establish the relationship between the value of self-sufficiency and the other variables in order to identify which variable is the most effective. To begin, we used a Chi-square test to see if the variables and self-sufficiency scores are linked or dependent on one another. The Chi-square test shows that one cell (25.0 %) have an expected count is less than 5. Fisher's Exact Test results are utilized when this rate is greater than 20%, and the frequency value in a 2x2 table is less than 5. (Suresh, 2019; UPENN, 2008). Additionally, a correlation analysis was carried out to verify the validity of the findings. If there is a correlation between two or more variables, the strength and direction of the association can be determined using this technique. Both methods are appropriate for this study's purpose. While the wheat self-sufficiency value is a dependent variable, the other values are the study's independent variables. In the first step, the goal is to identify the most important endogenous variables that contribute to self-sufficiency.

In the subsequent phase, we focus on exogenous variables such as climate change. There are three general kinds of research on wheat yields in Türkiye and four studies on specific regions, according to the literature. In this context, studies on Türkiye from all regions are uncommon. Due to this limitation, wheat yield values in this study were relied on average projections from regional studies. In order to make an assessment of climate change, basin-based temperature and precipitation forecasts in the TR2015-CC report (*Yeni Senaryolar ile Türkiye İklim Projeksiyonları ve İklim Değişikliği*) of the Turkish State General Directorate of Meteorology were used. We processed the temperature and precipitation projections data produced by the General Directorate of Meteorology for basins according to the RCP8.5 scenario and GFDL-ESM2M global model in the Arc-GIS 10.7 program. Temperature and precipitation projection data for the years 2050 and 2080 were used in the study since these are the

base years of the yield studies obtained from the literature (see Dellal, McCarl & Butt, 2011; Eruygur & Ozokcu, 2016; Dellal&Ünüvar, 2019). Using TURKSTAT (2021) high wheat production and yield statistics, great plain borders, regional yield studies, and climate change estimates, we analyzed climate change's effects on wheat yield/productivity in Türkiye. Great plain boundaries were constructed in ArcGIS 10.7 by digitizing plain boundary coordinates (X-Y coordinates) data obtained from the Turkish Ministry of Agriculture and Forestry's Agricultural Land Assessment and Management Automation (TADPortal) website.

3. Results and Discussion

As previously stated, in order to assess the association between wheat self-sufficiency and the other variables using TURKSTAT self-sufficiency data, the Fisher's Exact Test value was applied to the chi-square test table in the analyzing section. This test just displays the p-value that can be tracked on the exact sig column (2-sided).

Table 2. Results of Chi-Square Test

Variables	Value[a]	*df*	*p*
Wheat Production	13.747	1	**0.000**
Area Shown	0.043	1	1.000
Harvest Losses	3.834	1	**0.086**
Supply Use	0.043	1	1.000
Usable Production	13.747	1	**0.000**
Imports	0.043	1	1.000
Imports EU*	0.095	1	1.000
Domestic Use	2.376	1	0.198
Human Consumption	5.838	1	**0.030**
Seed Use	0.043	1	1.000
Animal Feed	1.173	1	0.395
Losses	3.834	1	**0.086**
Exports	1.173	1	0.395
Exports EU*	0.095	1	1.000
Change in Stocks	3.834	1	**0.086**
Human Consumption per capita (kg)	0.043	1	1.000

*Except for these variables [a.2 cells (50.0%)], Fisher's Exact Test a.1 cells (25.0%) have an expected count of less than 5. The minimum expected count is 4.76.

Wheat production and Useable production are significant at a 99 percent confidence level ($p=0.000$; $p<0.01$), and human consumption is significant at 95 percent ($p=0.030$; $p<0.05$), according to p-value data. In addition, losses, harvest losses, and stock change are all significant at 90% ($p=0.030$; $p<0.10$). According to the findings, wheat self-sufficiency is primarily determined by wheat production and usable production, with human consumption coming in second. Losses and stock changes are also significant, but only in the third degree (Table 2.).

The correlation analysis is used to determine the strength and direction of the association between wheat self-sufficiency and the factors. The value (r) can range between -1.0 and 1.0, indicating both a negative and positive correlation. Correlation is moderate if the value of r is $0.40 \leq r < 0.60$. If the number is $0.60 \leq r < 0.80$, this indicates a strong positive correlation. If the number is more than 0.8, it indicates that the variables have a very strong positive connection.

Table 3. The results of the correlation analysis

		Wheat Production	Usable Production	Losses	Harvest Losses	Human Consumption	Domestic Use	Degree of self sufficiency (%)
Degree of Self-Sufficiency (%)	Correlation Coefficient	**0.831****	**0.787****	**0.612****	**0.589****	**-0.529***	**-0.488***	1.000
	Sig.(2-tailed)	0.000	0.000	0.003	0.005	0.014	0.025	

**Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table 3. displays the values for which the association is significant at the 0.01 level (99 percent). The correlation of four variables (wheat production, harvest losses, useable production, and losses) is significant at the 0.01 level, according to correlation analysis. The r-values demonstrate a very high positive association between wheat production and wheat self-sufficiency. Then, a high positive link with usable production is discovered, as is a moderate positive correlation with harvest losses and losses. On the other hand, the correlation of two variables (human consumption and domestic use) is significant at the 0.05 level. Wheat self-sufficiency has a moderate negative correlation with human consumption and domestic use, as expected.

When the Chi-square test and the Correlation analysis are compared, it appears that wheat production is the most important variable in wheat self-sufficiency. Usable production is a significant factor as wheat production value. Both analyses share these two characteristics. Production and self-sufficiency are believed to have a positive and directly proportionate relationship. In parallel with the Chi-square test, harvest losses and losses have become prominent as another significant factor in the correlation analysis. This positive correlation is based on the relationship between production and losses. This outcome was anticipated because, as production increases, so make losses (Figure 7.). The main point is to repurpose all types of waste in the consuming process.

Figure 7. Variables of Wheat Production and Losses from 2000 to 2021, Türkiye (TURKSTAT, 2021).

In addition, consumption values, including human consumption and domestic use, are factors that negatively affect self-sufficiency. In the Fisher's Exact Test, human consumption is linked to wheat self-sufficiency. The correlation between human consumption and domestic use is significant at 0.05 level, and there is a moderate negative association (r= -0.529 and r= -0.488) with wheat self-sufficiency. This suggests that these elements have an inverse relationship with wheat self-sufficiency in the correlation analysis.

Both analyses show comparable results in terms of which variables have a greater impact on the degree of wheat self-sufficiency. The most essential component for the degree of self-sufficiency is all types of wheat production, including usable and total values. At the same time, all types of losses, including harvest and total values, emerged as important factors for wheat self-sufficiency in both analyses. Another important issue is that consumption and domestic use factors are negatively related to wheat self-sufficiency.

In terms of climate change, the increase in warmth and decrease in precipitation are predicted to have a negative impact on conditions, particularly in the Eastern Mediterranean, Eastern Anatolia, Southeastern Anatolia, and Central Anatolia regions where wheat production and yield are high. The maps for the years the 2050s and 2080s were made using the General Directorate of Meteorology's temperature and precipitation projections, as well as wheat yield estimation findings obtained from the regional studies, which were added to maps and integrated with large plain areas. The yield forecast estimates provided by the regional studies were compared to the temperature and precipitation forecasts to determine whether regions' wheat production conditions could be adversely affected. According to the findings of the studies, wheat yield is affected by temperature and, in particular, precipitation. While average yearly precipitation of 500-600 mm results in higher quality and more productive wheat, the average precipitation in Türkiye's regions has ranged from 400 (Central Anatolia) to 700 (Black Sea) mm over the last ten years. Wheat production is heavily dependent on precipitation, and when there is little precipitation, yield suffers.

The temperature will rise in all regions of the country in the 2050s, according to climate change forecasts shown in Figure 8., while precipitation will decrease in general except for the East Black Sea region and around Hatay province. It is projected that yield will be lower in areas where the temperature and precipitation are lower. However, the yield is expected to decline at a slower rate in the Black Sea and Aegean regions than in other regions. For example, while precipitation is anticipated to fall by up to 30 percent in the Konya Plain and its immediate surroundings, the study's findings reveal that yield will decline by 7.4 percent in the Central Anatolia region. With 7.2 percent, the Marmara area comes second in terms of yield value decrease (Eruygur and Özokçu, 2016; Dellal and Ünüvar, 2019). The South-Eastern Anatolia region is another key production area that will be impacted by climate change. In this region, where the huge plains are densely located, the average precipitation is projected to fall by up to 15%, and yield will fall by 5.9% on average (Figure 8.).

The average temperature in Eastern Anatolia and South-Eastern Anatolia is anticipated to rise by 5-6°C by the 2080s, according to forecasts shown in Figure 9. Following that, it is expected that temperatures in Central Anatolia, the Mediterranean, and the Southern Aegean will dramatically rise by 3.5 -4°C. Precipitation in Türkiye will decrease by more than 15% in the 2080s, while precipitation in the western Mediterranean region decrease by more than 30%. Furthermore, when all regions are considered, the regions with the greatest reduction in wheat output in the 2080s are South-Eastern Anatolia, Central Anatolia, and Eastern Anatolia. Wheat yield declines are expected to be less severe in the Aegean and Black Sea regions. South-Eastern Anatolia is one of the locations with high production and productivity, as well as tightly packed huge plains. However, as indicated in Figure 9., the yield in the South-Eastern Anatolia region is expected to fall the most, by an average of 11.85 percent in the 2080s (Eruygur and Özokçu, 2016; Dellal and Ünüvar, 2019).

The region of South-Eastern Anatolia is followed by the region of Central Anatolia, where both production regions are concentrated, and yields will decline by an average of 11.5 percent owing to climate change (Eruygur and Özokçu, 2016; Dellal and Ünüvar, 2019). The Konya Plain, one of Türkiye's largest plains, is located in Central Anatolia, which has the highest wheat production in the country. Furthermore, as temperatures rise in the Eastern Anatolia region, it is projected that production areas will suffer, and yields will plummet dramatically (Figure 9.).

In conclusion, the research findings first reveal that the most important variable affecting self-sufficiency is wheat production. Losses and human consumption are also significant variables. It is obvious that providing self-sufficiency is fundamentally dependent on the production and its relationship to other challenges. Second, current climate change forecasts and analyses reveal that the effects of climate change will steadily rise, and this shift will have a detrimental impact on wheat production. Wheat production will decline if adequate precipitation is not delivered and temperatures rise. According to forecasts for the 2050s and 2080s, the primary wheat production areas of Central

Anatolia, South-Eastern Anatolia, Eastern Anatolia, and Eastern Mediterranean will be the regions most affected by climate change in terms of wheat yield and production.

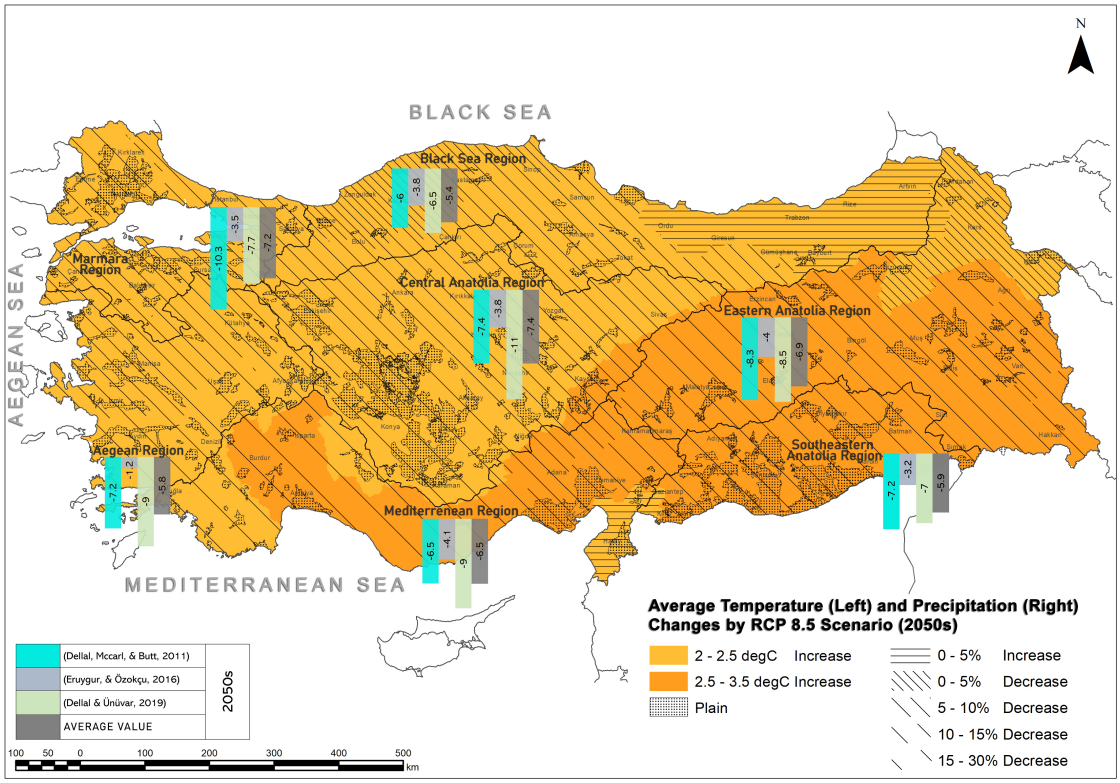


Figure 8. Average Annual Temperature and Precipitation Change by RCP 8.5 Scenario and Wheat Productivity Forecasts (2050s). *Source:* Prepared by the authors.

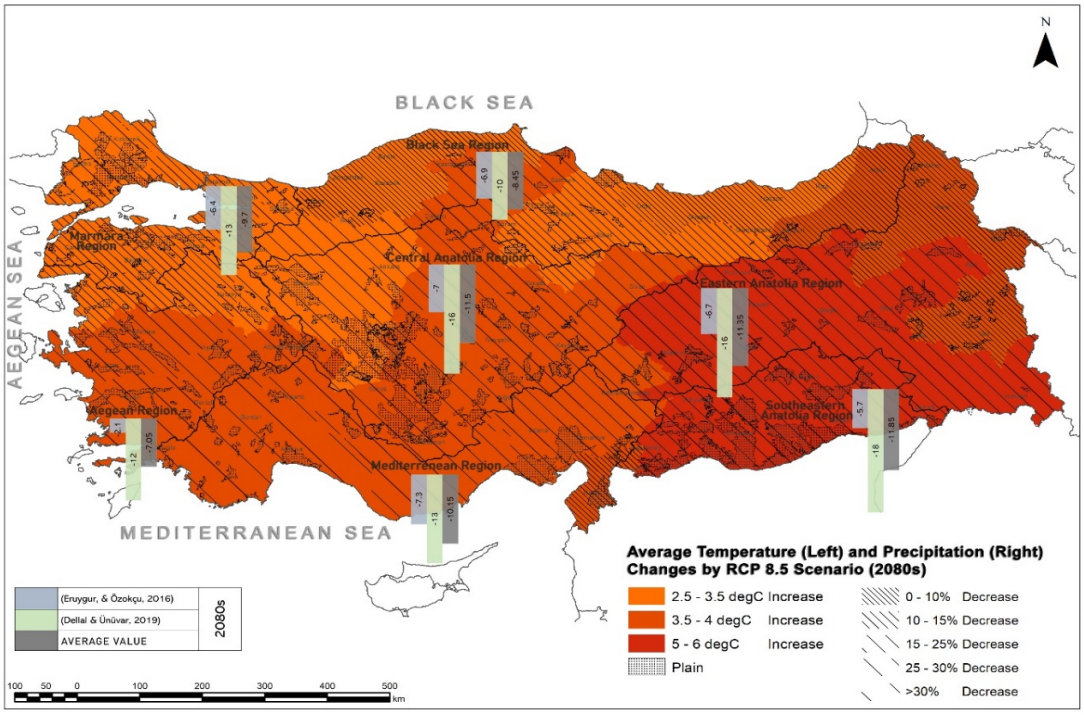


Figure 9. Average Annual Temperature and Precipitation Change by RCP 8.5 Scenario and Wheat Productivity Forecasts (2080s). Source: Prepared by the authors.

Conclusion and Recommendations

Due to the Covid-19 pandemic, which has been described as an unpredictable crisis, there has been a spike in the consumption of foods that can be stored at home for an extended period. Furthermore, essential foods such as wheat and wheat products such as bread, pasta, and flour are becoming more consumed products around the world. Self-sufficiency and fulfilling domestic food demands have become a major concern for many countries, particularly following recent food trade restrictions. Similarly, in terms of domestic demand and production potential in Türkiye, wheat has emerged as an important agricultural product. From this vantage point, whether wheat output fulfills domestic demand and/or what factors influence wheat self-sufficiency has emerged as a key research subject.

To explore wheat self-sufficiency, we focused on endogenous and external variables. To begin, we used TURKSTAT's wheat self-sufficiency statistics (2000-2020). The Chi-Square Test and Correlation Analysis were used to investigate the association between wheat self-sufficiency and the other factors. According to the findings of the analysis, wheat production has been the most important variable in terms of self-sufficiency. This production value encompasses all types of production, such as useable production. This finding is consistent with previous research that has highlighted the relationship between agricultural self-sufficiency and production. Aside from production, losses are a significant challenge for self-sufficiency. As stated in the literature, providing food security and self-sufficiency is dependent on the balance between agricultural production and losses. Second, productivity is an important factor in increasing output. Productivity is intimately tied to production; a decrease in productivity has a direct impact on self-sufficiency. Climate change, as an exogenous element, is quite effective at this stage. The analyses revealed that rising temperatures and decreasing precipitation would have a detrimental impact on wheat yield and productivity. Wheat yields are anticipated to fall in all regions of Türkiye in the 2050s and 2080s because of climate change.

In order to guarantee self-sufficiency and food security, arrangements should be established to ensure the long-term viability and productivity of regional wheat production throughout Türkiye. For instance, agricultural infrastructure should be developed from production through post-harvesting, including all linked issues, within agricultural policy. Furthermore, in terms of wheat policy, the government should help producers, and agricultural incentives for wheat should be expanded and administered at the level of developed countries. The buying price should be set at a greater level than the cost. Furthermore, input costs should be reduced. It could be solved with a solid technology foundation and agricultural innovation. Even if these new investments are overpriced, they offer significant potential for long-term production efficiency.

Under these conditions, increasing production and productivity is largely concerned with preserving all of their current values, such as soil, seed, and so on. The initial phase in this process is to create intervention forms to enhance cultivated areas while still protecting existing wheat production places such as large plains and agricultural lands. As is well known, arable areas in many countries have nearly surpassed their carrying capacity. Thus, while only a few countries may be able to expand their agricultural regions, Türkiye may be one of them. Despite the fact that Türkiye uses the majority of its agricultural land, it may be possible to use all arable land and reduce fallow lands. Existing agricultural land should be protected and prevented from being misused while arable land is being used, with no exceptions. According to this viewpoint, the framework or border of agricultural policy should be formed within the contexts of sustainability, food security, and self-sufficiency. Moreover, in a country like Türkiye with small and fragmented agricultural regions, land consolidation and mobilization of sustainable irrigation potential should be prioritized in terms of production efficiency.

In a nutshell, Türkiye, as a prominent agricultural country, should maintain present grain production potential for not only its own people but also for the global population. However, Türkiye's production is not at the expected level, despite the fact that it is self-sufficient in wheat. This research reveal providing self-sufficiency is fundamentally dependent on the production and its relationship to other challenges. It is obvious that climate change will also have a great long-term impact on productivity. Thus, arrangements should be established to ensure the long-term viability and productivity of regional wheat production throughout Türkiye. It can achieve this goal primarily by avoiding the loss of current agricultural areas suited for production and increasing productivity in accordance with climate change. A climate-change-compatible production model should be promoted in all locations where wheat production has already occurred or will occur, particularly in the regions of

South-Eastern Anatolia, Central Anatolia, Eastern Anatolia, and the Eastern Mediterranean. All of these reforms will secure the country's long-term food security and self-sufficiency. Finally, studies like this one, which is one of the priority projects for the relevance of agricultural production and its consequences in the pandemic process, should be expanded, particularly in agriculturally potential countries like Türkiye. These studies would benefit agricultural productivity in other producing countries throughout the world.

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Research Article

Cations-Base Application in Rubber Plantation: The Change of Calcium, Magnesium, and Potassium Status in the Soil and Leaves and Its Relation to Latex Yield

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Ultisols

Abstract: Nutrient balance in the soil support plant growth and yield. The objective of this study was aimed to obtain doses of calcium, magnesium, and potassium fertilizers in relation to the ratio of cations-base (Ca^{2+}, Mg^{2+}, K^{+}) to increase latex yield in rubber plants. The study was conducted on a rubber plantation in Dolok Masihul Sub-district, Serdang Bedagai District, North Sumatra, Indonesia, from January to August 2019. The treatment was used with three factors, including the first factor was $CaCO_3$ (0; 1 500 g/tree/year), the second factor of $MgSO_4.H_2O$ (0; 1 500; 3 000; 4 500 g/tree/year), and the third factor by KCl (0; 500; 1 000; 1 500 g/tree/year) in a Randomized Block Design (RBD) within three replicates. Results showed that the calcium of 1 500 g/tree/year increased the Mg-latex and latex yield by 160.70 g/tree/*tapping*. An increase in the three cations-base in soil, leaves, latex, and latex yield was also observed after the application of magnesium ranged by 1 500 to 4 500 g/tree/year. The potassium 500 - 1 500 g/tree/year increased the cations-base in soil, latex, and Ca-leaves. The interaction of calcium 1 500 + magnesium 1 500 - 4 500 and potassium 0-1 500 g/tree/year increased the *exchange-K*, Mg-latex, and also Mg- and K-leaves. The ratio of Ca:Mg:K in soil, leaves, and latex were 2: 1: 2 (optimum), 5: 1: 11 (high), and 1: 11: 32. The Ca, Mg, K in leaves and K-latex positively correlates and increases latex yield due to the three fertilizations.

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1. Introduction

Rubber plantations (*Hevea brasiliensis* Muell. Arg.) are the main commercial source of natural rubber (NR), an important raw material in several sectors, one of which is the tire industry (Vaysse et al., 2012). The area of rubber plantations worldwide is approximately 11.5 million ha, of which 90% was found in Southeast Asia countries, including Thailand, Indonesia, Malaysia, Vietnam, and India (Food and Agriculture Organization of the United Nations, 2016). However, most government-rubber

plantations in Indonesia are still monoculture. Meanwhile, the management of nutrients such as N, P, K, Ca, and Mg in monoculture rubber is a factor that influences growth and yield (Vrignon-Brenas et al., 2019). Thitithanakul et al. (2017) reported that the total nitrogen and total-P requirement of the clone RRIM600 rubber were 1191.08 and 112.52 mg/plant, while the clone RRIT251 required total-N and total-P were 1241.09 and 131.81 mg/plant, respectively. Moreover, Mokhatar et al. (2012) stated that N-P-K-Mg fertilization with a ratio of 10.7; 16.6; 9.5; 2.4 at 150% in the recommended dose or 28.13 kg ha^{-1} significantly increased the highest nutrient content of N, K, and Mg in the RRIM2001 clone rubber were 54.17; 61.90; and 92.86%, respectively compared to the control. Suchartgul et al. (2011) reported that the standard optimum requirements for macronutrients including N, P, K, Ca, and Mg in the leaves of clone RRIM600 rubber were 3.20; 0.25; 1; 1; >0.35%, respectively. Additionally, Correia et al. (2017) also reported that K_2O fertilization at a dose of 0.2 kg m^{-3} increased the total dry weight by 141.72 g/plant in the GT1 clone rubber.

The status and balance of nutrient in the soil considerably affect plant growth and yield (Öborn et al., 2003). However, certain nutrients have synergistic or antagonistic properties between ions. Voogt (1987) stated that the uptake of K, Ca, and Mg depends not only on the soil concentration but also on the content ratio; hence when one of the nutrients is in excess, this might lead to a deficiency of others. Furthermore, Nguyen et al. (2015) reported that a high concentration of Mg in the soil inhibits the absorption of K and Ca. Weerasuriya and Yogaratnam (1989); Singh et al. (2005) added that there is a strong antagonism between K and Mg uptake in rubber plants.

The existence of synergism or antagonism between Ca, Mg, and K ions in the soil cause an imbalance among the nutrients absorbed by plants. Therefore, balanced nutrient management, especially for K, Ca, and Mg in rubber plantations based on soil, leaf, and latex analysis, is needed. Balanced fertilization is expected to be used as a guideline for nutrient management based on nutrient ratios of K/Mg, K/Ca, and Mg/Ca in the soil, leaves, and latex of rubber plants. Therefore, this study was aimed to obtain doses of calcium, magnesium, and potassium fertilizers in relation to the cations ratio of Ca, Mg, and K to increase latex yield in mature-4 of rubber plants.

2. Material and Methods

2.1. Study area

This study was conducted at the afdeling-II, Sarang Giting Estate, PTPN-III, Serdang Bedagai District, North Sumatra, Indonesia, from January to August 2019. Sarang Giting Estate was selected due to its high latex productivity of 1,853.24 kg ha^{-1} $year^{-1}$ among other plantations with similar management of high metabolism clone RRIM712 aged 9 years and the ultisols soil type. Meanwhile, the sample plants were selected based on criteria for healthy plants with stem diameters ranging from 65 to 80 cm. References on the relationship between rubber stem diameter and nutrient uptake have not been reported, but Murbach et al. (2003) found that the nutrient uptake of K and Mg (73–86 and 27%) in rubber plants was higher in 13-years-old. Therefore, the selection of rubber plants in this study was based on plant age.

2.2. Initial soil analysis

Initial soil samples were collected using a soil drill with a depth of 0-30 cm in the weeding circle with a distance of 165-250 cm. A total of three samples were composited and analyzed in the analytical laboratory of PT. Socfin Indonesia (Table 1). Soil sampling depth was based on the findings of Song et al. (2022) that rubber plants absorb nutrients from a depth of 5–50 cm and then decrease as the depth increases.

Table 1. Initial soil characteristics of the study area

Soil characteristics	Method	Value	Classification*
Soil texture	Hydrometer	Sand= 31% Silt= 51% Clay= 18%	Silt loam
Total-N (%)	Khejdahl	0.15	Low
pH-H_2O	Electrometric	5.20	Acid
P_2O_5 (ppm)	Bray-II	40.80	Very high
Total-P (%)	Bray-II	0.035	Very low
CEC (me/100 g)	NH_4OAc (pH 7)	6.77	Low
Exchangeable-K (me/100 g)	NH_4OAc (pH 7)	0.40	Moderate
Exchangeable-Ca (me/100 g)	NH_4OAc (pH 7)	0.32	Very low
Exchangeable-Mg (me/100 g)	NH_4OAc (pH 7)	0.32	Very low
Exchangeable-Al (me/100 g)	KCl 1N	0.22	Very low

Note: *Soil Research Institute, (2009) with the criteria of total-N was low (0.1-0.2); pH 4.5-5.5 (acid); P_2O_5 >15 ppm (very high); total-P <5% (very low); cation exchange capacity (CEC) 5-16 me/100 g (low); exchangeable-K 0.4-0.5 me/100 g (moderate); exchangeable-Ca <2 me/100 g (very low); exchangeable-Mg <0.40 me/100 g (very low); exchangeable-Al <5 me/100 g (very low).

2.3. Study design

In this study, conversion dose requirements and a Randomized Block Design (RBD) with three factors were used. The first factor was calcium from calcium carbonate fertilizer ($CaCO_3$) at the rates of 0 (C_0) and 1 500 g/tree/year (C1). The second factor was magnesium from kieserite fertilizer ($MgSO_4.H_2O$) at the levels of 0 (M_0), 1 500 (M_1), 3 000 (M_2), and 4 500 g/tree/year (M_3). The third factor was potassium from KCl fertilizer at the rates of 0 (K_0), 500 (K_1), 1 000 (K_2), and 1 500 g/tree/year (K_3). All treatments were conducted by three replications. The rubber plants were fertilized by making an oval-row placement array 5 cm deep and then covered with soil. Fertilization was performed every two months alternately, with the first application at a distance of 2.5×1.65 m (zone-A) and the second at a distance of 5×3.3 m (zone-B) from the plant (Figure 1). The requirement for Mg and K fertilizers was based on the initial soil analysis shown in Table 1, while the Ca fertilizers are based on 1.5 times from exchangeable-Al. Furthermore, the results were converted to determine the dose of fertilizer/ha.

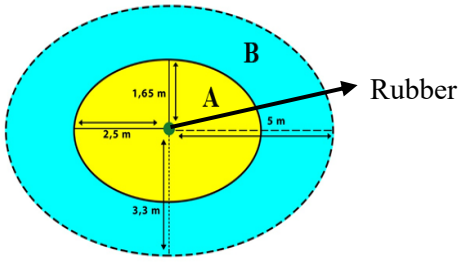

Figure 1. Fertilization design on rubber plants in the study area. The first (A) and the second (B) application zones.

2.4. Parameters and data analysis

The parameters in this study, including soil, plant tissue (leaves and latex), latex yield, cations-base ratio, and correlation analysis, were measured at eight Months After Fertilization (MAF). The soil samples were collected using a drill at a depth of 0-30 cm in the weeding circle area, while a total of three samples were composited. Afterward, soil exchangeable (Ca, Mg, K) were analyzed using the ammonium acetate saturation method pH 7. The leaves and latex sampling was implemented at 10.00-11.00 AM with 30 and 10 g samples, respectively. The leaves samples were initially soaked in 70% alcohol for several minutes and then dried, while the latex was only dried. Furthermore, the nutrient content of Ca, Mg, and K in the leaves and latex samples were analyzed using the ammonium acetate saturation method pH 7.

The latex yield was conducted by tapping every four days, followed by weighing the fresh latex in g/tree/tapping. Meanwhile, parameters of nutrient ratio for Ca, Mg, and K with latex yield were measured for the interaction of the three fertilizations with the highest latex yield. The ratios of K/Mg, K/Ca, and Mg/Ca in the soil and leaves were classified according to Suchartgul et al. (2011). The soil and leaves were consisted of low category ratios of K/Mg, K/Ca, and Mg/Ca (<2; <0.4; <0.2 and <3;

<0.8; <0.3), the optimum classified (2-6; 0.4-1.4; 0.2- 0.6; and 3.0-4.2; 0.8-1, 4; 0.3-0.5), and the high classified (>6; >1.4; >0.6 and >4.2; >1.4; >0.5). Data on the content of Ca, Mg, and K in soil, leaves, latex, and latex yield were analyzed using ANOVA, while the significance was further examined using the DMRT at $P<0.05$. Additionally, all data in natural logarithm and Pearson correlation analysis was performed using IBM SPSS v.20 software to obtain the relationship between the cations-base in soil, leaves, and latex on the latex yield.

3. Results

3.1. Cations-base (Ca, K, Mg) in soil

Fertilization with magnesium, potassium, and the third interaction of fertilizers significantly increased cations-base (Ca, Mg, K) in the soil, as shown in Tables 2 and 3. However, calcium fertilization was less effective in increasing soil cations at the 8 MAF. This is demonstrated in the control treatment, which provided higher soil exchangeable (Ca and Mg) compared to a dose of 1 500 g/tree/year. A similar result was also observed in the untreated magnesium fertilizer which had higher the exchangeable-Ca. Meanwhile, magnesium fertilization at doses of 4 500 and 1 500 g/tree/year increased soil exchangeable (Mg and K) by 20.41% and 1.33%, respectively, compared to the control.

Potassium fertilization with a dose of 1 500 g/tree/year showed the highest increase in the cations-base (Ca, Mg, K) in the soil at 9.72, 45.45, and 68.00% compared to the control. Meanwhile, the interaction of 0 g Ca+4 500 g Mg+1 500 g K and 1 500 g Ca+1 500 g Mg+1 500 g K produced the highest increase in the cations-base (Mg, K) in soil by 77.42 and 221.43%. However, the combined interaction was less effective in increasing the exchangeable-Ca in soil.

Table 2. Effect of calcium, magnesium, and potassium fertilization on cations-base in soil, nutrient content (in leaves and latex), and latex yield of mature-4 in rubber plants at the 8 Months After Fertilization (MAF)

Fertilization doses (g/tree/year)	Cations-base in soil (me/100 g)			Nutrient content in leaves (%)			Nutrient content in latex (%)			Latex yield (g/tree/tapping)
	Exch-Ca	Exch-Mg	Exch-K	Ca	Mg	K	Ca	Mg	K	
Calcium (Ca)										
0	0.76a	0.56a	0.73ns	0.93a	0.27ns	2.12ns	0.017a	0.08b	0.37ns	142.08b
1 500	0.71b	0.47b	0.71ns	0.87b	0.28ns	2.11ns	0.015b	0.10a	0.38ns	160.70a
Magnesium(Mg)										
0	0.79a	0.49b	0.75ab	0.96a	0.28ns	2.07ab	0.017a	0.09b	0.42a	138.88b
1 500	0.67c	0.45c	0.76a	0.91ab	0.28ns	2.20a	0.016a	0.08bc	0.39ab	158.70a
3 000	0.78a	0.52b	0.72b	0.85c	0.28ns	2.17a	0.013c	0.07c	0.32c	169.70a
4 500	0.70b	0.59a	0.65c	0.88bc	0.26ns	2.02b	0.019a	0.11a	0.38b	138.28b
Potassium (K)										
0	0.72b	0.44c	0.50d	0.93ab	0.28ns	2.14ns	0.016bc	0.10a	0.38b	148.51ns
500	0.70b	0.50b	0.74c	0.96a	0.27ns	2.10ns	0.016b	0.09a	0.36b	148.63ns
1 000	0.73b	0.47bc	0.80b	0.87bc	0.28ns	2.06ns	0.019a	0.08b	0.36b	152.81ns
1 500	0.79a	0.64a	0.84a	0.85c	0.27ns	2.15ns	0.014c	0.09a	0.42a	155.62ns

Note: the values followed by the same letter in the same column are not significantly different at the 5% level based on the DMRT. ns= not significant.

3.2. Nutrients content (Ca, Mg, K) in leaves

Fertilization with calcium and potassium only significantly increased the nutrients content of Ca, but it was insignificantly for Mg and K in the leaves. Meanwhile, magnesium fertilization significantly increased the nutrients content of Ca and K, but it was insignificantly for Mg-leaves. Moreover, the third interaction of fertilizers significantly increased the nutrients content for Ca, Mg, and K-leaves after 8 MAF is presented in Tables 2 and 3. The results also showed that calcium fertilization was less effective in increasing Ca-leaves. This is seen in the untreated calcium fertilizer which produced higher Ca levels compared to a dose of 1 500 g/tree/year.

A similar result was also observed in the untreated of magnesium fertilization which had higher Ca levels compared to another dose. Magnesium fertilization at a dose of 1 500 g/tree/year showed the

highest increase of K-leaves by 6.28%, while potassium with a dose of 500 g/tree/year caused the highest in Ca levels by 3.23%. Furthermore, the interaction of 0 g Ca+4 500 g Mg+1 000 g K showed the highest increase of Ca-leaves by 34.51%. Likewise, the interaction of 1 500 g Ca+1 500 g Mg+1 000 g K increased the highest of Mg and K-leaves by 93.75 and 30.19%, respectively.

Table 3. Effect of the third interaction of fertilization on cations-base in soil, nutrient content (in leaves and latex), and latex yield of mature-4 in rubber plants at the 8 Months After Fertilization (MAF)

Interaction (C×Mg×K)	Cations-base in soil (me/100 g)			Nutrient content in leaves (%)			Nutrient content in latex (%)			Latex yield (g/tree/tapping)
	Exch-Ca	Exch-Mg	Exch-K	Ca	Mg	K	Ca	Mg	K	
$C_0M_0K_0$	1.16a	0.62cd	0.42i	1.13bcd	0.32cd	2.12b-h	0.010j-p	0.13b-e	0.31h-j	109.83ns
$C_0M_0K_1$	0.56k-n	0.42fgh	0.76a-i	1.06b-e	0.30c-e	2.30a-f	0.018e-h	0.07g-k	0.35f-j	133.61ns
$C_0M_0K_2$	0.58k-n	0.41f-i	0.69a-i	0.92d-h	0.24e-k	2.18b-g	0.013i-p	0.06h-k	0.44c-g	115.39ns
$C_0M_0K_3$	0.61j-m	0.37ghi	0.82a-i	0.64i-l	0.28c-g	2.44a-d	0.014h-n	0.04j-k	0.49a-e	149.89ns
$C_0M_1K_0$	0.61j-m	0.38f-i	0.49ghi	0.88e-h	0.24d-k	1.88f-j	0.030b	0.09e-h	0.35f-j	148.22ns
$C_0M_1K_1$	0.79d-g	0.44fgh	0.79a-i	1.28ab	0.24d-k	2.13b-h	0.009p	0.06g-k	0.32g-j	114.06ns
$C_0M_1K_2$	0.49no	0.29i	0.71a-i	0.92d-h	0.19j-k	2.09b-i	0.021cde	0.06h-k	0.44c-g	151.83ns
$C_0M_1K_3$	0.85cd	1.01a	0.96a-e	0.82e-j	0.24d-k	1.92e-j	0.021def	0.16bc	0.58ab	149.44ns
$C_0M_2K_0$	0.71ghi	0.51def	0.62b-i	0.93d-h	0.30c-f	2.27b-f	0.011j-p	0.06g-k	0.37e-j	140.50ns
$C_0M_2K_1$	0.90bc	0.64cd	0.54c-i	0.95d-h	0.27c-i	2.50ab	0.014h-l	0.03k	0.18k	150.00ns
$C_0M_2K_2$	0.78d-g	0.58cde	1.29ab	0.56k-l	0.22f-k	2.12b-h	0.019efg	0.04i-k	0.16k	156.67ns
$C_0M_2K_3$	0.89bc	0.76b	0.86a-f	0.91d-h	0.52b	2.18b-g	0.014h-o	0.09e-h	0.27i-k	156.50ns
$C_0M_3K_0$	0.55lmn	0.36ghi	0.43i	0.59j-l	0.24d-k	2.03d-j	0.012i-p	0.04j-k	0.27i-k	154.67ns
$C_0M_3K_1$	0.45o	0.41f-1	0.45hi	0.77h-k	0.30c-f	2.04c-j	0.015g-k	0.09e-i	0.46b-f	129.61ns
$C_0M_3K_2$	1.09a	0.62cd	1.16a-d	1.52a	0.24e-k	1.64i-j	0.035a	0.16bc	0.36f-j	157.94ns
$C_0M_3K_3$	1.10a	1.10a	0.65b-i	1.03b-g	0.19k	2.03c-j	0.009p	0.10d-g	0.59a	155.17ns
$C_1M_0K_0$	0.84cde	0.49efg	0.58c-i	0.90d-h	0.28c-h	2.07b-i	0.024cd	0.08f-j	0.42c-h	137.44ns
$C_1M_0K_1$	0.96b	0.65bc	1.27abc	0.79g-k	0.22g-k	1.76g-j	0.026bc	0.17b	0.53abc	129.94ns
$C_1M_0K_2$	0.75e-h	0.38ghi	0.61b-i	1.21bc	0.28c-g	1.75g-j	0.015g-j	0.04i-k	0.37d-i	186.22ns
$C_1M_0K_3$	0.85cd	0.59cde	0.85a-g	1.05b-f	0.32cd	1.94e-j	0.011j-p	0.14bcd	0.40d-i	148.72ns
$C_1M_1K_0$	0.56k-n	0.37ghi	0.43i	1.05b-f	0.19j-k	2.08b-i	0.011j-p	0.07g-k	0.47a-f	172.50ns
$C_1M_1K_1$	0.68hij	0.40f-i	0.65b-i	1.03c-g	0.30c-e	2.24b-f	0.012i-p	0.04j-k	0.28i-j	183.00ns
$C_1M_1K_2$	0.73f-i	0.42fgh	0.67b-i	0.81f-j	0.62a	2.76a	0.012i-p	0.12c-f	0.50a-d	173.22ns
$C_1M_1K_3$	0.64i-l	0.31h	1.35a	0.50i	0.22f-k	2.53ab	0.013i-p	0.03k	0.19k	177.33ns
$C_1M_2K_0$	0.78d-g	0.45fg	0.56c-i	1.04b-g	0.32cd	2.20b-g	0.005p	0.05h-k	0.42c-h	174.44ns
$C_1M_2K_1$	0.74fgh	0.42fgh	0.77a-i	0.95d-h	0.20i-k	2.15b-h	0.014h-m	0.15bc	0.45b-g	215.00ns
$C_1M_2K_2$	0.81c-f	0.40f-i	0.58c-i	0.44l	0.19j-k	1.62j	0.011j-p	0.06g-k	0.28i-k	172.61ns
$C_1M_2K_3$	0.64ijk	0.38f-i	0.53ghi	0.99c-h	0.20h-k	2.31a-f	0.012i-p	0.08g-j	0.47a-f	191.89ns
$C_1M_3K_0$	0.54mn	0.36ghi	0.45hi	0.89d-h	0.34c	2.49abc	0.021def	0.24a	0.40d-i	150.44ns
$C_1M_3K_1$	0.54mn	0.65bc	0.67a-i	0.83e-j	0.30cde	1.71h-j	0.016f-i	0.12c-f	0.27j-k	133.78ns
$C_1M_3K_2$	0.61j-m	0.64cd	0.65b-i	0.57k-l	0.27c-j	2.34a-e	0.021cde	0.06g-k	0.28i-k	108.61ns
$C_1M_3K_3$	0.74fgh	0.59cde	0.70a-i	0.84e-i	0.21g-k	1.86f-j	0.019efg	0.08f-j	0.38d-i	116.00ns

Note: the values followed by the same letter in the same column are not significantly different at the 5% level based on the DMRT. ns= not significant. Calcium fertilization (C_0= 0; C_1= 1 500 g/tree/year); magnesium fertilization (M_0= 0; M_1= 1 500; M_2= 3 000; M_3= 4 500 g/tree/year); and potassium fertilization (K_0= 0; K_1= 500; K_2= 1 000; K_3= 1 500 g/tree/year).

3.3. Nutrients content (Ca, Mg, K) in latex

Fertilization with magnesium, potassium and the third interaction of fertilizers significantly increased the nutrients content of Ca, Mg, and K in the latex of mature-4 rubber plant at 8 MAF. Meanwhile, calcium fertilization significantly increased the nutrients content of Ca and Mg, but it was insignificantly of K-latex (Tables 2 and 3). The results showed that calcium fertilization was less effective in increasing Ca levels in latex. This is indicated in the untreated calcium fertilization which produced higher Ca levels, but a dose of 1 500 g/tree/year significantly increased Mg-latex by 25.00% compared to the control. A similar result was also observed in the control, which had higher K levels compared to another of magnesium. However, a dose of 4 500 g/tree/year produced the highest increase in Ca and Mg of 11.76 and 22.22%, respectively. Meanwhile, potassium fertilization at doses of 1 000 and 1 500 g/tree/year showed the highest increase in Ca and K levels of 18.75 and 10.53%, respectively, but it was less effective in increasing Mg-latex. The interaction of 0 g Ca+4 500 g Mg+1 000 to 1 500 g K and 1 500 g Ca+4 500 g Mg+0 g K showed the highest increase in Mg and K levels were 3.5 times; 90.32% and 84.62%, respectively.

3.4. Latex yield

Calcium and magnesium fertilization significantly increased latex yield, but potassium and the third interaction of fertilizers were insignificantly on the latex yield of mature-4 rubber plants (Tables 2 and 3). Fertilization of calcium at 1 500 g and magnesium at 3 000 g showed the highest latex yield of 13.11 and 22.19%, respectively, compared to the control. Although potassium had an insignificant effect, there was an increase in latex yield along with an increase in the doses of potassium fertilizer until 1 500 g/tree/year. Likewise, the highest latex yield was obtained with an interaction of 1 500 g Ca+3 000 g Mg+500 g K by 95.76% compared to the control.

3.5. Nutrients ratio

The nutrients ratio of Ca, Mg, and K in rubber plants with the highest latex yield were obtained from the interaction of calcium 1 500 g+magnesium 3 000 g+potassium 500 g as shown in Table 4. Based on the results, the nutrient ratios of Ca:Mg:K in soil, leaves and latex due to the three fertilizations were 2: 1: 2; 5: 1: 11; and 1: 11: 32, respectively. The ratio of K/Mg, and K/Ca was classified as optimum in the soil and high in the leaves, but Mg/Ca ratio was classified as low. These three ratios indicate that the optimal nutrient ratio in the soil support nutrient uptake both to the leaves and latex of the rubber plants. Moreover, the nutrients uptake of K and Mg tends to be abundant in latex compared to the leaves, while Ca was higher in the leaves.

Table 4. Nutrient ratios of Ca, Mg, and K in rubber plants with the highest latex yield at the interaction of calcium (1 500 g)+magnesium (3 000 g)+potassium (500 g)

Analysis	Nutrient content			Nutrient ratios					
	Ca	Mg	K	Ca/K	Ca/Mg	Mg/K	K/Mg	K/Ca	Mg/Ca
Soil (me/100 g)	0.74 (2)	0.42 (1)	0.74 (2)	1.00	2.00	0.50	2.00	1.00	0.50
Leaves (%)	0.95 (5)	0.20 (1)	2.15 (11)	0.45	5.00	0.09	11.00	2.20	0.20
Latex (%)	0.014 (1)	0.15 (11)	0.45 (32)	0.03	0.09	0.34	2.91	32.00	11.00

3.6. Correlation analysis

Correlation analysis between nutrients content of Ca, Mg, and K in soil, leaves, and latex on the latex yield are shown in Table 5.

Table 5. Correlation analysis between nutrients content of Ca, Mg, K in soil, leaves, and latex on the latex yield of mature-4 rubber plant

Correlation analysis	Ca	Mg	K	Latex yield
Soil				
Ca	1			
Mg	0.635**	1		
K	0.318**	0.263**	1	
Latex yield	0.053	-0.180	0.106	1
Leaves				
Ca	1			
Mg	0.262**	1		
K	0.108	0.414**	1	
Latex yield	0.225*	0.232*	0.324**	1
Latex				
Ca	1			
Mg	0.364**	1		
K	0.080	0.584**	1	
Latex yield	0.008	0.184	0.271**	1

Note: ** and *Correlation is significant at the 0.01 and 0.05 level (2-tailed). n= 96.

Nutrients content of Ca and K in soil positively correlated, while Mg negatively correlated to latex yield. In the leaves, the Ca, Mg, and K nutrients were significantly and positively correlated on the latex yield. Furthermore, K nutrient contributed higher in latex yield compared to Ca and Mg nutrients.

The K nutrient in the latex was significantly and positively correlated in latex yield, while Ca and Mg nutrients in latex were positively correlated, but it was an insignificant effect.

4. Discussion

4.1. Effect of calcium fertilization

Calcium fertilization statistically affected cations-base (Ca and Mg) in soil, Ca-leaves, Ca and Mg-latex, and also the latex yield (Tables 2 and 3). It also had a slight effect in increasing cations-base (Ca and Mg) in the soil, and Ca in leaves and latex of mature-4 rubber plants at 8 MAF. The calcium fertilization had a weak effect on latex yield indicated by a higher yield found in the untreated compared to a dose of 1 500 g/tree/year. The use of 1 500 g/tree/year increased Mg in latex and the yield by 25.00 and 13.11%, respectively. This is due to the K^+ ion, which has a lower valence electron and is highly absorbed compared to the Mg^{2+} and Ca^{2+} ions, even though calcium fertilization is applied. This result is linear with Ca nutrient, which had a positive correlation and highly significant (0.364**) to Mg in latex, and it had a positive correlation (0.008) for the latex yield (Table 5).

A similar result was reported by White (2001) that the cations uptake, such as Mg^{2+} and K^+, competed with Ca^{2+} in plant roots. Mengel et al. (2001) added that potassium ions are more efficiently absorbed than Ca or Mg due to the effectiveness of the H^+/K^+ symport enzyme, namely a protein that transports H^+ and K^+ simultaneously across the root cell membrane. Furthermore, White and Broadley (2003) reported that Ca activates various metabolisms in plant tissues, such as cell growth, cytoplasmic flow, mitosis, cytokinesis, and activator of enzymes associated with Ca-binding proteins. This calcium mechanism causes latex yield to increase by 13.11% (Table 4). Zhu et al. (2018) stated that calcium-dependent protein kinases play a key role in tissue interactions. This indicates that protein kinase and protein phosphorylation are important in ethylene signaling in rubber latex. Wang et al. (2015) also reported that the level of protein phosphorylation and expression of calcium-binding protein kinase in latex increased significantly after ethylene stimulation.

4.2. Effect of magnesium fertilization

Magnesium fertilization statistically affected the cations-base (Ca, Mg, K) in soil, the nutrients content of Ca and K in leaves, nutrients content of Ca, Mg, K in latex, and the latex yield of mature-4 rubber plants (Tables 2 and 3). Its application at a dose of 0 g/tree/year produced higher exchangeable-Ca in soil, Ca-leaves, and K-latex compared to other doses of magnesium, but at the dose of 1 500 g/tree/year increased exchangeable-K in soil and K-leaves by 1.33 and 6.28%, respectively. Similarly, magnesium at a dose of 4 500 g/tree/year increased the exchangeable-Mg, Ca, and Mg-latex by 20.41; 11.76; and 22.22%, respectively. Furthermore, the highest increase in latex yield of 22.19% was produced with a dose of 3 000 g/tree/year. Magnesium fertilization at a dose of 1 500 to 4 500 g/tree/year increased cations-base in soil, nutrients content in leaves and latex, as well as the latex yield of rubber plants. This indicates that there is a synergy between Mg and K nutrients in certain metabolic processes of plants.

These results are linear with Mg nutrients which had a positive correlation and highly significant with K in soil, leaves, and latex were 0.263**; 0.414**; and 0.584**; respectively, and Ca-latex of 0.364**. A similar content of Mg-leaves and latex correlated positively (0.232* and 0.184) on the latex yield (Table 5). According to Guo et al. (2010), Mg had a synergistic effect on potassium transport to the plant shoots, photosynthetic electron transport, photoassimilate formation, sucrose filling in the phloem, and nitrogen metabolism. Moreover, Karley and White (2009) added that K^+ and Mg^{2+} had different roles during transport in the xylem and phloem tissues, although both are highly mobile cations. Meanwhile, Tromp and van Vuure (1993) reported that Mg^{2+} is more easily absorbed by parenchymal cells than K^+ due to its higher valency. An unbalanced K/Mg ratio resulted in a higher concentration and transport rate of K^+ than Mg^{2+}. Based on the results, the ratio of K/Mg in soil, leaves, and latex were 2; 11; and 2.91, respectively (Table 4), which indicates that the concentration of K was higher compared to Mg. It has been reported by Hytönen et al. (2019) that the Mg content is higher in leaves and branches in a size less than 3 mm in rubber plants of 3.5 and 2.7 g kg^{-1}, respectively. Murbach et al. (2003) also added that Mg in latex of 13-year-old rubber plants was 0.4 kg ha^{-1} in Brazil.

4.3. Effect of potassium fertilization

Potassium fertilization statistically affected cations-base (Ca, Mg, K) in soil, Ca nutrient in leaves, and nutrients content (Ca, Mg, K) in latex, but it was insignificant to the latex yield of mature-4 rubber plant at 8 MAF (Tables 2 and 3). The dose of 500 g/tree/year produced the highest Ca of 3.23% in leaves, while the dose of 1 000 g/tree/year increased the highest Ca of 18.75% in latex compared to the control. Furthermore, potassium increased the highest cations-base (Ca, Mg, K) in soil and K-latex at 9.72, 45.45, 68.00, and 10.53%, respectively, but it was less effective in increasing Mg nutrient in latex. In general, potassium fertilization at a dose of 500 - 1 500 g/tree/year increased soil cations-base, nutrients in leaves and latex, but it was an insignificant effect on the latex yield of rubber plants. This is linear with the nutrient ratio of K/Ca in leaves and latex by 2.20 and 32, while K/Mg was 11 and 2.91, respectively (Table 4).

This indicates that the content of K nutrients in the leaves and latex tissue are more highly absorbed than Ca and Mg. Also, this finding authenticates that nutrient K had an insignificant effect on Ca-leaves by 0.108 and latex by 0.080. In contrast, K had a positive correlation and significance on the cations-base (Ca and Mg) in the soil of 0.318** and 0.263**, respectively (Table 5). This result is supported by Rhodes et al. (2018) ,who stated that potassium fertilization up to 300 kg ha^{-1} significantly increased the K concentration in the leaves, but an increase in the K concentration causes a decrease in Ca and Mg in the leaves. Fageria (1983) reported that a low concentration of K^+ stimulates Mg^{2+} absorption and vice versa. Furthermore, Xie et al. (2021) also reported that the antagonistic effect of K to Mg is stronger compared to Mg with K in root absorption and transport. Therefore, K and Mg fertilization must be balanced to reduce Mg deficiency. Amiri et al. (2022) added that potassium fertilization was highly significant for grain yield and harvest index of *Cyamopsis tetragonoloba*. Abu-Alama et al. (2022) also found potassium fertilizer dose of 80 kg/ha significantly increased the highest sugarcane yield by 9.93% compared to control.

4.4. Interaction of calcium, magnesium, and potassium fertilization

The interaction of Ca×Mg×K fertilization significantly increased the cations-base in soil, nutrient content (Ca, Mg, K) in leaves and latex, but it was insignificant to latex yield of mature-4 rubber plant at 8 MAF (Tables 2 and 3). The interaction of 0 g Ca+4 500 g Mg+1 000 g K increased the highest of Ca-leaves and latex by 34.51% and 3.5 times, while the interaction of 0 g Ca+4 500 g Mg+1 500 g K increased the Mg cation in soil and K-latex of 77.42 and 90.32%, respectively. In general, the combination untreated of calcium fertilizer+magnesium 4 500 g+potassium 1 000 to 1 500 g affected the cations-base in soil, nutrients content in leaves, and latex of mature-4 rubber plant. It was indicated that magnesium fertilization is synergistic with potassium, even untreated calcium fertilizer. This is demonstrated by the K/Mg, K/Ca, and Mg/Ca ratios in the soil of 2, 1, and 0.5 (classified as optimum), and this ratio can be seen in Tab 4. In addition, potassium was positively correlated and significantly with magnesium in the soil, leaves, and latex, but potassium was positively correlated and insignificant with calcium in leaves and latex (Table 5). These results are supported by Guo et al. (2010) that Mg nutrient synergizes with K-transport to the plant shoots, photosynthetic electron transport, photoassimilate formation, sucrose filling in the phloem, and nitrogen metabolism. Suchartgul et al. (2011) reported that the optimum range of K/Mg, K/Ca, and Mg/Ca ratios in the soil on rubber plants of RRIM-600 clones were 2-6; 0.4-1.4; and 0.2-0.6, respectively. Moreover, Mg content in leaves and exchangeable-K in soil increased the stem diameter of rubber plants were 43.14 and 35.78%, but the exchangeable-Ca in the soil only contributed by 6.04%.

The interaction of 1 500 g Ca+1 500 g Mg+1 500 g K and 1 500 g Ca+4 500 g Mg+0 g K increased the exchangeable-K and Mg content in latex by 221.43 and 84.62%, respectively. Moreover, the interaction of 1 500 g Ca+1 500 g Mg+1 000 g K increased the highest Mg and K content in the leaves by 93.75 and 30.19%. In general, the combination of calcium 1 500+magnesium 1 500 to 4 500+potassium 0 until 1 500 g/tree/year also affected the exchangeable-K in soil, Mg, and K-leaves, as well as Mg-latex of rubber plants. This is due to the nutrient ratio of K/Mg, K/Ca, and Mg/Ca in the soil were classified as optimum (2: 1: 0.5), while K/Mg and K/Ca ratios in the leaves were classified as high by 11: 2.2 (Table 4). It is supported by the positive correlation and significance between K and Mg content in soil, leaves, and latex, as well as the relationship between Mg and Ca (Table 5). According to Xie et al. (2021), the optimal ratio of K/Mg in soil and plant tissue is used to defend the nutrients and

support physiology processes to increase the plant yield. Furthermore, Suchartgul et al. (2011) also reported that the nutrients of K and Ca in the leaves increase the stem diameter of rubber plant clones RRIM-600 by 48.88 and 46.68%, respectively.

Conclusion

Fertilization with calcium (Ca) at a dose of 1,500 g/tree/year increased the Mg-latex and the latex yield by 25.00 and 13.11%. Meanwhile, magnesium (Mg) fertilizer at the dose of 1 500 g/tree/year increased the exchangeable-K in soil and K-leaves by 1.33 and 6.28%. Likewise, the dose of 3 000 g/tree/year had the highest latex yield of 22.19%. The 4 500 g/tree/year dose also increased the exchangeable-Mg in soil, Ca, and Mg-latex by 20.41; 11.76; and 22.22%. Furthermore, potassium (K) fertilizer at a dose of 500 g/tree/year increased the highest Ca-leaves by 3.23%, and the dose of 1 000 g/tree/year increased the highest Ca-latex by 18.75%, as well as the dose by of 1 500 g/trees/year, increased the highest cations-base (Ca, Mg, K) in soil, and K-latex by 9.72; 45.45; 68.00; and 10.53%, respectively.

The interaction untreated of calcium fertilization+magnesium 4 500+potassium 1 000 to 1 500 g/tree/year increased the exchangeable-Mg in soil, Ca-leaves, Ca, and K-latex. Meanwhile, the interaction of calcium 1 500+magnesium 1 500 to 4 500+potassium 0 until 1 500 g/tree/year increased the exchangeable-K, Mg-latex, Mg, and K-leaves. The interaction of calcium 1 500+magnesium 3 000+potassium 500 g/tree/year had the highest latex yield of 95.76% compared to the control. The nutrient ratios of Ca:Mg:K in the soil, leaves and latex due to the combined fertilizations were 2: 1: 2 (optimum), 5: 1: 11 (high), and 1: 11: 32. The cations-base of Ca and K in soil, nutrients content (Ca, Mg, K) in leaves and latex had a positively correlated with latex yield due to the third fertilizations.

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Research Article

Determination of the Effect of Salt Stress on Germination, Biochemical and Antioxidant Enzyme Activities in Linas Safflower Seeds

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Abstract: In this study, the germination and early seedling growth, biochemical and antioxidant enzyme activities (CAT, SOD, POD, and APX) of one-year, broad-leaved Linas safflower belonging to the Compositeae family were investigated at different salt concentrations (0, 50, 100, 150 and 200 mM). With increasing salt concentration, a 68.83% decrease in seedling length, 71% in stem length, 34% in germination rate, and 77% in fresh plant weight were determined. In addition, total phenolic content (267%), total flavonoid content (904%), CAT (462%), SOD (56%), POD (100%), and APX (381%) antioxidant enzyme activities were increased in parallel with the salt concentration. In addition, it was determined that as the salt stress increased, the water-soluble protein content decreased by 48%. In the study, it was determined that the seeds were relatively resistant to 100, 150, and 200 mM NaCl salt concentrations, and germination continued. As a result, it has been understood once again that our country has been feeling a negative impact lately, and the determination of alternative plants for growing oily plants has gained more importance in these days. Safflower, which is one of these plants, is a strategically important species both in terms of its oil content and being a source of biodiesel. This study carried out in this context will be a resource for our farmers regarding future studies on safflower seeds and which salt concentrations can be used for cultivation.

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1. Introduction

Most of the water resources in the world (70%) are salty. Therefore, considering that a quarter of the entire pedosphere (950 x 10^6 ha) is affected by salt, it shows up that 23% of the 1.5 x 10^9 ha cultivated land is saline (Glenn and O'Leary, 1985; Rhoades and Loveday, 1990; Flowers and Yeo, 1995). In addition, nearly half of the existing irrigation systems in the world (3 x 108 ha) are under the influence of secondary salinization, flooding, and alkalization, and therefore approximately 10 x 10^6 ha of irrigated land is abandoned each year (Szabolcs, 1987). Such unsuitable soils with low productivity are generally not suitable for agricultural production and cause yield loss. For these reasons, researchers have divided plants into halophytes and glycophytes. Halophytes are salinity tolerant plants that have adapted to saline environments and even benefit from high salt concentrations for optimum growth (Su

et al., 2020). On the other hand, glycophytes are salinity sensitive plants whose growth and development are adversely affected by soil salinity (Horie et al., 2012). Most cultivated plants are glycophytes. High salinity prevents the growth and development of glycophytes and severely limits productivity. Due to the increasing need for food production and the increasing distribution of salinity-affected soils, research on the response of plants to salinity has expanded rapidly in recent years. İncluding changes at the morphological, physiological, and molecular levels, studies of plant tolerance to salt stress cover many aspects of the effects of salinity on plant metabolism. In recent years, researchers have focused more on biotechnology, transgenic plants, improvement of breeding methodologies, and modification of the genetic structure of existing plants aimed at greater adaptation to salinity conditions. Physiological and biochemical changes caused by stress conditions have been frequently investigated by different researchers from past to present (Flowers et al., 1977; Greenway and Munns, 1980; Munns et al., 1983; Munns, 2002; Yildirim et al., 2021a; Yildirim et al., 2021b; Demir and Basayigit, 2021; Altun and Arslan, 2022).

Reactive oxygen species (ROS) synthesized under stress conditions increase free radicals in plant cells and cause oxidative stress in the cell. In such situations, plants develop antioxidant defense mechanisms to survive. Among these mechanisms, non-enzymatic antioxidants such as total phenolics, total flavonoids, and proline, and enzymatic antioxidants such as CAT, APX, SOD, and POD play an important role in the defense systems of plants against stress (Pérez-Pérez et al., 2012; Zhou et al., 2013; Shangguan et al., 2018).

Seed germination is the first and very important stage in the life cycle of plants. Germination is one of the most vital periods for a seed exposed to salinity (Dutta and Bera, 2014). Salinity prevents water absorption and seed germination by increasing the toxic effects of ions such as Na^+ and Cl^- (Turhan and Ayaz, 2004). Therefore, the salt tolerance of seeds during germination is critical for the growth of plants in saline soils (Khan et al., 1998). It is also known that high temperature interacts with salinity and increases the effect of stress conditions on the germination and development of many plants (Nedjimi, 2013). Therefore, the most basic approach to be followed in cases where the salinity problem in the soil can not be solved in the short term is to determine the salt tolerant species (Oral et al., 2019).

Safflower is an annual, broad-leaved, yellow, orange, red, white, and cream-colored oil plant from the family of Compositeae (Eryilmaz et al., 2014). In this study, it was purposed to determine the effect of salt stress on germination and antioxidant defense systems in Linas safflower seeds.

2. Material and Methods

In this study were used to Linas safflower cultivar seeds. The seeds were selected homogeneously, with uniform size, maturity, and appearance, and free from defects. The study was carried out on a total of 350 seeds, with 5 replications and 14 seeds in each replication. Plant seeds were first sterilized in 10% sodium hypochlorite for 10 minutes. Then, surface sterilization was performed with 80% ethanol and washed in distilled water for 3 x 5 minutes. Germination experiments of seeds were carried out in petri boxes containing double-layered sterile filter paper, 20/25ºC (in the dark) temperature, and blotter treated with different NaCl concentrations (0, 50, 100, 150, and 200 mM) for 7 days. Distilled water was used in the control treatment. Physiological parameters such as germination rate, seedling height, and stem length, and fresh weight were used to determine salt tolerance compared with control conditions. In addition, biochemical and antioxidant enzyme activities were also examined in plants during salt stress. Biochemical and antioxidant enzyme analyzes were carried out from the stem part of the seedling. For germination, the radicle length reached 10 mm (ISTA, 2019). For germination, the radicle was expected to reach a length of 10 mm (ISTA, 2019). The germinated seeds were counted every day to determine the germination percentage. Seedling length (cm), stem length (cm), germination rate (%), and fresh weight (mg/seedling) were determined at the end of the 7th day.

Total soluble protein content was determined according to the method of Hartree-Lowry (1972). Results were expressed as mg/ml

Total phenolic content was determined using the Folin-Ciocalteu method specified by Singleton and Rossi (1965). Results are expressed as mg GAE/g

Total flavonoid content was determined according to the method of Zhishen et al. (1999). Results are expressed as mg catechin/g.

POD activity was determined according to the method of Jiang et al. (2010). Results are expressed as Δ_{A460}/min/mg protein.

SOD activity was determined according to the method of Jiang et al. (2010). Results are expressed as U/mg protein.

APX activity was determined according to the method of Nakano vd. (1981). Results are expressed as mol/min/g protein.

CAT enzyme activity was determined according to the method of Beers vd. (1952). Results are expressed as U/mg protein.

The data obtained from the study were evaluated using the MINITAB package program. Tukey test was used to determine the differences between the means as a result of the evaluation.

3. Results and Discussion

In this study, the effect of salt stress on seedling length, stem length, germination, and fresh plant weight was found to be statistically significant ($p \leq 0.05$) (Table 1 and Figure 1). It was determined that there was a significant decrease in the seedling length as the salt concentration increased. The longest seedling length was obtained in the control treatment (13.83 cm), and the shortest seedling length was obtained in the treatment of 200 mM NaCl (4.31 cm). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 68% reduction in seedling length. Similarly, it was determined that the stem length decreased as the salt concentration increased. Accordingly, the longest stem length was obtained from the control treatment (13.33 cm) and the shortest stem length from the 200 mM NaCl treatment (3.80 cm). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 71% reduction in stem length. It is known that seed germination is inhibited in salty conditions, and they even lose their vitality in an environment containing high salt (Schmidhalter and Oertli, 1991; Tekin and Bozcuk, 1998). In our study, it was determined that there was a decrease in germination rates as the salt concentration increased. The highest germination rate was obtained from the control treatment at 92.5%, and the lowest germination rate was obtained from 200 mM NaCl treatment at 61%. When the control and 200 mM NaCl treatments were compared, it was determined that germination decreased by 34%. It was determined that the total fresh weight of the seedlings obtained as a result of seed germination decreased, similar to seedling length, stem length, and germination rate. While the total fresh weight of the seedlings obtained from the control treatment was 52.74 mg/seedling, it was determined as 12 mg/seedling in 200mm NaCl treatment. When the control and 200 mM NaCl treatments were compared, it was determined that there was a decrease of approximately 77% in fresh weight.

Table 1. The effect of salt applications on seedling height (cm), stem length (cm), germination percentage (%), and fresh plant weight (mg/seedling)

TREATMENTS	SEEDLING LENGTH (cm)	STEM LENGTH (cm)	GERMINATION RATE (%)	FRESH WEIGHT (mg/seedling)
Control	13.83±2.91^{a}	13.33±2.91^{a}	92.50±1.50^{a}	52.74±1.12^{a}
50 mM NaCl	12.70±3.16^{a}	12.01±3.15^{a}	82.50±2.50^{b}	42.69±2.21^{b}
100 mM NaCl	10.42±3.52^{b}	8.81±3.51^{b}	76.50±1.50^{c}	34.55±3,42^{c}
150 mM NaCl	8.13±1.77^{c}	6.83±1.77^{c}	70.00±1.85^{d}	19.26±2.23^{d}
200 mM NaCl	4.31±1.23^{d}	3.80±1.23^{d}	61.00±1.00^{e}	12.00±1.23^{e}

In this study, the effect of salt stress on total soluble protein, total phenolic, and total flavonoid content was found to be statistically significant ($p \leq 0.05$) (Table 2 and Figure 2). As a result of the excessive accumulation of ROS, protein degradation, DNA fragmentation, and cell death occur (Kusvuran et al., 2016). For this reason, it was determined that the amount of protein decreased as the salt concentration increased. The highest protein content was determined from the control treatment (2.65 mg/ml), and the lowest protein content was determined from the treatment of 200 mM NaCl (1.37 mg/ml). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 48% decrease. Phenolic and flavonoid contents are at the turn of antioxidant defense mechanisms that are effective in tolerance to stress conditions in plants (Naczk and Shahidi, 2004). Because the accumulation of polyphenols in the cell is seen as a response to biotic and abiotic stress conditions. For

this reason, in this study, it was determined that there were increases in total phenolic and total flavonoid contents at different salt concentrations. The lowest total phenolic content was determined from the control group (3.07 mg GAE/g), while the highest was determined from the treatment of 200 mM NaCl (11.26 mg GAE/g). It was determined that there was a 267% increase between the control and 200 mM NaCl treatments. Similarly, it was determined that the total flavonoid content increased in parallel with the increase in salt concentration. The lowest total flavonoid content was determined from the control (0.25 mg catechin/g), and the highest was determined from the 200 mM NaCl (2.51 mg catechin/g) treatment. It was determined that there was a 904% increase between the control and 200 mM NaCl treatments.

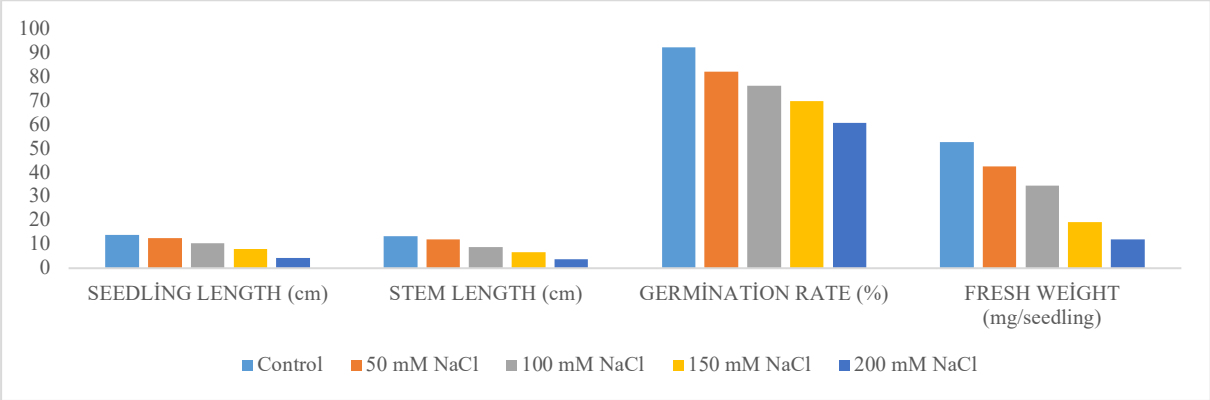

Figure 1. The effect of salt applications on seedling length (cm), stem length (cm), germination percentage (%), and fresh plant weight (mg/seedling).

Table 2. The effect of salt applications on total soluble protein (mg/ml), total phenolic content (mg GAE/g), and total flavonoid content (mg catechin/g)

TREATMENTS	TOTAL SOLUBLE PROTEIN (mg/ml)	TOTAL PHENOLIC CONTENT (mg GAE/g)	TOTAL FLAVONOID CONTENT (mg catechin/g)
Control	2.65±0.10^{a}	3.07±0.37^{d}	0.25±0.01^{c}
50 m MNaCl	2.21±015^{b}	4.88±0.37^{c}	0.36±0.01^{c}
100 mMNaCl	2.11±0.00^{b}	6.64±0.36^{b}	0.88±0.13^{b}
150 mMNaCl	1.69±0.21^{c}	7.24±0.16^{b}	1.15±0.13^{b}
200 mMNaCl	1.37±0.05^{c}	11.26±0.08^{a}	2.51±0.28^{a}

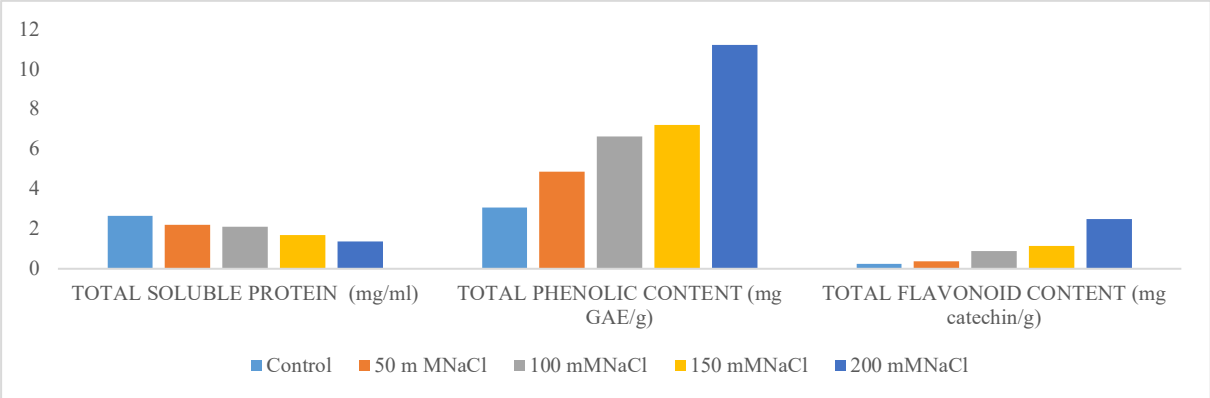

Figure 2. The effect of salt applications on total soluble protein (mg/ml), total phenolic content (mg GAE/g), and total flavonoid content (mg catechin/g).

In this study, the effect of salt stress on antioxidant enzyme activities was determined to be statistically significant ($p \leq 0.05$) (Table 3 and Figure 3). The highest activity in POD enzyme activity was determined from 150 mM NaCl (0.36 Δ_{460}/min/mg protein) treatment, while the lowest activity was

obtained from control and 50 mM NaCl treatments (respectively, 0.09 and 0.11 Δ A_{460}/min/mg protein). When the control and 150 mM NaCl treatments were compared, it was determined that there was a 300% increase. In SOD enzyme activity, it was determined that there was an increase in enzyme activity in parallel with salt concentration. The lowest SOD activity was similarly determined in the control treatment (6.29 U/mg protein) and the highest in the 150 mM NaCl treatment (11.13 U/mg protein). When the control and 150 mM NaCl treatments were compared, it was determined that there was an increase of approximately 77%. It was determined that there was an increase in the APX enzyme activity depending on the salt concentration, and the highest activity was obtained from the treatment of 200 mM NaCl (2.12 mol/min/g protein). When the control and 200 mM NaCl treatments were compared, it was determined that a 381% increase occurred. When plants are exposed to stress conditions, CAT antioxidant enzyme first contributes to the defense mechanism. It has been reported that the CAT enzyme clears high stability H_2O_2 and is the first antioxidant enzyme to respond at the onset of stress (Radi et al. 1991). In this study, the highest enzyme activity increase occurred in the CAT enzyme. The lowest enzyme activity was determined from the control treatment (8.07 U/mg protein) and the highest from the 200 mM NaCl (45.43 U/mg protein) treatment. When the control and 200 mM NaCl treatments were compared, it was determined that there was an increase of approximately 463%.

Table 3. The effect of salt applications on POD (Δ A_{460}/min/mg protein), SOD (U/mg protein), APX (mol/min/g protein), and CAT activity (U/mg protein)

TREATMENTS	SOD ACTIVITY (U/mg protein)	POD ACTIVITY (Δ A_{460}/min/mg protein)	APX ACTIVITY (mol/min/g protein)	CAT ACTIVITY (U/mg protein)
Control	6.29±0.64^{d}	0.09±0.00^{d}	0.44±0.08^{d}	8.07±1.76^{e}
50 mM NaCl	7.90±0.40^{c}	0.11±0.00^{d}	0.63±0.03^{d}	17.14±0.21^{d}
100 mM NaCl	9.02±0.51^{bc}	0.28±0.01^{b}	1.22±0.21^{c}	21.68±1.76^{c}
150 mM NaCl	11.13±0.55^{a}	0.36±0.01^{a}	1.71±0.16^{b}	32.74±1.68^{b}
200 mM NaCl	9.86±0.15^{ab}	0.18±0.02^{c}	2.12±0.16^{a}	45.43±0.95^{a}

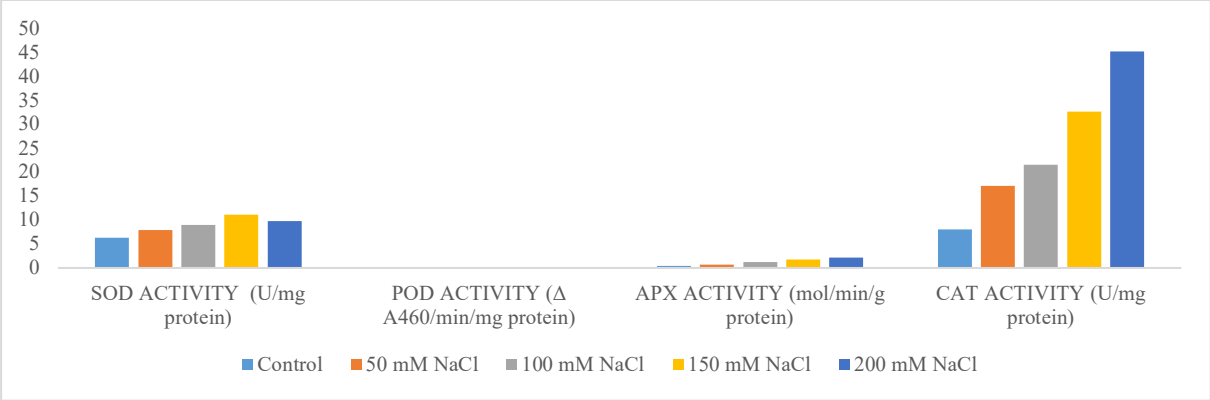

Figure 3. The effect of salt applications on SOD (U/mg protein), POD (Δ A_{460}/min/mg protein), APX (mol/min/g protein), and CAT activity (U/mg protein).

Salt stress occurs as a result of the excessive accumulation of soluble salts in the soil. The increase in salt content disrupts the ion and osmotic balance and suppresses plant growth and yield. (Parida and Das, 2005). Indeed, Yilmaz et al. (2022) reported that growth and development were adversely affected by the increase in salinity level. ROS accumulating in plant cells causes oxidative stress. (Jaleel et al., 2007). ROS accumulation is among the important factors that cause production and yield loss worldwide. ROS, oxidize proteins, damages nucleic acids, causes lipid peroxidation, and as a result, adversely affects many cellular functions (Foyer and Noctor, 2005). Indeed, Shah et al. (2021) reported that there was a significant decrease in germination percentage, seedling viability index, root and shoot lengths, and fresh and dry weights of seedlings under salt stress. Farhangi-Abriz and Torabian (2017) determined that different salt concentrations reduced shoot length and dry plant weight in bean seeds. Similarly, Paride and Das (2005) determined that seed germination and seedling growth were

negatively affected by salt stress. Huang et al. (2021) reported that salt stress decreased the germination percentage, emergence rate, and root and shoot length in sorghum seeds. As the salt concentration in the germination medium increases, the osmotic pressure increases, so the seed cannot receive the necessary water for germination (Day and Uzun, 2016). Therefore, it is known that plant germination is inhibited and germination percentage decreases in salty conditions (Bozcuk, 1989, Demir and Demir 1992; Dogan et al., 2008; Bahadorkhah and Kazemeini, 2014; Aydin and Atici, 2015; Kuscu et al., 2017; Toprak and Tuncturk, 2018; Kurtulus, 2020). In our study, it was determined that plant height, stem length, germination percentage, and plant fresh weight decreased as the salt concentration increased. Therefore, it was concluded that the obtained findings were compatible with previous studies and that salinity stress negatively affected plant growth (Abbasi et al., 2014, 2015; Liu et al., 2015). Plants develop some defense systems to resist the negative effects of ROS (Foyer et al., 1994). Antioxidant enzymes are the most important key elements of this system. ROS accumulation is neutralized by enzymatic antioxidant systems such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Mittler et al., 2004). SOD protects cells from damage by catalyzing oxygen radicals (O^{2-}) to hydrogen peroxide (H_2O_2). CAT, APX, and POD catalyze H_2O_2 into oxygen and water (Garratt et al., 2002). Shah et al. (2021) determined that there was an increase in total phenolic content, CAT, and SOD enzyme activities in parallel with the increasing salt concentration in corn seeds. It is reported that phenolic substances play an important role in scavenging free radicals, and their production increases under abiotic stresses (Król et al., 2014). In our study, it was determined that there was an increase in total phenolic and total flavonoid content with increasing salinity. Farhangi-Abriz and Torabian (2017) reported that different salt concentrations cause an increase in CAT, APX, SOD, and POD enzyme activities in bean seeds. Wang et al. (2021), determined an increase in SOD, POD, CAT and APX antioxidant enzyme activities in seedlings obtained from tomato seeds at different salt concentrations. Similarly, Altaf et al. (2021) reported that salt stress activates the antioxidant defense mechanism in plants and causes an increase in CAT, SOD, and APX enzyme activities. Many studies have been conducted in different plants regarding the antioxidant enzyme activities that take part in the plant defense mechanism under abiotic and biotic stress conditions, and as a result, it has been determined that there is an increase in enzyme activity (Bor et al., 2003; Li, 2009; Sharma et al., 2013; Turkhan et al., 2021; Yildirim et al., 2021a; Yildirim et al., 2021b; Boysan et al., 2022).

Conclusion

In this study carried out on Linas safflower seeds, 5 different NaCl concentrations (0, 50, 100, 150, and 200 mM) were applied, and the morphological, biochemical, and enzymatic responses of the seeds to salt stress conditions were investigated. In the study, it was determined that the seeds were relatively resistant to 100, 150, and 200 mM NaCl salt concentrations, and germination continued. In addition, it was determined that there were increases in biochemical and antioxidant enzyme results in parallel with salt concentration. It is thought that these increases in enzyme activity may be due to the defense mechanism developed to reduce the effect of stress. Due to the increasing world population, increasing salty soils, and decreasing freshwater resources, many studies have been carried out in recent years to identify new species that can adapt to adverse environmental conditions (Verma and Mishra, 2005; Ahmad et al., 2010; Maia et al., 2010; Zhang et al., 2013; García-Caparrós et al., 2019). In addition, it has been understood once again that our country has recently felt its impact negatively, and the determination of alternative plants for growing oily plants has gained more importance. Safflower, which is one of these plants, is a strategically important species both as an oil content and a source of biodiesel (Eryilmaz et al., 2014; Keskin, 2017). Therefore, more research is needed on this issue. This study carried out in this context will be a resource for our farmers regarding future studies on safflower seeds and which salt concentrations can be used for cultivation.

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Research Article

Performance of Local Bean (*Phaseolus vulgaris* L.) Genotypes for Agronomic and Technological Characteristics

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Abstract: The experiments were carried out in the field condition in 2019 and 2020, using 68 local bean genotypes and 5 cultivars (Akın, Akman 98, Göynük 98, Önceler 98, and Yunus 90) according to the Augmented design. Results showed that there were significant variations among dry bean cultivars and genotypes in terms of all the characteristics examined. In 2019, the vegetation period was 99.7-180.3 days, the first pod height 6.81-15.81 cm, pods per plant 9.90-60.92, seed per plant 20.2-268.9, the seed yield 1227-5970 kg ha^{-1}, the hundred-seed weight 18.99-112.8 g, water-uptake capacity 0.137 - 1.443 g and seed protein content varied between 13.95-23.15%. In 2020, the vegetation period was 99.4-159.2 days, the first pod height 4.42-15.76 cm, pods per plant 11.13-89.40, seed per plant 23.8-277.1, the seed yield 581.4-6801 kg ha^{-1}, the hundred-seed weight 23.5-135.5 g, water-uptake capacity 0.236-1.483g and the seed protein content varied between 17.85-24.79%. It was found that there were promising local genotypes superior to the cultivars in the seed yield and yield characteristics.

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Footnote: This study is derived from the doctoral thesis of first author and titled "characterization in terms of some physiological, morphological, agronomical and technological properties of local bean (*Phaseolus vulgaris* L.) populations collected from different provinces".

1. Introduction

Human life depends on plants, and the foodstuffs used in nutrition are provided either directly from plants or from animals that feed on plants. In addition to nutrition, plants are also used to meet people's daily needs such as shelter, medical-nutraceutical, and wear. Although many plant products can be produced artificially (such as synthetic fiber and rubber), it has not been synthetically possible to produce strategic crops accounting for the basic food of 75% of the world's population, such as wheat, corn, beets, potatoes, paddy, beans, etc. Nowadays, malnutrition problems are one of the most important agendas in the world. As a matter of fact, according to 2017 data, 821 million people in the world were malnourished and faced starvation (FAO, 2018). Malnutrition and death from hunger continue in Asia, sub-Saharan Africa, and Oceania. In most countries, billions of people do not have access to protein-based foods necessary for adequate and balanced nutrition (Ünal, 2017).

Dry beans are classified as legumes that have the most cultivation area in the world. In 2020, the total cultivation area of dry bean was 33 million ha, the total production amounted to 29 million

tons, and the average yield was 874.1 kg ha^{-1}. The highest dry bean producing countries in the world are Myanmar (5.8 million tons), India (5.3 million tons), and Brazil (2.9 million tons). Considering the food legumes in Türkiye, the cultivation and production of dried beans ranked third after chickpeas and lentils. In world bean production, Türkiye is ranked as the 21st country with approximately 280 thousand tons, obtained from 103 thousand ha cultivated area. The dry bean yield of Türkiye is above the world average, and its seed yield is 2715 kg ha^{-1} (FAO, 2020). In order to increase the production of beans both in the world and in Türkiye, along with the improvements in the growing techniques, new varieties with high yield and quality characteristics need to be developed.

While soil, water, and air are considered the main natural resources in crop production, "genetic resources" have been added to them as the fourth main natural resource. Plant genetic resources are extremely important for wide genotypic variation in plant breeding research. In this respect, one of the uses of local plant populations is the expansion of gene pools, which have been narrowed in modern cultivars. Nowadays, as modern cultivars that have high yields but narrow genetic bases lack genes for resistance (disease, pest, cold and arid), breeders are constantly looking for new sources of germplasm. In this respect, plant genetic resources are used directly or as bridge species to transfer quantitative characters in long-term programs and qualitative characters (disease resistance, etc.) in short or medium-term programs (Şehirali and Özgen, 1987). Furthermore, global problems manifested in the form of global warming and climate change have once again demonstrated the importance and value of genetic resources.

In recent years, numerous researches have been carried out in Türkiye to search for and discover new genetic resources. The ability to search and find a new genetic resource is unfortunately inadequate when it comes to its contribution to the economy. However, the most important contribution of this material can be achieved by using genes related to characteristics of economic importance. Considering diversity of plant gene resources in Türkiye, using the identified genes directly will be enormous asset for Türkiye.

The driving forces in dry bean breeding are consumer demand, yield, adaptation, disease-pest resistance, and especially rich nutritional content (Kobal Bekar et al., 2019). All of these breeding criteria are embedded in the genotype of local bean populations. There is a wide variation in beans that will allow selection among the varieties for yield and earliness. Numerous studies were conducted for this purpose, and successful results have been obtained from the development of early and also high-yielding bean varieties (Dreyer ve Wielpütz, 1998).

In some regions of Türkiye, factors such as geographical constraints require agriculture to continue in a way that we can describe as traditional agriculture, where the input is low and the variety is local, mostly for the need for families or local markets. These areas containing local materials (have came to today with natural selection and have stable and suitable taste) are considered a treasure. The breeders need more of these local populations in new variety development studies that take consumer demand into account.

In this study; it was aimed to record descriptive information of local dry bean populations by collecting from different provinces of Türkiye, to determine their agronomic and morphological characteristics, to identify high-yielding local genotypes, to create an infrastructure that can be useful in breeding studies, and to develop varieties suitable especially for semi-arid highland climate conditions. In addition, another purpose was to determine and suggest the appropriate parents for different regions resembling semi-arid highland climate.

2. Material and Methods

As a result of correspondence with the National Seed Genebank of Türkiye, it was determined that there were 24 provinces, including the provinces of Turkish Lakes Region,where material collection has not been thoroughly done yet and local dry bean populations having economic importance are grown. A total of 208 materials were collected for two years in 2017-2018. These materials were collected from provinces/sub-provinces of Isparta (101), Burdur (43), Konya (17), Eskişehir (9), Uşak (6), Karaman (6), Antalya (5), Denizli (4), Çorum (4), Manisa (3), Niğde (2), Kütahya (2), Balıkesir (1), Bolu (1), Bursa (1), Nevşehir (1), Kastamonu (1) and Erzincan (1). The six varieties, Yunus 90, Akman 98, Önceler 98, Akın, and Göynük 98, were used as control group in the experiment. Control cultivars

were obtained from the Republic of Türkiye Ministry of Agriculture and Forestry Transition Zone Agricultural Research Institute.

Table 1. Information on the local bean genotypes used in the study

Num	Code	Province	Town	Village	Growth habits
3	ISP3	ISPARTA	ATABEY	-	Dwarf
5	ISP5	ISPARTA	ATABEY	-	Dwarf
13	ISP13	ISPARTA	MERKEZ	GEYRAN	Dwarf
15	ISP15	ISPARTA	MERKEZ	GEYRAN	Dwarf
16	ISP16	ISPARTA	MERKEZ	GEYRAN	Climbing
17	ISP17	ISPARTA	MERKEZ	GEYRAN	Climbing
18	ISP18	ISPARTA	MERKEZ	KÜÇÜKGÖKÇELİ	Dwarf
19	ISP19	ISPARTA	MERKEZ	KÜÇÜKGÖKÇELİ	Dwarf
20	ISP20	ISPARTA	MERKEZ	KÜÇÜKGÖKÇELİ	Dwarf
21	ISP21	ISPARTA	MERKEZ	KÜÇÜKGÖKÇELİ	Dwarf
22	ISP22	ISPARTA	MERKEZ	BÜYÜKGÖKÇELİ	Dwarf
23	ISP23	ISPARTA	MERKEZ	BÜYÜKGÖKÇELİ	Dwarf
28	ISP28	ISPARTA	YALVAÇ	ÇETİNCE	Semi-Dwarf
29	ISP29	ISPARTA	YALVAÇ	ÖZGÜNEY	Climbing
30	ISP30	ISPARTA	YALVAÇ	ÖZGÜNEY	Climbing
31A	ISP31	ISPARTA	YALVAÇ	ÖZGÜNEY	Dwarf
31B	ISP32	ISPARTA	YALVAÇ	ÖZGÜNEY	Dwarf
32	ISP33	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Semi-Dwarf
33	ISP34	ISPARTA	ŞARKİKARAAĞAÇ	GÖKSÖĞÜT	Semi-Dwarf
34	ISP35	ISPARTA	ŞARKİKARAAĞAÇ	BEYKÖY	Semi-Dwarf
35	ISP36	ISPARTA	ŞARKİKARAAĞAÇ	BEYKÖY	Semi-Dwarf
38	ISP39	ISPARTA	EĞİRDİR	MAHMATLAR	Semi-Dwarf
52	ISP53	ISPARTA	GELENDOST	YEŞİLKÖY	Non-Uniform (S-C)
132	ISP60	ISPARTA	MERKEZ	DEREGÜMÜ	Climbing
136	ISP63	ISPARTA	ATABEY	-	Climbing
176	ISP72	ISPARTA	EĞİRDİR	AĞILKÖY	Dwarf
177	ISP73	ISPARTA	EĞİRDİR	AĞILKÖY	Dwarf
179	ISP75	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Non-Uniform (D-C)
180	ISP76	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Climbing
181	ISP77	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Dwarf
182	ISP78	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Climbing
185	ISP81	ISPARTA	ŞARKİKARAAĞAÇ	BEYKÖY	Dwarf
189	ISP85	ISPARTA	YENİŞARBADEMLİ	GÖLKONAK	Climbing
194	ISP90	ISPARTA	GELENDOST	YEŞİLKÖY	Climbing
196	ISP92	ISPARTA	GELENDOST	YEŞİLKÖY	Dwarf
197	ISP93	ISPARTA	GELENDOST	YEŞİLKÖY	Climbing
199	ISP95	ISPARTA	ŞARKİKARAAĞAÇ	YASSIBEL	Climbing
202	ISP98	ISPARTA	YALVAÇ	BAHTİYAR	Dwarf
205	ISP101	ISPARTA	MERKEZ	GELİNCİK	Dwarf
67	BUR14	BURDUR	MERKEZ	İLYAS	Dwarf
72	BUR19	BURDUR	MERKEZ	SALA	Climbing
73	BUR20	BURDUR	MERKEZ	KIŞLA	Dwarf
74	BUR21	BURDUR	ÇATAĞIL	-	Dwarf
96	ESK1	ESKİŞEHİR	MERKEZ	-	Dwarf
98	ESK3	ESKİŞEHİR	ALPU	KARAKAMIŞ	Dwarf
99	ESK4	ESKİŞEHİR	ALPU	KARAKAMIŞ	Semi-Dwarf
100	ESK5	ESKİŞEHİR	ALPU	-	Non-Uniform (D-S)
101	ESK6	ESKİŞEHİR	SEYİTGAZİ	SANCAR	Semi-Dwarf
102	ESK7	ESKİŞEHİR	SEYİTGAZİ	SANCAR	Semi-Dwarf
103	ESK8	ESKİŞEHİR	SEYİTGAZİ	KESENLER	Climbing
111	KON1	KONYA	EREĞLİ	-	Semi-Dwarf
115	KON5	KONYA	EREĞLİ	-	Dwarf
145	KON7	KONYA	ILGIN	-	Semi-Dwarf
164	KON15	KONYA	DOĞANHİSAR	YAZIR	Dwarf
165	KON16	KONYA	DOĞANHİSAR	BAŞKÖY	Dwarf
166	KON17	KONYA	BEYŞEHİR	ÜÇPINAR	Non-Uniform (S-S)
119	MAN1	MANİSA	DEMİRCİ	HOŞÇALAR	Semi-Dwarf
123	BRS1	BURSA	YENİŞEHİR	-	Dwarf
125	KÜT1	KÜTAHYA	SİMAV	-	Semi-Dwarf
155	DEN2	DENİZLİ	GÜRSU	-	Dwarf
206	DEN3	DENİZLİ	BOZKURT	HAYRETTİN	Climbing
207	DEN4	DENİZLİ	ÇAMELİ	ARIKAYA	Climbing
170	UŞK1	UŞAK	SİVASLI	PINARBAŞI	Climbing
171	UŞK2	UŞAK	BANAZ	AYRANCI	Semi-Dwarf
172	UŞK3	UŞAK	BANAZ	GÜRLEK	Climbing
173	UŞK4	UŞAK	BANAZ	YENİCE	Climbing
174	UŞK5	UŞAK	BANAZ	AYRANCI	Climbing
175	UŞK6	UŞAK	BANAZ	AYRANCI	Non-Uniform (D-S)

Each population was sown in plots having a length of 5m and 4 rows at the beginning of May 2018 to obtain seeds. Among the 208 materials, those showing climbing-spreading growth habits, severe disease and non-germination were eliminated and 68 of them were selected. Field trials were conducted in 2019-2020 in 4 replications according to the Augmented Experimental Design with 5 control cultivars (Yunus 90, Akman 98, Önceler 98, Akın and Göynük 98) and 68 local beans. The material was evaluated in augmented block design (Federer, 1956). The design consisted of 4 blocks containing 22 genotypes in each with 17 local bean and 5 control cultivars. Sowing was done by hand according to 60 x 10 cm sowing norm in plots (2.4 x 4 m and 4 rows) on May 10 in 2019 and on April 28 in 2020. Basic fertilization was applied with Diammonium Phosphate fertilizer at sowing [130 kg ha^{-1} Diammonium Phosphate (18:46:0)]. Pendimethalin was applied as a pre-emergence herbicide at 3000 ml ha^{-1} to trial plots. In order to ensure the field emergence, irrigation was done once and the plants were irrigated 5 times throughout the study with a drip irrigation system according to the needs of the plants.

The statistical analysis was done using the TARIST, TOTEMSTAT and MSTATC package programs according to the augmented design. LSD (Least Significant Difference); was calculated separately to compare the control cultivars and local genotypes (Peterson, 1994). The following formula was used to compare local genotypes with control cultivars.

$$LSD = t0.05\sqrt{\frac{(b+1).(k+1).HKO}{b.k}} \quad (1)$$

In the above formula; LSD: Least significant difference, HKO: Mean square error, b: Number of blocks; k: Number of control cultivars, t0.05: Two-tailed t-table value.

Isparta province, is located in the center of the Lakes Region; It is in the transition zone between the Mediterranean climate (semi-arid) and the continental climate. The altitude of Isparta is approximately 1050 m. Due to the geographical structure of the region, it has plateau and plain characteristics. For this reason, Lakes Region climate characterized by semi-arid highland climate. According to long-term climate records of Isparta province between April-November, the total precipitation is 275.3 mm, the average temperature is 16.6°C and the average relative humidity is 56.2%. In 2019, when the trial was conducted, it was determined that the average temperature (17.6°C) was higher than the long-term average, the relative humidity (55.8%) was almost the same as the long-term average, and the amount of precipitation (215.5 mm) was lower than the long-term average. In the second year, it is seen that the average temperature (18.9°C) was higher than the long-term average, while the average relative humidity (51.0%) and total precipitation (261.8 mm) were lower than the long-term average (Anonymous, 2021). The soil of experimental area is clayey-loam in terms of texture, calcareous rich (28.7%), poor in organic matter (1.54%), pH 7.66, poor in phosphorus (23.5 mg kg^{-1}) and rich in potassium (176.2 mg kg^{-1}).

3. Results and Discussion

In this study; vegetation period, first pod height, pods per plant, seeds per plant, grain yield, hundred-grain weight, water-uptake capacity and protein ratio characteristics of dry bean genotypes were discussed. These characteristics were examined according to the methods stated by Şener (2021). Means of genotypes and cultivars for vegetation period, first pod height, pods per plant, seeds per plant, seed yield, hundred-grain weight, water-uptake capacity and protein content were given in Table 2 and Table 3. Vegetation period, first pod height, pods per plant, seeds per plant values of dry bean genotypes are provided in Table 2 for 2019 and 2020 year. The differences among bean genotypes were significant for all characteristics examined.

In the first year of the experiment (2019), the average vegetation period ranged from 99.4 to 159.2 days. Among the genotypes, KON16 genotype had the shortest vegetation period, whereas ISP76 genotype had the longest vegetation period (Table 2). Among cultivars, Yunus 90 cultivars (cv.) had the shortest vegetation period with 108.3 days. However, from local genotypes 20 genotypes including ISP3, ISP5, ISP13, ISP15, ISP18, ISP19, ISP20, ISP21, ISP32, ISP73, ISP75, ISP81, ISP101, ESK5, KON1, KON15, KON16, MAN1, KÜT1, and UŞK6 had shorter than Yunus90 for vegetation period.

Table 2. Vegetation period, first pod height, pods per plant, seeds per plant, values of dry bean genotypes

	Vegetation period (day)		First pod height (cm)		Pods per plant (Pods plant^{-1})		Seeds per plant (Seeds plant^{-1})	
Genotype	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
AKIN	143.5	136.0	9.60	8.08	40.72	30.63	108.93	106.88
AKMAN98	120.8	128.5	11.43	8.53	53.20	43.49	196.50	191.31
GÖYNÜK98	118.0	120.3	10.31	8.65	35.70	30.23	122.45	105.35
ÖNCELER90	123.5	115.8	9.07	8.55	30.91	34.20	132.59	112.31
YUNUS90	108.3	124.8	11.00	10.00	27.82	30.80	68.86	82.46
ISP3	101.4	100.7	14.51	11.24	14.87	17.50	50.10	74.18
ISP5	100.4	102.7	12.71	11.84	15.67	16.70	57.30	78.88
ISP13	101.4	108.7	15.21	7.74	19.97	27.90	85.80	120.80
ISP15	101.4	109.7	15.81	9.64	22.36	17.70	82.63	73.88
ISP16	137.4	164.6	13.55	11.14	19.22	89.40	68.19	154.60
ISP17	139.4	137.6	9.36	12.34	20.10	16.70	53.30	67.28
ISP18	101.4	102.7	13.91	12.34	14.07	18.10	44.40	79.88
ISP19	102.4	103.7	10.91	9.74	17.57	18.80	60.20	74.38
ISP20	101.4	101.7	9.21	10.64	15.77	15.20	59.30	64.48
ISP21	101.4	103.7	11.61	10.74	14.97	18.60	43.30	82.28
ISP22	136.4	129.6	7.86	10.54	17.72	33.10	34.30	81.08
ISP23	129.4	103.7	6.81	9.64	17.87	14.80	47.30	67.68
ISP28	130.4	129.6	9.31	9.24	36.67	35.40	159.60	154.20
ISP29	137.4	137.6	9.99	10.54	18.14	22.23	74.63	112.30
ISP30	130.4	167.6	10.21	10.64	56.37	63.00	242.60	175.50
ISP31	110.4	129.6	12.91	10.84	26.77	22.90	68.20	56.88
ISP32	100.4	99.7	13.11	12.64	21.47	16.60	61.90	67.28
ISP33	113.2	106.8	8.66	12.16	28.19	23.15	87.70	98.57
ISP34	115.2	138.9	8.96	7.96	60.92	52.45	268.90	221.40
ISP35	115.2	116.8	11.06	5.56	26.12	30.80	101.30	133.50
ISP36	124.2	130.9	6.86	9.36	32.52	39.15	125.80	174.40
ISP39	138.2	127.8	8.46	11.86	28.62	28.75	74.58	99.37
ISP53	124.2	138.9	10.85	8.96	12.12	23.16	40.98	59.84
ISP60	133.2	138.9	6.86	9.86	32.08	33.65	119.10	131.10
ISP63	158.2	167.9	12.66	8.66	28.72	20.65	50.08	36.67
ISP72	123.2	108.8	10.35	10.76	33.72	29.85	105.90	103.70
ISP73	104.2	109.8	11.26	10.26	13.92	15.85	56.04	65.97
ISP75	102.2	138.9	11.85	7.36	19.52	20.95	65.11	27.57
ISP76	159.2	169.9	10.59	15.66	9.90	14.45	20.23	27.57
ISP77	137.2	131.9	8.34	11.36	14.96	12.45	42.23	47.27
ISP78	137.2	138.9	10.06	9.86	24.82	19.95	69.58	47.57
ISP81	102.2	106.8	15.06	15.76	19.02	21.85	82.98	74.28
ISP85	138.2	139.9	10.95	11.66	11.02	12.85	31.62	40.27
ISP90	114.2	131.9	8.46	10.36	37.62	34.95	134.10	150.30
ISP92	113.0	132.3	10.84	12.62	16.45	16.43	59.14	52.02
ISP93	127.0	132.3	9.47	8.92	10.95	12.81	42.21	44.30
ISP95	136.0	139.3	9.47	13.05	17.62	42.93	55.32	118.40
ISP98	130.0	113.3	10.55	9.02	16.51	29.87	71.98	95.53
ISP101	101.0	100.3	10.64	10.32	14.15	14.63	53.24	53.62
BUR14	109.0	118.3	13.04	9.82	26.35	28.53	94.74	101.10
BUR19	157.0	180.3	9.74	4.42	17.95	26.73	41.44	53.12
BUR20	129.0	132.3	8.24	9.32	15.38	11.13	76.11	44.82
BUR21	110.0	105.3	10.84	11.22	22.28	12.53	74.92	49.62
ESK1	113.0	139.3	11.17	10.72	22.39	11.53	58.76	26.62
ESK3	124.0	113.3	9.34	8.02	36.95	23.93	137.90	84.82
ESK4	123.0	132.3	8.84	9.12	23.39	23.73	76.24	55.72
ESK5	103.0	106.3	11.04	10.92	27.35	19.53	101.50	65.82
ESK6	113.0	132.3	8.84	11.92	37.51	39.43	95.24	111.90
ESK7	123.0	133.3	8.34	8.72	53.56	20.76	165.90	58.53
ESK8	113.0	133.3	8.04	9.14	30.75	23.43	124.80	75.64
KON1	102.0	133.3	9.54	7.12	23.15	31.73	86.14	83.72
KON5	123.4	125.3	15.80	12.58	38.05	21.33	109.90	62.03
KON7	120.4	105.3	7.23	5.95	25.83	25.23	91.01	106.60
KON15	100.4	126.3	12.23	8.88	24.85	19.53	54.88	37.13
KON16	99.4	115.3	13.80	8.18	32.95	24.33	92.29	75.23
KON17	109.4	100.3	10.10	9.48	38.49	27.13	127.50	107.50

**: Significant at P≤0.01 level.

Table 2. Vegetation period, first pod height, pods per plant, seeds per plant values of dry bean genotypes (continued)

	Vegetation period (day)		First pod height (cm)		Pods per plant (Pods plant^{-1})		Seeds per plant (Seeds plant^{-1})	
Genotype	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
MAN1	101.4	101.3	11.80	10.80	37.35	24.63	133.80	87.43
BRS1	109.4	132.3	13.90	9.38	25.45	16.03	76.08	36.53
KÜT1	100.4	134.3	11.20	8.85	21.05	11.34	66.39	23.82
DEN2	113.4	132.3	10.20	9.48	29.15	27.73	108.30	101.40
DEN3	123.4	126.3	8.99	8.48	52.05	29.52	213.50	138.20
DEN4	123.4	126.3	9.99	8.28	53.15	45.23	263.10	215.40
UŞK1	135.4	142.3	9.43	14.28	51.75	49.63	199.30	212.70
UŞK2	111.4	126.3	8.40	7.08	33.05	54.83	141.20	225.60
UŞK3	112.4	126.3	10.20	9.68	33.75	28.13	136.80	106.20
UŞK4	113.4	130.3	10.70	11.58	40.65	47.03	147.20	178.90
UŞK5	110.4	126.3	9.10	7.28	50.95	61.93	193.40	277.10
UŞK6	101.4	132.3	11.20	10.88	19.85	25.03	70.99	105.10
LSD	**7.893**	**16.69**	**1.579**	**0.8061**	**3.143**	**5.430**	**20.14**	**25.81**
CV	**%2.41**	**%4.99**	**%5.74**	**%3.45**	**%3.12**	**%6**	**%5.99**	**%8.07**
F-Value	**76.553****	**6.192****	**10.883****	**23.214****	**286.954****	**30.48****	**151.06****	**74.35****

**: Significant at P≤0.01 level.

In the second year of the experiment (2020), the average vegetation period ranged from 99.7 days in ISP32 genotype to 180.3 days in BUR19 genotype. Among the control cultivars, Önceler98 had the shortest vegetation period with 115.8 days (Table 2). In 2020, even though ISP3, ISP5, ISP13, ISP15, ISP18, ISP19, ISP20, ISP21, ISP23, ISP32, ISP33, ISP72, ISP73, ISP81, ISP98, ISP101, BUR21, ESK3, ESK5, KON7, KON16, KON17, and MAN1 local genotypes had numerically shorter vegetation period than Önceler98, this difference was not significant. The fact that the genotypes showed different responses to different climatic conditions, this is an indication of air temperature having an effect on the vegetation period of the genotypes. In regions where the vegetation period is short; high seed yield is obtained from varieties that are early in terms of germination and flowering. Lower seed yield is obtained from varieties with a long vegetation period (Akçin, 1974). Varieties having less temperature sum requirement flower and mature in a shorter time (Bıyıklı et al., 2021; Soydemir, 2021). Temperature, day length and day length × temperature affect flowering and maturation period (Sepetoğlu, 2002). At the same time, the earliness characteristic is important in terms of growing second crops and crop rotation. Earlier studies showed that the vegetation period was between 63.75 and 149.33 days (Düzdemir, 1998; Pekşen, 2005; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019; Topal, 2019; Tunalı, 2019; Soydemir, 2021).

The first pod height ranged between 6.81 and 15.81 cm in 2019 and between 4.42 and 15.76 cm in 2020 (Table 2). In 2019, the highest first pod heights were in ISP3, ISP13, ISP15, ISP81, and KON5 local genotypes, and these genotypes did not differ from each other. Among the control cultivars, the highest first pod height (11.43 cm) was in Akman98, and 18 local genotypes (ISP3, ISP5, ISP13, ISP15, ISP16, ISP18, ISP21, ISP31, ISP32, ISP63, ISP75, ISP81, BUR14, KON5, KON15, KON16, MAN1 and BRS1) had higher first pod height than this cultivar (Table 2). In 2020, the highest first pod height was in ISP81 and ISP76 local genotypes. In the same year, Yunus90 cv. had the highest first pod height among the control cultivars with a 10.00 cm however 33 local genotypes (ISP3, ISP5, ISP16, ISP17, ISP18, ISP20, ISP21, ISP22, ISP29, ISP30, ISP31, ISP33, ISP33, IPS39, ISP72, ISP73, ISP76, ISP77, ISP81, ISP85, ISP90, ISP92, ISP95, ISP101, BUR21, ESK1, ESK1, ESK5, ESK6, KON5, MAN1, UŞK1, UŞK4 and UŞK6 genotypes) had higher than its (Table 2). Akın had the lowest first pod height (8.08 cm) among the control cultivars, whereas 10 local genotypes had a lower than its. According to our results, the significant variations was observed among genotypes and years for the first pod height related to genetic and environmental factors. Considering that the first pod height is essential in mechanized agriculture, it can be said that there are promising genotypes in this study. Actually, it has been reported that the first pod height varied between 5.0 and 50.3 cm (Pekşen, 2005; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019; Topal, 2019; Tunalı, 2019).

The pods per plant ranged from 9.90 to 60.92 in 2019 year. ISP76 and ISP34 genotypes had the lowest and highest value for this trait. Among the control cultivars, Akman98 (53.20) had the highest value and genotypes such as ISP30 (56.37), ISP34 (60.92), and ESK7 (53.56) had higher than Akman98 (P≤0.05; Table 2). Local genotypes including ESK7, DEN3, DEN4, UŞK1, and UŞK5 had similar value with Akman98 (P≤0.05).

The pods per plant ranged from 11.13 to 89.40 in 2020. BUR20 genotype had the lowest pods, while ISP16 genotype had the highest pods. Among the control cultivars, Akman98 had the highest value and genotypes including ISP16, ISP30, ISP34, DEN4, UŞK1, UŞK2, UŞK4, and UŞK5 had higher than Akman98 ($P \leq 0.05$; Table 2). Local genotypes such as ISP36, ESK6, DEN4, UŞK4, and ISP95 had similar value with Akman98 ($P \leq 0.05$; Table 2). The number of pods per plant is an important characteristic for the yield and plant breeding. In this study, it was observed wide variation in number of pods per plant. Some research, number of pods per plant in dry beans varied between 1 and 163 (Çiftçi and Şehirali, 1984; Yorgancılar, 1995; Bozoğlu and Sözen, 2007; Ceyhan et al., 2009; Pekşen, 2005; Varankaya and Ceyhan, 2012; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019; Topal, 2019; Tunalı, 2019; Soydemir, 2021). Meshram (1977) reported that the most important yield components affecting seed yield of mung beans were grain weight and number of pods per plant and emphasized that these two characteristics should be taken as the most important selection criteria. The determination of local genotypes superior to control cultivars in terms of the number of pods per plant, which is an important feature in terms of yield, will also increase the chances of success in the advanced breeding stages. In research, it was detected that this characteristic is affected by environmental factors. The First flowers of bean have a high pod filling ratio. Therefore, temperature during the flowering period is very significant. The temperature above 23 °C causes low number of pods and seeds per plant (Sepetoğlu, 2002; Adak, 2021). Bozoğlu (1995) reported that environmental conditions affect the number of pods per plant in dry beans. Additionally, Şehirali (1988) and Düzdemir (1998) reported that the number of pods per plant was one of the most important yield components affected seed yield in dry beans was.

The number of seeds per plant of local dry bean genotypes was provided in Table 2 and it varied between 20.23 and 268.9. The lowest number of seeds was in ISP76 (20.23) genotype and the highest number of seeds was in DEN4 (263.1) and ISP34 (268.9) genotypes. Akman98 had the highest number of seeds (196.50), however, 5 local genotypes (ISP30, ISP34, DEN3, DEN4, and UŞK1) had higher than Akman98. Local genotypes such as UŞK5 (193.4), UŞK1 (199.3), and DEN3 (213.5) had similar number of seed with Akman98 ($P \leq 0.05$). In 2020, the number of seeds per plant ranged from 23.82 to 277.1, the lowest and highest values were in the KÜT1 and UŞK5 genotypes, respectively (Table 2). In the second year, Akman98 had the highest value, however 5 local genotypes (ISP34, DEN4, UŞK1, UŞK2, and UŞK5 genotypes) had higher than this cultivar. It was found that some local genotypes had higher seeds than control cultivars. The significant variations were observed related to the genotypes and years for this trait. It was thought that these variations caused by genetic and environmental factors.

The day length, temperature and relative humidity are the most important environmental factors affecting the number of seeds per plant. Temperature above 30 °C and relative humidity below 50% can reduce the number of grain per plant of genotypes, which are not sensitive to the day length (Sepetoğlu, 2002; Akbulut et al., 2014; Adak, 2021). Some researchers reported dry bean was affected by environmental conditions for this trait (Bozoğlu, 1995; Ülker, 2008). Sözen et al. (2014) indicated that the number of seeds per plant of beans is one of the agronomic characteristics that positively and significantly affect the yield. Anlarsal et al. (2000) stated that seed yield positively related to the number of pods, number of filled pods, number of seeds per plant, and seed weight per plant in climbing bean types.

The seed yield is more affected by environmental and agronomic factors. Therefore, the number of pods and seeds per plant should be considered as priority selection criteria in regions where ecology is suitable for bean cultivation (Önder, 1992; Özdemir, 2006; Adak, 2021). The seed per plant ranged from 11.03 to 167.30 (Düzdemir, 1998; Varankaya and Ceyhan, 2012; Serengül, 2019; Topal, 2019). The findings our study, were corresponded with related to researcher.

Table 3. Seed yield, hundred-grain weight, water-uptake capacity, and protein content values of dry bean genotypes

	Seed yield (kg ha^{-1})		Hundred-seed weight (g)		Water-uptake capacity (%)		Protein content (%)	
Genotype	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
AKIN	3885	3769	43.94	43.37	0.473	0.515	21.91	22.57
AKMAN98	4754	5307	26.92	33.52	0.308	0.367	17.25	20.01
GÖYNÜK98	4591	4178	39.73	43.02	0.442	0.475	18.53	19.57
ÖNCELER90	3011	4311	27.91	31.8	0.340	0.383	19.78	22.24
YUNUS90	2212	2954	35.07	43.35	0.413	0.543	19.24	22.18
ISP3	2349	2474	46.13	40.73	0.453	0.422	14.48	18.13
ISP5	2498	3019	45.72	42.06	0.433	0.382	18.42	17.86
ISP13	2067	3759	24.77	35.12	0.233	0.342	17.64	22.34
ISP15	2633	2524	34.41	36.51	0.343	0.282	16.98	22.07
ISP16	2474	6545	37.54	49.69	0.403	0.472	20.26	20.28
ISP17	1429	1920_	47.31	39.58	0.463	0.422	19.41	21.71
ISP18	1789	3224	40.97	43.58	0.413	0.422	17.29	19.81
ISP19	2477	3563	44.10	40.85	0.503	0.462	20.25	19.81
ISP20	2311	2301	39.64	36.80	0.433	0.402	19.48	19.71
ISP21	2217	3555	43.93	43.36	0.443	0.432	21.86	19.07
ISP22	1227	3372	49.96	46.32	0.573	0.562	22.33	23.74
ISP23	1537	2786	33.88	36.17	0.343	0.402	18.93	20.02
ISP28	4415	4069	29.40	35.99	0.283	0.392	17.50	21.59
ISP29	2938	3829	45.22	49.68	0.603	0.482	20.77	23.10
ISP30	4950	3747	18.99	25.48	0.233	0.272	16.29	23.64
ISP31	2683	2378	42.32	44.17	0.453	0.533	18.08	21.96
ISP32	3070	2664	49.81	40.71	0.553	0.472	20.09	19.69
ISP33	2900	3555	31.09	34.77	0.367	0.378	17.14	17.85
ISP34	4828	4602	19.75	23.74	0.207	0.238	16.51	18.78
ISP35	2517	5098	27.89	41.24	0.287	0.468	17.42	24.28
ISP36	3414	5976	31.75	38.77	0.317	0.378	17.76	21.56
ISP39	2506	3773	37.03	43.18	0.387	0.508	19.92	22.09
ISP53	1230	1939	29.52	38.03	0.307	0.468	13.95	21.90
ISP60	3207	3469	26.88	31.53	0.137	0.378	16.76	21.10
ISP63	3156	2986	73.64	84.57	0.617	0.948	20.75	22.15
ISP72	3512	3971	41.26	40.24	0.467	0.438	17.49	21.12
ISP73	2799	2835	48.33	44.19	0.517	0.428	19.22	20.24
ISP75	1838	3403	31.72	33.26	0.407	0.348	16.54	17.88
ISP76	1542	3403	107.30	127.50	1.367	1.469	21.34	22.65
ISP77	1246	1369	32.76	33.59	0.327	0.388	22.01	22.03
ISP78	2625	1644	39.85	43.62	0.437	0.488	21.28	20.46
ISP81	1984	2917	39.28	36.98	0.447	0.398	17.62	22.27
ISP85	1349	2196	48.44	65.31	0.597	0.789	20.18	23.97
ISP90	3195	3268	27.02	28.93	0.227	0.288	20.39	20.98
ISP92	2081	2267	41.28	46.80	0.423	0.502	19.81	20.93
ISP93	1532	1806	43.72	43.05	0.433	0.523	17.11	18.01
ISP95	2130	3982	43.53	49.83	0.443	0.372	22.23	21.79
ISP98	2353	3317	39.31	42.77	0.303	0.523	19.01	19.62
ISP101	1770	1994	40.04	43.58	0.383	0.432	20.45	20.00
BUR14	2962	3807	34.54	45.45	0.393	0.523	17.29	22.79
BUR19	3114	6801	112.80	135.50	1.443	1.483	18.34	20.71
BUR20	2610	1707	34.81	39.47	0.413	0.442	21.57	21.48
BUR21	2953	2283	42.38	44.38	0.513	0.502	20.05	20.62
ESK1	2003	1160	36.65	43.68	0.423	0.513	20.62	22.57
ESK3	3881	2620	33.10	33.01	0.383	0.392	18.92	19.13
ESK4	2125	1977	30.88	40.39	0.343	0.523	18.07	22.24
ESK5	3075	2185	33.28	38.84	0.333	0.482	16.32	19.11
ESK6	2669	5300	33.78	63.85	0.413	0.672	16.46	20.67
ESK7	5970	2618	37.17	51.22	0.443	0.573	16.90	21.03
ESK8	3643	2922	34.53	49.82	0.483	0.502	16.70	21.97
KON1	2549	3061	34.44	43.40	0.423	0.533	16.98	21.29
KON5	4829	3033	44.90	55.82	0.527	0.676	18.88	21.52
KON7	2235	2843	31.42	28.76	0.397	0.316	20.74	18.01
KON15	2315	1603	39.74	44.53	0.357	0.496	19.61	24.56
KON16	4019	3068	40.99	43.54	0.417	0.476	16.80	21.34
KON17	3234	6525	28.74	35.07	0.337	0.396	16.07	21.84

**: Significant at P≤0.01 level.

Table 3. Seed yield, hundred-grain weight, water-uptake capacity, and protein content values of dry bean genotypes (continued)

	Seed yield (kg ha^{-1})		Hundred-seed weight (g)		Water-uptake capacity (%)		Protein content (%)	
Genotype	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
MAN1	4256	3198	37.69	39.28	0.417	0.376	17.26	19.82
BRS1	2497	1135	39.85	36.83	0.437	0.506	19.53	23.33
KÜT1	2149	581	35.22	29.51	0.407	0.406	15.85	20.05
DEN2	3023	2612	33.74	28.80	0.397	0.336	23.15	24.79
DEN3	4349	3886	26.08	33.97	0.237	0.326	18.77	21.42
DEN4	5222	5072	26.92	25.17	0.257	0.236	18.13	18.99
UŞK1	3353	3730	23.40	23.94	0.217	0.256	18.27	20.38
UŞK2	2499	4821	23.36	25.69	0.207	0.266	16.54	20.02
UŞK3	2927	2505	25.28	29.55	0.227	0.316	15.60	20.61
UŞK4	3180	4974	26.03	34.63	0.297	0.346	17.31	20.91
UŞK5	2992	4748	21.83	23.53	0.217	0.306	16.68	20.64
UŞK6	1582	2799	23.38	29.70	0.247	0.296	14.80	20.17
LSD	**51.25**	**48.76**	**6.960**	**5.885**	**0.0845**	**0.08450**	**2.177**	**3.077**
CV	**%5.2**	**%4.45**	**%7.5**	**%5.64**	**%5.81**	**%8.17**	**%4.21**	**%5.4**
F-Value	**125.838****	**88.009****	**32.051****	**27.879****	**36.551****	**17.657****	**17.859****	**5.986****

**: Significant at P≤0.01 level.

In 2019, the seed yield ranged from 1227 to 5970 kg ha^{-1}. The lowest and highest value was determined in ISP22 and ESK7 genotype, respectively. Akman98 had the highest seed yield, whereas 5 local genotypes (ISP30, ISP34, ESK7, KON5, and DEN4) performed better than this cultivar (Table 3). In 2020, the seed yield varied between 581 kg ha $^{-1}$ (KÜT1) and 6801 kg ha $^{-1}$ (BUR19). The genotypes such as ISP16, ISP36, BUR19, and KON17 had higher seed yield than Akman98, while ISP35, ESK6, DEN4, UŞK2, and UŞK4 had similarity with Akman98 (Table 3). İyigün and Kayan (2019) reported that precipitation and temperature affected seed yield. For our study, we can infer that seed yield of genotypes differed between years as precipitation and temperature differed in 2019 and 2020. However, some researcher stated that seed yield of dry beans was affected components such as plant length, number of pods per plant, number of seeds per pod, seed weight, hundred-seed weight, and number of plants per unit area, and these characteristics differed to varieties (Westermann and Crothers, 1977; Önder and Özkaynak, 1994; Yorgancılar et al., 2003; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019). Additionally, Bozoğlu and Gülümser (1999) determined that seed yield of beans was significant and positive bilateral relations with plant length, number of pods per plant, 1000 seed weight, harvest index, biological yield, seed size index, and flowering period characteristics. In our study, we found that genotypes had higher number of pods per plant, number of seeds per plant, and hundred-seed weight, seed yield. From findings our study, we can infer that some of the local genotypes had promising. Ecological conditions and cultivation practices cause significant variation in yield (Çakır, 2019; Sümbül and Sönmez). Variations in the yield of genotypes vary related to photoperiod response characteristics, temperature, soil and air humidity. Day length and temperature are the main factors that affect bean flowering and fertile pod setting. It recommended that the optimum temperature for bean growth is 18-24 °C, and the relative humidity especially during the flowering period was above 50% (Adak, 2021). In other studies, it was reported that the seed yield of beans is highly affected by genetic structure (Önder, 1992; Önder and Şentürk, 1996; Düzdemir, 1998; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019). The other researcher reported that genotype, environment, and environment × genotype interaction on seed yield is very significant (Şehirali, 1980; Bozoğlu and Gülümser, 2000). In other studies, seed yield was between 657.0 and 4007.4 kg ha^{-1} (Mishra and Dash 1991; Bozoğlu, 1995; Yorgancılar, 1995; Düzdemir, 1998; Bozoğlu and Gülümser, 2000; Ceyhan et al., 2009; Varankaya and Ceyhan, 2012; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019; Topal, 2019; Soydemir, 2021).

In 2019, hundred seed weight ranged from 18.99 to 112.8 g. BUR19 genotype had the highest, and ISP30 genotype lowest values. Among the control cultivars, Akman98 (26.92 g) and Akın (43.94 g) had the lowest and highest value. Local genotypes such as ISP3, ISP5, ISP17, ISP19, ISP22, ISP29, ISP32, ISP63, ISP73, ISP76, ISP85, BUR19, and KON5 had higher value than Akın, while ISP21 (43.93 g) and ISP93 (43.72 g) local genotypes had similar.

In 2020, the hundred-seed weight it was between 23.53 (UŞK5) and 135.5 g (BUR19) (Table 3). Akın had the highest value, however, local genotypes including ISP16, ISP18, ISP22, ISP29, ISP31, ISP63, ISP73, ISP76, ISP78, ISP85, ISP92, ISP95, ISP101, BUR14, BUR19, BUR21, ESK1, ESK6, ESK7, ESK8, KON1, KON5, KON15, KON16 had higher than Akın. Aydoğan, (2019) reported that

the hundred-seed weight of chickpeas significantly affected by ecological and genetic factors. Additionally, Çancı and Toker (2009) determined that the hundred-seed weight of chickpeas varied depending on environment and sowing date. Anlarsal et al. (2000) detected that there was positive significant relationship between seed yield and hundred-seed weight in dwarf forms of beans. Malhotra et al., 1974 reported that the selections will be successful taking into account the hundred-seed weight, the number of pods plant^{-1}, and the number of seeds per pod. In our experiments, we can say that the variations observed in hundred-seed weight can be one of the criteria to be used in the determination of promising genotypes in the advanced breeding stages. According to the results of our study, the hundred-grain weight shows a wide variation. In other studies, the hundred-grain weight varied between 14.74 and 135.0 g (Çiftçi and Şehirali, 1984; Bozoğlu, 1995; Yorgancılar, 1995; Düzdemir, 1998; Ceyhan et al., 2009; Peksen, 2005; Varankaya and Ceyhan, 2012; Ekincialp and Şensoy, 2013; Özbekmez, 2015; Serengül, 2019; Taşkesen, 2019; Topal, 2019; Tunalı, 2019).

In 2019, BUR19 genotype had the highest (1.443 g) for water-uptake capacity, whereas ISP60 genotype had the lowest values (0.137 g). Akın had the highest water-uptake capacity, however its value was lower than those of 12 local genotypes (ISP19, ISP22, ISP29, ISP32, ISP63, ISP73, ISP76, ISP85, BUR19, BUR21, ESK8, and KON5).

In 2020, the water-uptake capacity varied between 0.236 (BUR19 and ISP76) and 1.483 (DEN4) g. Yunus90 had the highest water-uptake capacity, while local genotypes including ISP22, ISP63, ISP76, ISP85, BUR19, ESK6, ESK7, and KON5 had higher than Yunus90. Yunus90 had similarity with local genotypes such as ISP31, ESK1, and KON1 ($P \leq 0.05$; Table 3). Cengiz (2007) stated that it varied between 0.168 and 0.487 g, whereas Özbekmez, (2015) and Soydemir, (2021) detected that it ranged from 0.146 to 0.809 g. The cooking time of legumes is associate by water-uptake capacity, and varieties with hard seed coats uptake less water than varieties with normal coat hardness (Williams et al., 1986). In addition, it was stated that the hundred-seed weight had a positive relationship with the water-uptake capacity (Karasu, 1993).

The seed protein content ranged from 13.95% (ISP53) to 23.15% (DEN2). Akın had the highest protein content, however this value was lower than those of local genotypes (ISP22, ISP77, ISP95, and DEN2). In 2020, The seed protein content ranged from 17.85% (ISP33) to 24.79 % (DEN2). Akın had lower value than 11 local genotypes (ISP22, ISP29, ISP30, ISP35, ISP76, ISP85, BUR14, ESK1, KON15, BRS1, and DEN2) (Table 3). It thought that this case due to the genetic structure, environmental and agricultural conditions. It has been reported that factors such as genotype, environment (temperature, precipitation, and day-length), cultivation area, soil and climate characteristics, diseases and pests, storage conditions have a strong effect on the technological characteristics of the varieties (Cengiz, 2007). The values obtained from this study ranged within the limits of the previous studies. In other studies, it was reported that the seed protein content ranged from 17.13 to 29.8% (Yorgancılar, 1995; Düzdemir, 1998; Kahraman, 2008; Coelhoa et al., 2009; Varankaya and Ceyhan, 2012; Ghanbari et al., 2013; Özbekmez, 2015; Türkmen, 2020; Soydemir, 2021). As dry bean, which significant food legume crop, has cheap, high in protein content, and digestibility, determination of genotypes with a high protein content is significant.

Conclusion

In this research, phenological observations such as vegetation period, number of pods plant^{-1}, number of seed per plant and hundred-seed weight, etc. have a direct contribution to seed yield, some quality, and technological characteristics particularly seed yield. The many local genotypes had similar performances compared to control cultivars and some genotypes were better than control cultivars. For the number of pods plant^{-1}, number of seeds per plant, and seed yield. It was found that local genotypes such as ISP5, ISP13, ISP18, ISP19, ISP21, ISP22, ISP23, ISP31, ISP32, ISP34, ISP35, ISP72, ISP98, BUR14, BUR20, BUR21, ESK3, KON5, MAN1, DEN2, KON16, and KON17 had better performance than control cultivars. The obtaining promising genotypes that stand out in terms of yield and yield components revealed the importance of this study. Consequently, we can say that these materials have a high potential to be used in advanced breeding levels and variety development studies. The continuing the experiments in the following years may result in developing high-yielding cultivars, high in protein content. Additionally, it is thought that local bean genotypes collected from different regions of Türkiye

and wide variations will make a significant contribution to institutions and organizations that carry out breeding research.

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Research Article

Identification of Some Paddy Rice Diseases Using Deep Convolutional Neural Networks

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Abstract: In modern digital agricultural applications, automatic identification and diagnosis of plant diseases using artificial intelligence is becoming popular and widespread. Deep learning is a promising tool in pattern recognition and machine learning and it can be used to identify and classify diseases in paddy rice. In this study, 2 different paddy rice diseases, including rice blast and brown spot, were investigated in the district of İpsala in the province of Edirne between the 2020 and 2021 production seasons by collecting 1569 images. These diseases are very common and important in Edirne province and surrounding rice production areas. Therefore, practical methods are needed to identify and classify these two diseases. A Convolutional Neural Network (CNN) model was created by applying pre-processing techniques such as rescaling, rotation, and data augmentation to the paddy rice disease images. The classification model was created in Google Colab, which is a web-based Python editor using Tensorflow and Keras libraries. The CNN model was able to classify rice blast and brown spot diseases with high accuracy of 91.70%.

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1. Introduction

There are many fungal diseases that affect the yield and quality of paddy rice. Two major diseases that can cause economic losses in Türkiye are rice blasts (*Pyricularia oryzae*) and brown spot (Helmithosporium oryzae). If these diseases spread, the plant may dry out completely, and sometimes, no crop may be harvested. With the developments in computer science in parallel with technology, the detection of such diseases in a shorter time and with minimal experience has become possible (Arnal Barbedo, 2013).

Artificial intelligence (AI) or machine learning is one of the important tools that is used in image-based identification and classification. During the classification of images, computational complexity should be avoided, and the working time should be greatly reduced. For this purpose, Deep learning (DL) is used to learn characteristic hierarchies, increase representation capacity and provide better prediction performance (Affonso et al., 2017; Zhang et al., 2019; Wang et al., 2020). The coding of the model can be done through computer models that offer interactive as well as web-based platforms. Google Colaboratory is the web-based framework for writing and executing Deep Learning and Machine Learning codes. It can upload larger datasets directly from Google Drive servers at very high speed (Kanani and Padole, 2019).

Convolutional Neural Network (CNN) is generally used in image-based AI applications. It is a neural network approach that consists of image acquisition, pre-processing, fragmentation, extraction of features from images, detection, and classification (Rawat and Wang, 2017; Kadhim and Abed, 2018). By optimizing the model parameters, the difference between the actual and predicted output values can be minimized in the classification. Many studies have been carried out on plant diseases using the advantages of CNN (Kawasaki et al., 2015; Boulent et al., 2019; Ferentinos, 2019; Priyadharshini et al., 2019; Gokulnath and Usha Devi, 2021).

Due to the inadequacy of the appropriate methods to detect rice plant leaf diseases, paddy rice production has been decreasing gradually in recent years. Thus, a user-friendly and fast recognition system is needed. CNN is a useful tool that can be considered in this sense (Pinki et al., 2017; Mique and Palaoag, 2018; Vardhini et al., 2020). Lu et al., (2017) reported the performance of CNN in paddy rice disease detection by using 500 images. They showed that comparing the alternative approaches to predict diseases, and CNN is the best of the classification models considered. Asfarian et al. (2013) diagnosed four significant rice plant diseases such as brown spots, leaf blight, leaf blast, and tungro. According to the results of the study, CNN is the fastest and most accurate classifier. Xiao et al. (2018) showed that CNN is superior to the current machine learning technique with great accuracy in differentiating the impact of rice diseases. Vanitha (2019) used CNN to detect leaf blight, bacterial blight, and stem rot diseases in paddy rice. They conducted the study with 500 images, both diseased and healthy. They stated that the model could effectively detect three rice diseases with an accuracy of 99.53%. Tawde et al. (2021) used CNN as a feature extractor and support vector machine (SVM) as a classifier for the identification of rice diseases. They reported that rice diseases could be successfully identified early with this approach with an accuracy rate of 96%. In this study, a model based on a deep CNN was developed to identify two 2 major fungal rice diseases in the Edirne province of Turkey.

2. Material and Methods

The study was carried out on paddy rice fields in the İpsala district of Edirne province/Turkey in 2020 and 2021. İpsala district is located at latitude 40.8865 and longitude 26.3712 (Figure 1). Approximately 14% of the rice production in Turkey is made in İpsala. Images of healthy and infected rice samples from cultivars such as Casanova, Güneş-Cl, and Keşhan were acquired to identify blast (*Pyricularia oryzae*) and brown spot *(Helmithosporium oryzae*) diseases. The symptoms of the blast in the plant are seen in leaves, nodes, clusters, peduncles, and husks. These symptoms are generally lozenge-shaped with two pointed ends, gray in the middle, and brown-reddish spots around it. On the stem, it looks like an oil stain, and green mold develops. It causes no grains to form in the cluster and the formation of white husks (Devi and Neelamegam, 2018). Leaves infected with brown spots have many large spots on the leaves that may kill the entire leaf. The rapid spread and development of the disease are fostered by continuous rainfall, clouds, and higher daytime temperatures. The yield can be reduced by up to 45% in cases of severe illness and by 12% in cases of mild infection (IRRI, 2012).

Image acquisition started from the second week of July (the first appearance of the diseases) in 2020 and 2021 and continued until the end of August in both years. Images were obtained from 15 infected, and 5 healthy rice fields were used. A total of 1569 RGB images were acquired using Redmi Note 9 Pro.

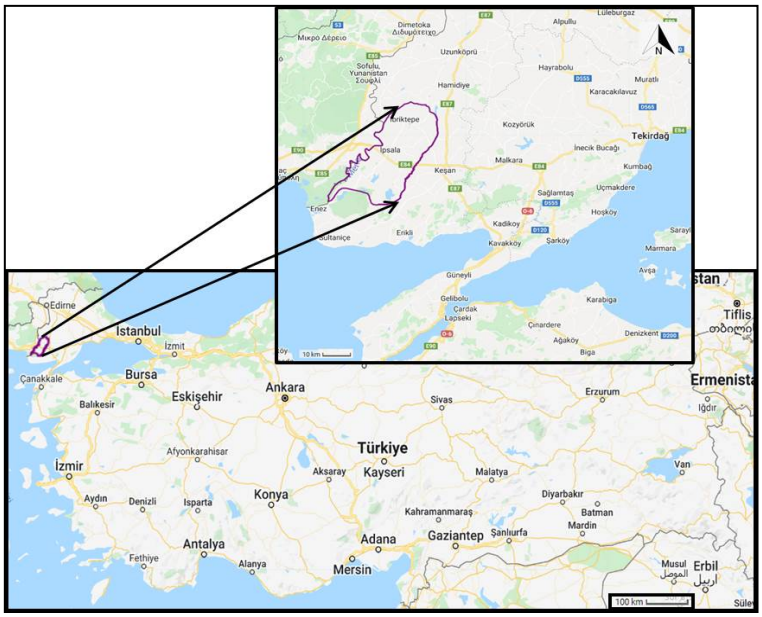

Figure 1. Study area.

2.1. Pre-processing of images

To reduce model operation time and noise disturbance, paddy rice images were passed through a number of pre-processing steps. Firstly, each image was labeled as healthy, rice blast, or brown spot (Figure 2). The image size was decreased from 4640 × 3472 to 256 × 256 in order to reduce the size of the total image data. Since the entire paddy pictures are colored, they are compressed to the 0-1 range, and their backgrounds are colored gray in order to gain stability and prevent noise. Later, in order for the CNN model to process the data set more comprehensively, the horizontal and vertical ratio of the images was chosen as 0.2, and the images were inverted.

Healthy Rice Blast Brown Spot

Figure 2. Sample paddy rice images.

2.2. Modelling

The rice diseases detection CNN model was developed in the Google Colaboratory (Colab). The TensorFlow, matplotlib.pyplot, IPython.display, GPU, Sequential, compile, model.fit, and sklearn.metrics libraries were used in classification. The dataset consisting of 1569 images for the proposed CNN model was uploaded to Google Drive and later transferred into Google Colab. The percentages of training, validation, and testing were 70, 20, and 10%, respectively. The reason for choosing these ratios is to increase the learning speed and accuracy score of the model. The epoch number was set to 100 in order to ensure that the model was not over-fitting and appropriately reflected the validation accuracy. The flowchart of the model is given in Figure 3.

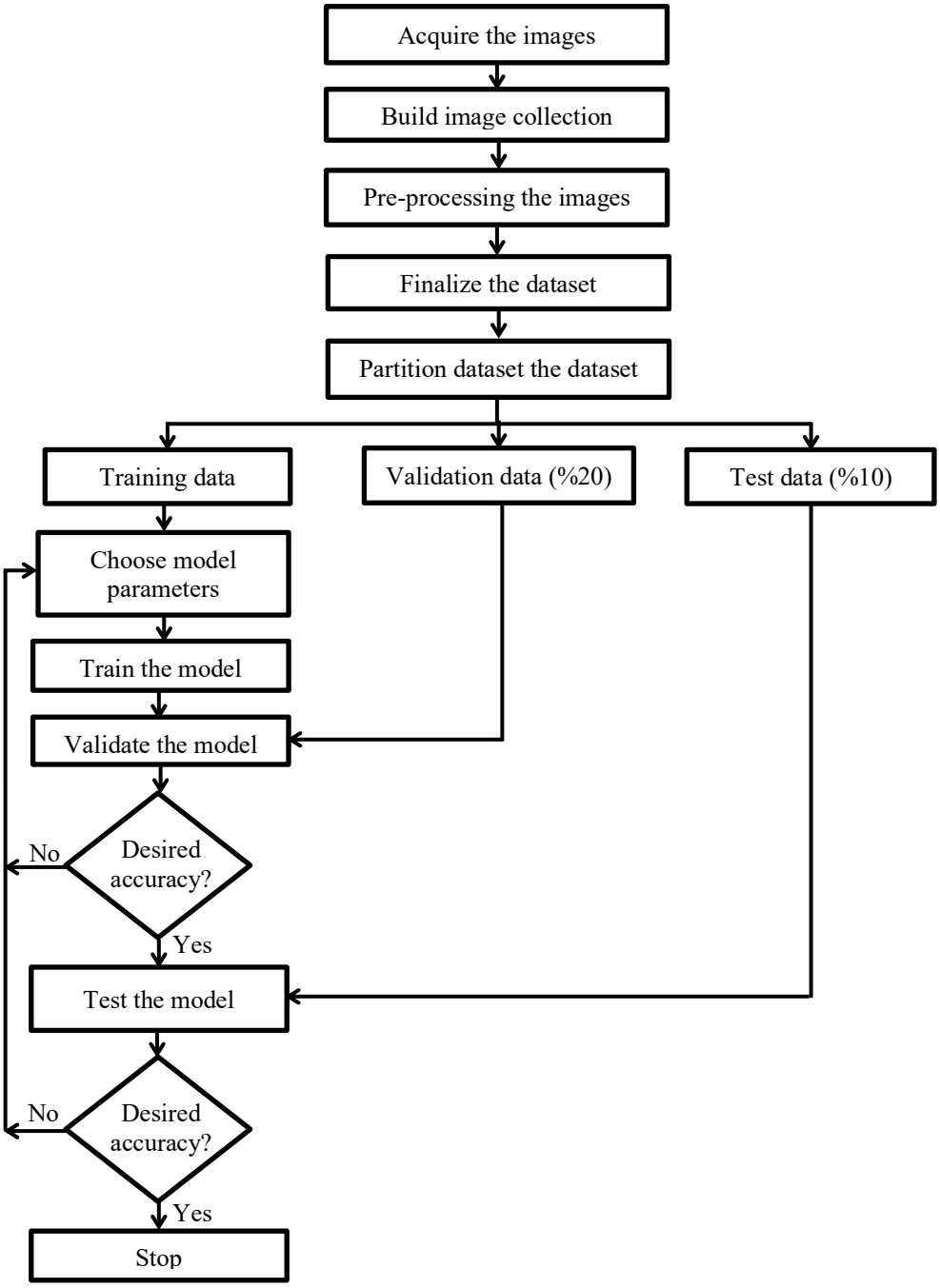

Figure 3. Flowchart of the model.

Values that yield the highest classification accuracy were applied in the selection of model parameters. In CNN, the simplest technique to build a model is sequential. In this technique, the model is built layer-by-layer (Russakovsky et al., 2015). Conv2D was used to add layers to the model. A total of 8 layers was used, including 6 convolutions + rectification layers (Figure 4). The input images were processed by Conv2D layers (Rajaraman et al., 2018). The number of nodes in each layer was determined as 32 and 64. The number can be adjusted to be larger or smaller in accordance with the model's accuracy score by trial and error method (Russakovsky et al., 2015). The ReLU activation function was used as a rectifier to increase the non-linearity of the images. Also, this function only activates a few neurons at a time. So it makes the model sparse and easy to perform (Siddiqi, 2019). In the modeling 2×2 maximum pooling method was applied. Pooling increases the NN's flexibility to recognize features that belong to the same class but are rotated, distorted, squeezed, etc. It also gets rid

of the majority of the information that is not related to the feature that should be recognized. However, it still preserves the textural or locational information of the feature even if they are different in various locations. By applying pooling, the size of the image can be reduced about %75. In max-pooling, the maximum value is selected from all available values in the window, thereby incorporating only the highest feature of this pixel (Scherer el al., 2010). The softmax function compresses the output of each class between 0 and 1. Thus, it provides the probability that an input belongs to a particular class. After sequential modeling, two dense layers were added to the end of the CNN (Figure 4). Dense layers extract characteristics from the convolution and pooling layers (Siddiqi, 2019). The second dense layer generated the classification. Given that the model was designed to solve a classification problem, Sparse Categorical was selected entropy as the loss function, and adam was selected as the optimizer (Anonymous, 2022).

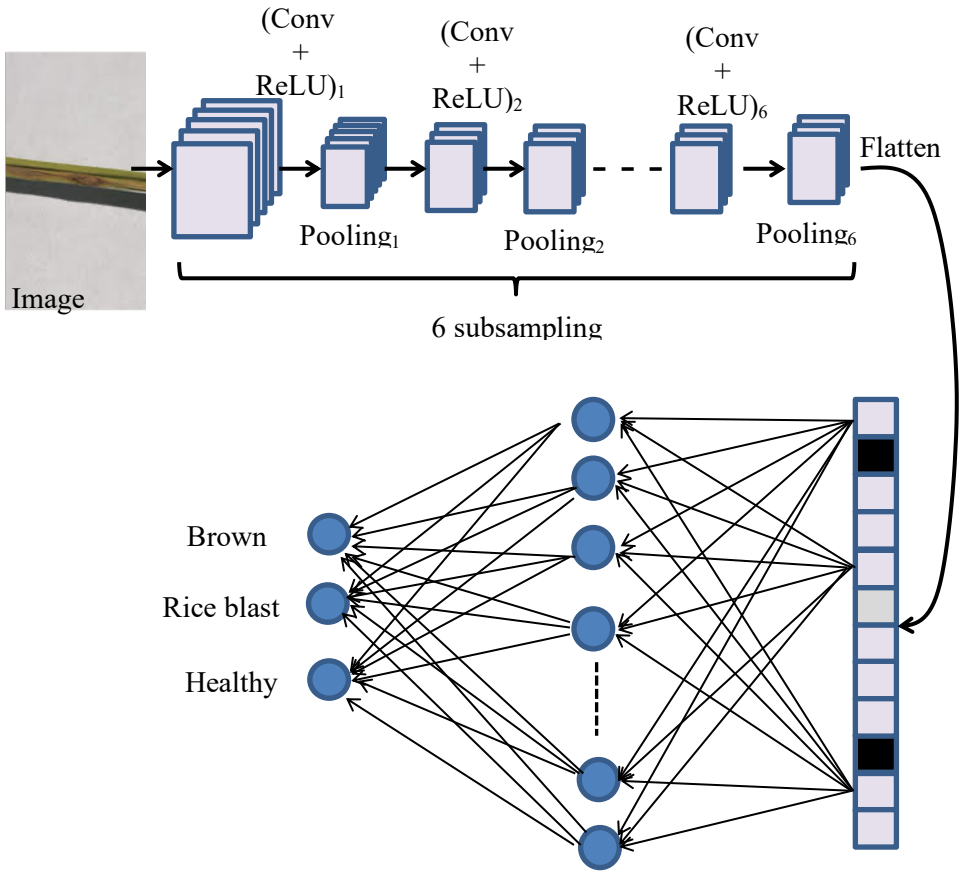

Figure 4. CNN model structure.

3. Results and Discussions

In this study, 70/20/10 percentages of training, testing, and validation were applied for the best results in generalizing the model. A total of 1098 images were used for the training process. The model was validated after the completion of the training phase. Twenty percent of the dataset was randomly selected for validation. The training step value (epoch) for this model 100, batch size 32 was chosen. These numbers can be changed until the highest accuracy is achieved. The change in model accuracy and loss factor as the epochs increase in training and validation steps are plotted in Figure 5. As expected, the accuracy increases and stabilizes after the 90th epoch while the loss factor gains the lowest values for each step. A higher accuracy of 92% was achieved in the training and validation steps.

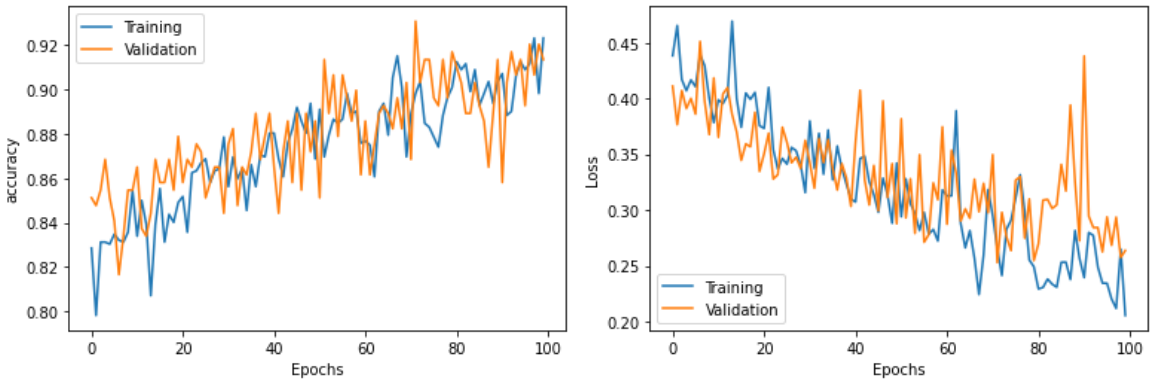

Figure 5. Relationship among epoch, accuracy, and loss factor.

One of the methods used to determine the classification performance of each algorithm is the creation of a confusion matrix. The matrix gives information about how often an observation belonging to a certain class is detected correctly and how often it is determined as another class (Ruuska et al., 2018). The confusion matrix of the model is given in Figure 6.

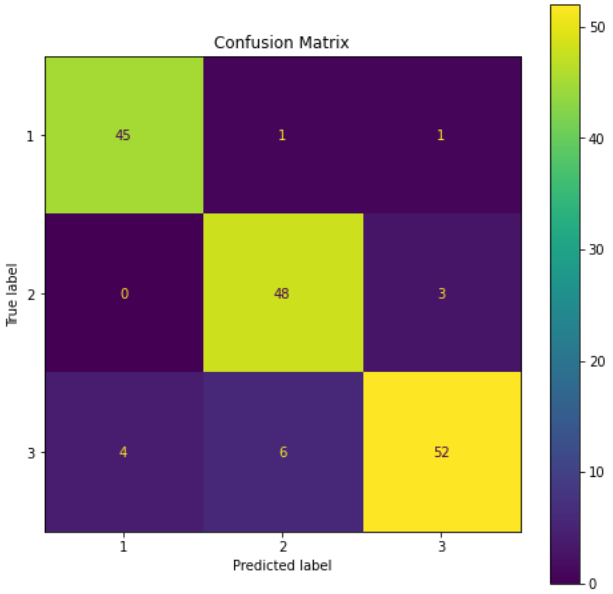

Figure 6. Confusion matrix of the model.

In the creation of the confusion matrix, the test data, which corresponds to 10% of the database, was used. A total of 160 images was evaluated, including 47, 51, and 62 rice blasts, brown spots and healthy, respectively. Based on the data obtained from the confusion matrix, 45 of 47 rice blasts, 48 of 51 brown spots and 52 of 62 healthy images were correctly classified. Classification accuracies of each class are also summarized in Table 1. Higher classification accuracies were obtained for two diseases (>0.94). The overall classification performance of the model on test data was also calculated to be 0.91.

Table 1. Summary of model accuracies

Class	Accuracy
Rice blast	0.96
Brown spot	0.94
Healthy	0.84

In Table 2, various studies with the CNN method on the detection of rice diseases are given. The overall accuracies of the studies given in Table 2 are compatible with this study. Vanitha 2019 found the highest model result and used 350 datasets. The reason why the accuracy rate of the proposed

study is lower than other studies is that the number of an epoch is limited to 100, and the dataset consists of 1569 images. The accuracy rate of the model can be increased by using a higher epoch number and a less comprehensive dataset, but this may cause overfitting in the model. This problem lowers the predictive score of the model in the test dataset.

Table 2. Comparative analysis of CNN techniques for rice diseases

Author	Dataset	Diseases	Overall accuracy
Lu et al., 2017	500	Rice Blast and Brown Spot	0.95
Shrivastava et al., 2019	619	Rice Blast, Bacterial Leaf Blight, and Sheat Bligh	0.91
Vanitha, 2019	350	Brown Spot, Sheath Rot, and Bacterial Blight	0.99
Anadhan and Singh, 2021	Over 1500	Rice Blast, Bacterial Leaf Blight, Sheat Blight, Leaf Streak	0.87
Tawde et al., 2021	Not specified	Rice blast, Rice blight, Brown spots, leaf smut, tungro, and sheath blight	0.96

Conclusion

Deep CNN has been used in various applications in agriculture and other disciplines. In this study, it was aimed to re-evaluate the classification performance of a 8-layer CNN model in major paddy rice diseases in the İpsala district of Edirne province in Türkiye. The local cultivars were used as the study material. The rice blast and brown spot fungal diseases were identified using RGB images collected from paddy rice fields in the district. The results showed that the CNN algorithm could be successfully applied to local paddy rice cultivars. Developing of a database for training, validating, and testing the model is relatively easy with use of current technologies. Also, cloud-based modeling environments such as Google Colab provide useful, fast and effective tools in model development. Such work can be conducted without downloading larger sophisticated software on the computer free of charge. However, there are still challenges in the use of deep learning algorithms such as CNN. The main challenge is the decision and optimization of model parameters, structure (number of layers, etc.), epoch numbers, etc. The use of the trial error method is often time consuming and requires former experience in model building. Our future work includes expanding the number of diseases observed in the region affecting the local cultivars that can be identified by CNN. Even though classification using a CNN model stored in a could system performs well, it is not yet practical. A tool should be developed that doesn't require the use of the Google Colab environment. Therefore, in the next step, a user-friendly mobile smartphone application will be developed that can be used by the local farmers. It is also planned to use aerial imagery acquired by a drone to identify such diseases on field scale.

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Research Article

Effects of Crab Shellmeal Inclusions to Fishmeal Replacement on the Survival, Growth, and Feed Utilization of Mangrove Crab *Scylla serrata* (Forsskal 1775)

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Crab shellmeal,
Fishmeal,
Growth,
Mangrove crab,
Survival

Abstract: Mangrove crab *Scylla serrata* is associated with mangroves throughout the Indian and Pacific oceans. This species is crucial to aquaculture and fishing. As wild stocks decline and demand rises, mangrove crab aquaculture has become increasingly popular. However, feed development research and its quality are still meager in the industry. This study examined the interactive effects of different levels of crab shellmeal (CSM) to fishmeal (FM) replacement on proximate composition, feed utilization, carcass composition, growth, and survival performance of mangrove crab *S. serrata*. Four formulated diets were prepared, and one for chopped trash fish (TF) supplement: 30% FM and 0% CSM (Diet 1) as a negative control, 20% FM and 10% CSM (Diet 2), 10% FM and 20% CSM (Diet 3), 0% FM and 30% CSM (Diet 4), and TF as a positive control (Diet 5). Experiments were conducted in each group for 30 days with ten replicates. Results revealed that formulated diets using different levels of CSM and FM did not significantly affect mangrove crabs' growth and survival rates as well as feed utilization. However, the proximate composition of Diet 4 was significantly higher among other experimental diets. Moreover, the crab's whole body composition (ash, moisture, carbohydrates, crude protein, crude fat, and calories) with different levels of CSM and FM was significantly improved. Hence, it is possible to enhance the carcass composition and proximate composition by supplementing CSM; however, it has no effect on feed utilization, as well as the growth and survival rates of mangrove crab *S. serrata*.

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1. Introduction

Mangrove crab *S. serrata* (Forsskal, 1775), also known as green crab or mud crab, is one of the largest portunid species that lives along the coast and in mangrove swamps all along the Indo-Pacific coastline (Barnes et al., 2002; Le Vay et al., 2007; Shelley and Lovatelli, 2011). It has become increasingly popular in aquaculture due to its economic importance and is currently admired seafood all over the world because of its savory sweet flesh, larger size, and nutritional value (Sathiadhas and Najmudeen, 2004; Ali et al., 2011). As a matter of fact, mangrove crab is one of the most commonly

used ingredients in a number of dishes (Mirera, 2011). Together with shrimp and mollusks, it already ranks among the top most in-demand aquaculture products globally (Sathiadhas and Najmudeen, 2004). In 2020, crustacean production accounted for nearly 11 237 metric tons in world aquaculture, of which 159.4 metric tons came from crabs (FAO, 2022). Furthermore, aside from the delicious and nutritious crab meat, its shells are also demanded in the pharmaceutical industry for its high levels of chitin, which has a variety of applications such as water-repellent adhesive, oral mucoadhesive liquid, and wound dressings (Kato et al., 2003). Aquaculture is also exploring the possibility of using shells as a formulated feed ingredient because they contain chitin like krill (Ringo et al., 2012).

Mangroves' crabs are initially harvested in the wild (Agbayani, 2011; Mirera, 2011; Quinitio, 2017; Hasanuzzaman et al., 2014; Basu and Roy, 2018). Mangroves provide a high level of salinity and temperature and protect young against cannibalism and predators, which is why these species thrive in these areas (Baylon, 2010; Alberts-Hurbatsch et al., 2014). In addition to its undeniable importance to society, it is an integral part of the mangrove ecosystem since it is critical in nutrient cycling and energy transfer (Mirera, 2011). Thus, wild mangrove crabs are increasingly being collected, leading to increased concern that a decrease in their abundance might negatively affect their ecosystems (Alberts-Hubatsch et al., 2016). Furthermore, habitat destruction has been a serious concern resulting from the hunting of these wild species (Shelley, 2008). Due to high demand in the market, even juveniles of mangrove crabs (crab farming's main source of crab seeds) are still extracted despite rising concerns (Lindner, 2005). Due to its higher survival rate and resistance to disease than shrimps, the mangrove crabs trade is a reliable source of income and is also a good alternative for shrimp farmers (Marichamy and Rajapackiam, 2001). As a result, commercial mangrove crab culture initiatives are being developed (Williams and Field, 1999). Different techniques have been developed to achieve the best possible method for fattened crabs to survive and grow (Shelley, 2008; Agbayani, 2011; Petersen et al., 2011; Quinitio, 2017). In addition, there is an ongoing effort to develop alternative feeds that would produce better quality mangrove crabs, both in size and consistency (Alber, 2003).

Mangrove crabs consume fish, mollusks, crustaceans, sand, and detritus (Viswanathan and Raffi, 2015). However, as the culture of this product has grown, the supply of feeds has become a major problem (Alber, 2003). Particularly, trash fish, one of the fishmeal feeds to mangrove crabs, have competing demands with the growing human food consumption (Gasco et al., 2018). Therefore, mangrove crab culture may not be worthwhile due to the high prices of trash fish in response to the competing demand over the years. As a result of this problem, researchers have worked on formulating alternative feeds to fatten mangrove crabs, such as plant-based, algae, animal-based, insects, and crustacean feeds (Alber, 2003; Krogdahl, 2016; Gasco et al., 2018). Crab shellmeal has been used as a replacement for fishmeal in poultry and livestock (Vijayalingam and Rajesh, 2020). Due to the high chitin content of crustacean by-products such as shrimp heads and shells and crab shells, the use of these products in feed has gained an increasing amount of attention in recent years (Gasco et al., 2018). In food and pharmaceutical manufacturing, chitin is a biopolymer with various uses (Kato et al., 2003). Toppe et al. (2006) stated that crab by-products could be used as a feed in diets for the fish Atlantic cod, *Gadus morhua*. In crustaceans, such as prawn *Macrobrachium rosenbergii,* chitin diets are shown to advance growth (Kumar et al., 2006). In addition, increasing survival with chitin diets has been linked to removing potentially pathogenic bacteria from adult male shore crab *Carcinus maenas* (Powell and Rowley, 2007). However, researchers are still investigating whether crab shells can be used as a feed ingredient in aquaculture (Ringo et al., 2012). Considering this, the study examined the effectiveness of formulated feed incorporated with crab shells as a meal for mangrove crab *S. serrata* on survival, growth, proximate composition, feed utilization, and carcass composition.

2. Material and Methods

2.1. Study site and duration

This study was conducted at the Multi-Species Hatchery of the College of Fisheries, Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao Tawi-Tawi, Philippines (Figure 1) for a duration of 30 days.

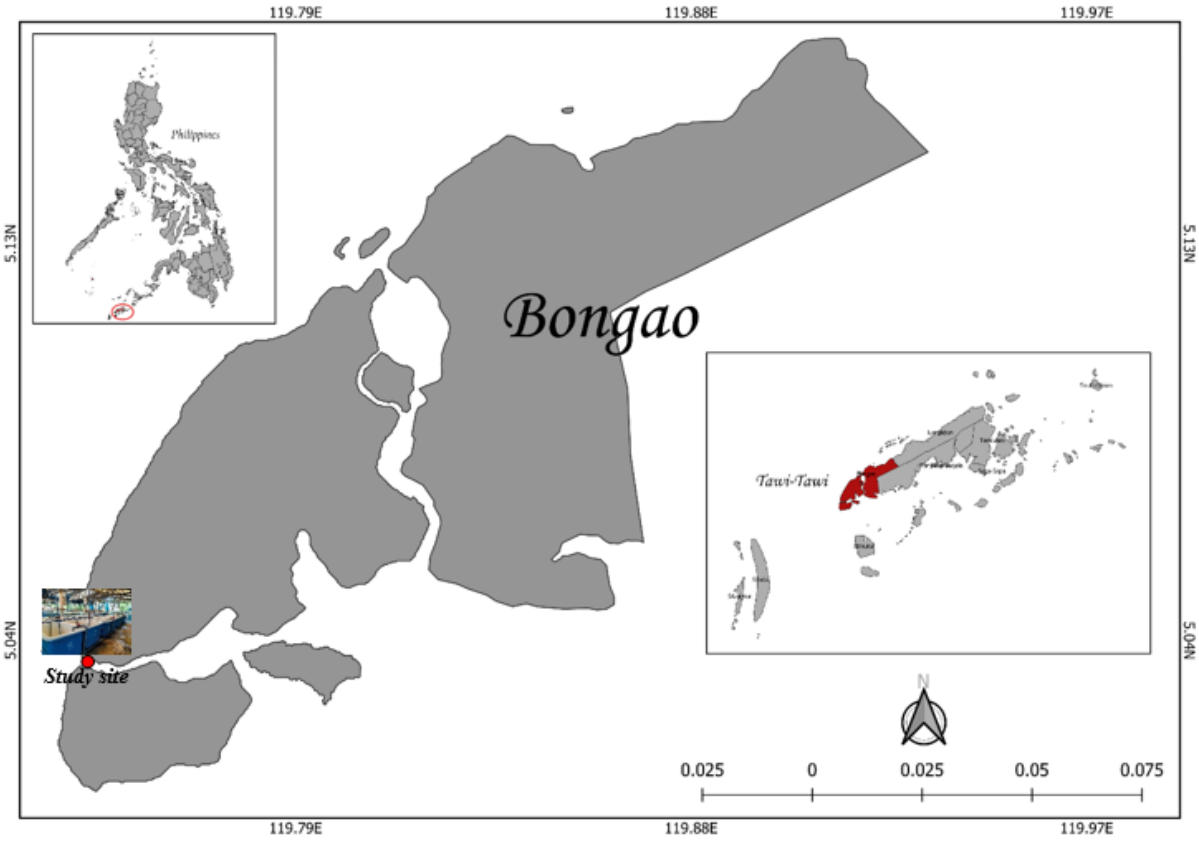

Figure 1. Map of the study site.

2.2. Source of crab shells and feed ingredients

Blue swimming crab (*Portunus pelagicus*) shells were used as the source of powdered crab shells from the carapace and pincers only because these parts of crab shells could be cleaned and powdered easily. These were collected from Crab Meat Processing Plant in Tubig Boh, Bongao, Tawi-Tawi, Philippines. Roughly 1.4 tons (wet weight) of crab shells (Figure 2) were discarded monthly by this processing plant (Pers. Com.). In addition, the feed ingredients used in the experiment were purchased from Bongao Public Market, as well as the fresh *Sardinella* sp. that was used for the positive control diet.

Figure 2. Discarded crab shell in Crab Meat Processing Plant, Bongao, Tawi-Tawi, Philippines.

2.3. Experimental Species

Mangrove crab *S. serrata* was used as an experimental animal, purchased from fisherfolk in Panglima Sugala, Tawi-Tawi, which was previously collected from mangrove areas. The crabs were transported by pump boat to Bongao, Tawi-Tawi, and landed at the MSU-TCTO Multi-purpose Hatchery.

Figure 3. Experimental animals.

2.4. Preparation of powdered crab shells

Blue swimming crab (*P. pelagicus*) shells were cleaned thoroughly and air-dried within five days until they could be crushed easily and ground using a grinder. The ground crab shells were sieved, packed in ziplock plastic, and stored at room temperature to be used to formulate experimental diets.

2.5. Preparation of treatment diets

Table 1 presents the ingredients of the experimental diet. Using locally available ingredients, crab shells were gradually substituted for fishmeal in a feed formulation. They were dried and processed, along with other ingredients for animal feed. The ingredients were powdered in an osteorizer and sieved through a 0.5 mm mesh siever. In an electric blender, with the binder dissolved in 500 mL of purified water, the ingredients were mixed according to the formula. The mixture was kneaded into a dough and formed into a spherical ball approximately 2.0 cm in diameter by hand (Ali et al., 2011). The spherical balls were steamed at atmospheric pressure for five minutes and dried in an electric oven at 60 ˚C for 12 hours to remove excess water. The dried feeds were stored in sealed plastic containers until used. There were five diets prepared, four experimental diets incorporated with 0% (negative control), 10%, 20%, and 30% powdered crab shells $kg^{-1,}$ respectively, and positive control (chopped *Sardinella* sp.). All treatment diets were evaluated using proximate analysis, which was done at Davao Analytical Laboratories Inc. (DALI) in Davao City and the Department of Science and Technology (DOST) in Region IX, Zamboanga City, Philippines.

Table 1. Composition of the treatment diets in crab shellmeal as replacement of fishmeal (g kg^{-1})

Ingredients	**Inclusion level**			
	0% Crab shell	10% Crab shell	20% Crab shell	30% Crab shell
Crab shell meal (g)	0	100	200	300
Fishmeal (g)	300	200	100	0
Copra meal (g)	300	300	300	300
Rice Bran (g)	300	300	300	300
Corn starch (g)	40	40	40	40
Palm Oil (mL)	40	40	40	40
NaCl (g)	19.5	19.5	19.5	19.5
Vitamin Premix (g)	0.5	0.5	0.5	0.5
Total	1 000	1 000	1 000	1 000

2.6. Stocking of crabs

Experimental animals to be considered were a young adult male and female mangrove crabs that have already reached sexual maturity (approximately 150 – 350 grams in weight). Prior to stocking, 50 crabs were used and acclimatized for one week with salinity ranging from 30 to 33 ppt and temperature from 26 to 30 °C. The crabs were subjected to an intensive environment where feeds were provided, the water was replenished, and waste was removed regularly. During the experiments, the crabs were randomly weighed (207.24 ± 7.66g), assigned, and stocked in 30-L plastic containers (one crab per container). Each treatment had ten replicates.

2.7. Water maintenance

The temperature and salinity of the water were monitored using a thermometer and refractometer. Prior to feeding each day, both parameters were checked before and after changing the water in the plastic containers. Every week, approximately 80% of the water was changed. Siphoning the container every early in the morning was done before changing the water to remove uneaten feeds.

2.8. Feeding

There were five dietary treatments used in the experiment: Diet 1 (30% FM and 0% CSM) as the negative control, Diet 2 (20% FM and 10% CSM), Diet 3 (10% FM and 20% CSM), Diet 4 (0% FM and 30% CSM crab), and Diet 5 (chopped *Sardinella* sp.) as the positive control. The experimental feeds and chopped *Sardenella* sp. were weighed and supplemented based on the weight of each crab per plastic container. Feeding was done once a day in the afternoon with 5% of the average body weight.

2.9. Experimental design

This study used a Completely Randomized Design (CRD) with five experimental diets and ten replicates for each diet (Figure 4). The experiment was conducted using a 50 rectangular clear plastic container (30 L volume capacity) half-filled with seawater with holes on its cover to enable air passage and another hole on its side that serve as excess water passage from the container.

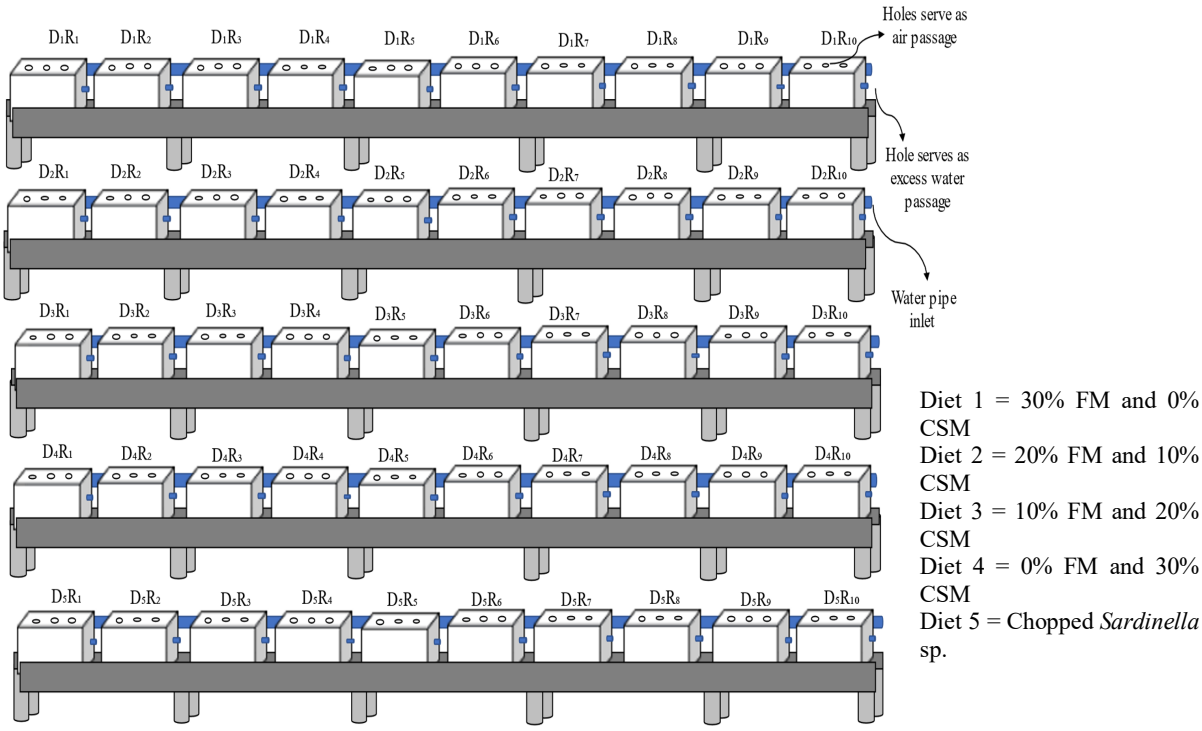

Figure 4. Experimental set-up of the study.

2.9. Sampling

Sampling was done every 15 days to determine the survival and growth rates. Analysis of the body composition was done at the end of the culture period. In order to avoid scaping the crab, a plastic bowl was used, which was weighed using a weighing scale, then tared to zero weight before placing the crab inside. Samples of treatment diets and body composition of experimental animals were sent to DOST, Zamboanga City, and DALI, Davao City, for proximate and carcass analysis such as ash, moisture, carbohydrates, crude fat, crude protein, crude fiber, and calorie. Proximate and carcass analysis was conducted after 30 days of the culture period. The survival rate, specific growth rate (SGR), and feed utilization were computed using the following formula (Kader et al., 2017):

A. Survival rate

$$Survival\ rate = \frac{Final\ number\ of\ stocks}{Initial\ number\ of\ stocks} \times 100 \quad (1)$$

B. Growth rate

$$WG\ (g) = ABWf - ABWi \quad (2)$$

$$SGR\ (\%\ Day) = \frac{\ln(ABWf) - \ln(ABWi)}{DOC} \times 100 \quad (3)$$

Where: WG = weight gain
ABWf = average body weight final
ABWi = average body weight initial
DOC = days of culture

C. Feed utilization

$$FCR = \frac{FI}{WG} \quad (4)$$

$$PER = \frac{WG}{FI} \times Diet\ CP \quad (5)$$

Where: FCR = feed conversion ratio
FI = feed intake
WG = weight gain
PER = protein efficiency ratio

2.10. Statistical analysis

The collected data were presented as mean ± standard error of the mean (SEM) and subjected to a one-way analysis of variance (ANOVA) using IBM SPSS 20.0 software package to identify significant differences in the different treatments in terms of growth, survival rate, and feed utilization at $\alpha = 0.05$. Levene's Test was used to test for homogeneity of variance, and Duncan's Post-Hoc Test was used to rank the mean.

3. Results

3.1. Proximate composition treatment diets

The proximate composition of treatment diets is shown in Table 2. Levels of ash content and carbohydrates varied from 5.57 ± 0.12 % – 15.23 ± 0.13 % and 1.16 ± 0.87 % – 38.23 ± 0.27 %,

respectively, indicating that Diet 4, which contains 30 % crab shellmeal inclusion significantly higher ($p<0.05$) among all diets. Moisture content ranged from 15.09 ± 0.04 % – 20.38 ± 0.18 %, where Diet 2, Diet 3, and Diet 4 were significantly lower ($p<0.05$) than Diet 1 and Diet 5. Crude fat levels varied from 9.60 ± 0.06 % – 28.47 ± 0.23 %. Diet 1 was significantly greater ($p<0.05$) in all diets in terms of crude fat. Levels of crude protein in all experimental diets ranged from 8.93 ± 0.22 % – 57.60 ± 0.70 %, where Diet 5 was significantly higher ($p<0.05$) among all diets. Crude fiber levels varied from 0.46 ± 0.04 % – 9.79 ± 0.41 %, and Diets 3 and 4 were significantly higher ($p<0.05$) in all diets.

Table 2. Proximate composition of experimental diets (%)

[1]Diet	Ash	Moisture	Carbohydrates	Crude fat	Crude protein	Crude fiber
Diet 1	5.57 ± 0.12^{e}	17.30 ± 0.10^{b}	28.63 ± 0.58^{d}	28.47 ± 0.23^{a}	20.03 ± 0.18^{b}	3.91 ± 0.27^{d}
Diet 2	9.33 ± 0.03^{d}	15.25 ± 0.05^{d}	31.73 ± 0.26^{c}	25.90 ± 0.17^{b}	17.77 ± 0.09^{c}	6.62 ± 0.44^{c}
Diet 3	11.67 ± 0.03^{b}	16.90 ± 0.08^{c}	35.80 ± 0.44^{b}	24.27 ± 0.43^{c}	11.30 ± 0.06^{d}	9.50 ± 0.59^{ba}
Diet 4	15.23 ± 0.13^{a}	15.09 ± 0.04^{d}	38.23 ± 0.27^{a}	22.53 ± 0.12^{d}	8.93 ± 0.22^{e}	9.79 ± 0.41^{a}
Diet 5	11.27 ± 0.12^{c}	20.38 ± 0.18^{a}	1.16 ± 0.87^{e}	9.60 ± 0.06^{e}	57.60 ± 0.70^{a}	0.46 ± 0.04^{e}

[1]Values are measures of triplicates. Means with the same letters within a row do not differ significantly (P> 0.05), n = 15.

3.2. Growth and survival performance and feed utilization

The gain weight and specific growth rate (SGR, % day^{-1}) of *S. serrata* are shown in Figures 4 and 5. The gain weight of Diet 1, Diet 2, Diet 3, Diet 4, and Diet 5 groups were 19.08 ± 7.48 g, 16.04 ± 2.93 g, 15.96 ± 1.91 g, 21.75 ± 7.91 g, and 23.87 ± 8.99 g, respectively. All experimental diets were not significantly different ($p>0.05$) in terms of weight gain after 30 days of the culture period. However, mangrove crab fed with Diet 4 was higher than Diet 2, Diet 3, and the negative control diet. Moreover, Diet 1, Diet 2, Diet 3, Diet 4, and Diet 5 achieved SGR of 0.26 ± 0.09 % day^{-1}, 0.26 ± 0.07 % day^{-1}, 0.26 ± 0.05 % day^{-1}, 0.35 ± 0.11 % day^{-1}, and 0.42 ± 0.18 % day^{-1}, respectively. All experimental diets were not significantly different ($p>0.05$). Nevertheless, Diet 4, which contains a 30 % diet of crab shellmeal inclusion fed on mangrove crab *S. serrata* obtained higher SGR, although there was no significant difference.

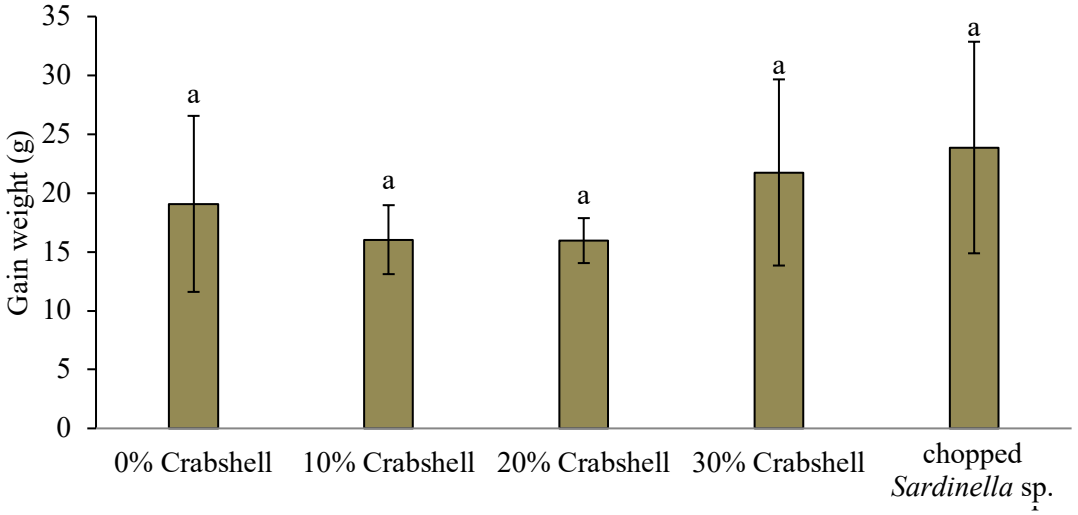

Figure 4. Gain weight performance of mangrove crab *S. serrata* fed with different diets after 30 days of culture. Diet 1 = 0% Crabshell, Diet 2 = 10% Crabshell, Diet 3 = 20% Crabshell, Diet 4 = 30% Crabshell, Diet 5 = Copped *Sardinella* sp. Bars with the same letters do not differ significantly (P> 0.05). Values are means ± S.E.M (standard error mean), n= 6 – 40.

The survival rate (SR, %) of Diet 1, Diet 2, Diet 3, Diet 4, and Diet 5 groups were 80.00 ± 13.33 %, 80.00 ± 13.33 %, 60.00 ± 16.33 %, 80 ± 13.33 %, and 100.00 ± 0.00 % (Figure 6). Inclusions of all levels of crab shellmeal fed on mangrove crab *S. serrata* were not significantly different among experimental diets in terms of SR. Moreover, Table 3 shows the feed intake (FI), feed conversion ratio

(FCR), and protein efficiency ratio (PER) values of mangrove crab *S. serrata.* The combination of fishmeal and crab shellmeal had no significant effects in terms of FI, FCR, and PER. However, the FI and FCR of all experimental diets were significantly higher ($p<0.05$) than those of trash fish (chopped *Sardinella* sp.), whereas the PER of trash fish was significantly higher ($p<0.05$) than that of all experimental diets.

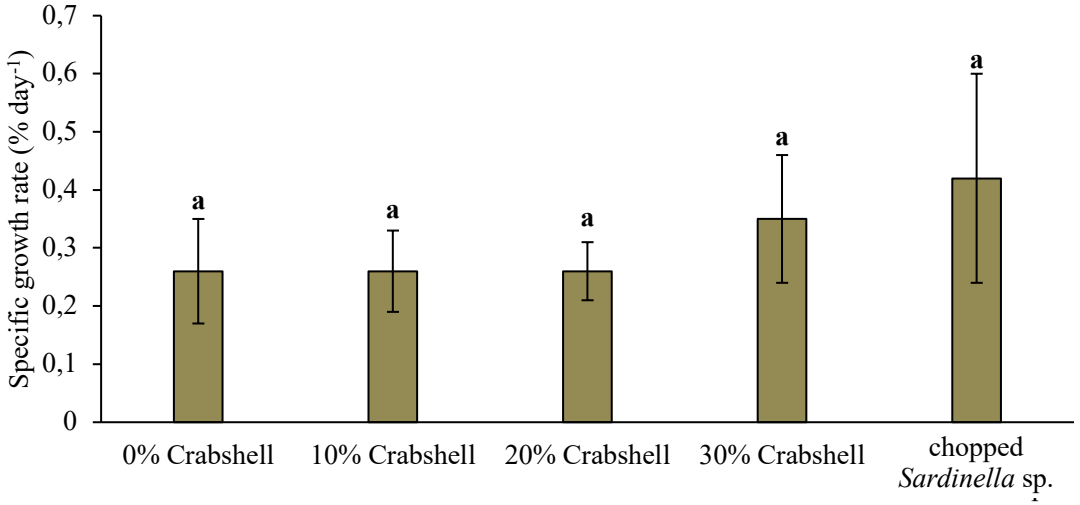

Figure 5. Growth performance (SGR) of mangrove crab S. serrata fed with different diets after 30 days of culture. Diet 1 = 0% Crabshell, Diet 2 = 10% Crabshell, Diet 3 = 20% Crabshell, Diet 4 = 30% Crabshell, Diet 5 = Copped Sardinella sp. Bars with the same letters do not differ significantly (P> 0.05). Values are means ± S.E.M (standard error mean), n= 6 – 40.

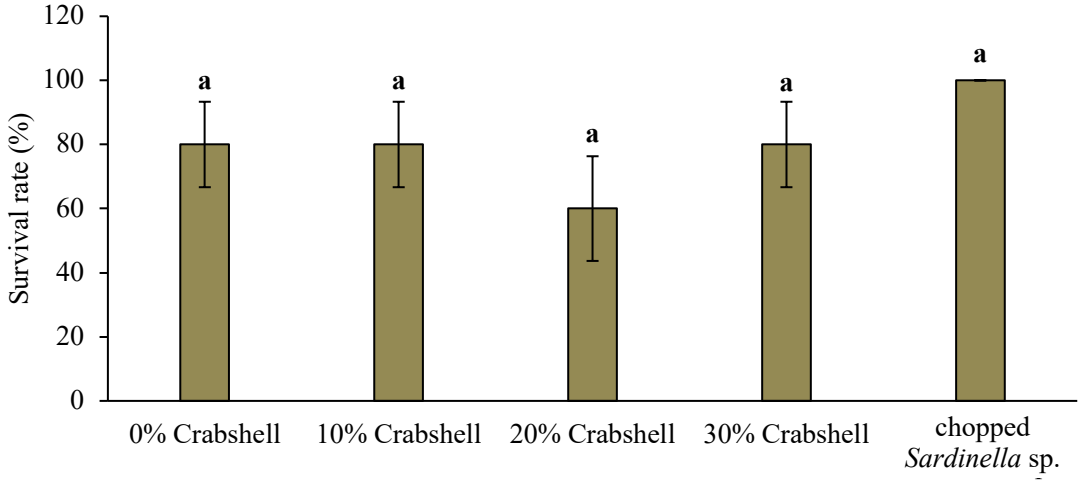

Figure 6. Survival rate of mangrove crab *S. serrata* fed with different diets after 30 days of culture. Diet 1 = 0% Crab shellmeal, Diet 2 = 10% Crab shellmeal, Diet 3 = 20% Crab shellmeal, Diet 4 = 30% Crab shellmeal, Diet 5 = Copped *Sardinella* sp. Bars with the same letters do not differ significantly (P> 0.05). Values are means ± S.E.M. (standard error mean), n = 10 – 50.

Table 3. Feed utilization of mangrove crab *S. serrata*

[1]Diets	FI	FCR	PER
Diet 1	261.94 ± 34.19^{a}	22.17 ± 3.73^{a}	1.41 ± 0.49^{b}
Diet 2	285.12 ± 27.81^{a}	22.23 ± 4.12^{a}	1.15 ± 0.33^{b}
Diet 3	265.43 ± 29.13^{a}	18.31 ± 3.40^{a}	0.75 ± 0.16^{b}
Diet 4	253.45 ± 14.17^{a}	21.30 ± 6.43^{a}	0.75 ± 0.25^{b}
Diet 5	115.71 ± 21.75^{b}	7.71 ± 1.99^{b}	161.81 ± 140.44^{a}

[1]Values are means ± S.E.M. (standard error mean). Means with the same letters within a row do not differ significantly (P> 0.05), n = 40.

3.3. Whole body proximate composition of mangrove crab

Proximate compositions of mangrove crabs are shown in Table 4. Ash content and moisture content varied from 21.53 ± 0.22 % – 31.60 ± 0.20 % and 7.69 ± 0.04 % – 21.48 ± 0.05 %, respectively, where crab fed with Diet 3 was significantly (P<0.05) greater among all diets. Carbohydrates ranged from 13.00 ± 0.10 % – 34.07 ± 0.87 %, indicating that crab fed with Diet 4 was significantly higher (P<0.05) in all experimental diets. Levels of crude fat ranged from 1.73 ± 0.18 % – 5.03 ± 0.07 %, where crab fed with Diet 2 and Diet 4 were significantly lower (P<0.05) than crab fed with Diet 1, Diet 3, and Diet 5. In addition, crude protein varied from 21.83 ± 0.73 % – 37.70 ± 0.12 %, where crab fed with Diet 2 was significantly greater (P<0.05) among all diets. Levels of calories ranged from 218.33 ± 1.45 % – 275.00 ± 1.15 %, indicating that crab fed with Diet 5 was significantly higher (P<0.05) among all experimental diets.

Table 4. Whole body composition of mangrove crab *S. serrata* (%)

[1]Diet	Ash	Moisture	Carbohydrates	Crude Fat	Crude Protein	Calorie
Diet 1	26.67 ± 0.20^{c}	16.33 ± 0.09^{b}	26.57 ± 0.35^{c}	3.00 ± 0.15^{b}	27.43 ± 0.24^{d}	243.00 ± 0.58^{c}
Diet 2	26.10 ± 0.15^{d}	21.48 ± 0.05^{a}	13.00 ± 0.10^{e}	1.73 ± 0.18^{d}	37.70 ± 0.12^{a}	218.33 ± 1.45^{e}
Diet 3	31.60 ± 0.20^{a}	7.69 ± 0.04^{e}	28.77 ± 0.20^{b}	2.47 ± 0.09^{c}	29.47 ± 0.19^{c}	255.33 ± 0.33^{b}
Diet 4	29.40 ± 0.23^{b}	12.79 ± 0.13^{d}	34.07 ± 0.87^{a}	1.90 ± 0.26^{d}	21.83 ± 0.73^{e}	241.00 ± 2.52^{d}
Diet 5	21.53 ± 0.22^{e}	16.09 ± 0.07^{cb}	25.07 ± 0.78^{d}	5.03 ± 0.07^{a}	32.30 ± 0.60^{b}	275.00 ± 1.15^{a}

[1]Values are means ± S.E.M. (standard error mean). Means with the same letters within a row do not differ significantly (P> 0.05), n = 15.

4. Discussion

CSM supplementation can enhance the proximate composition of experimental diets, thereby improving mangrove crab's whole body composition. However, CSM supplementation had no significant effect on feed utilization, as well as the growth and survival rates of mangrove crabs. In mangrove crab farming, shrimp diet and trash fish are commonly used. Therefore, developing an appropriate mangrove crab feed formulation is a priority area of research (Say and Ikhwanuddin, 1999; Zhao et al., 2016; Dayal et al., 2019; Aaqillah-Amr et al., 2022). A farmer can further enhance the quality of the feed by understanding the composition and reformulating the feed if the nutrients are not adequate to sustain the crabs. The crab's body is made of claws and legs, and its back shell is made of hard, opaque tissue called chitin (Cornwall, 2014). Chitin is a polymer that is found in arthropods' exoskeletons, such as crabs (Guerrero, 2000; Brock et al., 2003). Among the most abundant organic compounds in nature, chitin is made up of sugar molecules arranged in a network with a variety of uses, such as agriculture, aquaculture, and biotechnology (Beundia, 1999; Shamshina et al., 2020). About 10% of the shell of the mangrove crab *S. tranquebarica* contains chitin (Thirunavukkarasu and Shanmugam, 2009). Using underutilized by-products in the seafood industry as a substitute for costly FM in mangrove crab diets, the present study aims to develop a more cost-effective diet. By substituting FM with 30% CSM, it is evident that the survival and growth of mangrove crab *S. serrata* can be boosted, although not significantly different. In aquaculture nutrition, the replacement of FM with alternative protein sources has long been well-established for many crustacean and fish species, and research on crab species is meager. Species of crabs such as *Eriocheir sinensis* (Luo et al., 2011; Jiang et al., 2013), *S. serrata* (Nguyen et al., 2014), and *S. paramamosain* (Suwirya et al., 2009) have been studied to replace FM with different vegetable sources of protein. In addition, the fish bone meal (FBM) is regarded as a significantly improved source of dietary protein that can be used to replace 45% of FM in Atlantic cod diets. Luo et al. (2011) stated that it is possible to replace 40% of FM with rapeseed meal and soybean meal in the diet of *E. sinensis*. In addition, *S. paramamosain*, soybean meal, and corn gluten meal could replace 20 – 40% of FM (Suwirya et al*.,* 2009). In our study, 10 – 20% FM could be replaced with CSM for *S. serrata.* The higher level of FM could be replaced by animal protein sources, possibly

due to higher protein content, fatty acids, balanced amino acids, palatability, and less toxin or antinutrients (Kader et al., 2017).

FBM tended to increase dietary phosphorus, calcium, and ash contents, which helped to enhance Atlantic cod growth and feed consumption (Toppe et al., 2006). A crabstick (farm-made feed) developed for crab fattening increased moisture, crude protein, crude lipids, crude fiber, and total ash, thereby enhancing FI and FCR in blue swimming crab *P. pelagicus* (Kalidas et al., 2020). In the present study, increasing supplementation of CSM significantly affects dietary ash, moisture content, carbohydrates, crude fat, crude protein, and crude fiber. The nutrient composition of feed, such as ash, moisture content, carbohydrates, fat, protein, and fiber, can control the metabolism of crustaceans, shrimps, and other fishes, provide energy, and contribute to a living cell's functioning (Krogdahl et al., 2005; Ndome et al., 2010). For aquafeed ingredients, the PER is an effective parameter for measuring protein quality (Goytortua-Bores et al., 2006; Luo et al., 2011). In the present study, the FCR, PER, and FI showed no effect with different levels of CSM supplementation; hence, the feed utilization, growth, and survival of the mangrove crab *S. serrata* were not affected. In other studies, different levels of phospholipids enhanced feed utilization for kuruma shrimp (Michael et al., 2008), blue swimming crab (Li et al., 2014), and blant snout bream (Li et al., 2015), and black tiger shrimp (Kumaraguruvasagam et al., 2005).

CSM replacement changed the body composition of the *S. serrata*, such that ash, carbohydrates, moisture, crude fat, crude protein, and calories were significantly different ($p<0.05$). Kader et al. (2017) stated that there were no significant effects of replacing FM with FBM on the composition of whole bodies of *S. paramamosain.* However*,* the inclusion of phospholipids significantly improved crude lipids and crude protein content. Moreover, researchers have observed that dietary phospholipids positively affect protein retention in *Megalobrama amblycephala* (Li et al., 2015) and *Seriolla dumerilli* (Uyan et al., 2009). As shown in the body composition analysis, diets based on CSM (Diet 2, Diet 3, and Diet 4) contain higher levels of ash. Conversely, dietary FBM did not influence the whole body ash composition of *S. paramamosain* (Kader et al., 2017)**,** *Megalobrama amblycephala* (Li et al., 2015), *Penaeus monodon (*Kumaraguruvasagam et al., 2005), *Litopenaeus vannamei* (Gonzalez-Felix et al., 2002) and *Gadus morhua* (Toppe et al., 2006).

Conclusion

The results of this study suggest that fishmeal may be replaced with crab shellmeal from mangrove crab diet formulations. Supplementing crab shellmeal to the diets of mangrove crabs can enhance their whole body composition; however, it did not affect feed utilization, growth performance, and survival rate of the mangrove crab. By utilizing the by-products and waste products of crab meat processing plants, crab shellmeal can be the most cost-effective and efficient option for aquatic animal feed as it contains chitin and protein.

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Short Communication

Sex Reversal of the Giant Freshwater Prawn *Macrobrachium dacqueti* through Androgenic Gland Ablation

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Sex reversal

Abstract: The male *Macrobrachium* species (giant freshwater prawn) typically achieve better growth and a larger harvest size than females. Hence, it is clear that the monosex culture of all-male prawn populations would be inexpensively advantageous. This study aimed to determine whether androgenic gland (AG) ablation induces sex reversal of giant freshwater prawn *Macrobrachium dacqueti*. The AG of the prawn was ablated through bilateral microsurgery (AG ablation) and let them recover for two months. The weight and length were also measured every 15 days. Results revealed that the removal of AG from the males of immature *M. dacqueti* resulted in sex reversal, with 70% female differentiation. Successful neo-female prawns exhibited the development of an ovary with orange coloration as it matures. Andrectomized *M. dacqueti* did not develop the appendix masculina in the second pleopod, an indicator of a suspected neo-female prawn. A significant increase in weight and length was observed within two months compared to the control. Based on the result of the study, sex reversal of *M. dacqueti* is possible through AG ablation. Therefore, sex-reversed (neo-females) *M. dacqueti* can be used to breed with normal males to produce all-male progenies since both parents possess male hormones, thereby rendering a huge advantage for prawn culture.

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1. Introduction

The farming of giant freshwater prawns (*Macrobrachium* spp.) is important and is practiced worldwide, which contributed significantly to aquaculture production in many Southeast Asian countries such as Malaysia, Thailand, and Indonesia (Romana-Eguia et al., 2006). About 472 000 metric tonnes, or 5% of the total world crustacean aquaculture production, were contributed by *Macrobrachium* species in 2018 (FAO, 2020). This decapod has become a world food source owing to its great protein content, palatability, and bigger size (Tan and Wang, 2022). *Macrobrachium dacqueti* was previously known as *M. rosenbergii dacqueti* and was recently separated into different species by taxonomists (De

Bruyn et al., 2004; Wowor and Ng, 2007; Iketani et al., 2011). *M. dacqueti* is one of the most commercially significant crustaceans and is famously farmed all around the world (Wowor and Ng, 2007; Iketani et al., 2011). In the Philippines, *M. dacqueti* is among the important freshwater prawn with high economic value (Eguia et al., 2009). In recent years, this species of freshwater prawn has been not only extensively farmed but also fished and researched species from Indochina, South Asia, and Southeast Asia (Shy et al., 2013).

In the course of increasing production of giant freshwater prawns due to the increasing demand, the monosex culture (i.e., all-male populations) is more desirable and advantageous due to its fast-growing characteristics that can reach greater biomass than females during the harvest period (Aflalo et al., 2006; Aflalo et al., 2014; Levy et al., 2018). Recently, the use of monosex farming of crustaceans has been considered as a promising approach to increase production yield, especially when there are huge differences in growth rates and behavioral patterns between females and males (Tan et al., 2020).

Androgenic gland (AG) ablation as a sex reversal potential for monosex mariculture has been well explored in different species of decapods (Alfaro-Montoya et al., 2016; Tropea et al., 2011). AG ablation plays an important role in male differentiation among decapods (Tropea et al., 2011; Dunn et al., 2020; Tan et al., 2022). The AG is responsible for prawn sexual differentiation and is important for the male primary and secondary sexual characteristics (Tan et al., 2020). For instance, when the males of *M. rosenbergii* ablated at a very early development stage, sex-reversal was accomplished, making the males transformed into completely functional females (neo-females) (Sagi and Aflalo, 2005; Aflalo et al., 2006).

AG's role in the sex differentiation of Malacostraca has been largely studied, primarily employing two alternative techniques; AG implantation in females and andrectomy (AG ablation) in males. The alteration of AG is viable since, in male crustaceans, the endocrine and gametogenic functions are segregated into different organs, the AG and the testis, respectively. Hence, sex differentiation can be determined by removing the AG without impairing the gonads (Tropea et al., 2011), and by aberrating the male AG activity, de-masculation of crustaceans can be achieved (Ford, 2008). It is believed that the insulin-like AG produced by the male's AG in decapods governs the male sex differentiation, behavior as well as growth (Sroyraya et al., 2010; Huang et al., 2014).

The microsurgical ablation of the AG, also known as "andrectomy," is accomplished by the removal of the fifth pair of walking legs together with the AG and pulling off a significant portion of the sperm duct to ensure that the AG has been fully removed. Bilateral androgenic gland ablation can be performed as early as the formation of the post-larval stage of the prawn (Aflalo et al., 2006; Tan et al., 2020). To perform AG microsurgical removal, skillful personnel is needed, and under farm conditions, this can be realistically applied without any sophisticated instruments. Using this procedure, it is likely that the production of neo-females is high, and all-male prawn post-larvae mass production can be attained commercially when neo-female breed with normal males (Tan et al., 2020). Previous studies only focused on the sex reversal of *M. rosenbergii* and other crustaceans. Hence, this study aimed to determine whether sex reversal for *M. dacqueti* would be possible through AG ablation. Additionally, the weight and length of successfully sex-reversed individuals were also monitored.

2. Material and Methods

2.1. Study site

The study was conducted at the Fish Health Laboratory, Iloilo State College of Fisheries (ISCOF)-Main Tiwi Campus, Barotac Nuevo, Iloilo, Philippines.

2.2. Acquisition and acclimatization of experimental animal

Giant freshwater prawn *M. dacqueti* specimens were acquired from the Bureau of Fisheries and Aquatic Resources - National Integrated Fisheries Technology Development Center (BFAR-NIFTDC), Dagupan City, Pangasinan, Philippines. These were transported to the study site and acclimatized for one week.

2.3. Andrectomy process (AG ablation)

M. dacqueti prawns (30-day-old post larvae, PL_{30}) were separated into two groups: control and experimental. The control prawns (mixed male and female, n=100) were kept in normal culture, and the experimental group (all male, n=100) underwent the andrectomy process (AG ablation). Each of the experimental prawns was ablated through bilateral microsurgery of their androgenic glands. This was done by the removal of the fifth pair of walking legs together with the AG and pulling off a significant portion of the sperm duct to ensure that the AG had been entirely removed. The andrectomized prawns were placed in the recovery aquarium. A total of 100 andrectomized prawns were used in the experiment, and 70 individuals survived after two months of culture.

2.4. Culture and maintenance

The andrectomized and control prawns were stocked in 20 aquariums (10 for control and 10 for andrectomized prawns) with a stocking density of 10 prawns per aquarium. The prawns were fed at a 5% feeding rate, same water management and supply of aeration were applied. The prawns were maintained at 27°C temperature, 0 ppt salinity, 6.5-6.8 pH, and satiated dissolved oxygen (6-9 ppm). The duration of the culture was two months after the andrectomy.

2.5. Sampling

Sampling was done every 15 days for two months. Once no mortality was observed after AG ablation (1-7 days), monitoring of weight and length was commenced. All individuals were sampled for measurements of length and weight using a vernier caliper and an electronic top-loading balance scale, respectively. A total of 70 andrectomized prawns and 90 control prawns survived after the culture period.

2.4. Data analysis

The data on the weight and length of two sets of groups were subjected to a t-test using SPSS software version 20. The significant difference was set at 0.05.

3. Results and Discussion

Successful neo-female prawns developed an ovary. The ovary is located distal behind the eyes and is dorsally visible on the prawn's cephalothorax. As a prawn's ovary matures, its orange color is noticeable. In this study, out of the 100 ablated prawns, all 70% (70 individuals) of *M. dacqueti* that survived possessed light orange-colored gonads (Figure 1).

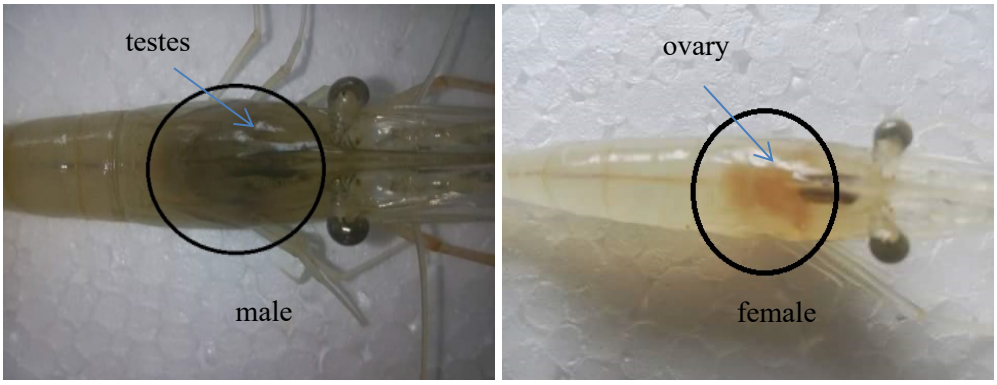

Figure 1. Gonadal development of *Macrobrachium dacqueti* before (left) and after AG ablation (right).

Mortalities (30% or 30 individuals) were caused by both the procedure and cannibalism. According to Aflalo et al. (2006), there is a possible ovarian development if a light orange-colored gonad is visible through the prawn's transparent cuticle, as observed in their study. This suggests that the AG-ablated *M. dacqueti* samples in this study successfully underwent sex reversal from male to neo-female

individuals. AG-ablated (andrectomized) *M. rosenbergii* (PL_{15} and PL_{30}) developed ovaries after 105 days of succeeding metamorphosis (Aflalo et al., 2014).

The appendix masculina (AM) is an accessory organ of a male situated medially on the second pair of pleopods between the appendix interna and endopodite. The absence or presence of an AM is the simplest way to determine females and males in most prawns (Hobbs et al., 1977). Studies use the existence of AM after ablation as an indicator of failed sex reversal. The absence of the AM, therefore, indicates a suspected neo-female prawn. As observed after andrectomy (AG ablation), the ablated prawns did not develop AM on their second pleopod (Figure 2 A). In the study of Aflalo et al. (2006), about 32% of the andrectomized *M. rosenbergii* males did not develop appendix masculina after 30 days, which indicated that sex reversal was completed.

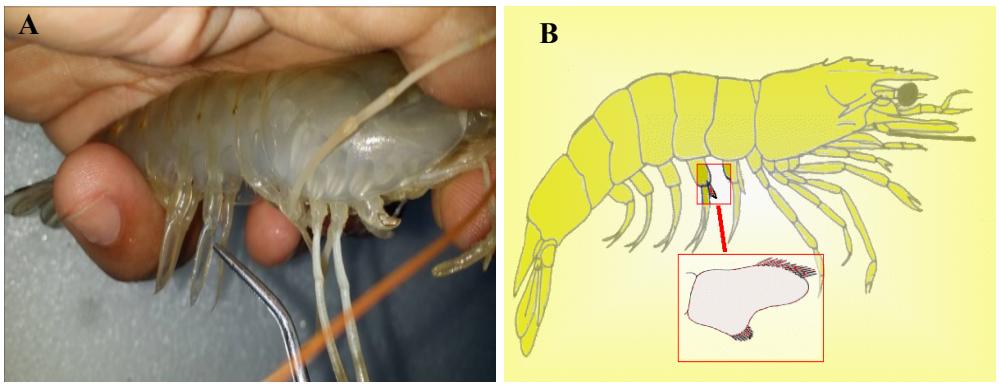

Figure 2. Appendix masculina (AM) of *M. dacqueti*. Absence of AM after AG ablation (left). Normal AM in unoperated prawn (right) (https://aquaculture.ugent.be).

In decapods, the first successful sex reversal was produced with *M. rosenbergii*. In order to obtain neo-males, the AG tissues were implanted into immature *M. rosenbergii* females. By crossing these neo-males, all-female offspring were produced (Nagamine et al., 1980; Tan et al., 2020). When the males of *M. rosenbergii* AG ablated at a very early development stage, sex reversal was accomplished, making the males transformed into entirely functional females (neo-females) (Sagi and Aflalo, 2005; Aflalo et al., 2006). In this study, sex reversal was successful at a higher rate (70% or 70 individuals), evident from the presence of an ovary and the absence of an appendix masculina. The mechanism behind successful sex reversal from males to neo-females of *M. rosenbergii* is attributed to different signaling pathways (e. g., Hippo, Rap1, PI3K, thyroid hormone, oxytocin, and apoptosis), which play essential roles in the sex reversal process by regulating gonad development, cell proliferation, and maintaining homeostasis (Tan et al., 2022). However, in other decapods, the use of AG ablation did not successfully accomplish the sex reversal in *Litopenaeus vannamei* (Alfaro-Montoya et al., 2016).

The AG is normally found associated with the terminal male gamete duct's portion. As stated by Diwan (2005), the bilateral andrectomy of AG in some shrimp has an effect on sex reversal. Specifically, shrimp males who have undergone andrectomy have lost secondary sexual characteristics and demonstrated a lack of sperm in the lumen of their testicular acini. AG in decapod is not necessary for spermatogenesis completion, and their non-existence leads only to the reduction of spermatogenesis intensity (Diwan, 2005). AG controls the male sex differentiation, growth, and behavior in crustaceans, where insulin-like AG plays a crucial role (Sroyraya et al., 2010; Huang et al., 2014; Sun and Li, 2021). The absence of AG in *Penaeus indicus* showed to impede spermatogonial differentiation (Mohamed and Diwan, 1991). In terms of the androgenic hormone's chemical nature, some studies indicated that the AG has the ability to produce various compounds, such as proteins and the hexahydroxy farnesylacetone, terpenes, and farnesylacetone, and the precise role of these compounds is still unknown (Laufer and Landau, 1991). Charmantier et al. (1997) stressed that the AG hormone regulates spermatogenic activity in the testis and is accountable for maintaining and developing secondary sexual characteristics in male crustaceans.

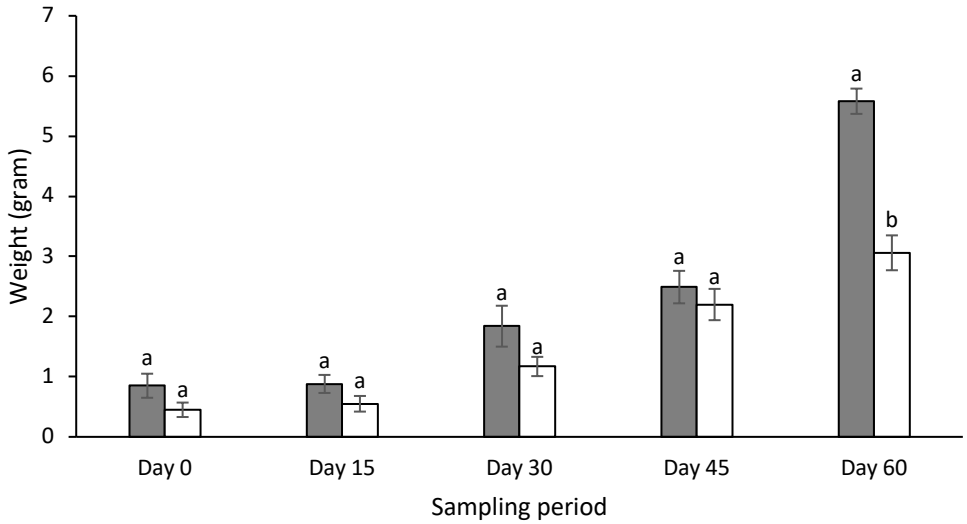

Figure 3. Weight of sex-reversed (dark gray bar) and control (light bar) *M. dacqueti* after 60 days.

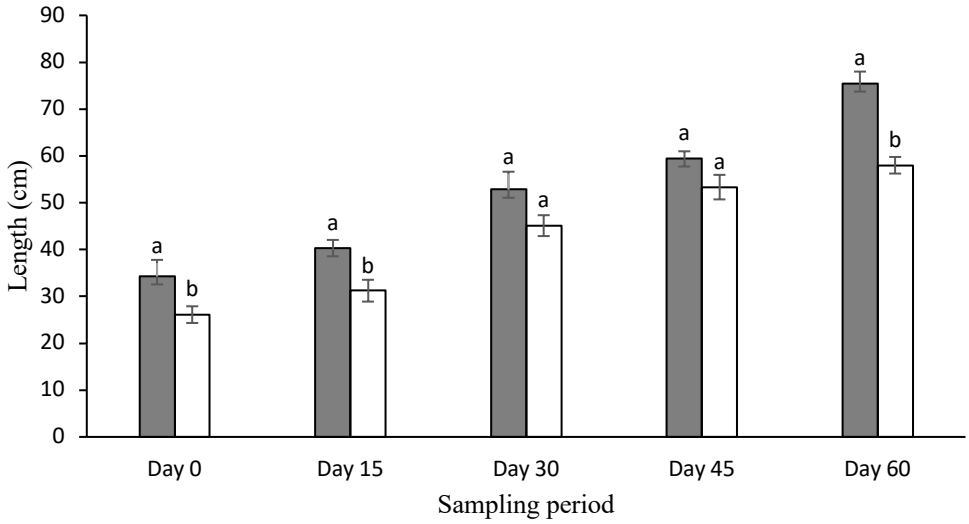

Figure 4. Length of sex-reversed (dark gray bar) and control (light bar) *M. dacqueti* after 60 days.

The weight and length of sex-reversed *M. dacqueti* after 60 days in this study were significantly higher (p<0.05) than the control prawns (Figures 3 and 4). The result of the study shows that the AG-ablated prawns grow faster than the control prawns because they still possess male genes. Earlier reports under the same genus, like in *M. rosenbergii*, indicated AG-ablated males resulted in lower growth significantly compared to unoperated male groups (Sagi et al., 1990). In the present study, since the control prawns were mixed (male and female), the female prawns may have affected the result of the growth of the control. In other decapods, the AG ablation of the *Cherax quadricarinatus* males had no influence on the somatic growth parameters (growth increment and specific growth rate) (Tropea et al., 2011). In *Litopenaeus vannamei*, although sex reversal was unsuccessful, andrectomized males were significantly higher compared to control both in terms of body weight and length four months after surgery of PL_{70} (Alfaro-Montoya et al., 2016).

Conclusion

In conclusion, our study demonstrated that sex reversal of *Macrobrachium dacqueti* is feasible through AG ablation. This practical approach can be used in a monosex culture by producing an all-male population through mating neo-females with normal male individuals, thereby enhancing the

production yield in prawn culture. However, the relationship between AG ablation and the growth of the prawn should be given attention in future studies.

Compliance with Ethical Standards

Authors' Contributions

R.C.V.: Design the experiment.
P.P.T., M.A.V., and R.C.V.: Conducted the experiment and collected the data.
R.C.V., J.O.A., and A.B.T.: Wrote the initial and final draft of the manuscript.
R.C. V. and A.B.T.: Analyzed the data.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Research Article

Extraction and Identification of Volatile Compounds in *Rosa laxa* Retz var *harputense* T. Baytop "Kişmiri rose"

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Abstract: The gardening of ornamental plants comes back to ancient times (Urartians). Nowadays the gardening of ornamental plants is done worldwide as in the Van provinces of Türkiye. Old and traditional gardens can be seen in different regions of this province naturally. Among these plants, *Rosa laxa* Retzius var. *harputense* T. Baytop (Kişmiri rose), is an exotic plant coming from Central Asia. Kişmiri rose has semi-double and miniature flowers and blooms for about five months in the ecological conditions of Van. It has not only highly decorative but also has a light, pleasant, and enthusiastic fragrance. As far as we know there is no previously published on the volatile profile of *Rosa laxa* Retz var. *harputense* (Kişmiri rose). For this purpose, in this study, "Headspace Solid Phase Micro Extraction Gas Chromatography-Mass Spectrometry" (HS/SPME/GC/MS) was applied for the detection of volatile compounds of Kişmiri rose flowers. A total of 31 compounds of Kişmiri rose were identified and quantified using Gas Chromatography-Mass Spectrometry (GC/MS). Among these identified compounds, phenyl ethyl alcohol (26.59%), cis-3-hexenyl acetate (18.573%) was detected as the major ones. According to the obtained results, it is concluded that our species has also a Chinese origin.

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1. Introduction

Roses are grown and consumed as food, perfumes, cosmetics, and pharmaceutical aspects and have a big potential for the ornamental plant industry (Guney, 2020). Native plants including *Rosa* species can be used for aesthetic studies besides functional landscape renewals such as biological repair works (Erzurumlu and Erzurumlu, 2021). The importance of roses is increasing recently worldwide because of containing natural fragrances and aromas, which make them an economically precious plant (Lavid et al., 2002; Kovacheva et al., 2010).

The rose plant, which has been used for medicinal purposes for over 5000 years, is mostly of Western Asian and partly European origin. It is common in Middle East and European countries,

especially in Iran, Afghanistan, Türkiye, and Bulgaria. Due to the pharmacological effect of the Rose, it is accepted as an important medicinal and aromatic plant. Besides, it is also widely used in the food and cosmetic industry. Studies have shown that the leaves and fruits of rose have health-supporting properties due to their anti-cancer, anti-inflammatory, anti-mutagenic, anti-microbial, and anti-depressant properties.

R. damascena Mill., *R. gallica* L., *R. moshata* Herrm, and *R. centifolia* L. are the four main *Rosa* species that are used for the production of essential oil in the industry all over the world. Different significant products, including concrete, water, oil, and absolute, are extracted from oil-bearing rose (Koksal et al., 2015).

Previous reports demonstrated over 400 volatile compounds which are obtained from the floral scent of various rose cultivars. It is well known that the main components of perfumery rose oil are linalool, citronellol, nerol, and geraniol. Volatiles reported from *Rosa* spp are classified into five major compounds: terpenes, esters, alcohols, acids, and others (Lavid et al., 2002). So far, volatile compounds of *Rosa damascena* Mill. of rose oil have been studied by some research groups (Babu et al., 2002; Rezaei et al., 2003; Nowa et al., 2005; Koksal et al., 2015).

In recent research carried out in Van's old garden, a new garden rose was determined; *Rosa laxa* Retzius var. *harputense* T. Baytop. Because of prominent characteristics such as shape, color, and scent, it has been admired since ancient times (Alp and Koyuncu, 2008 and 2010). This study aimed to detect flavor profiles of newly signed rose genotypes using Headspace Solid Phase Micro Extraction "Gas Chromatography-Mass Spectrometry Techniques" (HS-SPME/GC/MS).

2. Material and Methods

2.1. Material

Flowers of *Rosa laxa* Retzius var. *harputense* T. Baytop that are grown in a traditional garden were used as a material in the study. *R. laxa* var. *harputense* is a shrubby plant of 1-4 meters in height. The flowers are milky white with a base of lemon yellow. Flowers have a very pleasant fragrance and can be formed both singly or in 2-6 groups, and are semi-double with about 2.5 cm diameter. The blooming period may last from May to October, based on the ecological conditions. The blooming period of *R. laxa* var. *harputense* in Van province is about 5 months where the flowers were collected for analysis. Flowers of used rose are highly decorative with a pleasant fragrance (Alp and Koyuncu, 2008). Flower samples were taken at the blooming time (June 2014) in Van province.

2.2. Methods

2.2.1. HS-SPME analysis of volatile compounds

The extraction of the volatile compounds was performed via HS-SPME technique. Fresh flowers were homogenized with 5M calcium chloride to release volatiles through HS-SPME. Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) with 60 µm coated fused-silica fiber was used for absorbing the volatile compounds. The fiber was immersed through headspace and incubated for 30 min at 30ºC while extraction of volatiles was continued by stirring with a magnetic stirrer. After equilibration, the fiber was injected into the GC/MS port for 10 min set at 250ºC to thermally desorb the volatile compounds. The analyses were carried out in triplicate.

2.2.2. GC/MS analysis

A Perkin Elmer GC/MS (Clarus 600) equipped with HP-5 MS (30 m × 0.25 mm × 0.25 µm), fused-silica capillary column was used. The carrier gas was helium, with a flow rate of 1 ml/min. The injector temperature was 250ºC. The initial column temperature was set at 60ºC by increasing 5 ºC/min to 260 ºC and remained for 20 min. The mass spectra for the identification of volatiles were screened through Wiley and NIST GC-MS Libraries. The percentage of related picks for each identified compound was calculated using the total ion chromatograms equipped with the computerized integrator.

3. Results and Discussions

Gas chromatography is a popular basic analytical technique for analyzing volatile compounds in plants due to its efficiency, versatility, and sensitivity. However, different types of plant compounds only can be analyzed by GC when they are vaporized in normal conditions or can be vaporized at a suitable temperature. Solid Phase Micro Extraction (SPME) is also a fast and solventless technique for analyzing the volatiles between the headspace above the sample and a stationary phase coated on a fused-silica fiber (Zarifikhosroshahi et al., 2022). However, the volatile extraction and analysis methods may affect the apparent overall aroma composition. Moreover, new compounds can be formed before and during the analysis is possible (Kafkas et al., 2012; Guney et al., 2015; Keles and Erturk, 2021). The geographic and climatic conditions of the Van Region have revealed a unique garden culture, and R. laxa var. harputense grown in Van has taken an important place because of the smell in traditional Van garden culture. The percent of Volatile compounds in R. laxa var. harputense determined by HS-SPME/GC/MS technique are presented in Table 1.

Table 1. The percentage of volatile compounds of *R. laxa* var. *harputense*

Retention Time	Compounds	Content (%)
	Esters	
8.05	cis-3-hexenyl acetate	18.57
1.74	Methyl acetate	9.99
15.27	Benzyl acetate	9.19
9.28	Hexyl acetate	1.88
22.16	Methyl 9-octadecenoate	1.16
14.45	Citronellol acetate	0.93
2.18	Ethyl acetate	0.69
20.31	Methyl pentadecanoate	0.22
15.92	β-Phenethyl formate	0.11
	∑ **Esters**	**42.74**
	Alcohols	
17.24	Phenylethyl alcohol	26.59
16.83	Benzyl Alcohol	8.68
10.53	1-Hexanol	1.96
7.42	1-Penten-3-ol	0.06
14.22	Levomenthol	0.56
7.01	3-Hexenol	0.14
	∑**Alcohols**	**37.99**
	Aldehydes	
8.41	2-Hexenal	0.28
5.73	Hexanal	0.24
	∑**Aldehydes**	**0.52**
	Terpenes	
4.12	a-Phellandrene	0.59
13.707	Caryophyllene	0.27
19.88	a-Cadinol	0.18
7.92	D-Limonene	0.16
6.54	a-Pinene	0.16
8.92	3-Carene	0.07
	∑**Terpenes**	**1.43**
	Acids	
2.78	Formic acid	0.18
	Others	17.14

Totally, 24 volatile compounds, including 9 ester compounds, 6 alcohol compounds, 2 aldehyde compounds, 6 terpene compounds, 1 acid, and other compounds, were detected in Rose kesmiri (Table 1). Alcohols were detected as the major compounds, and the highest value was obtained from phenyl ethyl alcohol (26.59%) and benzyl alcohol (8.68%), respectively. Hethelyi et al., (2010) pointed out that phenyl ethyl alcohol was the principal component of fragrant rose flowers among the 13 rose varieties.

They also implied that the main compounds in white and pink rose varieties were phenyl ethyl alcohol and orcinol dimethyl ether. Phenylethanols show antioxidant, antiviral, and anti-inflammatory properties (Zarifikhosroshahi et al., 2021). The role of antioxidant capacity in the prevention of diseases such as cancer, cellular aging, cardiovascular diseases, and inflammation is proven (Keles, 2020; Gundesli et al., 2021, Ergun, 2021). Rusanov et al., (2011a) demonstrated that the main compound on flower extract of five oil-bearing rose genotypes was phenyl ethyl alcohol (7.99-8.44%) and followed by nonadecane, heneicosane, 9-nonadecene, heptecosane, tricosane, nonacosane, beta-citronellol, nerol, trans geraniol, n-heptedecane, pentacosane. Antonelli et al., (1997) reported that significant differences were seen in the composition of the major components in 24 old rose cultivars. Except for five cultivars, 2-phenyl ethanol was an almost major component in roses. Similarly, in this paper, we detected phenyl ethyl alcohol as the major component of *R. laxa* var. *harputense* of fresh petals. Piccone et al., (2004) also implied that 2-phenyl ethanol was the major volatile emitted of *Rosa damascena* var *semperflorens*. Rusanov et al., (2011b) demonstrated that major volatile compounds of *R. damascene* flower were designated by β-citronellol, trans-geraniol, phenyl ethyl alcohol, heneicosane, nonadecane, heptedecane. Similar results to previous papers were obtained in this study. However, various compounds were also detected in this paper. These differences can be due to using various extraction techniques. However, the aromatic compounds in ornamental plants also varies depending on the climatic conditions and the genotype (Georgieva et al., 2022).

Esters are one of the most valuable flavor compounds and cis-3-hexenyl acetate, Methyl acetate, Benzyl acetate, and acetic acid hexyl ester were identified as major ester compounds. Volatile components identified in the alcohols were determined to be phenyl ethyl alcohol, 1-hexanol, Levomenthol, 1-penten-3-ol, benzyl alcohol, 3-hexenol.

The results obtained in this study are in agreement with Joichi et al., (2005) in which Chinese rose species and varieties ascertained cis-3-hexenyl acetate and Citronellol acetate. The obtained results revealed that our species might come from China or Chinese origin. This study is important due to the first report in our country on *R. laxa* var *harputense*.

Conclusion

Rosa is a kind of cut-flower used in the cosmetic industry due to having a strong odor. As far as we know, there is no study on the volatile compounds of Kismiri Rose. Different numbers of volatile compounds were detected in Kismiri Rose flowers from Türkiye. Phenylethyl alcohol, cis-3-hexenyl acetate, and benzyl alcohol were the main aroma components for the studied rose flower. Due to increasing demands for cosmetic product besides natural ones versus synthetic perfumes, investigating flower scenes from naturally grown plants are of interest. However, agricultural aspects need plants that have various applications, such as medicine and landscape. Therefore, this study may be a light for achieving these purposes in the future because it is unavoidable to transform our genetic resources into economic values and to ensure the sustainable use of these resources.

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Research Article

The Effect of Inorganic Fertilizer and Biofertilizer Applications on Some Quality and Biochemical Properties of Safflower (*Carthamus tinctorius* L.)

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Biochemical

Abstract: This study was carried out in irrigated conditions during the summer growing season of 2020 and 2021 to determine the effects of biofertilizer applications and inorganic fertilization on some quality and biochemicals properties of safflower (*Carthamus tinctorius* L.) in Van ecological conditions. The experiment was set up as a randomized block design in 3 replicates at the Faculty of Agriculture, Van Yüzüncü Yıl University. The mixture of five different biofertilizers (*Frateuria aurantia* (B_1), *Bacillus megaterium* (B_2), *Azospirillum lipoferum* (B_3), *Chlorella saccharophilia* (B_4), and a *mixture of Lactobacillus casei + Rhodopseudomonas palustris + Saccharomyces cerevisiae + Lactococcus lactis microorganisms* (B_5)) different NP (nitrogen+phosphorus) fertilizer doses (control, 100% NP (NP_{100}) as full dose (optimum) 15 kg of pure nitrogen (Ammonium sulfate (21%) and 8 kg of pure phosphorus (TSP (42%)) per decare); % 7.5 kg of pure nitrogen (Ammonium sulfate (21%) and 4 kg of pure phosphorus (TSP (42%)) were applied as 50 NP (NP_{50}) reduced dose per decare. Some quality and biochemical Parameters including petal yield, crude oil rate, crude oil yield, total dyestuff ratio, total phenolic substance content, total flavonoid substance content and total antioxidant activity were measured. According to the results of the research; In both experimental years, the best results for crude oil yield and petal yield were obtained from NP_{100} applications, while the best results for total flavonoid substance content and total antioxidant activity were obtained from NP_0 applications. B_4 biofertilizer applications for crude oil ratio, B_1 biofertilizer applications for petal yield, and B_5 biofertilizer applications for total phenolic content were the biofertilizer applications with the best results in both years.

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1. Introduction

Due to the insufficient production and high cost of animal fats, there has been a tendency for vegetable oils, and vegetable oils have had a larger share in human nutrition than animal fats. Oil plants have great importance in both human and animal nutrition due to the protein, mineral substance, fat, carbohydrate, and vitamins they contain (Kolsarıcı et al., 2015). The amount of energy that the consumed foods provide to the body varies. It is known that the energy provided by the same amount of

fat is more than twice as much as protein and carbohydrate (Bahar, 1999). Oils; It is used as a raw material in many industrial establishments such as cleaning, construction, medicine, cosmetics, plastic, and biodiesel production (Arıoğlu, 2016). It was reported that the total production amount of oil crops worldwide in 2020 was 610.1 million tons and increased by 3.8% compared to 2019 (FAO, 2021). It is reported that the 2020 oil crops production area in our country is around 8.9 million decares, and the production amount is around 3.5 million tons (TUIK, 2021). The production area and quantities of the first five provinces, respectively, where safflower cultivation is carried out in our country, are given in Table 1.

Safflower, which is known to have originated in South Asia, spread to the Middle East and Mediterranean coasts, and has been cultivated in India, Egypt, Japan, Iran, and China and it is a species with a history of approximately 3500 years (Johnson et al., 2001). Safflower was first brought to our country by the Turks who immigrated from Central Asia, and its cultivation started in the 1940s. Safflower breeding was first started in the 1930s (Berber, 2007). Safflower, known as false saffron, zaferan, or painter's saffron, is an annual plant with wide leaves and flowers in colors such as yellow, orange, red, white, and cream, with thorny or thornless forms and with a height of up to 100 cm. It is known that the thorny forms have a higher oil rate and lower flower amount than the thornless forms. Safflower is a plant suitable for growing in arid regions, containing 30-50% oleic and linoleic acids in its seeds (Babaoğlu, 2005).

Table 1. According to TUIK 2020 data, the production area and quantities of the first five provinces where safflower cultivation is carried out, respectively, in our country

Provinces	**Production area (decare)**	**Amount of production (kg decare^{-1})**
Ankara	40 479	5 675
Muş	18 200	3 270
Aksaray	17 957	2 483
Konya	13 648	1 812
Gümüşhane	7 000	921

HSYA (Hydroxy Safflor Yellow A) constitutes most of the color pigments of the safflower flower. Since HSYA determines the majority of the therapeutic effects of safflower flowers, it is especially included in the Chinese pharmacopoeia (Ao et al., 2018).

It is reported that HSYA amounts of flower samples of different safflower varieties obtained from many countries are 0.05 mg-14.99 mg g^{-1}. It is reported that the environment in which the safflower plant grows, the color scale of the flower, and the harvest time are among the most important environmental factors affecting the HSYA rate (Zhao et al., 2020). It is reported that HSYA in safflower flowers decreases from dark colors to light colors (Xu et al., 2018), and the highest HSYA value is obtained in the morning hours, 4 or 5 days after the beginning of flowering (Tian et al., 2008).

Recently, it has been known that the colorants obtained from safflower flowers are used as natural colorants in the coloring of cosmetic products, face creams, shampoo, body lotion, and hair creams (Al-Snafi, 2015). Considering the frequently consumed beverages, foods such as candy, and pharmaceutical products such as syrup and tablets used in treatment, the importance of natural colorants is understood (Babaoğlu, 2005).

As a result of pharmacological studies, safflower flower extracts are used; In the protection of endothelial (inner surface cells of the vessels) cells, in the prevention of cerebral ischemia, dementia, parkinsonism, and traumatic brain damage from nervous system disorders, in acute lung inflammation, pneumonia and healing of wounds, which are among lung diseases, in coagulation, high blood pressure and heart enlargement from cardiovascular diseases. It is reported that it can be used in the prevention of circulatory failure, connective tissue growth, and tissue damage, which are liver diseases, and in the treatment of cancer disease and metabolic disorders (Ao et al., 2018).

Safflower seed contains less than 10% saturated fatty acids and more than 90% unsaturated fatty acids. It has been reported that palmitic and stearic fatty acids constitute the majority of saturated fatty acids in safflower seeds, while the majority of unsaturated fatty acids are composed of oleic (omega-9) from monounsaturated fatty acids and linoleic (omega-6) fatty acids from polyunsaturated fatty acids (Gürbüz, 1987). It is reported that safflower seeds contain 1.5-2.4% stearic fatty acid, 5.7-7.9% palmitic fatty acid, 13.5-17.6% oleic acid and 72.5-77.4% linoleic acid (Joshan et al., 2019). Safflower seeds

contain higher omega-6 fatty acids than olive, sunflower, rapeseed, corn, and hazelnut (Coşge et al., 2007).

Unsaturated fatty acids have important contributions to brain development, prevention of cardiovascular diseases, and strengthening of the body's defense system (Eseceli et al., 2006). In addition, unsaturated fatty acids are reported to be effective in increasing blood circulation and preventing congestion, reducing menstrual pains, lowering cholesterol levels, reducing insulin resistance and heart attack risk by causing an increase in the substance called adinopectin in the blood, and reducing obesity and hypertension (Nagao et al., 2003; Pischon et al., 2004; Guo, 2011).

Depending on the increase in the world population, the need for agricultural products and especially for plant production, is increasing day by day. Increasing crop production is mostly related to the use of synthetic fertilizers in sufficient quantities and at the right time. However, depending on the increase in the use of chemical fertilizers, the ecological balances deteriorated and the harmful aspects along with their benefits began to emerge, and the search for alternative fertilizers emerged. Biofertilizers, which are one of the leading alternative fertilizers, have recently started to be used in plant production due to their low cost, easy application, and being suitable for ecological agriculture. Microbial fertilizers (biofertilizers) are living organisms that contribute to the healthy growth and development of the plant in the root zone where it is applied, help reduce the damage caused by pathogens, facilitate the uptake of plant nutrients from the soil, and have a direct or indirect effect on reducing the damage caused to the plant by environmental stress conditions (Elnahal et al., 2022).

Determining the relationship of biofertilizers with the plant and determining their possible contributions to the plant is of great importance for the adoption and sustainability of organic agriculture. This study was carried out to determine the effects of different nitrogen and phosphorus combinations and environmentally friendly biofertilizer applications on some quality and biochemical parameters of the safflower plant, which has an important place in closing the oil deficit of our country in Van ecological conditions.

2. Material and Methods

The study was carried out in irrigated conditions in a summer cottage in the field crops trial area of Van Yüzüncü Yıl University Faculty of Agriculture in 2020 and 2021. In the study, as seed material, the registered oleic type safflower variety “Asol” in the spiny form obtained from Trakya Agricultural Research Institute was used.

According to Table 2, it is seen that the total precipitation in 2020 (336.8 mm) and the total precipitation in 2021 (206.2 mm) in the province of Van, where the research was conducted, are lower than the long term average (406.2 mm). It is seen that the average temperature of the 2020 trial year (10.76 ºC) and the 2021 trial year's average temperature (11.73 ºC) are higher than the long-term average (10.05 ºC). It is seen that the average humidity rate in 2020 (59.16%), in which the experiment was conducted, was above LTA (Long-Term Average), while the average humidity rate in 2021 (53.29%) was below LTA.

According to the results of the analysis of the soil sample in which the experiment was conducted, it was determined that it was a calcareous, slightly alkaline reaction, unsalted, sufficient in potassium, low in phosphorus, and weak in terms of organic matter, according to both research years.

Biofertilizer promotes plant growth in Van ecological conditions (B_0: Control, B_1=*Frateuria aurentia*, B_2=*Bacillus megaterium*, B_3=*Azospirillum lipoferum*, B_4=*Chlorella saccharophilia* (microalgae), B_5=*Lactobacillus casei* + *Rhodopseudomonas palustris* + *Saccharomyces cereviscociae* + *Lactococcus lactis*) inorganic fertilization (control, 50% NP reduced dose =7.5 kg da^{-1} pure $(NH_4)_2SO_4$; 4 kg da^{-1} pure P_2O_5, 100% NP full dose=15 kg da^{-1} pure $(NH_4)_2SO_4$; 8 kg da^{-1} pure P_2O_5) and It was aimed to determine some quality and biochemical characteristics of Asol safflower variety by applying different combinations of these. This study was organized according to the "Divided Plots in Random Blocks Trial Design" with 3 replications. The experiment was planned with inorganic fertilizer (NP) doses to the main plots and biofertilizer to the sub-plots. The trial field was left for the winter after deep plowing was done in the autumn, and it was made ready for planting by making surface plowing just before planting in the spring.

Table 2. Average temperature (°C), total precipitation (mm) and average humidity (%) values for 2020, 2021, and long years of Van province where the experiment was conducted

	AT (°C)			TP (mm)			AH (%)		
Months	**2020**	**2021**	**LTA**	**2020**	**2021**	**LTA**	**2020**	**2021**	**LTA**
January	-2.5	- 0.7	-2.5	43.8	13.0	33.2	74.5	67.1	66.7
February	-1.7	0.8	-1.5	79.9	12.9	31.5	77.1	73.3	67.2
March	4.9	3.7	2.8	44.3	39.9	47.7	72.5	67.0	65.4
April	8.6	11.7	8.4	51.8	5.0	57.4	65.4	48.8	59.3
May	14.5	16.7	13.4	27.8	20.2	45.3	54.0	46.4	55.1
June	19.3	21.6	18.8	13.7	0.2	16.4	44.4	32.0	47.1
July	23.0	24.2	22.7	17.6	4.6	6.9	46.4	38.4	42.3
August	21.6	23.5	22.9	10.0	1.4	5.3	44.5	38.0	40.5
September	20.1	18.8	18.3	5.6	6.3	20.4	41.3	40.6	43.9
October	13.3	12.3	12	1.8	50.2	48.2	53.0	51.0	57.3
November	6.7	7.0	5.1	12.8	23.1	48.8	65.4	69.6	64.2
December	1.4	1.2	0.2	27.7	29.4	45.1	71.5	67.3	67.5
Average	10.76	11.73	10.05				59.16	53.29	56.37
Total				336.8	206.2	406.2			

***** Meteorological data of the experimental area were obtained from the Van Meteorology Regional Directorate.
LTA: Long-Term Average, TP: Total Precipitation, AT: Average Temperature, AH: Average Humidity.

In the experiment, a distance of 2m was left between the blocks and 1m between the plots. The research plots are 3m x 1.8m = 5.4m^2 in size, and each plot is arranged with 30 cm row spacing and 6 rows. The total area of the experiment was 13m x 49.4m= 642.2 m^{2,} and 54 parcels were included in the trial. The sowing process was carried out manually at a depth of 3 cm in the lines opened with the marker so that 3 kg of seeds per decare were used in both years. Biofertilizer applications, from biofertilizers per 1 kg of seeds (according to the manufacturer's instructions for use); Seeds in bacterial suspensions prepared as B_1 = 10 ml L^{-1} distilled water, B_2 = 10 ml L^{-1}distilled water, B_3 = 10 ml L^{-1} distilled water, B_4 = 50 ml L^{-1} distilled water and B_5 = 10 ml L^{-1} distilled water, were added separately, and the coating process was carried out in this way.

Covered seeds were filtered and placed on blotting papers to dry in the shade for 30 minutes. Bacteria-covered seeds prepared for sowing were sown early in the morning to avoid the negative effects of sun rays on bacteria, and the seeds were immediately covered during sowing. In the rosette period, when the plants had 3-4 leaves (10-15 cm), thinning was done to 15 cm above the row. After thinning, biofertilizer solutions were prepared at the concentrations given above, and the prepared biofertilizer solutions were applied to the root zone of the plants, with an average of 125 ml per row in the plot. The application of Bio Fertilizer solutions to the plant root zone was made at sunset so that the biofertilizers would not be affected by light.

During the rosette period, when the plants had 3-4 leaves (10-15 cm), misfires were done on the row, with one plant in every 15 cm. In the experiment, 15 kg pure nitrogen (Ammonium sulfate (21%) and 8 kg pure phosphorus (TSP (42%)) as 100% NP full dose (optimum) per decare; 7.5 kg pure nitrogen per decare as 50% NP reduced dose (Ammonium sulfate (21%) and 4 kg of pure phosphorus (TSP (42%)) were applied (Tunçtürk, 2003). All of the phosphorus was given with the planting. Half of the nitrogen was given in the sowing and the other half in the plant elongation period. In both experimental years, the water needs of the plants were met with the sprinkler irrigation method due to insufficient rainfall after the plants emerged, during the plant elongation period, and in the pre-flowering period. After the plants germinated, weeds in the plots were removed by mechanical control, and hoeing was carried out before the plant elongation and flowering periods. Since disease or pest was not observed in the experiment, chemical control was not applied. Harvesting was done when the petals of the plants were completely dry, the grains turned white, and the leaves turned brown. All the measurements were

carried out on the remaining 2.4 m^2 (1.2m x 2m) area after the 6 rows forming the plot were excluded from the observation as a side effect of 50 cm from each side and the top of the row.

2.1. Petal yield (kg da^{-1})

The total weight of the petals of the plants in one m^2 harvested from the plots was determined as kg da^{-1} by weighing with an accuracy of 0.001 g (Kızıl. 1997).

2.2. Crude oil ratio (%)

Crude oil was obtained by using hexane as a solvent in Soxallet type extractors. The results were determined as % over dry matter (Tunçtürk, 2003).

2.3. Crude oil yield (kg da^{-1})

Crude oil yield values were calculated as a result of simple mathematical multiplication of % crude oil ratio values with seed yield values obtained from the same plot (Tunçtürk, 2003).

2.4. Total dyestuff ratio (%)

Total dyestuff determination was determined by modifying the method developed by Harborne (1973) for polar flavonoids. For dyestuff extraction, 0.5 grams of flower samples were taken, 10 ml of 2M HCL (37%) was added and left in a heated shaker for 2 hours. 15 ml of ethyl acetate was added to each sample taken from the shaker, vortexed for 15-20 seconds, and then filtered with the help of a Bühner funnel. The obtained filtered samples were taken into tared petri dishes and kept at 40 ^oC for 2 days, the evaporation process was completed, the final weights of the petri dishes were determined, and the amount of dyestuff was calculated as %.

2.5. Total phenolic substance amount (mg GA 100^{-1} g)

In the determination of the total amount of phenolic substance, the method developed by modifying the FolinCicaltea spectrophotometric method specified by (Obanda et al., 1997) was used. The Folin-Cicaltea solution was diluted at 1:3. Saturated sodium carbonate (35%) solution; 87.5 g of sodium carbonate was dissolved in distilled water, made up to 250 ml, and left overnight and then filtered. Gallic acid stock solution (500 µg ml^{-1}); was prepared by dissolving 50 mg of gallic acid in 100 ml of distilled water. Gallic acid working solution; Each 500 µg ml^{-1} gallic stock solution was prepared in 5 ml measuring balloons as 9 separate solutions with concentrations ranging from 0-55 µg ml^{-1}. 1 ml of these solutions was taken and mixed with 1 ml of FolinCicaltea solution. After waiting for 5 minutes, 2 ml of sodium carbonate was added and shaken, and diluted with 2 ml of water. After this mixture was kept in the dark for 30 minutes, the absorbance value was read in the spectrometer at a wavelength of 700 nm. A calibration curve was obtained by graphing the absorbance values read against these different concentrations of gallic acid (r^2= 97.47).

2.6. Total flavonoid substance amount (mg QE 100^{-1} g)

Determination of the total amount of flavonoid substances; Total flavonoid substance determination was specified according to the method that (Quettier-deleu et al., 2000) developed. 2 ml of 2% $AlCl_3$ was added to 2 ml of extract and left in the dark for 1 hour at room temperature. The total flavonoid contents of the extracts were measured with a spectrophotometer at a wavelength of 415 nm by performing 2 parallel studies in each sample and calculated in mg QE 100^{-1} g using the calibration curve prepared using standard quarcetin.

2.7. Total antioxidant activity (mg TE g^{-1})

Determination of total antioxidant activity; After weighing 2 g of safflower petal and adding 4 ml of methanol, the material passed through the homogenizer was centrifuged at 10000 rpm for 10 minutes, and the supernatant remaining on top was taken. Also, after preparing 300 mM acetate buffer (pH 3.6), 10 mmol L^{-1} 2,4,6-tripyridyl-s-triazine (TPTZ) prepared by dissolving in 40 mM HCl and 20 mmol L^{-1} $FeCl_3.6H_2O$ solutions, FRAP reagent was prepared by mixing them at a ratio of 10:1:1,

respectively. The mixture prepared for the analysis of 2850 µL of FRAP reagent and ABTS (2.2-Azinobis 3-ethyl-benzothiazoline-6-sulfonic acid) on safflower petal was diluted 50 times with ethanol, then 150 µL of the sample was mixed and kept at room temperature for 30 minutes. The resulting ferrous tripyridyltriazine complex was measured at 593 nm in the spectrophotometer, and the results were reported as mg Trolox g^{-1} (Lutz et al., 2011). Trolox concentration range has been studied as 0-500 ppm.

2.8. Statistical analysis

The data obtained from the study were subjected to variance analysis with the Costat 6.303 package program according to the "Divided Plots in Random Blocks Experiment Pattern" in terms of separate years, and the averages obtained were grouped according to the LSD (0.05) multiple comparison test. However, in terms of united years, the data were subjected to variance analysis with the Costat 6.303 package program according to the "Divided Plots Trial Pattern in Random Blocks" and the averages obtained were grouped according to the LSD (0.05) multiple comparison test.

3. Results and Discussions

As a result of the study, it was determined that the effect of inorganic fertilizer × biofertilizer interaction and inorganic fertilizer × biofertilizer × year interaction on all parameters examined was statistically significant according to the results of the analysis of variance performed in 2020, 2021, and the united years average.

3.1. Crude oil ratio

According to the results of the analysis of variance, the differences between years in terms of crude oil ratio were found to be statistically significant. The average crude oil rate was 31.09% in the 2020 research year and 25.24% in the second year (Table 3). It is thought that the precipitation falling in the vegetation period of 2020 is higher than the precipitation in the vegetation period of 2021, causing the average oil rate to increase significantly. Öztürk et al. (2008) reported that crude oil ratios of safflower cultivars were higher in a year with high annual precipitation. The effect of inorganic fertilizer applications on the crude oil ratio was not statistically significant in 2020, 2021, and the united years. The crude oil rate was found within the range of 30.19-31.55% in 2020, 24.71-25.86% in 2021, and 27.45-28.70% in united years averages (Table 3). In previous studies. It has been reported that the effect of different nitrogen doses on the crude oil ratio in safflower is statistically insignificant (Akış, 2013; Buçak, 2019; Ünsal, 2020), and NP applications do not cause a significant effect (Çelik, 2017; Demir and Karaca, 2018).

The effect of biofertilizer applications on the crude oil ratio was not statistically significant according to the united averages of the two years with the 2020 and 2021 trial years. Crude oil ratio average values were determined as 30.27-31.82% in 2020, 24.20-26.25% in 2021, and 27.29-29.04% according to the united averages of the two years (Table 3).

3.2. Crude oil yield

According to the results of the analysis of variance, the differences between years in terms of crude oil yield were found to be statistically significant. The average crude oil yield was 56.39 kg da^{-1} in the first year of the study and 41.01 kg da^{-1} in the second year (Table 3). The effect of inorganic fertilizer applications on crude oil yield was found to be statistically significant according to 2020, 2021, and united averages of two years. According to the average of 2020 and 2021 trial years and united years, the highest crude oil yield values were obtained from NP_{100} applications with 74.45-49.66-62.06 kg da^{-1}, respectively. According to the average of 2020 and united years, the lowest values were determined from the control applications with 41.09-38.56 kg $da^{-1,}$ respectively. Compared to the 2021 average, the lowest crude oil yield of 36.02 kg da^{-1} was determined from the control applications and was statistically in the same group with the NP_{50} applications (Table 3). It has been reported that crude oil yield in safflower increases in direct proportion to nitrogen doses but is not affected by phosphorus doses (Sezer, 2010; Karaca, 2017).

The effect of biofertilizer applications on crude oil yield was found to be statistically significant compared to the averages of 2020, 2021, and the united years. Compared to 2020, the highest crude oil

yield was achieved in B_5 applications with 65.05 kg da^{-1}, and the lowest crude oil yield was achieved in control applications with 44.12 kg da^{-1}. The highest crude oil yield in 2021 was obtained from B_1 applications with 46.98 kg da^{-1}, and the lowest value was obtained from B_3 applications with 24.29 kg da^{-1}. According to the united years' average, the highest crude oil yield of 53.28 kg da^{-1} was determined from B_5 applications and was in the same statistical group with B_1, B_2, and B_4 applications. The lowest crude oil yield was determined from the control applications with 40.86 kg da^{-1} and it was in the same statistical group with the B_3 applications. (Table 3). It is known that potassium accumulation occurs in the soil as a result of the activity of the bacterium Frateuria aurantia (B_1), which is effective in dissolving potassium in the soil and making it useful (Salem, 2020; Şelem et al. 2021). The increase in potassium in the soil causes an increase in the seed fullness and seed yield in the generative phase of the plant. It has been reported that crude oil yield increases in sunflowers due to increased potassium doses (Altınparmak, 2016; Yağmur and Okur, 2017). It is reported that the *Rhodopsudomonas palustris* bacterium (bacteria found in B_5 biofertilizer) fixes nitrogen to the soil, and accordingly, the soil is enriched with nitrogen (Sakpirom et al., 2019). It has been reported that the fixed oil yield of black cumin increases due to increasing nitrogen and potassium doses (Kızılyıldırım and Gedik, 2021), and the crude oil yield increases due to the increased nitrogen dose in sunflower (Yıldız, 2014). As a result of our study, it is thought that the increase of nitrogen and potassium minerals in the soil due to B_1 and B_5 applications and, consequently, the crude oil yield may have increased, as supported by the literature.

Table 3. Crude oil ratio (%), crude oil yield (kg da^{-1}), petal yield (kg da^{-1}), and total dyestuff ratio (%) values of different inorganic fertilizer doses and biofertilizer applications in safflower in 2020, 2021, and comined years

	COR (%)			COY (kg da^{-1})			PY (kg da^{-1})			TDR (%)		
İF	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**
NP_0	31.53	25.86	28.70	41.09^{c}	36.02^{b}	38.56^{c}	14.94^{b}	10.08^{c}	12.51^{b}	2.83^{a}	2.08	2.46^{a}
NP_{50}	31.55	25.14	28.35	53.64^{b}	37.35^{b}	45.49^{b}	17.61^{a}	11.89^{b}	14.75^{a}	2.67^{c}	2.11	2.39^{b}
NP_{100}	30.19	24.71	27.45	74.45^{a}	49.66^{a}	62.06^{a}	18.01^{a}	13.02^{a}	15.52^{a}	2.78^{b}	2.05	2.41^{b}
LSD (0.05)	ns	ns	ns	7.28	7.04	4.20	1.64	1.05	0.81	0.02	ns	0.02
B	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**
B_0	30.89	25.35	28.12	44.12^{c}	37.60^{bc}	40.86^{b}	15.74^{c}	9.76^{c}	12.75^{d}	2.68^{cd}	1.95^{c}	2.32^{b}
B_1	30.27	24.31	27.29	57.57^{ab}	46.98^{a}	52.28^{a}	20.38^{a}	13.38^{a}	16.88^{a}	2.78^{a-c}	2.09^{b}	2.44^{a}
B_2	31.81	26.22	29.01	57.52^{ab}	43.18^{ab}	50.35^{a}	15.99^{bc}	10.16^{bc}	13.07^{d}	2.75^{b-d}	2.21^{a}	2.47^{a}
B_3	30.77	24.20	27.48	54.61^{b}	24.29^{c}	44.45^{b}	15.45^{c}	11.87^{ab}	13.66^{cd}	2.82^{ab}	2.10^{b}	2.46^{a}
B_4	31.82	26.25	29.04	59.49^{ab}	42.49^{ab}	50.99^{a}	17.10^{b}	11.62^{ab}	14.36^{bc}	2.89^{a}	2.07^{b}	2.48^{a}
B_5	30.99	25.09	28.04	65.05^{a}	41.50^{ab}	53.28^{a}	16.46^{bc}	13.20^{a}	14.83^{b}	2.65^{d}	2.08^{b}	2.36^{b}
LSD (0.05)	ns	ns	ns	7.09	5.70	4.45	0.99	1.76	0.99	0.11	0.08	0.06
CV (%)	3.09	9.31	6.52	11.54	14.44	13.73	5.45	15.68	10.42	4.11	4.34	4.27
YA	31.09	25.24	28.16	56.39	41.01	48.70	16.85	11.66	14.25	2.76	2.08	2.42

*There is no statistical difference between the values in the same column and written with the same lowercase letters.

LSD (P<0.05), CV: Coefficient Value, YA: Year Average, İF: İnorganic Fertilizer, NP: Nitrogen + Phosphorus, NP_0: Control, NP_{50}: Half dose, NP_{100}: Full dose, B: Biofertilizer, B_0: Control, B_1: *Frateuria aurantia,* B_2: *Bacillus megaterium*, B_3: *Azospirillum lipoferum*, B_4: *Chlorella saccharophilia*, B_5: *Lactobacillus casei + Rhodopseudomonas palustris + Saccharomyces cerevisiae + Lactococcus lactis.* ns: non significant

COR: Crude Oil Ratio, COY: Crude Oil Yield, PY: Petal Yield, TDR: Total Dyestuff Ratio, UY: United Years.

3.3. Petal yield

According to the results of the analysis of variance, the differences between years in terms of petal yield were found to be statistically significant. The average petal yield was 16.85 kg da^{-1} in the 2020 year and 11.66 kg da^{-1} in the second year (Table 3). The effect of inorganic fertilizer applications on petal yield was found to be statistically significant in 2020, 2021, and united years. According to the average of 2020, 2021, and the united years, the petal yield values obtained from the NP_{100} applications were better than the other applications with 18.01-13.02-15.52 kg da$^{-1,}$ respectively. The lowest petal values were determined from NP_0 applications with 14.94-10.08-12.51 kg da^{-1} according to 2020, 2021, and united years' average ranking (Table 3). It has been reported by various researchers that there is an increase in flower yield due to increased nitrogen applications in safflower (Amırgaı and Koç, 2016;

Andırman and Karaaslan, 2021), while phosphorus applications increase flower yields in studies conducted on different plants (Çavuşoğlu, 2015; Muktar, 2021).
The effect of biofertilizer applications on petal yield was found to be statistically significant compared to the experimental years of 2020, 2021, and united years. According to the average of 2020 and united years, the highest petal yield was determined from B_1 applications with 20.38-16.88 kg $da^{-1,}$ respectively. In 2021, the highest petal yield with 13.38 kg da^{-1} was obtained from B_1 applications and was in the same statistical group with B_5 applications. According to the data of 2020, the lowest petal yield was determined at 15.45 kg da^{-1} from B_3 applications, and no statistical difference was observed between with control applications. In 2021, the lowest petal yield was obtained from B_0 applications with 9.76 kg da^{-1}. According to the united years' average, the lowest petal yield was obtained from B_0 applications with 12.75 kg da^{-1}, and there was no statistical difference between B_2 applications (Table 3).

3.4. Total dyestuff ratio

According to the results of the analysis of variance, the differences between years in terms of total dyestuff ratio were found to be statistically significant. The average total dyestuff rate was 2.76% in the 2020 year and 2.08% in the second year (Table 3). While the effect of inorganic fertilizer applications on the total dyestuff ratio was statistically significant compared to the averages of 2020 and the united years, it was found to be insignificant in 2021. According to the average of 2020 and united years, the highest total dyestuff ratio was obtained from NP_0 applications with 2.83-2.46%, respectively. The lowest total dyestuff ratio in 2020 was obtained from NP_{50} applications with 2.67%. According to the united years average, the lowest total dyestuff ratio was determined as 2.39% in NP_{50} applications, and it was in the same statistical group with NP_{100} applications. The total dyestuff ratio in 2021 was in the range of 2.05-2.11% (Table 3). The reason why the highest dyestuff ratio is obtained from the control applications, according to the average of 2020 and the united years, is that the plant is stressed by showing a mineral substance deficiency due to the lack of inorganic fertilizer. As a result, the amount of flavonoid substances, which increased in many plants under stress, caused an increase in the amount of dyestuff in the flavonoid structure in our study and the corresponding increase in the total dyestuff ratio. Studies have been conducted on the increase of various substances in the flavonoid structure due to mineral substance deficiency in various plants (Heimler et al., 2017; Mokgehle et al., 2017; Şahin, 2018).

The effect of biofertilizer applications on the total dyestuff ratio was found to be statistically significant in the averages of 2020, 2021, and two years. In 2020, the highest total dyestuff ratio was obtained from B_4 applications with 2.89%, and the lowest value was obtained from B_5 applications with 2.65%. In 2021, the highest total dyestuff ratio was obtained from B_2 applications with 2.21%, and the lowest value was obtained from B_0 applications with 1.95%. According to the united years average, the highest total dyestuff ratio with 2.48% was determined from B_4 applications, but it was statistically in the same group with B_1, B_2, and B_3 applications. The lowest value was determined from B_0 applications with 2.32%, and there was no statistical difference between with B_5 applications (Table 3). It is estimated that microalgae contain minerals, hormones, and vitamins necessary for plants (Piwowar and Harasym, 2020) and can activate many metabolic activities, such as pigment production. Accordingly, we think that the rate of dyestuff may have increased with the increase in color pigments.

3.5. Total phenolic substance amount

According to the results of the analysis of variance, the differences between years in terms of the total amount of phenolic substances were found to be statistically significant. In the first research year, the average total amount of phenolic substances was found 122.76 mg GAE 100^{-1} g, and in the second year, it was 79.48 mg GAE 100^{-1} g (Table 4). The effect of inorganic fertilizer applications on the total amount of phenolic substances was not found to be statistically significant averages of 2020, 2021, and two years. The total amount of phenolic substances range in 2020 was determined as 121.13-124.52 mg GAE 100^{-1} g, in 2021, it was 75.33-86.67 mg GAE 100^{-1} g, and according to the averages of two years, it was determined between 98.78-104.66 mg GAE 100^{-1} g (Table 4).

The effect of biofertilizer applications on the total amount of phenolic substances was found to be statistically significant in 2020, 2021, and united years. In 2020, the highest total phenolic substance content was determined with 128.21 mg GAE 100^{-1} g from B_5 applications and was included in the same

statistical group with B_3 applications. The lowest value was obtained from B_2 applications with 112.33 mg GAE 100^{-1} g. In 2021, the highest total phenolic substance amount was determined from B_5 applications with 86.38 mg GAE 100^{-1} g, and the lowest value was determined from B_0 applications with 72.86 mg GAE 100^{-1} g. According to the averages of two years, the highest total phenolic substance content was determined from B_5 applications with 107.29 mg GAE 100^{-1} g. the lowest total amount of phenolic substance was determined in united years from B_0 applications with 97.25 mg GAE 100^{-1} g, and it was in the same statistical group with B_2 applications (Table 4). According to the trial years and united years, it is seen that B_5 applications are more effective than other biofertilizers in increasing the total phenolic content values (Table 5). It has been reported that *Rhodopsudomonas sp.* increases secondary metabolites in phenolic and flavonoid structures in different plants (Lee et al., 2009), and *Rhodopseudomonas palustris* bacteria application in cucumber grown under salt stress is effective in reducing salt stress by increasing phenolic compounds (Ge and Zhang, 2019).

Table 4. Average total phenolic substance amount (mg GAE 100^{-1} g), total flavonoid substance amount (mg QE 100^{-1} g), and total antioxidant activity (mg TE 100^{-1} g) values of biofertilizer applications with different inorganic fertilizer doses in safflower between 2020-2021 and two years

	TPSA (mg GAE 100^{-1} g)			TFSA (mg QE 100^{-1} g)			TAA (mg TE g^{-1})		
İF	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**
NP_0	122.65	86.67	104.66	11.87^{a}	9.96^{a}	10.78^{a}	11.95	9.05	10.50^{a}
NP_{50}	124.52	75.33	99.92	7.81^{b}	8.84^{b}	8.33^{c}	10.82	8.17	9.50^{b}
NP_{100}	121.13	76.44	98.78	10.89^{a}	8.71^{b}	9.80^{b}	10.21	8.20	9.20^{b}
LSD (0.05)	ns	ns	ns	1.29	0.55	0.58	ns	ns	0.84
B	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**	**2020**	**2021**	**UY**
B_0	121.63^{b}	72.86^{c}	97.25^{c}	8.36^{b}	8.32^{c}	8.34^{c}	10.65^{bc}	7.96^{b}	9.30^{cd}
B_1	122.39^{ab}	77.03^{bc}	99.71^{bc}	9.08^{b}	8.16^{c}	8.62^{bc}	9.77^{c}	8.31^{ab}	9.04^{d}
B_2	112.33^{c}	79.35^{a-c}	95.84^{c}	8.83^{b}	8.64^{bc}	8.73^{bc}	10.71^{bc}	8.64^{a}	9.67^{bc}
B_3	125.58^{a}	80.07^{a-c}	102.82^{ab}	12.44^{a}	10.94^{a}	11.69^{a}	11.75^{ab}	8.64^{a}	10.19^{ab}
B_4	124.45^{ab}	81.20^{ab}	103.82^{ab}	9.19^{b}	9.16^{b}	9.18^{b}	12.30^{a}	8.56^{a}	10.43^{a}
B_5	128.21^{a}	86.38^{a}	107.29^{a}	13.25^{a}	9.27^{b}	11.26^{a}	10.78^{bc}	8.74^{a}	9.76^{bc}
LSD (0.05)	5.73	7.87	4.76	1.20	0.73	0.69	1.11	0.56	0.61
CV (%)	4.72	10.28	7.07	8.56	8.42	10.76	10.77	6.92	9.45
YA	122.76	79.48	101.12	10.19	9.08	9.63	10.99	8.47	9.73

*There is no statistical difference between the values in the same column and written with the same lowercase letters.

LSD (P<0.05), CV: Coefficient Value, YA: Year Average, İF: İnorganic Fertilizer, NP: Nitrogen + Phosphorus, NP_0: Control, NP_{50}: Half dose, NP_{100}: Full dose, B: Biofertilizer, B_0: Control, B_1: *Frateuria aurantia,* B_2: *Bacillus megaterium*, B_3: *Azospirillum lipoferum*, B_4: *Chlorella saccharophilia*, B_5: *Lactobacillus casei + Rhodopseudomonas palustris + Saccharomyces cerevisiae + Lactococcus lactis,* ns: non significant

TPSA: Total Phenolic Substance Amount, TFSA: Total Flavonoid Substance Amount, TAA: Total Antioxidant Activity, UY: United Years.

3.6. Total flavonoid substance amount

According to the results of the analysis of variance, the differences between years in terms of the total amount of flavonoid substances were found to be statistically significant. In 2020, the average total amount of flavonoid substance was found to be 10.19 mg QE 100^{-1} g, and in the second year 9.08 mg QE 100^{-1} g (Table 4). The effect of inorganic fertilizer applications on the total amount of flavonoid substances was determined to be statistically significant compared to the averages of 2020, 2021 and united years. In 2020, the highest total amount of flavonoid substance was observed in NP_0 applications with 11.87 mg QE 100^{-1} g, and it was in the same statistical group with NP_{100} applications. The lowest value was obtained in NP_{50} applications with 7.81 mg QE 100^{-1} g. According to the 2021 and united years average, the highest total flavonoid substance amounts were determined in NP_0 applications with 9.96-10.78 mg QE 100^{-1} g, respectively. In 2021, the lowest total flavonoid substance amount was determined with 8.71 mg QE 100^{-1} g from NP_{100} applications and was statistically in the same group with NP_{50}. According to the united years' average, the lowest total flavonoid substance content was determined with 8.33 mg QE 100^{-1} g from NP_{50} applications (Table 4). It has been reported that flavonoids increase under stress situations such as limited water, high amounts of salt, and insufficient nutrients (AlKahtani et al., 2021). When our study results are examined, it is seen that the total flavonoid amounts are higher in applications without nitrogen and phosphorus fertilization than in other applications due to insufficient nutrients in the soil.

The effect of biofertilizer applications on the total amount of flavonoid substances was found to be statistically significant according to 2020, 2021, and united years. In the 2020 year, the highest total flavonoid substance content was determined with 13.25 mg QE 100^{-1} g from B_5 applications and was in the same statistical group as B_3 applications. The lowest total flavonoid substance amount in 2020 was obtained from B_0 applications with 8.36 mg QE 100^{-1} g, and there was no statistical difference between B_1, B_2, and B_4 applications. In the 2021 year, the highest total flavonoid substance amount was determined from B_3 applications with 10.94 mg QE 100^{-1} g, the lowest total flavonoid substance amount was determined from B_1 applications with 8.16 mg QE 100^{-1} g, and it was in the same statistical group with B_0 applications. According to the united years' average, the highest total amount of flavonoid substance was obtained from B_3 applications with 11.69 mg QE 100^{-1} g, and it was in the same statistical group as B_5. The lowest total flavonoid substance amount in united years average was obtained from B_0 applications with 8.34 mg QE 100^{-1} g (Table 4). *Azospirillum sp.* It is reported that flavonoid application causes an increase in flavonoid compounds in rice (Chamam et al., 2013). It is reported that the application of *Azospirillum lipoferum* in chickpea increased phenolic and flavonoid compounds (El-Esawi et al., 2019). The literature results are in agreement with our findings.

3.7. Total antioxidant activity

According to the results of the analysis of variance, the differences between years in terms of total antioxidant activity were found to be statistically significant. The average total antioxidant activity was found to be 10.99 mg TE g^{-1} in 2020 and 8.47 mg TE g^{-1} in the second year of the study (Table 4). While the effect of inorganic fertilizer applications on total antioxidant activity was not statistically significant in 2020 and 2021, it was found to be significant compared to the averages of the united years. The total antioxidant activity range for 2020 and 2021 was determined as 10.21-10.95 and 8.17-9.05 mg TE g^{-1}, respectively. In the united years, the highest total antioxidant activity value was determined in NP_0 applications with 10.50 mg TE g^{-1}. The lowest total antioxidant activity value in united years was observed in NP_{100} applications with 9.20 mg TE $g^{-1,}$ and it was in the same statistical group with NP_{50} applications (Table 4).

The effect of biofertilizer applications on total antioxidant activity was found to be statistically significant compared to the experimental years of 2020 and 2021 and the averages of two years. According to the 2020 and united years average, the highest total antioxidant activity was obtained from B_4 applications with 12.30-10.43 mg TE g^{-1}, respectively, and the lowest total antioxidant activity was obtained from B_1 applications with 9.77-9.04 mg TE g^{-1}, respectively. The total antioxidant activity value of 8.74 mg TE g^{-1} obtained from B_5 applications in 2021 gave better results than other applications. The lowest value was determined in B_0 applications with 7.96 mg TE g^{-1} in 2021 (Table 4). Secondary metabolite production in plants is directly related to the macronutrients in the soil (Zhang et al., 2017), and it is reported that microalgae applications increase the uptake of micro and macro nutrients in the soil (Gatamaneni et al., 2020). It has been reported that microalgae application in basil increases antioxidant activity (Hristozkova et al., 2017), while growth and secondary metabolites increase due to microalgae applications in different studies (Garcia-Gonzalez and Sommerfeld, 2016; Wuang et al., 2016).

Conclusion

This research, which was carried out in Van ecological conditions in the summer growing season of 2020 and 2021, was carried out to determine the effects of biofertilizer applications and inorganic fertilization on some quality and biochemical properties of safflower (*Carthamus tinctorius* L.). According to the average of both trial years and united years, it was determined that the petal yield and crude oil yield increased depending on the increase in NP doses, and the crude oil ratio and the total phenolic substance content were not affected. Depending on the increase in NP doses, it was determined that there were decreases in parameters such as total dyestuff content, total flavonoid substance content, and total antioxidant activity in 2020, 2021, and united years.

According to the average of 2020, 2021, and united years, it was determined that the biofertilizer applications had a statistically significant effect on all parameters except the crude oil ratio. Considering the average values of the trial years and united years, more positive results were obtained in NP fertilizer doses and biofertilizer applications compared to the control plots. It has been observed that NP

fertilization gives better results than biofertilizer applications for many parameters. It is estimated that the fact that the highest values in the investigated parameters were obtained from different biofertilizer agents according to the years, different soil temperatures, climatic conditions, and partially small changes in soil pH may be due to the inability to reach the optimum level in bacterial colonization. Likewise, it is thought that the values that vary according to the years in the examined parameters may be caused by climatic conditions such as precipitation, temperature, and humidity.

As a result, It has been concluded that biofertilizer applications cannot replace NP applications and will be insufficient when applied alone, but the best results can be obtained when applied together with NP. It was determined that the most suitable nitrogen-phosphorus (NP) dose to be recommended for safflower cultivation in Van conditions was NP_{100}. B_1 (*Frateuria aurantia*) for petal yield, B_5 (*Lactobacillus casei* + *Rhodopseudomonas palustris* + *Saccharomyces cerevisiae* + *Lactococcus lactis*) for crude oil yield and total phenolic content, and B_4 (*Chlorella saccharophilia*) for other parameters are the best biofertilizer agents to be recommended.

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Research Article

The Effect of Pre-Sprouting and Planting Time on Different Sized Potato Tuber Yields (*Solanum tuberosum* L.)

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Yield

Abstract: The purpose of this study was to see how pre-sprouting and planting dates affected potato tuber yields of varied sizes. The Split-split Plot was used as the experimental design, with four replications. The effect of years on medium, small, and discard tuber yields was significant, while the effect of planting times on total, big, small, and discard tuber yields per decare was not. The impact of pre-sprouting and planting timings on the traits studied were not statistically significant. There was no difference in total, medium, small, and discarded tuber yields per decare between the varieties; however, there was a difference in big tuber yield. According to the study's findings, the Binella variety should be pre-sprouted on March 23 (2 080.5 kg da^{-1}, 1169.3 kg da^{-1}) and the Slaney variety on April 13 (2 022.9 kg da^{-1}, 1207.5 kg da^{-1}), and both should be planted on May 15. The Binella variety should be pre-sprouted on April 13 (701.0 kg da^{-1}), and the Slaney variety on April 3 (892.4 kg da^{-1}) for medium tuber yield, and both should be planted on May 15. Plant the Binella variety without pre-sprouting (243.0 kg da^{-1}) and the Slaney variety with pre-sprouting on April 3 (223.8 kg da^{-1}) on May 5th for small tuber output. The Binella variety should be pre-planted on April 13th and planted on May 5th (123.4 kg da^{-1}) for the production of discarded tubers, but the Slaney variety should be planted on May 25th without pre-sprouting (113.8 kg da^{-1}).

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1. Introduction

Potatoes are an important food because of their high quantity of dry matter, carbohydrates, protein, minerals, and vitamins (Esendal, 1990). It has a significant role in agricultural enterprises, and its production necessitates more labor than other field goods, such as hilling and hoeing throughout the growing season, allowing employment in agricultural enterprises.

Almost half of the world's potato production is consumed fresh in various forms by humans (cooking in the oven, boiling, chips). The remaining portion is used to make processed foods (such as frozen chips), animal feed, industrial starch, and seed. The starch-rich shells of potatoes and other debris left behind after processing are liquefied and used to make ethanol.

Potatoes are grown in 79 percent of the world's countries. The global potato producing area is 19,3 million ha, with a total production of 388 million tons and a yield per decare of 2 042 kg. China

(26%) produces half of the world's output, followed by India (12%), Russia (8%), and Ukraine (2%). Turkey ranks 14th in world potato output with a share of 1.24 percent. In 2018, potatoes were grown on around 136 thousand hectares in 71 provinces, yielding 4.55 million tons. Potato intake per capita in our country is 50 kilograms per year (Anonymous, 2020).

Erzurum province is one of our country's major potato production centers with 3570.5 hectares of production area, 88.725 tons, and a yield per decare of 2485 kg (Anonymous, 2019). Due to the short and cool growing season in Erzurum, as well as the unavailability of various growth techniques, yield per unit area is low. In our region, the major cultivation techniques for increasing potato output per unit area are pre-sprouting and planting periods.

According to Davies and Allaby (1971), pre-sprouted plants may produce more marketable tubers than non-sprouted plantings.

Arslan and llisulu (1976) found that pre-sprouting treatments resulted in 14 days faster emergence than regular plantings, with a significant yield increase that varied by variety.

In a study conducted in Erzurum conditions by Günel (1982), it was discovered that the proportion of large tubers in planting on May 3 was 70% of total tubers, while this rate decreased by 53% in 17 June planting, the medium tuber ratio increased from 25% to 43%, and the small tuber ratio increased from 3% to 5%.

Pre-sprouting treatment in seed potato tubers, according to Günel (2002), enhances tuber production by promoting early emergence and robust plant development, in addition to using healthy seeds for high tuber yield.

In a study conducted by Kara and Sebahattin (1991), seed tubers with a diameter of 3.5-5.0 cm were pre-sprouted on March 20, April 4, and April 19 and planted on May 5 with the control. As a result of the research, they discovered that pre-sprouting dates had a substantial influence on medium tuber (3.5-5.0 cm) yield but not on tiny tuber (3.5 cm) or big tuber (> 5.0 cm) production.

Calışkan and Arıoğlu (1997) found that postponing the planting date enhanced tuber ratios and yields for small (10-30 mm), medium (30-50 mm), and big tuber (> 50 mm) tubers. During the 2004-2005 growing season, Khan et al. (2011) planted potatoes on four distinct dates (24 September, 1 January, 7 January, and 15 January) to identify the growing duration of the potato and the best planting dates. According to them, since the planting date was postponed, the number of stems in the plant rose. The percentage of large tubers (> 55 mm) was highest on September 24, the earliest planting date, while the percentage of tiny tubers (35 mm) rose when plantings were delayed. They discovered that the earliest planting (September 24) produced the maximum tuber production (15 t ha-1) and that the planting delay influenced dry matter buildup. The planting on January 15 yielded the greatest dry matter (18.31%).

In Erzurum, yield is low per unit area due to the short and cool growing season and also the lack of some cultivation techniques. Pre-sprouting and planting times are the main cultivation techniques to increase the yield of potatoes per unit area in our region.

Potatoes do not contain generative seeds and require a different technique to grow than other vegetables. One of these is pre-sprouting therapy, in which seed tuber potatoes are maintained in a warm environment with natural or artificial light before planting. This results in the production of short, robust, green sprouts and, as a result, quicker plant growth (Smith-Heavenrich, 2007). This treatment is critical for earlier plant emergence and production (Essah et al., 2004; Mikitzel and Wattie, 2000). Therefore, the purpose of this study was to look into the effects of pre-sprouting dates and planting timings on tuber yields of different sizes of potatoes.

2. Material and Methods

2.1. Material

This research was carried out at the experimental field of Atatürk University's Faculty of Agriculture in 2015 and 2016. Binella and Slaney cultivars were selected in the experiments due to their excellent adaptability and yield, disease resistance, and technical features. Binella is a cooking type, an early variety with a large yield, tuber shape oval, shell color yellow and smooth, interior color light yellow, and eye depth surface. Slaney cultivar is of the cooking type, plant height is long and very late, tuber form is short oval, tuber eye depth is surface, shell color is yellow, tuber inner color is cream,

starch content is 11.9-14.8 percent, and dry matter content is 17.9-21 percent. For fertilization, 24 kg of nitrogen, 6 kg of phosphorus, and 5 kg of potassium were administered as pure substances per decares (Ilisulu, 1986; Ozturk, 2001).

2.1. Climate and Soil Characteristics of the Study Field

2.1.1. Climate Characteristics

Total rainfall in Erzurum between May and September, which is the potato vegetation period, was 285.1 mm in 2015, 303.7 mm in 2016, and 195.5 mm in the long term average. The average temperature in 2015 was 14.80 °C, 14.80 °C in 2016, and 14.5 0 °C in the long term average; relative humidity was 60.4% in 2015, 53.3% in 2016, and 57.0% in the long term average (Anonymous, 2017).

2.1.2. Soil characteristics

The soil in the study field was clay and loamy, with pH values ranging from 7.20 to 7.73, low organic matter (1.04 to 2.28 percent), available phosphorus ranging from 8.70 to 11.93 kg da^{-1}, and potassium ranging from 136 to 154.8 kg da^{-1}.

2.2. Method

2.2.1. Study pattern

The Split-Split Plot was used as the experimental design, with three planting dates (May 5, May 15, and May 25) as main plots, four pre-sprouting treatments (no pre-sprouting, starting pre-sprouting on March 23, April 3, and April 13) as subplots, and two cultivars (Binella and Slaney) as sub-sub plots with four replications (Yıldız, 1994). Hills were planned with 70 cm inter-row spacing and 35 cm intrarow spacing in the plants (Şenol, 1976). Each plot consisted of four lines with ten hills on each line. There were 96 plots, each measuring 9.8 m^2 (2.8 m x 3.5 m), with a total experimental area of 2 507.76 m^2.

Seed tuber potatoes of egg size with three eyes were treated to pre-sprout after the dormancy period. The seed tuber potato should be kept at a warm, consistent temperature, such as room temperature until it sprouts strong green shoots. Seed tubers were hand-sewed at various times after this procedure. A few centimeters of soil were hilled up around the base of the stem after the potato plants produced stems to prevent developing tubers at shallow levels from being exposed to sunlight and to encourage the plant to continue generating tubers.

2.2.1. The investigation properties

2.2.1.1. Tuber yield (kg da^{-1})

Tubers were collected, weighted, and estimated tuber yield per decare in all plots.

2.2.1.2. Tuber yields with different sizes

Tubers were classified into four sizes (Günel, 1976). The harvested potato tubers from each plot were weighed after being passed through 5.0, 3.5, and 2.8 cm sieves, and the tubers in each tuber size class were weighed, and their yields per decare were determined.

2.2.2. Statistical Analysis

The Split-Split Plot was used as the experimental design, with three planting dates, four pre-sprouting treatments, two cultivars, and two years with four replications. 4 way ANOVA was used to analyze all parameters and interactions. Therefore Duncan multiple comparison test was used the compare the means.

3. Results and Discussion

Table 1 shows data from potato cultivars that were pre-sprouted and planted at various dates, and Table 2 shows the findings of associated variance analyses.

3.1. Total Tuber Yield (kg da^{-1})

In terms of total tuber yield per decare, although there was a numerical difference between trial years, pre-sprouting, and planting times, there was no statistical difference (Tables 1 and 2). The total tuber yield per decare was 1 552.6 kg in 2015 and 1 882.4 kg in 2016 (Table 2).

The maximum total tuber yield was determined on April 3 (1 861.1 kg da^{-1}), followed by 13 April yield (1 739.9 kg da^{-1}), Control (1 729.8 kg da^{-1}), and finally, March 23 (1 512.2 kg da^{-1}) according to the pre-sprouting timings (Table 2).

The maximum total tuber yield per decare (1 929.1 kg da^{-1}) was determined on May 15, followed by plantings on May 5 (1 727.1 kg da^{-1}) and May 25 (1727.1 kg da^{-1}) (1496.5 kg da^{-1}).

The total tuber production of the Binella variety was determined to be 1 645.9 kg, whereas the Slaney variety yielded 1 789.3 kg (Table 2).

Because total tuber yield did not exhibit consistency across years, pre-sprouting dates, and planting times, the interactions year x pre-sprouting date, pre-sprouting time x variety, planting time x pre-sprouting date, and variety were significant ($p<0.05$) (Table 1, Figer 1, 2, 3).

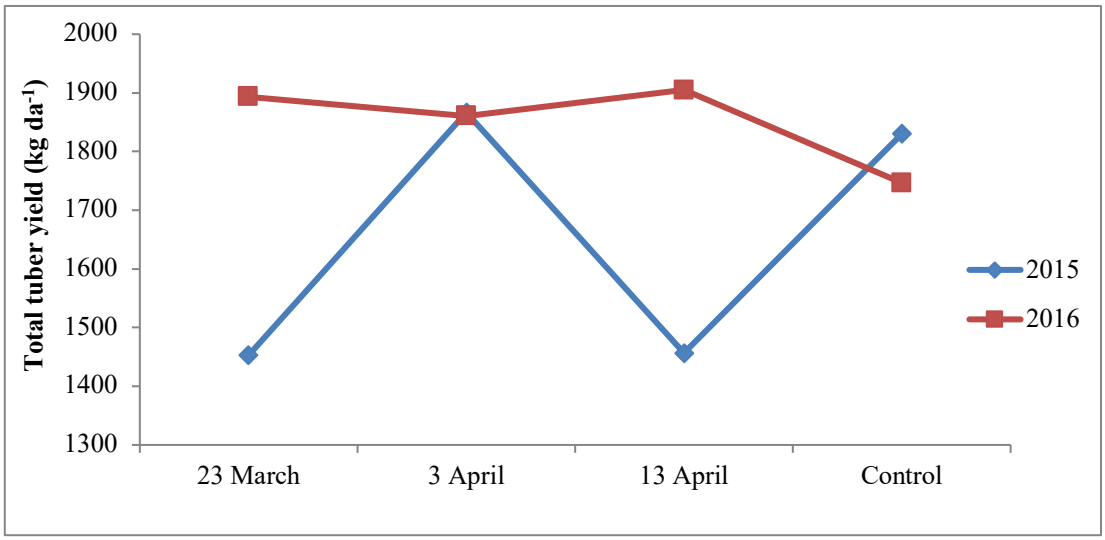

Figure 1. Year x pre-sprouting date interaction related to tuber yield per decare of years' average.

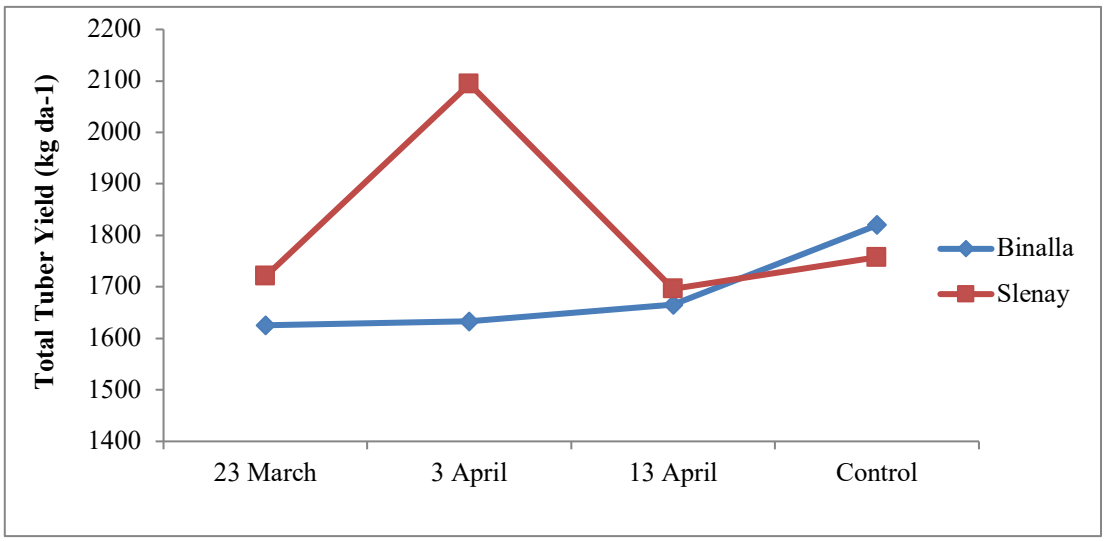

Figure 2. Pre-sprouting date x variety interaction regarding tuber yield per decare in years average.

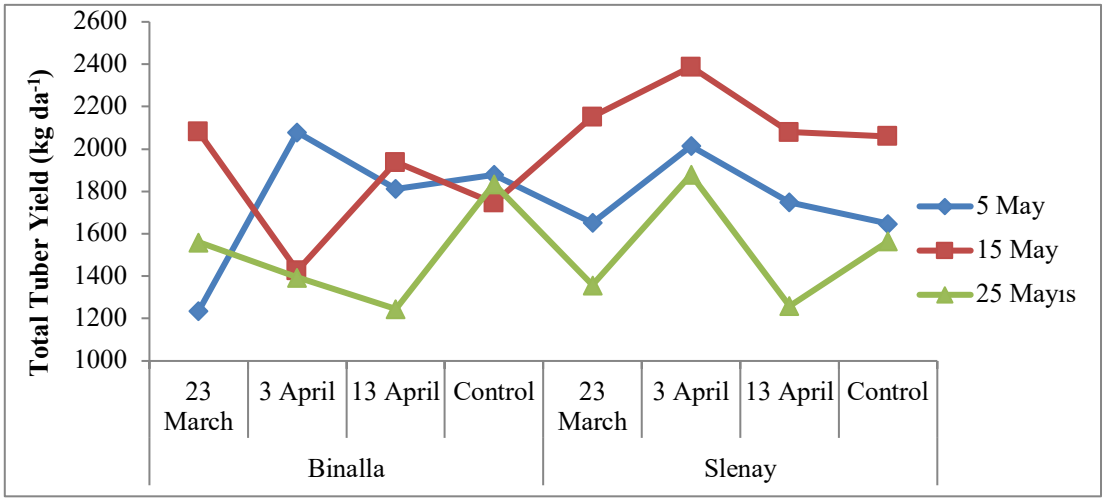

Figure 3. Planting time x pre-sprouting date x variety interaction in annual average of tuber yield per decare of cultivars.

3.2. Large tuber (>50 mm) yield per decare (kg da^{-1}) and its share in total tuber yield

Although there was a numerical difference in tuber yield per decare between the trial years, pre-sprouting, and planting dates, there was no statistical difference (Tables 1 and 2). Significant variations between the cultivars, on the other hand, were found at the p>0.05 level. The production of big tubers per decare was 995.4 kg (64.1%) in 2015 and 811.4 kg (43.1%) in 2016. (Table 1).

The maximum tuber yield per decare was calculated using data from the pre-sprouting application on April 3 (1011.0 kg da^{-1}, 54.3 percent), the application on April 13 (949.7 kg da^{-1}, 53.7 percent), the control (889.3 kg da^{-1}, 51.4 percent), and the application on March 23 (763, 7 kg da^{-1}, 50.5 percent). (Table 2).

According to the planting schedules, the 15 May planting produced the maximum tuber production per decare (1 051.0 kg da^{-1}, 54.5%), followed by the 5 May planting (898.8 kg da^{-1}, 51.5%) and the 25 May planting (769. kg da^{-1}, 51.4%). (Table 1).

The Binella variety yielded 842.0 kg of large tubers, whereas the Slaney cultivar yielded 964.8 kg. Because the cultivars' large tuber yield was not consistent from year to year, the pre-sprouting and planting times (year x pre-sprouting time, year x planting time x pre-sprouting time, planting time x pre-sprouting time, planting time x pre-sprouting time x variety, year x planting time x pre-sprouting time x variety) interaction was statistically significant (p<0.05) (Table 1, Figure 4, 5, 6 and 7).

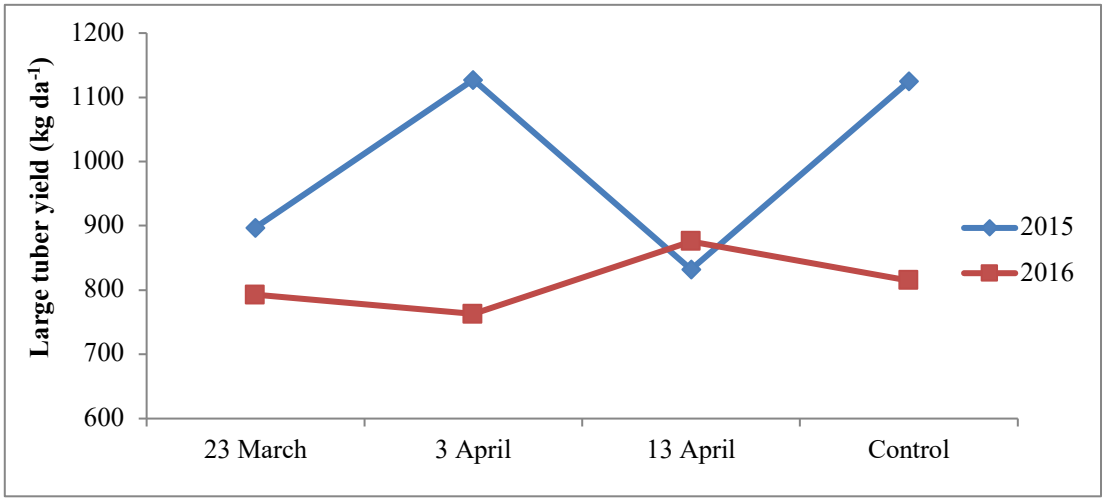

Figure 4. Interaction of year x pre-sprouting date related to the yield of big tuber per decare of years' average.

Table 1. Analysis of variance results of total, large, medium, small and discarded tuber yields per decare of pre-sprouted and planted potatoes at different times

Variation Source	df	Total tuber yields	Large tuber yield	Medium tuber yield	Small tuber yield	Discard tuber yield
Year (A)	1	4.8	3.2	35.9*	39.5*	279.1**
Error[1]	3	-	-	-	-	-
Planting time (B)	2	1.2	1.0	2.4	2.6	0.5
(A) x (B)	2	1.7	1.4	4.2*	0.9	0.2
Error$_2$	12	-	-	-	-	-
Pre-Sprouting date (C)	3	2.1	2.8	0.9	1.0	0.5
(A) x (C)	3	2.8*	4.4*	1.5	1.4	0.3
(B) x (C).	6	0.7	0.6	1.2	0.7	2.8*
(A) x (B) x (C).	6	2.1	2.4*	0.8	0.7	2.5*
Error	54	-	-	-	-	-
Variety (D)	1	3.5	5.0*	0.2	0.01	0.4
(A)x (D)	1	0.1	0.02	0.4	0.7	0.02
(BxD)	2	2.3	0.9	3.1	1.1	0.3
(C)x(D)	3	2.8*	0.9	5.5	0.4	0.2
(A)x(B)x(D)	2	1.7	2.9	1.1	0.2	0.002
(A)x(C)x(D)	3	0.6	0.03	4.3*	0.3	0.4
(B)x(C)x(D)	6	2.4*	2.4*	1.2	2.9*	0.9
(A)x(B)x(C)x(D)	6	2.0	2.3*	0.8	1.5	1.3
Error$_4$	72	-	-	-	-	-

** Marked F values are 1%, * marked F values are significant at 5% level.

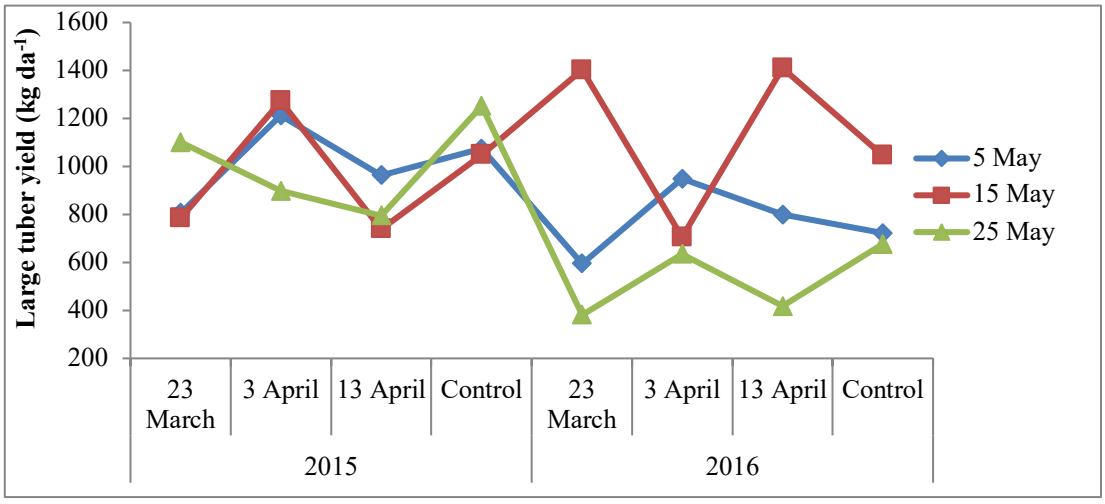

Figure 5. Interaction of year x planting time x pre-sprouting date related to large tuber yield per decare of years' average.

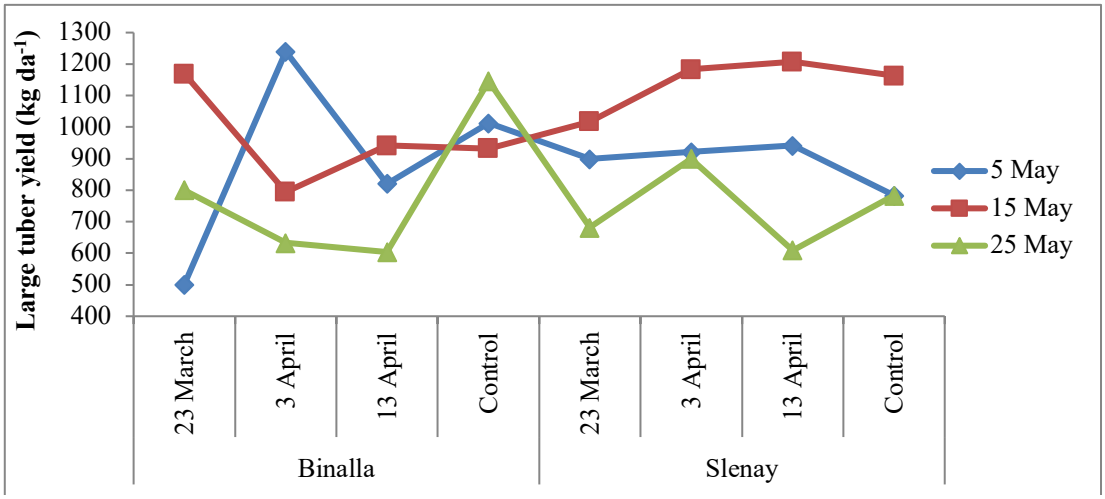

Figure 6. Planting time x pre-sprouting dates x variety interaction related to large tuber yield per decare of the year average of the cultivars.

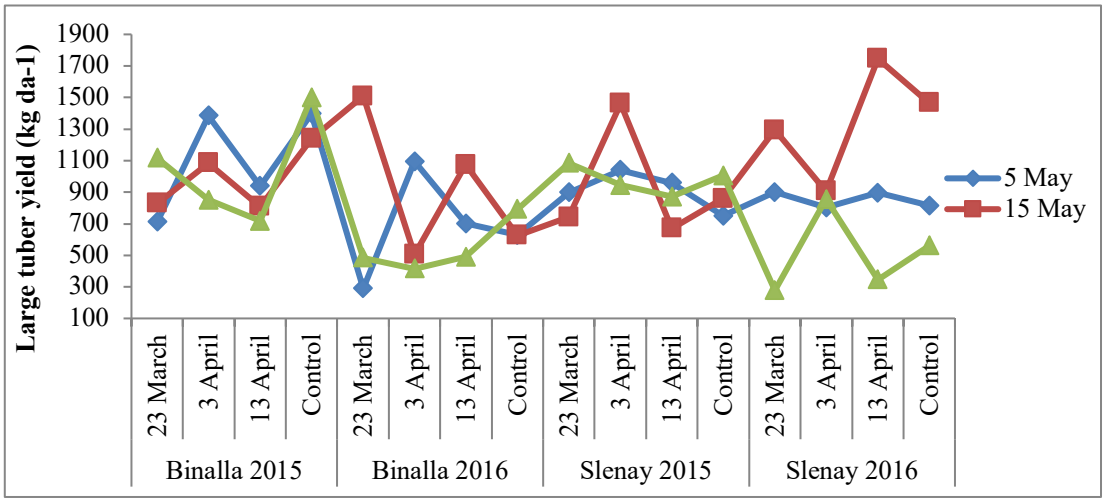

Figure 7. Year x planting time x pre-sprouting dates x variety interaction related to big tuber yield per decare the years average of cultivars.

3.3. Medium tuber (50 mm) yield per decare (kg da^{-1}) and its share in total tuber yield

The difference between the trial years in terms of mean tuber yield per decare was statistically significant ($p<0.05$), but no significance was found for pre-sprouting and planting dates or types (Tables 1 and 2). The medium tuber yield was 406.7 kg (26.2%) per decare in the first year of the experiment and 696.1 kg (37.0%) in the second year. This might be owing to the fact that the soils in the second trial year are nutrient-richer than those in the first (Table 2).

The maximum yield of medium tuber per decare was obtained on April 3 (601.7 kg da^{-1}, 32.3 percent), control (564.5 kg da^{-1}, 32.6 percent), April 13 (541.4 kg da^{-1}, 30.6 percent), and March 23 (498.0 kg da^{-1}, 32.9 percent) during the pre-sprouting period (Table 2).

The highest mean tuber yield per decare was found on May 15 (643.3 kg da^{-1}, 33.3 percent), followed by May 5 (552 kg da^{-1}, 32.0 percent) and May 25 (458.9 kg da^{-1}, 30.7 percent) according to planting timings (Table 2).

The experimental variety Binella yielded 543.9 kg per decare, whereas the Slaney variety yielded 558.9 kg per decare (Table 2).

Because the variety's medium tuber production per decare was not consistent across years, pre-sprouting, and planting periods, statistically significant ($p<0.05$) year x planting time, year x pre-sprouting time x planting time relationships emerged (Table 1, Figure 8, 9).

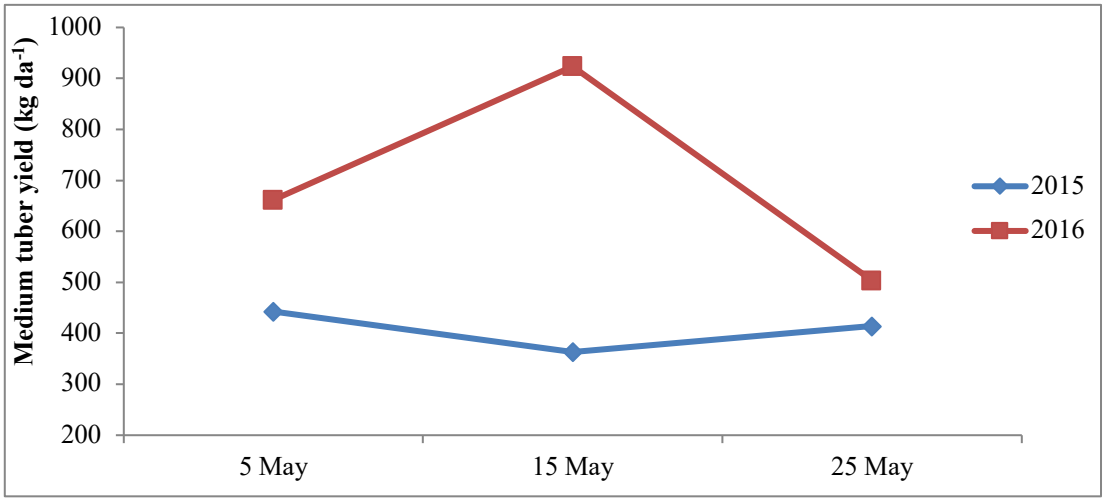

Figure 8. Interaction of year x planting time related to medium tuber yields per decare of years average.

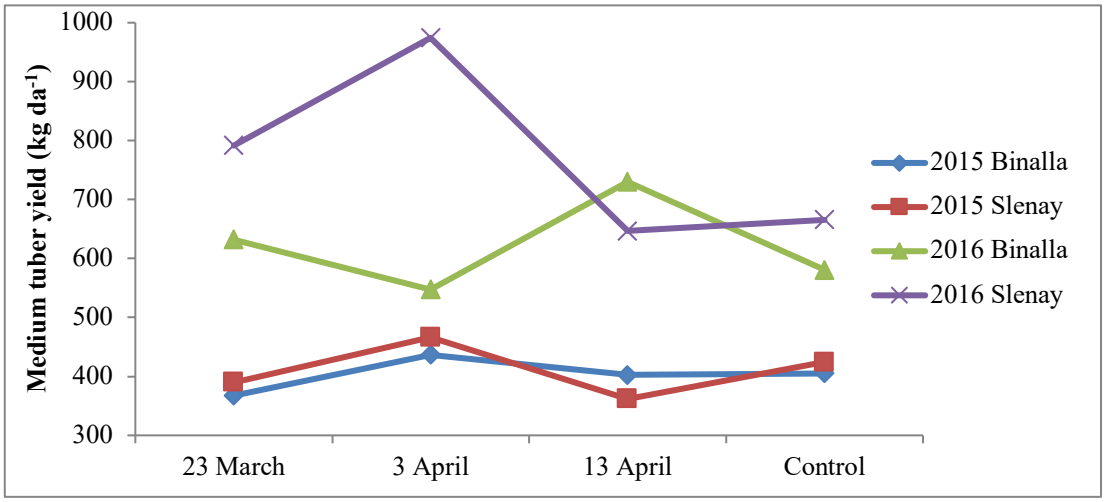

Figure 9. Year x pre-shooting date x variety interaction related to medium tuber yield per decare of years' average.

3.4. Small tuber (size 28-50 mm) yield per decare (kg da^{-1}) and its share in total tuber yield

The difference in small tuber yield per decare across trial years was statistically significant ($P<0.05$). In the case of decar tiny tuber yields, however, no correlation was found between pre-sprouting and planting periods and cultivars. Small tuber yield per decare was 116.2 kg in the first year of the experiment (7.5%) and 247.7 kg in the second year (13.2%). (Tables 1 and 2). This might be because the number of tubers per plant in the second trial year (8.5 counts) was greater than in the first year.

The interaction of variety x pre-sprouting time x planting time was significant ($p<0.05$) because the tuber yield per decare of the cultivars was not consistent according to pre-sprouting and planting periods. Tubers that were pre-sprouted on April 13 (192.3 kg da^{-1}, 10.9 percent) had the maximum small tuber yield, followed by control (189.5 kg da^{-1}, 11.0 percent), April 3 (173.7 kg da^{-1}, 9.3 percent), and pre-sprouted tubers (172.5 kg da^{-1}, 11.4 percent) on March 23 (Table 2).

In terms of planting dates, the May 5 planting had the largest tuber yield per decare (200.1 kg da^{-1}, 11.6 percent), followed by May 25 planting (184.4 kg da^{-1}, 12.3 percent) and May 15 planting (161.3 kg da^{-1}, 8.4 percent) (Table 2). The Binella variety yielded 181.5 kg per decare, whereas the Slaney variety yielded 182.4 kg (Table 2).

Table 2. The total and different size tuber yields in pre-sprouting and different planted time treatments

Treatments		Total Tuber Yield (kg da^{-1})	Large Tuber Yield		Medium tuber yield		Small tuber yield		Discard tuber yield	
			(kg da^{-1})	%	(kg da^{-1})	%	(kg da^{-1})	%	(kg da^{-1})	%
Year	**2015**	1552.8	995.4	64.1	406.7 **b**	26.2	116.2 **b**	7.5	34.5 **B**	2.2
	2016	1882.4	811.4	43.1	696.1 **a**	37.0	247.7 **a**	13.2	127.2 **A**	6.8
Mean		**1717.7**	**903.4**	**52.6**	**551.4**	**32.1**	**182.0**	**10.6**	**80.9**	**4.7**
Pre-sprouting date	**23 March**	1512.2	763.7	50.5	498.0	32.9	172.5	11.4	78.0	5.2
	3 April	1861.6	1011.0	54.3	601.7	32.3	173.7	9.3	75.2	4.0
	13 April	1767.2	949.7	53.7	541.4	30.6	192.3	10.9	83.8	4.7
	Control	1729.8	889.3	51.4	564.5	32.6	189.5	11.0	86.5	5.0
Mean		**1717.7**	**903.4**	**52.6**	**551.4**	**32.1**	**182.0**	**10.6**	**80.9**	**4.7**
Planting time	**5 May**	1727.1	889.8	51.5	552.0	32.0	200.1	11.6	85.2	4.9
	15 May	1929.1	1051.0	54.5	643.3	33.3	161.3	8.4	73.5	3.8
	25 May	1496.5	769.4	51.4	458.9	30.7	184.4	12.3	83.8	5.6
Mean		**1717.7**	**903.4**	**52.6**	**551.4**	**32.1**	**182.0**	**10.6**	**80.9**	**4.7**
Varieties	**Binella**	1645.9	842.0 **b**	51.2	543.9	33.0	181.5	11.0	78.5	4.8
	Slaney	1789.3	964.8 **a**	53.9	558.9	31.2	182.4	10.2	83.2	4.6
Mean		**1717.7**	**903.4**	**52.6**	**551.4**	**32.1**	**182.0**	**10.6**	**80.9**	**4.7**
5 May x 23 March		1401.3	670.0	47.8	463.9	33.1	186.3	13.3	81.1	5.8
5 May x 3 April		1970.6	1080.8	54.8	615.1	31.2	204.0	10.4	70.7	3.6
5 May x 13 April		1810.9	880.9	48.6	608.0	33.6	212.5	11.7	109.5	6.0
5 May x Control		1696.3	897.8	52.9	521.1	30.7	197.7	11.7	79.7	4.7
15 May x 23 Mart		2098.2	1093.3	52.1	723.5	34.5	188.2	9.0	93.2	4.4
15 May x 3 April		1839.6	988.8	53.8	635.6	34.6	158.1	8.6	57.1	3.1
15 May x 13 April		1951.8	1074.5	55.1	646.0	33.1	159.1	8.2	72.2	3.7
15 May x Control		1827.3	1047.5	57.3	568.2	31.1	140.0	7.7	71.6	3.9
25 May x 23 March		1437.8	740.9	51.5	448.8	31.2	159.7	11.1	88.4	6.1
25 May x 3 April		1615.4	766.1	47.4	567.6	35.1	190.8	11.8	90.9	5.6
25 May x 13 April		1194.3	606.4	50.8	351.7	29.4	168.2	14.1	68.0	5.7
25 May x Control		1738.1	964.4	55.5	467.5	26.9	219.0	12.6	87.2	5.0
5 May x 23 March x Binella		1205.4	500.6	41.5	366.7	30.4	226.9	18.8	111.2	9.2
5 May x 23 March x Slaney		1657.1	899.3	54.3	561.1	33.9	145.7	8.8	51.0	3.1
5 May x 3 April x Binella		2079.2	1240.0	59.6	560.0	26.9	220.5	10.6	58.7	2.8
5 May x 3 April x Slaney		1861.9	921.6	49.5	670.2	36.0	187.4	10.1	82.7	4.4
5 May x 13 April x Binella		1777.8	820.5	46.2	632.7	35.6	201.2	11.3	123.4	6.9
5 May x 13 April x Slaney		1843.8	941.2	51.0	583.2	31.6	223.8	12.1	95.6	5.2
5 May x Control x Binella		1795.0	1012.4	56.4	452.2	25.2	243.0	13.5	87.4	4.9
5 May x Control x Slaney		1597.4	783.1	49.0	590.0	36.9	152.3	9.5	72.0	4.5
15 May x 23 March x Binella		2080.5	1169.3	56.2	634.0	30.5	183.4	8.8	93.8	4.5
15 May x 23 March x Slaney		2115.8	1017.3	48.1	813.0	38.4	192.9	9.1	92.6	4.4
15 May x 3 April x Binella		1344.3	794.2	59.1	378.8	28.2	126.0	9.4	45.3	3.4
15 May x 3 April x Slaney		2334.6	1183.3	50.7	892.4	38.2	190.1	8.1	68.8	2.9
15 May x 13 April x Binella		1880.5	941.4	50.1	701.0	37.3	154.9	8.2	83.2	4.4
15 May x 13 April x Slaney		2022.9	1207.5	59.7	591.0	29.2	163.2	8.1	61.2	3.0
15 May x Control x Binella		1690.1	932.4	55.2	536.5	31.7	160.9	9.5	60.3	3.6
15 May x Control x Slaney		1964.2	1162.5	59.2	599.8	30.5	119.0	6.1	82.9	4.2
25 May x 23 March x Binella		1542.7	800.8	51.9	498.5	32.3	160.1	10.4	83.3	5.4
25 May x 23 March x Slaney		1332.6	681.0	51.1	399.0	29.9	159.2	11.9	93.4	7.0
25 May x 3 April x Binella		1428.7	632.7	44.3	536.9	37.6	173.9	12.2	85.2	6.0
25 May x 3 April x Slaney		1801.9	899.4	49.9	598.3	33.2	207.6	11.5	96.6	5.4
25 May x 13 April x Binella		1250.3	603.7	48.3	364.7	29.2	198.3	15.9	83.6	6.7
25 May x 13 April x Slaney		884.6	609.0	68.8	83.3	9.4	138.0	15.6	54.3	6.1
25 May x Control x Binella		1532.8	1145.8	74.8	93.4	6.1	233.1	15.2	60.5	3.9
25 May x Control x Slaney		1187.0	783.0	66.0	85.2	7.2	205.0	17.3	113.8	9.6

Capital letters are significant at %1, and small letters are significant at %5 level.

The interaction of variety x pre-sprouting time x planting time was found to be significant ($p<0.05$) due to the inconsistency of tuber yield per decare of the cultivars when pre-sprouting and planting times were included (Table 1, Figure 10).

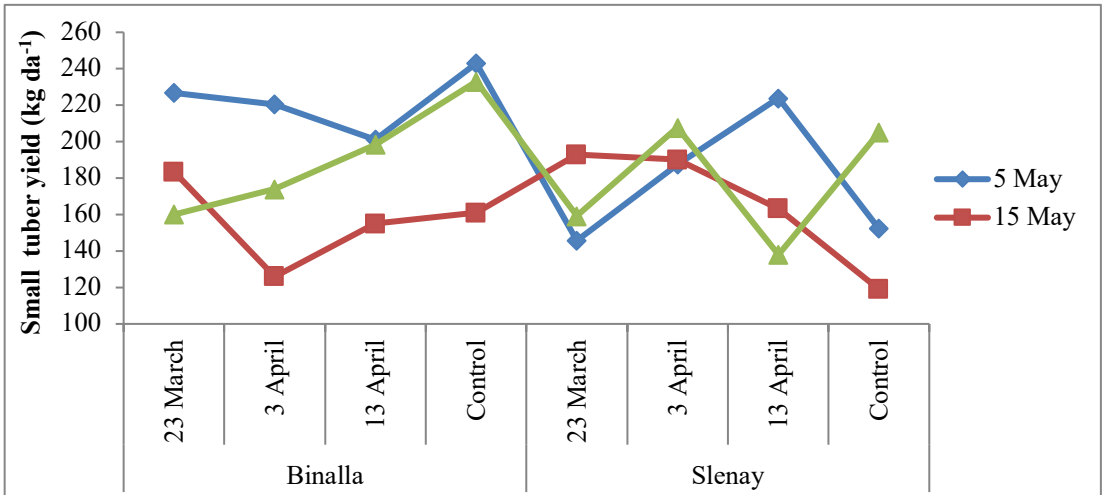

Figure 10. Pre-sprouting date x planting time x variety interaction related to small tuber yield per decare of cultivars over the years.

3.5. Discard tuber (< 28 mm) yield per decare (kg da^{-1}) and its share in total tuber yield

Between study years, there was a statistically significant ($p<0.01$) difference in discarded tuber yields (Table 1). The discarded tuber yield in the first research year was 34.5 kg da^{-1} (2.2%), while in the second year, it was 127.2 kg da^{-1} (6.8%) (Table 1). This might be due to greater tuber counts per plant in the second year compared to the first.

The control (86.5 kg da^{-1}, 5.0 percent) treatment had the greatest discarded tuber yield per decare among the pre-emergence dates, followed by the pre-emergence dates on April 13 (83.8 kg da^{-1}, 4.7 percent), March 23 (78.0 kg da^{-1}, 5.2 percent), and April 3 (75.2 kg da^{-1}, 4.0 percent) (Table 2).

The potatoes planted on May 5 had the highest output of discarded tuber per decare of all the planting periods (85.2 kg da^{-1}, 4.9 percent). Following this, planting on May 25 (83.8 kg da^{-1}, 5.6 percent) and May 15 (73.5 kg da^{-1}, 3.8 percent) took place (Table 2).

The discarded tuber yield of the Binella variety was found to be 78.5 kg per decare, whereas the Slaney variety yielded 83.2 kg (Table 2).

Because the discarded tuber yield per decare varied by year, pre-sprouting, and planting times, the interaction of pre-sprouting x planting time, year x pre-sprouting x planting time, and year x pre-sprouting x planting time was statistically significant ($p<0.05$) (Table 1). Pre-sprouting x planting time, year x pre-sprouting x planting time x interaction was statistically significant ($p<0.05$) because the yield of discarded tuber per decare was not constant across years, pre-sprouting, and planting periods (Table 1, Figure 11, 12).

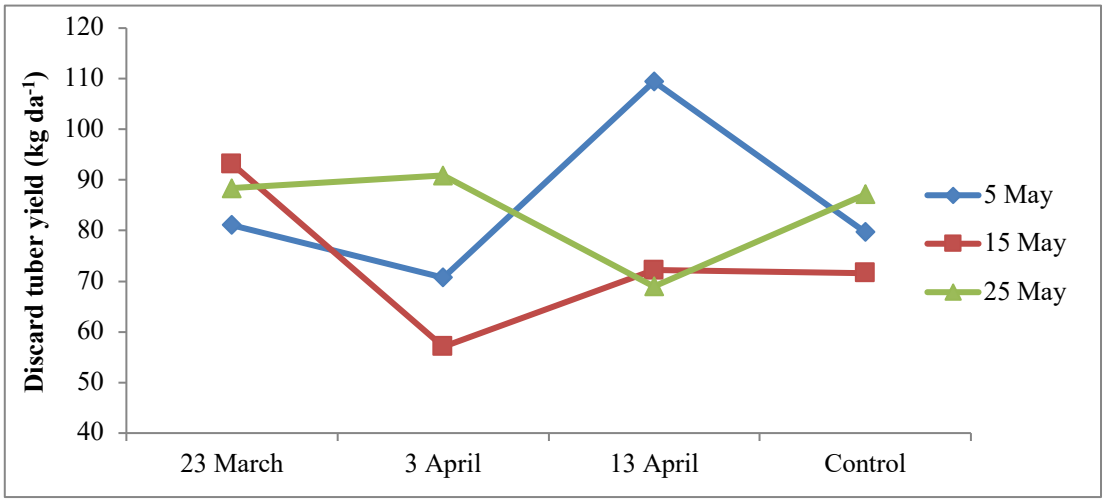

Figure 11. Pre-sprouting date x planting time interaction related to discarded tuber yield per decare on average of years.

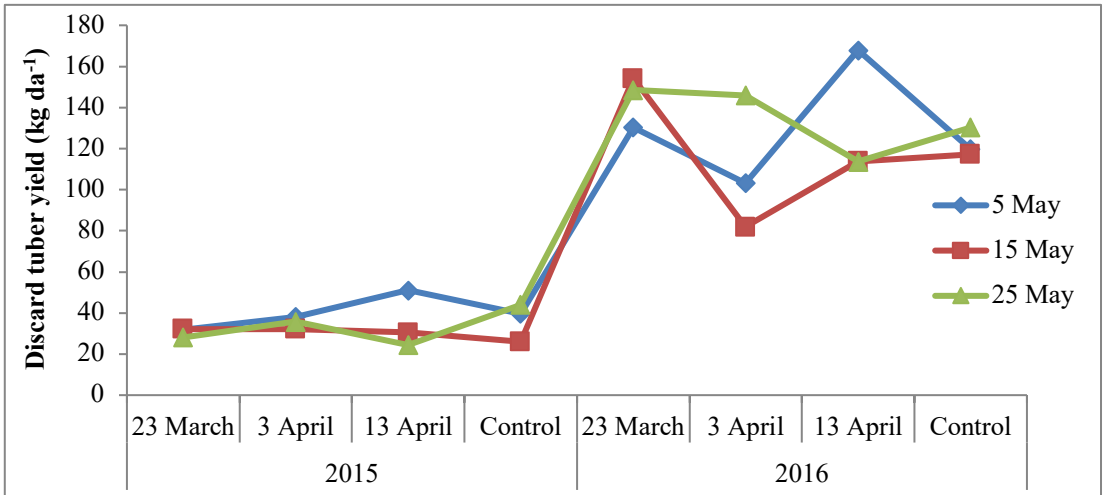

Figure 12. Year x pre-sprouting date x planting time interaction related to discarded tuber yield per hectare of years average.

Conclusions

As a consequence, the Binella variety should be pre-sprouted on March 23 (2 080.5 kg da^{-1}, 1 169.3 kg da^{-1}) and the Slaney variety should be pre-sprouted on April 13 (2 022.9 kg da^{-1}, 1 207.5 kg da^{-1}) for total and big tuber production, and both should be planted on May 15. The Binella variety (701.0 kg da^{-1}) and the Slaney variety (892.4 kg da^{-1}) should be pre-sprouted on April 13 and planted on May 15, respectively, for medium tuber output. The Binella variety should be pre-sprouted (243.0 kg da^{-1}) for small tuber production, whereas the Slaney variety should be pre-sprouted (223.8 kg da^{-1}) and planted on May 5 for small tuber output. The Binella variety should be pre-sprouted on April 13 and planted on May 5 (123.4 kg da^{-1}), but the Slaney variety should be planted on May 25th without pre-sprouting (113.8 kg da^{-1}) for discarded tuber production.

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Research Article

Novel g.2055A>C and g.2064T>A Polymorphisms of KISS1 Gene and Its Association with Reproductive Traits in Local Indonesian Goats

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SNP

Abstract: Kisspeptins are known as neuropeptides encoded by the KISS1 gene, which potentiates GnRH neuron excitability. Unfortunately, the role of the KISS1 gene in reproductive traits remains unclear in Indonesian native goat breeds. The current study purposed to detect the genetic variation of the KISS1 gene and investigate the association with reproductive traits in goats to acquire marker-assisted selection (MAS) for the breeding program. Further, ninety blood samples from randomly selected animals were used for DNA isolation and SNP genotyping. The blood serum was collected from sixteen treated goat does to assess FSH levels using ELISA method. The differences between studied parameters were determined using independent samples T-test from SAS Software. Two SNPs, g.2055A>C (SNP1) and g.2064T>A (SNP2) were discovered in intron 1 of KISS1 gene in Indonesian native goat breeds. Two genotypes were identified from each SNPs: AA and AC at SNP1 and TT and TA at SNP2. The allele A (0.96) at SNP1 and allele T (0.93) at SNP2 were discovered as dominant alleles in the population. Furthermore, chi-square test showed that both SNPs were under Hardy-Weinberg equilibrium. The variants of KISS1 gene have no effect on litter size ($p>0.05$). Moreover, AA genotype of SNP1 had a higher FSH level than that observed in AC genotype ($p<0.0001$), especially in the follicular phase. SNP2 does not correlate with FSH level relatively ($p=0.23$). Furthermore, using of AA genotype at SNP1 of KISS1 gene as MAS for the breeding selection program could escalate the reproductive traits in goats.

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1. Introduction

In many circumstances, most of goats are reared in extensive/semi-extensive low-input systems with a relatively slight environmental influence (Peacocka and Sherman, 2010) leading low productivity (Bingöl and Bingöl, 2018). Reproduction, i.e., ovulation rate (OR) and litter size (LS), is critical to a profitable sheep or goat business. Further, animal selection based on genetic potency for reproductive traits could be a prominent strategy to increase animal performance (Notter, 2008; Li et al., 2009; Redden and Thorne, 2020). Unfortunately, the heritability for reproductive efficiency is low, causing hard fast genetic advancements (Sarma et al., 2019).

High fecundity in livestock species usually equals greater economic efficiency. Fecundity is the capability of animals to deliver live offspring (Notter, 2008). Fertility traits are complex quantitative traits managed by a few major genes and many minor genes (Wang et al., 2022). The KISS1 gene is recognized as an essential regulatory task in reproduction (Pinilla et al., 2012). KISS1 gene in caprine was discovered in chromosome 16, consisting of two coding regions (exons) and one non-coding region (intron). The length of the transcript is 408 bp and encodes 135 amino acids. This gene attains around 2.62 kilobases (ENSCHIT00000037363.1). Mulyono et al. (2019) and Hardyta et al. (2021) reported that there was no relationship among SNPs of the KISS1 gene with reproductive traits in local Etawah Grade (EG). Febriana et al. (2022) found g.2055A>C (SNP1) and g.2064T>A (SNP2) as novel SNPs of the KISS1 gene in Indonesian native goat breeds. Unfortunately, the correlation between these two SNPs with reproductive traits remains unclear.

Kisspeptins are neuropeptides encoded by the KISS1 gene (Pinilla et al., 2012). Nowadays, Kisspeptin–G-protein-coupled receptor 54 (GPR54) is a signaling pathway required for normal fertility, primarily located in GnRH neurons (Irwig, et al., 2004; Herbison et al., 2010; Mayer and Boehm, 2011; Kirilov et al., 2013). Kisspeptin has potent effects on GnRH neuron excitability (Han et al., 2005; Zhang et al., 2008; Dumalska et al., 2008; Pielecka-Fortuna and Moenter, 2008; Liu et al., 2008), indicating that this is a location for kisspeptin's actions within the HPG axis. The secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) out of the anterior pituitary was induced by the GnRH, which is needed to initiate puberty and maintain reproductive function (de Roux et al., 2003; Funes et al., 2003).

However, due to these traits' poor heritability and sex-limited nature, a traditional selection based on polygenic quantitative approaches for increasing reproductive traits in small ruminants becomes problematic (Janssens et al., 2004). Molecular tools combined with the traditional breeding technique are necessary to reach better results in animal breeding programs (Olsen et al., 1999). Further, breeding strategies based on molecular techniques and marker-assisted selection (MAS) as genetic information are critical for enhancing reproductive efficiency (El-Tarabany et al., 2017). The current study purposed to detect the genetic variation of the KISS1 gene, investigate the association with goat reproductive traits, and acquire marker-assisted selection for a breeding program.

2. Material and Methods

2.1. Experimental animal and phenotypic data

All experiment procedures implicating the animals and samples were under supervision by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia, numbered 57-06/A-4/KEP-FPP, where the research was established. This research was carried out from August – November 2020. The animals in this research were selected randomly from three different regions. Blood samples (3 mL each) were taken from ninety uncorrelated does (thirty each from Kacang (KC) goats, Kejobong (KJ) goats, and Senduro (SD) goats) and stored in EDTA vacutainers. The goat data recording was collected, including litter size, parity (first to fifth parities), doe age, and the origin farms.

2.2. Polymerase Chain Reaction (PCR) and DNA sequencing of KISS1 gene

DNA isolation and PCR were used to amplify the particular fragment. The genomic DNA was extracted from whole-blood samples using a GeneJET Genomic DNA Purification Kit (Thermo

Scientific, USA). The quantity and purity of genomic DNA extraction were determined by 1% agarose gel electrophoresis and visualised under ultraviolet light in a gel documentation system.

A 227 bp fragment of the KISS1 gene at intron 1 was amplified by PCR using the following set of primers (F: 5'-GGACTCCACGACAAGAGGAG-3'; R: 5'-TCCCTTACCCAGAAAGAGCA-3') designed with Primer3 Plus web program based on the GenBank sequences of goat KISS1 gene (GU142847.1) The total volume of PCR amplification is 50 µL consisting of 4 µL DNA extraction containing 20-30 ng/µL, 1 µL for each primer (10 pmol/µL), 19 µL ddH2O and 25 µL of MyTaq Red Mix Bioline (1st BASE). The protocol for PCR was 5 min at 95°C for initial denaturation, followed by 35 cycles of denaturation at 94°C at 30 s, annealing at 59°C at 30 s, extension at 72 °C at 30 s, and final extension at 72 °C at 7 min. The amplicons were then sequenced in forward and reverse direction. The chromatograph could be used to detect the SNPs and heterozygosity.

2.3. Hormonal assay

Sixteen goat does with various LS were administered with 0.30 mg medroxyprogesterone acetate (MAP) intravaginal sponges during 14 days. The blood serum was obtained five times (0, 3, 6, 9, and 12 hours) after the intravaginal sponge was taken. Three milliliters of whole blood were stored in plain vacutainer tubes. The blood samples were then centrifuged (3000 rpm/5 min) to collect blood serum. The enzyme-linked immunosorbent assay (ELISA) method was used to assess FSH levels.

2.4. Statistical and phenotypic association

2.4.1. SNP genotyping

The MEGA X software (version 10.1) was used to align multiple sequences with the sequence from the GenBank (GU142847.1) to screen the presence of gene variants.

2.4.2. Linkage disequilibrium, genotype frequencies and allele frequencies.

Linkage disequilibrium (D') was estimated using DnaSP 6th version. Genotype frequencies were calculated using the direct counting methods (Nei and Kumar, 2000).

$$X_{ii} = \frac{n_{ii}}{n} \quad (1)$$

The allele frequencies were calculated according to the following formula (Nei and Kumar, 2000).

$$X_i = \frac{\left(2n_{ii} + \sum_{j \neq 1} n_{ij}\right)}{2n} \quad (2)$$

Where X_i is allele frequency of the i^{th} allele, X_{ii} is genotype frequency of the ii^{th} genotype, n_{ii} is the total number of samples with genotype ii, n_{ij} is the total number of samples with genotype ij and n is the number of total samples.

2.4.3. Hardy–Weinberg equilibrium, heterozygosity and association analysis

The Arlequin Software Package version 3.5. was used to count observed heterozygosity (Ho), expected heterozygosity (He), and Hardy–Weinberg Equilibrium (HWE) test (Excoffier and Lischer, 2010). The differences between studied parameters were determined using independent samples T-test from SAS Software (SAS® University Edition, 2018).

3. Result and Discussion

3.1. Detection of PCR amplicons and SNPs.

The KISS1 gene was amplified successfully; further, the PCR amplicons were separated using 2% agarose gels (Figure 1). It demonstrated that the length of amplified fragments and target DNA

fragments were consistent, and the specificity of the amplicon was apparent. Therefore, the amplicons could be directly analyzed by DNA sequencing (1st BASE Asia). The DNA sequences in Indonesian native goat breeds were 227 bp long and 99.8% identical to KISS1 gene of *Capra hircus*, which is registered on the GenBank database (GU142847.1). This condition indicated that the PCR products were targeted fragments of KISS1 gene.

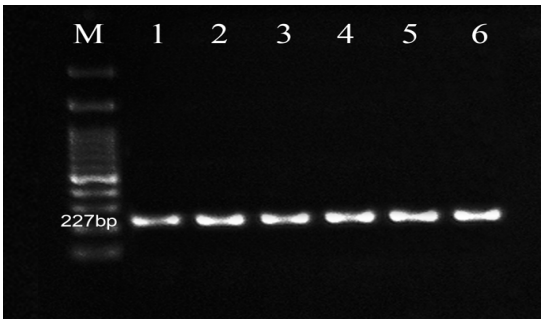
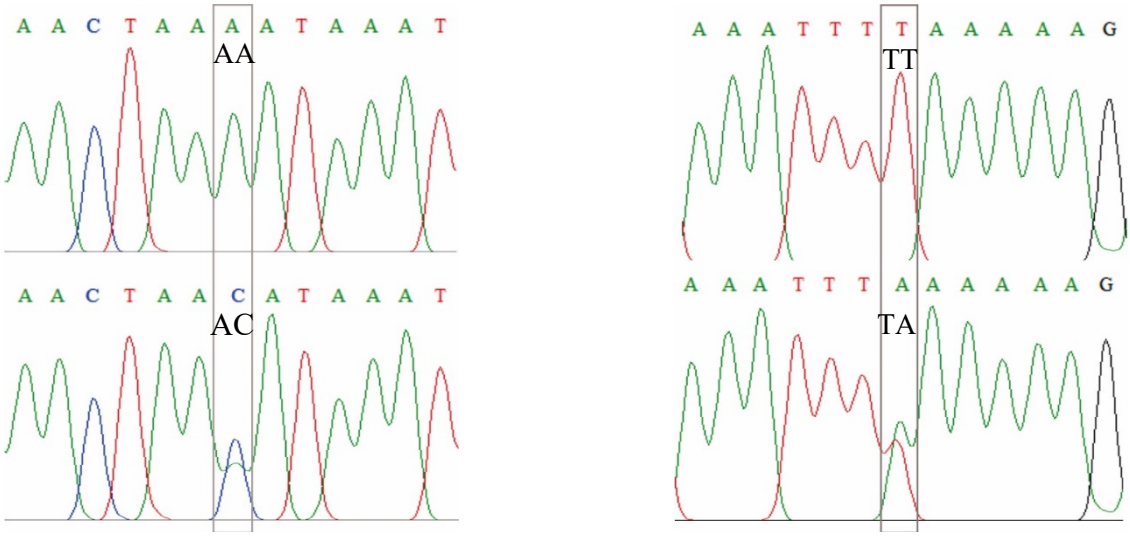


Figure 1. A 227 bp of KISS1 gene amplicon; M: Marker (DNA ladder 100 bp); Lane 1 and 2: Kacang goats; Lane 3 and 4: Kejobong goats; Lane 5 and 6: Senduro goats.

In the present study, two SNPs (SNP1 and SNP2) were discovered in intron 1 of KISS1 gene in Indonesian native goat breeds by screening the sample sequences and alignment with the database from GenBank (GU142847.1). Further, two genotypes were identified from each SNPs, namely AA and AC at SNP1 also TT and TA at SNP2 (Figure 2).



a) AA and AC genotype at SNP1 of KISS1 gene

b) TT and TA genotype at SNP2 of KISS1 gene

Figure 2. Chromatograms of SNP1 and SNP2 of KISS1 gene.

3.2. Genotype distribution

Table 1 summarizes the genotype and allele frequencies in three Indonesian native goat breeds based on the nucleotide sequence of KISS1 gene intron 1. The genotypic frequencies of AA and AC at SNP1 were obtained to be 0.86 and 0.14 in KC goats, 0.87 and 0.13 in SD goats, and 0.91 and 0.09 in overall population, respectively. Furthermore, the respective genotypic frequencies of TT and TA at SNP2 were found to be 0.75 and 0.25 in KJ, 0.86 and 0.14 in SD goats, and 0.87 and 0.13 in the overall population. SNP1 in KJ goats and SNP2 in KC goats were monomorphic. The allele A (0,96) at SNP1 and allele T (0,93) at SNP2 were discovered as dominant alleles in Indonesian native goat breeds. The allelic frequencies and genotypic frequencies among three native goat breeds showed similar

distribution, except in monomorphic goat breeds. According to Singh et al. (2015), these discrepancies in allelic frequencies could be due to the fact that various breeds reared under dissimilar environmental conditions are subject to varying degrees of evolutionary forces. Furthermore, sampling variations also contributed to variances in allelic frequencies between breeds and populations.

Table 1. Allele and genotype frequency of KISS1 gene intron 1

Loci	Allele	Allele frequency				Geno type	Genotypic frequency			
		Population					Population			
		KC	KJ	SD	Overall		KC	KJ	SD	Overall
SNP1	A	0.93	1.00	0.94	0.96	AA	0.86 (26)	1.00 (30)	0.87 (26)	0.91 (82)
	C	0.07	0.00	0.06	0.04	AC	0.14 (4)	0.00 (0)	0.13 (4)	0.09 (8)
SNP2	T	1.00	0.88	0.94	0.93	TT	1.00 (30)	0.75 (23)	0.86 (26)	0.87 (78)
	A	0.00	0.12	0.06	0.07	TA	0.00 (0)	0.25 (7)	0.14 (4)	0.13 (12)

KC: Kacang goats; KJ: Kejobong goats; SD: Senduro goats; Overall: genotypic and allelic frequencies in all population. The number of genotyped individuals are represented in the brackets.

Furthermore, the current research showed that the chi-square test for both SNPs in the population was under Hardy-Weinberg equilibrium ($\chi^2 = 3.81$; $p>0.05$). Recent situations suggest that breeding selection for litter size had little effect on genotypic frequencies (Alim et al., 2019) and due to a deficiency of significant heterozygosity (El-Tarabany et al., 2017). The χ^2 value and heterozygosity are presented in Table 2.

Table 2. Heterozygosity and Chi Square of SNPs discovered at intron 1 of KISS1 gene

Loci	N	KC			KJ			SD			Overall		
		Ho	He	χ^2	Ho	He	χ^2	Ho	He	χ^2	Ho	He	χ^2
SNP1	90	0.14	0.13	0.04	0.00	0.00	-	0.13	0.18	0.04	0.09	0.08	0.05
SNP2		0.00	0.00	-	0.25	0.22	0.16	0.13	0.12	0.04	0.12	0.12	0.11

Ho: observed heterozygosity; He: expected heterozygosity; (-): the $\chi 2$ value could not be counted because the goat breed was monomorphic; χ^2 distribution table (0.05; df = 1) = 3,841; Overall: the value in all sample population; KC: Kacang goats; KJ: Kejobong goats; SD: Senduro goats; N: the number of sample.

3.3. Effects of KISS1 genotypes on litter size and FSH profile in goats

In the current research, Table 3 showed that the variants of KISS1 gene have no impact with litter size ($p>0.05$). In contrary, Budisatria et al. (2012) reported that Indonesian native goat breeds have high prolific traits. In addition, previous research reported that a polymorphism of KISS1 gene is associated with reproduction traits (Cao et al., 2010; Hou et al., 2011; An et al., 2013; El-Tarabany et al., 2017; Mekuriaw et al., 2017; Sahoo et al., 2019; Jeet et al., 2022), which in turn could escalate litter size. This finding indicated a presence of other SNPs, which affected large litter size in Indonesian native goat breeds. Febriana et al. (2022) reported that a SNPs at g.2425C>G, g.2436A>G, and g.2459G>A of KISS1 gene have an effect on larger litter size in Indonesian native goat breeds.

Table 3. Litter size based on different genotype of KISS1 gene on Indonesian native goat breeds

SNPs	Genotype	N	Means ± SE	P value
SNP1	AA	82	2.36±0.13	0.67
	AC	8	2.50±0.17	
SNP2	TT	78	2.38±0.13	0.86
	TA	12	2.33±0.25	

SE: standard error; N: the quantity of sample.

Kisspeptins are known to play a vital role in determining reproductive activities in various species, especially at the hypothalamus level of the gonadotropic axis (Tu et al., 2014). In mammals, the follicle stimulating hormone (FSH) is a pituitary gonadotropin that leads to gonadal function and follicle growth (Bartlewski et al., 2009, Aerts and Bols, 2010). The FSH is in charge of the survival and

proliferation of follicular somatocyte, further the cyclic recruitment of ovarian follicles through maturation for the early antral stage until ovulation (McGee and Hsueh, 2000). The association of various genotype of KISS1 gene with FSH level in Indonesian native goat breeds were provided in Table 4.

Table 4. Means of FSH level on different estrus phase based on genotype of KISS1 gene intron 1

Loci	Genotype	N	Means ± SE		
			Overall	**Luteal phase**	**Follicular phase**
			----------mIU mL^{-1}----------		
SNP1	AA	82	3.16 ± 0.38^{b}	2.77±0.43	3.34 ± 0.64^{b}
	AC	8	1.35 ± 0.07^{a}	2.59±0.65	1.38 ± 0.02^{a}
p value			<0.0001	0.89	0.005
SNP2	TT	78	2.63±0.29	2.54±0.38	2.76±0.44
	TA	12	4.26±1.27	3.65±1.29	5.19±2.65
p value			0.23	0.40	0.43

Overall: means of FSH level in all population; Different superscript letters at the same column and SNP indicated substantial difference ($p<0.05$); SE: standard error; N: the amount of sample.

Analytical statistics revealed that AA genotype of SNP1 had a higher FSH level than that observed in AC genotype ($p<0.0001$), especially in the follicular phase ($p=0.005$). SNP2 has no correlation with FSH level relatively ($p=0.23$). Further statistical analysis showed there was no differences ($p> 0.05$) in FSH level between genotypes of SNP2, neither the luteal phase ($p=0.40$) nor the follicular phase ($p=0.43$). Fleming et al. (1996) reported that slight discrepancies in the FSH plasma concentrations between the genotypes in the present study could be led by small number of animals used that did not adequately represent the population's condition. In addition, SNP2 might be associated with other reproductive traits. For instance, SNP rs1213704663 C/G at 3'UTR region of KISS1 gene may be involved in estradiol hypersecretion, which may be responsible for deregulating the mechanism of GnRH secretion and stimulating LH hypersecretion with an elevated LH-FSH ratio (Ali, 2015; Zhu et al., 2019).

Previous research in Kaligesing goats in Indonesia showed that the polymorphism of KISS1 gene has no differences on both 17β-estradiol and progesterone levels (Hardyta et al., 2021). On the other hand, El-Tarabany et al. (2017) discovered that TT genotype at T121A of KISS1 gene in Egyptian goat breeds had higher 17β-estradiol and a greater amount of progesterone than TA genotype. According to Febriana et al. (2021) reported that goat breed, litter size, genotype, and haplotype have a significant association with FSH level.

Basically, prolificacy is governed primarily by the ovulation rate in small ruminants, which is determined by the development of preovulatory ovarian follicular (Nett et al., 2002). FSH concentrations are increased in livestock with larger litter size (Wikins et al., 1997). According to El-Tarabany et al. (2017), polymorphisms of KISS1 gene have a connection with fertility in small ruminants particularly; thus, the present research might lead to a crucial and applicable contribution to breeding selection.

Conclusion

The present study revealed polymorphisms of the KISS1 gene, leading to an association of the genotype of the KISS1 gene on the FSH level. As the sample number selected for the research was tiny, other researchers were required to determine the relationship between the SNPs of the KISS1 gene and FSH level in Indonesian goat breeds. AA genotype at SNP1 of the KISS1 gene is correlated with the best level of FSH in goats. Furthermore, the utilization of the AA genotype at SNP1 in the KISS1 gene for the breeding selection program could escalate the reproductive traits in goats.

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Research Article

Genetic Diversity and Phylogenetic Position of Traditional Rice (*Oryza sativa* L.) Landraces: A Case Study of South Kalimantan in Indonesia

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Abstract: Traditional rice (*Oryza sativa* L.) landraces provide many essential genes for improving yield, disease resistance, abiotic stress tolerance, and other parameters for future rice breeding. This study aimed to analyze the genetic diversity and determine the phylogenetic position of the traditional rice landraces from the tidal swamp areas of South Kalimantan, Indonesia, compared to other rice germplasm, including wild relatives, obtained from the GenBank database, using a cpDNA-*rbc*L marker. In this case, six traditional rice landraces from this region were collected and analyzed molecularly using the *rbc*L marker and compared with 16 similar others and 25 wild relatives from the GenBank database. The genetic diversity of this germplasm was determined using the nucleotide diversity index (π), whereas the phylogenetic analysis by maximum likelihood with bootstrap for 1 000 replicates. The principal component analysis (PCA) was employed to confirm this grouping. Based on this marker, the traditional rice landraces have a genetic diversity of 0.38, lower than intra-species and inter-species levels, i.e., 0.44 and 0.83, respectively. The phylogenetic analysis shows that this germplasm has separated from most *O. sativa* rice cultivars and their wild relatives, except for the 'GBVN' and 'NARC' (comparison cultivars obtained from GenBank). This information has substantial implications for future rice breeding and conservation efforts, locally and globally.

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1. Introduction

Over the last 10 000 years, rice, an important staple crop for over half of the world's population, has been massively domesticated (Hu et al., 2018). As a result, it is unsurprising that this crop has been distributed far from the center of its diversity and cultivated in various ranges of environmental systems and conditions, i.e., from monoculture (single-crop) in temperate zones to the tropics with rainfed and irrigated polycultures (Reig-Valiente et al., 2016). Even rice is widely cultivated in more than 120 countries, from 35ºS to 53ºN geographic ordinate, and harvested from 163 million hectares per year, guided by China, India, Bangladesh, Thailand, and Indonesia (Wei and Huang, 2018). Domestication has also led to various cultivated rice with different traits. In national and international germplasm collections, over 400 thousand rice cultivars have been stored and recorded (Wei and Huang, 2018).

In general, two species of cultivated rice with a unique historical background of domestication, namely *Oryza sativa* (better known as Asian rice) and *Oryza glaberrima* (African rice), are known (Sweeney & McCouch, 2007). According to Hu et al. (2018), *O. sativa* and *O. glaberrima* have domesticated independently from the AA genome wild *Oryza* rice in Asia and Africa. By the cytological analysis, the genus *Oryza* comprises 22 wild relatives and separates into four species complexes, i.e., the *O. sativa*, *O. officinalis*, *O. ridleyi*, and *O. granulata* (Wei and Huang, 2018). *O. sativa* complex contains two domesticated species: *O. sativa* and *O. glaberrima*, and six wild species, including *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*, *O. nivara*, and *O. rufipogon* (Wei and Huang, 2018; Xu and Sun, 2021).

According to Izawa (2008), all cultivated and wild rice have different distributions. *Oryza sativa*, for example, is distributed around the world with a concentration in Asia, while *O. glaberrima* is in Africa. Barth's rice (*O. barthii*) and long-stamen rice (*O. longistaminata*) are African ones, where the first is endemic to West Africa, whereas the second is to the entire of Africa. In contrast, *O. glumaepatula* is endemic in Central and South America, and *O. meridionalis* is native to Australia. So both are known as South American and Australian wild rice, respectively. Finally, *O. rufipogon* (usual wild rice) and *O. nivara* (annual wild rice) are present throughout Asia and Oceania (Sweeney and McCouch, 2007; Wei and Huang, 2018).

Besides the various rice germplasm, rice breeding is production or yield-oriented. On the other hand, domestication and contemporary rice breeding created a wide genetic bottleneck. Consequently, the superior genes or alleles that contribute to yield, and other variables, like disease resistance and abiotic stress tolerance, might be lost in the modern rice cultivars because of this effect. Meanwhile, these superior genes were present in the wild progenitors and traditional rice landraces (Reig-Valiente et al., 2016). However, due to human interference destroying their habitats, wild species are in danger of going extinct, and farmers' preference for high-yield commercial cultivars has resulted in the disappearance of traditional landraces (Mursyidin et al., 2017; Wei and Huang, 2018). Thus, collecting, documenting, analyzing, or characterizing wild and cultivated (traditional) rice cultivars are indispensable (He et al., 2021).

South Kalimantan in Indonesia is an urgent route for the domestication and distribution of traditional rice cultivars worldwide (Mursyidin et al., 2017). In this region, hundreds of traditional rice cultivars are found and still preserved, also maintained by the local farmers (Mursyidin et al., 2017). Previously, we have successfully characterized this germplasm by morphological markers, both at the macro (Mursyidin et al., 2018; Mursyidin and Khairullah, 2020) and micro-structure levels (Mursyidin et al., 2019, 2021). While these markers are essential in evaluating rice germplasm, they were strongly affected by environmental variables (Nadeem et al., 2018). Besides, these traits have certain disadvantages, such as time-consuming, low polymorphism, late expression, and low heritability (Anumalla et al., 2015).

Recently, the *rbc*L marker, part of the cpDNA genome, has been valuable and urgent in characterizing rice germplasm, including their domestication history. According to Izawa (2008), following this method, the order of most DNA changes, such as single nucleotide polymorphisms during domestication, can be inherited due to relatively low mutation rates. As a result, this study aimed to analyze the genetic diversity and determine the phylogenetic position of the existing traditional rice landraces from the tidal swamp areas of South Kalimantan in Indonesia and compared to other (*O. sativa*) cultivars and wild relatives (*Oryza* spp.) obtained from the GenBank database, using the *rbc*L marker. In this study, we hypothesized that traditional rice landraces from this region have low diversity. Thus, our results provide urgent guidance to support future rice breeding and conservation efforts, locally and globally.

2. Material and Methods

2.1. Plant materials

A total of 47 samples of rice (*Oryza* spp.) comprise six traditional rice (*O. sativa*) landraces from the tidal swamp areas of South Kalimantan in Indonesia, including 16 other commercial rice cultivars (*O. sativa*) and 25 wild relatives, obtained from GenBank, were used in the study (Table 1).

Table 1. Rice samples (*O. sativa*), including wild relatives, used in the study, their accession number and nucleotide length of *rbc*L

Rice samples	Origin	Accession Number	Nucleotide Length (bp)
Landrace (*O. sativa*)			
'Lakatan Gadur'	Sungai Tabuk, Banjar, South Kalimantan	MZ198245	608
'Raden Rata'	Tabunganen, Barito Kuala, South Kalimantan	MZ198246	606
'Lemo Putih'	Kertak Hanyar, Banjar, Kalimantan Selatan	MZ198247	605
'Pandak Kembang'	Barambai, Barito Kuala, South Kalimantan	MZ198248	605
'Siam Mutiara'	Aluh Aluh, Banjar, South Kalimantan	MZ198249	608
'Karang Dukuh'	Barambai, Barito Kuala, South Kalimantan	MZ198250	607
Commercial cultivar (*O. sativa*)[1]			
'Shuhui498'	Republic of China	CP018170.1	607
'RP Bio-266'	Republic of India	KU705873.1	607
'Basmati'	Republic of India	KT289403.1	607
'Anonymous-1'	Saudi Arabia	JN861110.1	607
'93-11'	Republic of China	AY522329.1	607
'Hassawi'	Kingdom of Saudi Arabia	JN861109.1	607
'Anonymous-2'	Japan	X15901.1	607
'Nipponbare'	Japan	AY522330.1	607
'Anonymous-3'	Republic of China	KT289404.1	607
'Anonymous-4'	Republic of China	MW001303.1	607
'TN1'	Republic of Korea	KM103369.1	607
'IR8'	Republic of Korea	KM103367.1	607
'NARC'	Islamic Republic of Pakistan	KP827660.1	601
'N22'	Republic of Korea	MG252500.1	607
'HSAGSDYD1802'	Republic of China	MT653617.1	607
'GBVN15800'	Socialist Republic of Vietnam	KR073275.1	592
Wild relatives[1]			
O. alta	Republic of China	MT726934.1	607
O. australiensis	Republic of China	MT726929.1	607
O. barthii	Republic of China	KF359904.1	607
O. brachyantha	Republic of China	MT726939.1	607
O. coarctata	Republic of China	MT726931.1	607
O. eichingeri	Republic of China	KX085496.1	607
O. glaberrima	Republic of China	KF359903.1	607
O. glumipatula	Republic of China	KF359905.1	607
O. grandiglumis	Republic of China	MT726928.1	607
O. granulata	Republic of China	KF359920.1	607
O. latifolia	Republic of China	KF359915.1	607
O. longiglumis	Republic of China	MT726933.1	607
O. longistaminata	Republic of China	KM881642.1	607
O. malampuzhaensis	Republic of China	MT726935.1	607
O. meridionalis	Republic of China	KF359906.1	607
O. meyeriana	Republic of China	KF359921.1	607
O. minuta	Republic of Korea	KU179220.1	607
O. neocaledonica	Republic of China	MT726926.1	606
O. nivara	Japan	AP006728.1	607
O. officinalis	Republic of China	MT726930.1	607
O. punctata	Republic of China	MT726932.1	607
O. rhizomatis	Republic of China	KX085497.1	607
O. ridleyi	Republic of China	MT726937.1	607
O. rufipogon	Republic of China	KF359902.1	607
O. schlechteri	Republic of China	MT726927.1	607

[1]Obtained from GenBank database.

2.2. DNA isolation and other molecular assays

DNA isolation was performed using a commercial kit from Geneaid Biotech, Taiwan (Plant genomic DNA mini kit, GP100) against the young leaves of each rice sample. DNA samples were then quantified using the spectrophotometric UV-Vis method and amplified using a pair of specific rbcL primers (Gholave et al., 2017), i.e., 5'-ATGTCACCACAAACAGAGACTAAAGC-3' (Forward) and 5'-GTAAAATCAAGTCCACCRCG-3' (Reverse). The amplification reaction follows the instructions of Gholave et al. (2017), using a PCR machine from Applied Biosystem, USA (SimpliAMP Thermocycler PCR), with a total volume of 25 µL. The volume reaction consists of PCR mix (22 µL, MyTaq HS Red Mix, Bioline, UK), forward and reverse primer (10 µM, 2.0 µL), and DNA template (10 ng, 1.0 µL). The formed target DNA (rbcL) was then separated by the electrophoresis method (2% gel agarose in 1xTBE Buffer and GelRed dye) and observed in a UV transilluminator. After that, it was isolated and re-purified for sequencing at 1st Base Ltd., Malaysia, using the Sanger (bidirectionally) method. The sequence results were deposited in Genbank with accession numbers MZ198245- MZ198250 (Table 1).

2.3. Data analysis

By the MEGA-X program, all *rbc*L sequences were examined, modified, and put together (Kumar et al., 2018). Utilizing the nucleotide diversity index (π) approach, the genetic diversity was determined and classified into three categories: low (0.1 to 0.4), medium (0.5 to 0.7), and high (0.8 to 2.0) (Nei & Li, 1979). Multiple sequence alignments of the sequences were carried out using Clustal-Omega for phylogenetic analysis (Sievers et al., 2020), and they were manually altered using a similar program to the one previously used to create an unbias sequence alignment. The Consortium for the Barcode of Life (CBOL) provided instructions for the use of Kimura 2-Parameter (K2) distances in MEGA-X to determine interspecific genetic divergences (Kumar et al., 2018). Maximum likelihood was used for the phylogenetic analysis (ML) (Lemey et al., 2009), followed by the bootstrap for 1000 replicates (Mursyidin et al., 2019) and the PCA (Kumar et al., 2022).

3. Results and Discussion

Information on genetic diversity is valuable in crop improvement and conservation efforts (Lloyd et al., 2016). Based on the *rbc*L marker, traditional rice landraces from the tidal swamp areas of South Kalimantan in Indonesia has lower genetic diversity (π% = 0.38) than at intra- and inter-species levels, i.e., 0.44 and 0.83, respectively (Table 2). However, compared to other studies with similar markers, this diversity is higher, such as *Durio zibethinus* and its wild relatives (Mursyidin et al., 2022). Teixeira and Huber (2021) contend that a high genetic diversity level is essential for population survival and ensuring natural populations' capacity for adaptation in the face of constantly shifting environmental stressors.

Table 2. Characteristics of the *rbc*L sequence of *Oryza* germplasm, including traditional rice landraces of South Kalimantan, Indonesia[1]

Parameter	Traditional rice population (*O. sativa*)	*O. sativa* population (intra-species)	Global rice population (inter-species)
Sequence length (bp)	605-608	592-608	502-608
Polymorphic sites (*S*)	6	10	26
Akaike Information Criterion (AICc)	1823.853	1980.191	2400.480
Bayesian Information Criterion (BIC)	1885.781	2311.761	3178.071
Maximum Likelihood Value (ln*L*)	-901.896	-945.953	-1105.932
Transition/transversion bias value (*R*)	0.75	0.86	0.94
Guanine-Cytosine/GC content (%)	43.55	43.55	43.47
Nucleotide diversity (π%)	0.38	0.44	0.83
Tajima test of neutrality (D)	-0.64	-0.03	-0.44

[1]Following Kimura 2-parameter model.

Table 3. Polymorphic sites (mutations) are present in the *rbc*L region of traditional rice (*O. sativa*) landraces of South Kalimantan, Indonesia

Landraces	Accession Number	Nucleotide Position									
		2[a]	3b[c]	4[c]	15[c]	19[b]	588[c]	600[c]	601[b]	610[bc]	611[a]
'Lakatan Gadur'	MZ198245	-	T	T	.	.	.	.	.	C	.
'Raden Rata'	MZ198246	-	-	.	.	.	.	.	.	.	-
'Lemo Putih'	MZ198247	-	-	.	.	.	.	.	.	.	-
'Pandak Kembang'	MZ198248	-	-	.	.	.	C	.	.	.	-
'Siam Mutiara'	MZ198249	.	A	A	C	-	.	A	.	-	-
'Karang Dukuh'	MZ198250	-	T	.	C	.	C	A	-	.	-
Consensus		A	C	G	A	A	T	G	A	A	A

a = insertion, b = deletion, c = transversion.

According to Frankham et al. (2004), the low level of diversity strongly correlates to a mutation occurring in this region. In this study, the traditional rice landraces have six polymorphic or mutation events. In contrast, at the intra- and inter-species levels, the number of polymorphic sites was 10 and 26, respectively (Table 2). In this case, the mutations present in the *rbc*L of rice are substitution (transversion) and indels (insertion-deletion) (Table 3). Referring to Aloqalaa et al. (2019), the transition is more common in these sequences than in transversions. Furthermore, in the course of molecular evolution, there is frequently a pattern where nucleotide transitions repeatedly occur over transversions (Stoltzfus and Norris, 2016).

Further, several factors, such as the founder effect, natural selection, genetic isolation, population bottleneck, and inbreeding, can all contribute to a low level of diversity (Gao et al., 2017). In this case, inbreeding is the most likely cause of this condition (Mursyidin et al., 2017). Local farmers of this region usually select and replant their seeds from the previous crop season. Furthermore, they do both depending on the grain shape and color only. As a result, this rice is homogenous and has a relatively identical genetic background (Mursyidin et al., 2017). In other words, most rice is autogamous (self-pollinated), and inbreeding resulting from this type of reproduction also contributes to low genetic diversity (Jain and Kharwal, 2004).

The Tajima neutrality test (D) has also supported this condition, where this germplasm has an indicating the expansion of population size (for example, after a purifying selection or bottleneck) because all sequences have negative values ($D<0$) (Korneliussen et al., 2013). Consequently, future rice improvement must be oriented to outcrossing, as was employed by Niruntrayakul et al. (2009) and Bierschenk et al. (2020). According to Gaikward et al. (2021), most wild rice relatives have novel beneficial alleles for improving cultivated rice varieties, such as better adaptation to different ecological regimes and biotic and abiotic stresses. Allier et al. (2020) added that broadening the genetic diversity or gene pool of germplasm could be employed by hybridization, introgression, mutation, or genetic engineering.

According to historical records, the low level of rice diversity due to inbreeding is strongly related to the evolutionary pathway and distribution of this plant in this region. Kiple and Ornelas (1999) have predicted that rice plants evolved and spread from Sumatra Island to this region about 300 years before the century. During this period, inbreeding is unavoidable and may reduce genetic diversity (Gao et al., 2017). However, the last parameter is indispensable in generating a foundation population for natural selection or general evolutionary processes (Govindaraj et al., 2015). In other words, this parameter plays a significant role in the future evolutionary direction and adaptive changes. As a result, it has substantial consequences for future conservation and breeding efforts (Lloyd et al., 2016).

The phylogenetic analysis shows the unique relationship of rice germplasm globally. Following the maximum likelihood (ML), this germplasm was separated into three distinct clades (Figure 1). In this case, the traditional rice landraces of South Kalimantan, Indonesia, are separated from most *O. sativa* rice cultivars and their wild relatives. According to Gascuel et al. (2015), these grouping and separation may correlate with ecological factors and landscape dynamics, where each sample is obtained. Similarly, environmental factors and evolutionary history may shape this phylogenetic relationship (Saladin et al., 2019). From the global perspective, dispersal and niche evolution may jointly shape the geographic turnover of phylogenetic clades across continents (Eiserhardt et al., 2013).

The substantial variance in species richness or composition among distinct groupings at the broadest evolutionary scales is due to some intricate and interrelated causes. The most basic assumption behind this research is that clade age should be connected only with this occurrence in extant clades. In other words, older clades will have more time to develop variety than younger clades under the same conditions (Rabosky et al., 2012).

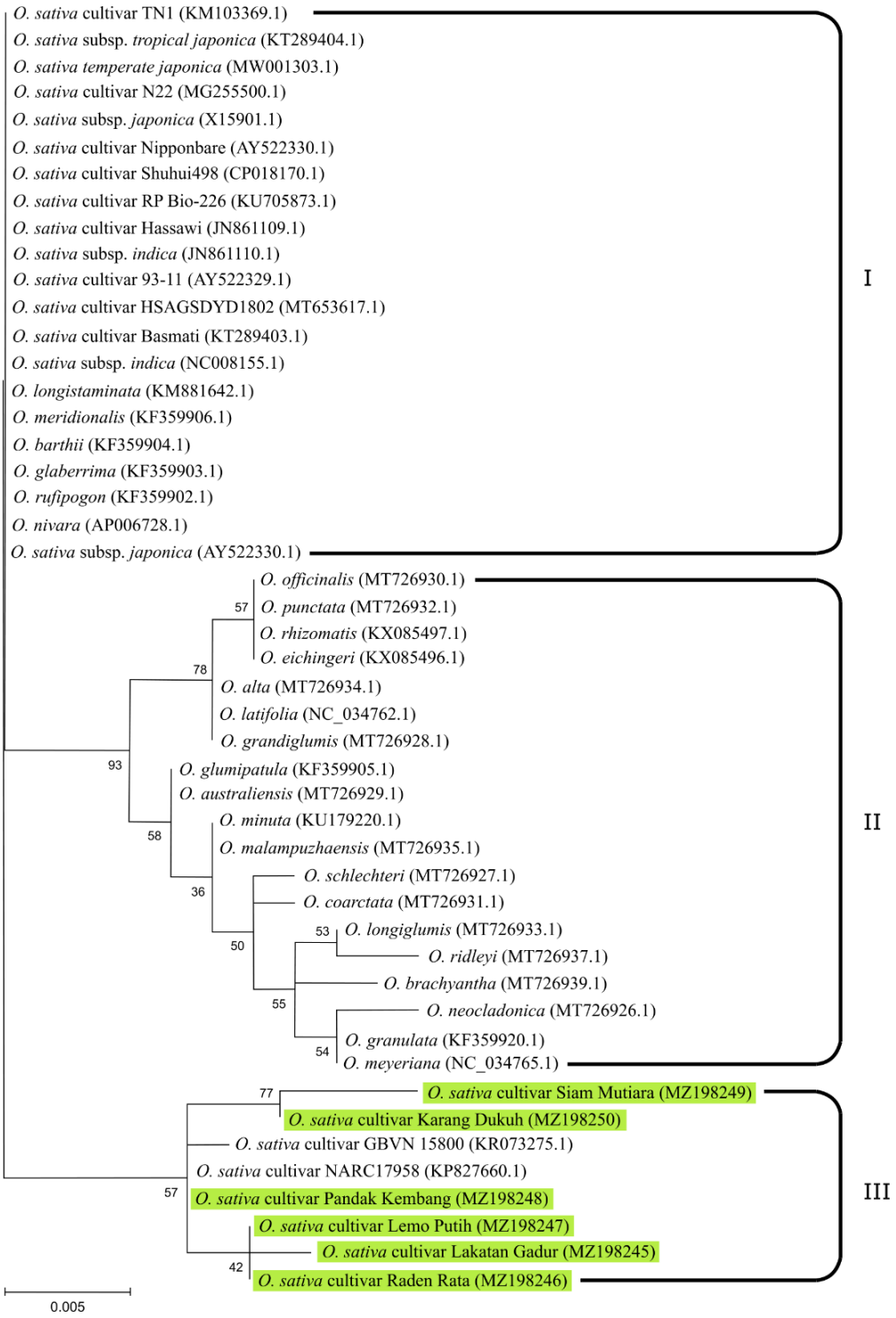

Figure 1. Phylogenetic relationship of tidal swamp rice (O. sativa) landraces of South Kalimantan, Indonesia (highlight), compared to others, including wild relatives (Oryza spp.), based on the ML. The numbers above branches are bootstrap values of 1000 replicates.

Interestingly, two comparison cultivars obtained from GenBank, namely 'GBVN' and 'NARC', are difficult to distinguish because they are joined or clustered together or have the closest related to this traditional rice germplasm (Figure 1), except for the PCA result (Figure 2). In the PCA (Figure 2), this germplasm was clustered into four groups, where the traditional rice landraces were separated into two different groups (i.e., II and III). Furthermore, the 'NARC' is still joined with most tidal swamp rice, whereas the 'GBVN' was formed as a new one (IV).

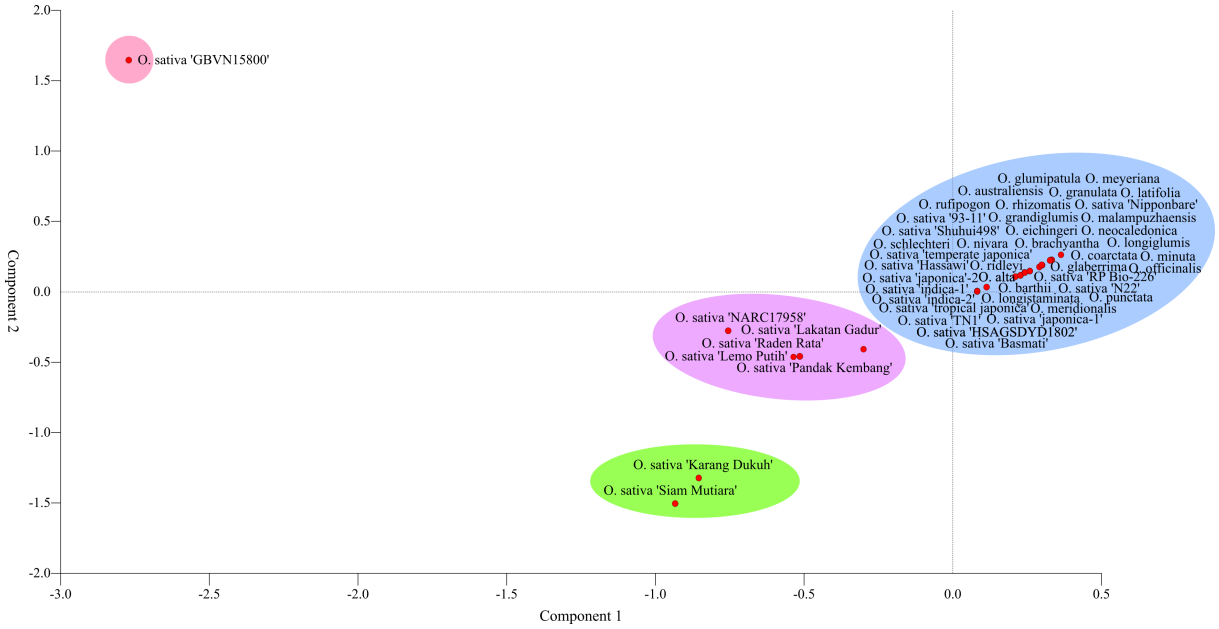

Figure 2. Grouping of traditional rice (*O. sativa*) landraces from South Kalimantan in Indonesia, compared to others, including wild relatives (*Oryza* spp.), based on the PCA.

Philippe et al. (2011) hypothesized that two factors significantly share this case. First, when speciation events occur at close intervals, the quantity of phylogenetic signals produced is generally limited, resulting in short internal tree branches that are difficult to resolve. Second, if the events of interest are old, the terminal nodes are likely to be lengthy and brimming with many substitutions at the same position, known as homoplasy (Philippe et al., 2011). However, the primary cause of this grouping may be an identical single-nucleotide polymorphism (SNP) on the sixth glutamic acid triplet (GAA/GAG). It suggests that the Southeast Asian region may have been the origin of the germplasm ancestors (Hernández-Soto et al., 2022).

As a result, information on this phylogenetic relationship has substantial implications for future rice breeding and conservation efforts, both locally and globally (Flint-Garcia, 2013). Concerning conservation, the phylogenetic study can help resolve species delimitation, gene flow, and genetic differentiation and infer species and their evolutionary history (Fernández-García, 2017). The use of the phylogenetic relationship is also the main focus of current research due to its objective parameters for conservation in previous evolutionary history, the genetic status of present species, and management for future species (Fernández-Garca, 2017).

Finally, the breeding program also depends on the phylogenetic relationship since it forecasts the genetic variety of progeny when individuals mate (Acquaah, 2015). In theory, when distantly related individuals cross out, their descendants may have a broad genetic diversity. In other words, the chances of transgressive segregation increase when individuals from different groups or with a known genetic distance cross. As a result, there is a better chance that distant genotypes will provide unique, desirable alleles at specific loci (Koide et al., 2019). However, crosses between rice subspecies usually have a high rate of infertility. Then, some limitations to this strategy emerge (Guo et al., 2016). In contrast, when closely related individuals cross, the genetic heterogeneity of their progeny may be limited (Turner-Hissong et al., 2020). Breeders and researchers have avoided crossing individuals with a close relationship since the progeny will most likely be inbred.

Briefly, our results complement our previous study that used morphological markers to characterize traditional rice cultivars in the swamp areas of South Kalimantan, Indonesia, as discussed in the introduction section (Mursyidin et al., 2018, 2019, 2021; Mursyidin and Khairullah, 2020). Thus, these findings have novel information and could be valuable as a reference in supporting the rice conservation and breeding program in the future, both locally and globally.

4. Conclusion

In conclusion, the traditional rice landraces naturally growing in tidal swamp areas of South Kalimantan, Indonesia, has lower genetic diversity than other *O. sativa* cultivars (intra-species) and their wild relatives (inter-species). However, it tends to increase along with the taxonomic level observed. Following the phylogenetic analyses used (ML and PCA), this germplasm has a unique relationship, shown by its composition and position where this germplasm is grouped. In this case, all were joined together in a similar clade and separated distinctively from other *O. sativa* cultivars and their wild relatives. Thus, these findings have novel information and could be valuable as a reference in supporting the rice conservation and breeding program in the future, both locally and globally.

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Research Article

Determination of Phytochemicals, Antimicrobial, Antioxidant and Allelopathic Effects of *Fagonia cretica* L., collected from Jamshoro, Pakistan

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Abstract: Medicinal plant *Fagonia cretica* L., is well known in traditional medicines for curing various complaints of human beings from ancient times and is locally known as Dhamasa. Previous many studies have reported the presence of many phytochemicals, antimicrobial, and antioxidant properties in the various parts of this plant. Therefore, here in this study, we have presented a comprehensive study on the presence of similar medicinal and chemical properties of Dhamsa found in Jamshoro District, Pakistan. For this study, extracts of the root, stem, leaf, and pod of the plant were prepared separately from three different solvents, water, ethanol, and methanol. Then the amount and presence of various phytochemicals, antimicrobial, antioxidant properties, and allelopathic effects were determined in all the extracts. The obtained results of this study confirm the presence of medicinal important phytochemicals in the plant extracts. The antimicrobial testing of this plant proved its highest activity against *E. coli* (16 ±1.4mm), *Salmonella typhi* (18±0.7mm), and *Pseudomonas aeruginosa* (15±1.4mm) in methanol, water, and ethanol extracts respectively. The presence of antioxidant activities was also observed in the ethanolic extract of the leaf at about 0.98 mg/ml. While this plant showed allelopathic effects on the growth of radish and spinach plants. So, we have concluded this study that *Fagonia cretica* L., collected from Jamshoro has the same or more important properties compared to the same plant from other regions, which proves the similar significant value of the *Fagonia cretica* plant of Jamshoro in various fields of medicinal sciences.

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1. Introduction

The *Fagonia cretica* L., is a well-known traditional medicinal plant, locally called Dhamasa. Mostly used as an astringent, and febrifuge in different regions of the world and use as a prophylactic drug for various diseases like smallpox, liver trouble, stomach ache, dysentery, fever, typhoid, vomiting, piles, skin diseases, toothache and cancer (Rawal et al., 2004; Hussain et al., 2007; Khan Marwat et al.,

2008; Akhtar and Begum, 2009; Ali, 2017; Charan et al., 2021). It worked as a blood purifier and a laxative to release constipation (Chourasia et al., 2014). Its extracts from roots and bark are applied for scabies (Baquar, 1989). Leaves and twigs are applied for snakebites (Prasad et al., 2007). The boiled extracts of various parts are used for induction of abortion, and leaf extracts and pastes are effective against tumors and swelling on the neck if applied externally (Rawal et al., 2004; Hussain et al., 2007). It has presented antimicrobial activities against various pathogens and showed antioxidant activities in various studies. Various kinds of important phytochemicals have also been found in the different parts of this plant (Shi et al., 2004). Phytochemicals are important chemicals that help to prevent some common diseases and ailments (Chung et al., 1998).

Allelopathy is the beneficial or harmful effects of one plant on another plant (Turk and Tawaha, 2003). It occurs due to the release of certain chemical substances from a plant through root decomposition, leaching, residue exudation, and some other courses, in both natural and agricultural systems. These chemical substances are known as allelochemicals, and often cause growth inhibition or delay in seed germination (Turk and Tawaha, 2003). In this study, we have also tried to evaluate what may be the possible allelopathic effects of *Fagonia cretica* on other plants.

Fagonia cretica L. counting as a small spiny under-shrub, distributed around the hilly regions of district Jamshoro, mostly found in dry calcareous rocks in the vicinity. Local Hakims and herbal medicine practicians collect it to use for curing various ailments of Humans (Panhwar and Abro, 2007). Although a vast scientific study is present of *Fagonia cretica* of different regions, however, the scientific study of *Fagonia cretica* of district Jamshoro like its antibacterial, antioxidant, presence of important phytochemicals and allelopathy, and agricultural significance in detail is also important to evaluate. So that it can help to determine, that the local *Fagonia cretica* possesses the same pharmaceutical potential as present in the other regional *Fagonia cretica*.

For these studies, 10% extracts of four different parts i.e., pods, leaves, stems, and roots of the *Fagonia cretica* L. were obtained in different solvents like water, methanol, and ethanol to carry out various tests. The basic aim of this study is to evaluate phytochemicals, antimicrobial activities, antioxidant properties, and allelopathic effects of *Fagonia cretica* L. collected from district Jamshoro, Pakistan. The obtained results of the current study confirm the presence of important phytochemicals, antioxidant activities, antimicrobial properties, and allelopathic effects of *Fagonia cretica* L. of district Jamshoro.

2. Material and Methods

2.1. Collection and Identification of Plant Materials

The samples collected from the vicinity of district Jamshoro, Pakistan, were washed and dried at room temperature and were taxonomically verified and identified as *Fagonia cretica* L. (Dhamasa) from the Institute of Plant Sciences, University of Sindh, Pakistan. After one week, the dried pods, leaves, stems, and roots were finely ground in an electric grinder and stored in a dry and cool storeroom.

2.2. Preparation of 10% Extracts

Samples of 10% extracts were extracted by our previously described method (Tunio et al., 2022). Briefly, 2.0g of powder was dissolved in 10ml solvent (water, 70% methanol, and 70% ethanol) and centrifuged at 6000rpm for 30 minutes. Then obtained supernatant was collected in beakers separately, finally, the volume of the sample was leveled at 20ml by adding the respective solvent and stored at 4°C in the freezer, before any practical proceedings.

2.3. Antimicrobial Activity

The antimicrobial activity of different extracts of the plant was observed through the agar well diffusion method. In brief, different precultured, bacterial species (*Salmonella typhi, E. coli,* and *Pseudomonas aeruginosa*) and fungal species (*Aspergillus nigar, Rhizopus sp,* and *Mucor piriformis*) obtained from IBGE (Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan) were used to determine the antimicrobial activities. Luria Bertani media for bacteria and potatoes dextrose media for fungi were used to inoculate microbes, while wells of 8mm

diameter were constructed through sterile borer to fill drug of study, as described in our previous works (Charan et al., 2022; Rahu et al., 2021).

2.4. Determination of Total Antioxidant Activity

The total antioxidant activity of different extracts of the plant was observed, as described in previous work (Rahu et al., 2021; Tunio et al., 2022). Briefly, 0.2ml sample and 2ml of reagent solution were mixed and incubated in an Eppendorf tube for 90 minutes at 95 ºC. The total antioxidant activity was observed at 695 nm absorbance in UV-spectrophotometer compared through the obtained standard curve of α-tocopherol and ascorbic acid, respectively.

2.5. Quantification of Total Protein, Total Sugar, Reducing Power and Reducing Sugar

All the extracts of the plant were analyzed quantitatively for the total protein, total sugar, and reducing sugar, by applying the methods mentioned in previous works (Rahu et al., 2021). Total sugar was estimated by obtaining the glucose standard curve. The reducing power of the sample extracts was estimated by applying Bajaj's method (Bajaj et al., 1981). Estimation of reducing sugars was performed by the dinitro salicylic acid (DNS) method. Estimation of total proteins performed by reacting the sample with alkaline copper reagent and Folin-Ciocalteu reagent.

2.6. Qualitative Screening of Phytochemicals

All the extracts of *Fagonia cretica* were qualitatively tested for various phytochemicals such as alkaloids, Coumarin, steroids, phalobatanin, and saponins by applying the standard methods of Soni and Sosa (Soni and Sosa, 2013). Cardiac glycosides were qualitatively detected through the reported method of Vaghasiya et al. (Vaghasiya et al., 2011). Terpenoids and quinones were tested through the reported methods of Edeoga et al. and Khan et al. respectively. (Edeoga et al., 2005; Khan et al., 2020).

2.7. Quantification of Total Phenolics, Total Flavonoids, Total Flavonol, and Total Tannins

Total phenolics from all extracts were estimated using the Folin-Ciocalteu method (Tunio et al., 2022). Total flavonoids were estimated on a spectrophotometer by the aluminum chloride method of Djeridane et al (Djeridane et al., 2006). Total flavonol contents were estimated by the method reported by Kumaran et al. (Kumaran and Joel Karunakaran, 2007). The estimation of total tannins from extracts was analyzed through the reported method of Tamilselvi et al. (Tamilselvi et al., 2012).

2.8. Allelopathic Effects

The allelopathic effects of *Fagonia cretica* were observed by adding water extract of leaves at the time of germination of radish and spinach in Petri dishes. Briefly four samples D1, D2, D3, D4 of (extract : water) 1:0, 1:1, 1:3, 0:1 respectively were prepared for experiment purpose. All the samples were added in the same quantity directly in soil and seed-filled Petri-dishes at the time of sowing of radish and spinach. The experiment diagram is presented in Figure 4. The germination of plants and survival rate (frequency) was calculated through the following equation (1) and (2) (Gnankambary et al., 2019).

$$Germination\ \% = \frac{Total\ number\ of\ germinated\ seeds}{Totral\ number\ of\ seeds\ of\ experiment} \times 100 \quad (1)$$

$$Survival\ = \frac{Total\ number\ of\ survived\ seedling}{Totral\ number\ of\ germinated\ seeds} \quad (2)$$

3. Results and Discussion

3.1. Antimicrobial activity

In vitro antibacterial activity from all the extracts of the *Fagonia cretica* plant was checked against *Salmonella typhi, E. coli,* and *Pseudomonas aeruginosa.* The maximum inhibitory activity against *E. coli* was observed from methanolic root extract (16±1.414mm). The water extract of the leaf presented high activity against *Salmonella typhi* (18±0.707mm). While *Pseudomonas aeruginosa* showed maximum sensitivity (15±1.4mm) for ethanolic extract of root. All the results in detail are presented in Table 1.

Table 1. Detailed, results of Antibacterial activities of *Fagonia cretica* against bacterial species

Solvent extract[X]	Antibacterial activity of *Fagonia cretica***				Bacterial species
	Pods	Leaf	Stem	Root	
Water	7 ±0.3*	9 ±1.4*	9 ±1.2*	5 ± 0.1*	
Ethanol	11 ±0.7*	6 ±0.1*	10 ±1.3*	10 ±0.7*	*E. coli*
Methanol	12 ±0.4*	14 ±0.7*	12 ±1.3*	16 ±1.4*	
Water	14±0.5*	18±0.5*	16±2.1*	13±1.4*	
Ethanol	6±0.4*	8±0.4*	10±2.1*	7±1.4*	*Salmonella typhi*
Methanol	12± 0.1*	8±0.2*	8±1.4*	12±2.1*	
Water	7±1.4*	9±0.7*	5±0.2*	11±0.7*	
Ethanol	10±0.1*	7±0.3*	12±1.4*	15±1.4*	*Pseudomonas aeruginosa*
Methanol	7±0.7*	11±0.6*	6±0.7*	13±2.1*	

* Zone of inhibition was measured in mm and + standard deviation.
** Given values are means of triplicated determination (n=3) + standard deviation.
X 10% extracts of dried powder of pods, leaves, stems, and roots were prepared in water, ethanol, and methanol separately.

Table 2. Detailed, results of antifungal activities of *Fagonia cretica*

Solvent extract[X]	Antifungal activity of *Fagonia cretica***				Fungal species
	Pod	Leaf	Shoot	Root	
Water	10±0.4*	10±0.2*	11±0.2*	14±0.5*	
Ethanol	5±0.3*	7±0.1*	14±0.3*	12±0.1*	*Aspergillus nigar*
Methanol	13±0.1*	12±0.3*	15±0.2*	19±0.2*	
Water	9±0.1*	5±0.2*	7±0.1*	14±0.3*	
Ethanol	12±0.3*	8±0.1*	5±0.2*	17±0.1*	*Rhizopus* sp
Methanol	14±0.2*	8±0.1*	11±0.1*	21±0.2*	
Water					
Ethanol	Negative in all parts and solvents				*Mucor piriformis*
Methanol					

* Zone of inhibition was measured in mm and + standard deviation.
** Given values are means of triplicated determination (n=3) + standard deviation.
X 10% extracts of dried powder of pods, leaves, stems and roots were prepared in water, ethanol and methanol separately.

The antifungal activity of all the extracts was checked against *Aspergillus nigar, Rhizopus sp,* and *Mucor piriformis.* The maximum inhibitory activity against *Aspergillus nigar* was observed from methanolic root extract (19±1.2mm). The water extract of the root presented high activity against *Rhizopus sp* (17±0.5mm). While, against *Mucor piriformis*, we observed all negative results. The results of antifungal activities in detail are presented in Table 2.

After observing all the obtained results, it was concluded that the native *Fagonia cretica* has excellent antibacterial capacity against various bacteria and fungi, Which proved the similar potential of native *Fagonia cretica* compared to other *Fagonia cretica* from different regions presented in previous studies. However, we observed that all the extracts of the samples present a negative effect against *Mucor piriformis*, which suggest that this specie of plant has a variable effect on various organisms.

3.2. Determination of total antioxidant activity

The antioxidant activity from all the extracts was determined in this study. According to obtained results presented in Figure 1, the ethanolic extract presented maximum activity 0.98 mg/ml, while the methanolic extract of the leaf showed the second highest activity (0.81). in this study, all extracted samples of the plant presented antioxidant activity, these results are shown in Figure 1. The obtained results also justify that the Fagonia cretica, collected from the Jamshoro district has antioxidant properties and can be presented as an alternative anticancer drug in herbal medicine.

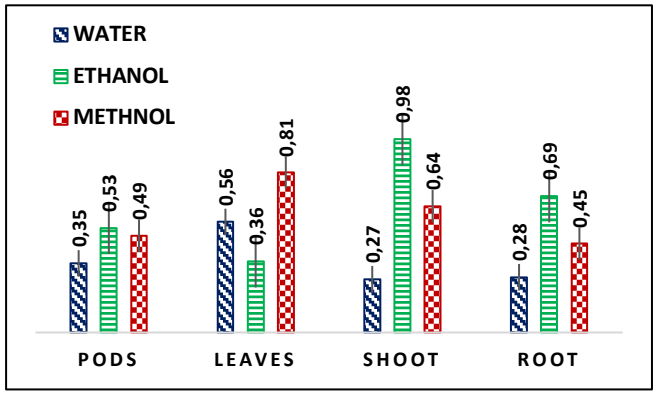

Figure 1. Results of total antioxidants activity from different parts of the plant Fagonia cretica, presented in the comparative clustered column chart.

3.2 Quantification of Total Protein, Total Sugar, Reducing Power and Reducing Sugar

The total soluble sugars, reducing sugar and total protein from all the extracts of *Fagonia cretica* were estimated. The obtained results show the presence of a variable amount of these biochemicals in all the extracts of the plant like the total proteins found in the variable range from 1.26±0.1 mg/ml in water extract of the shoot to the highest 1.84±0.06 mg/ml in ethanol extract of the leaf. The total sugar ranges from 19.6±0.8 mg/ml in the root extract of ethanol to the highest 39.6±1.2 mg/ml in the leaf extract of ethanol. While the ethanolic extract of leaves presented the highest value of reducing power at about 3.8 mg/ml. The reducing sugar found from the lowest 13.1±0.6 mg/ml amount to the highest 32.65±0.68 mg/ml in ethanol of root and methanol of leaf extracts respectively. The results are present in clustered columns in Figure 2.

3.3 Qualitative Screening of Phytochemicals

The various extracts of *Fagonia cretica* plants were qualitatively screened for the presence of important phytochemicals. The results were developed by observing any recommended change in color in samples as compared to the control (colorless solution). The intensity of color was observed as light color, intermediate color and dark color and presented as +, ++, and +++ respectively, representing the different amounts of the specific phytochemical present in the extracts, while no change in color or no intensity in color presented as – means absent of particular chemical in extracts. The detailed results of the quantitative screening of phytochemicals are presented in Table 3. The observed screening results present an absence of phlobatanin and alkaloids in solvent extracts of *Fagonia cretica.*

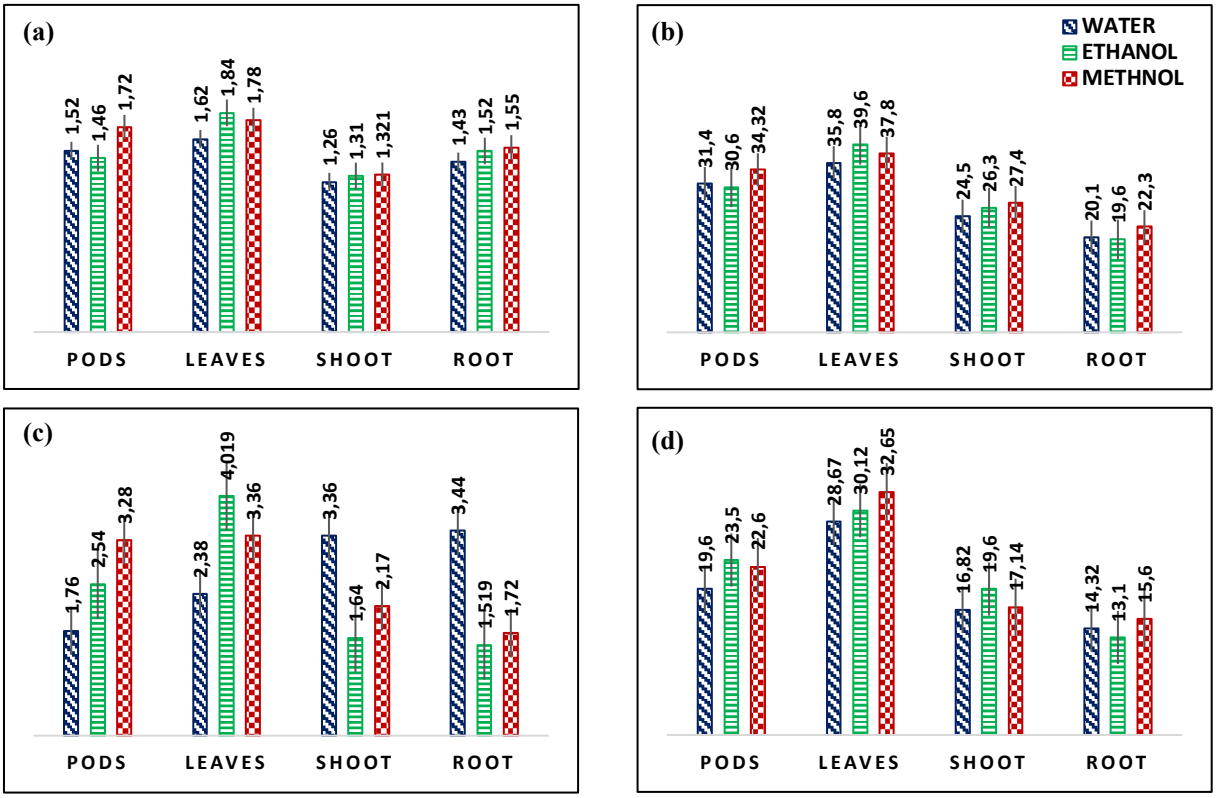

Figure 2. Various results of the study (a) total protein, (b) total sugar, (c) reducing power and (d) reducing sugar.

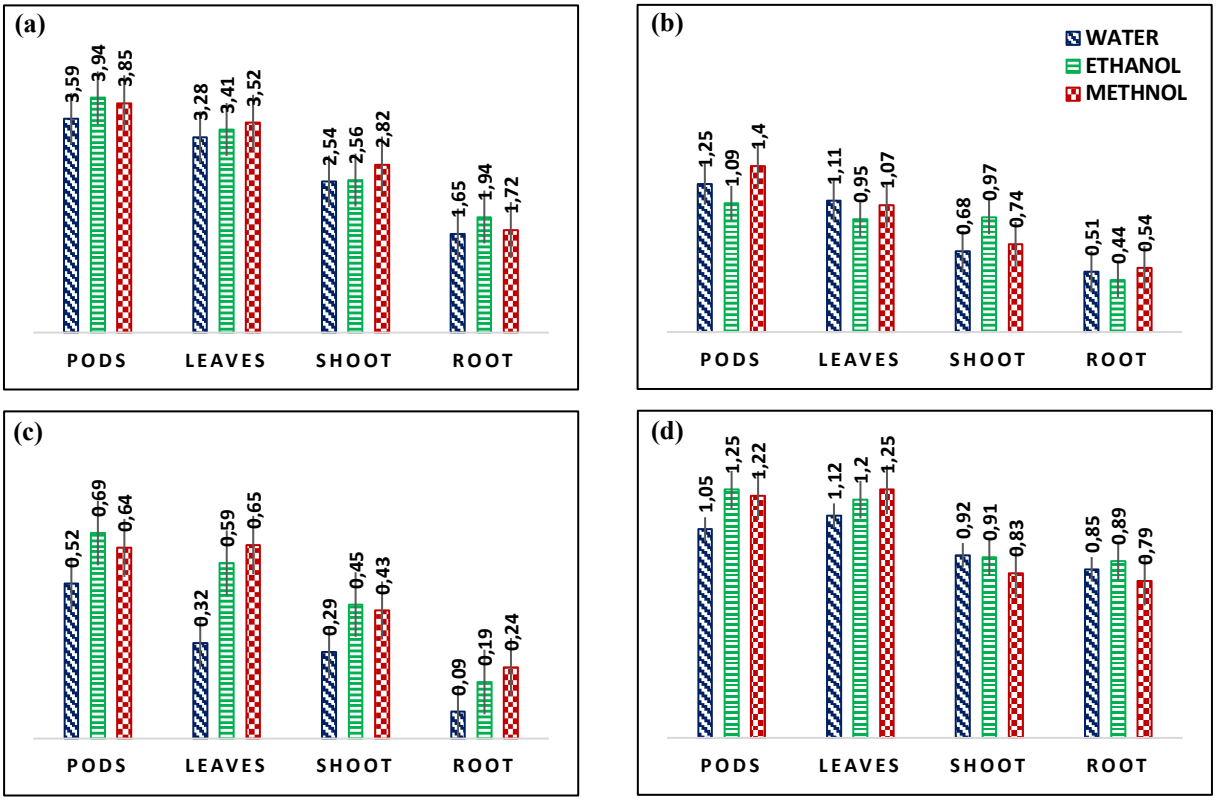

Figure 3. Various results of study (a) total phenolics, (b) total flavonoids, (c) total flavonol and (d) total tannins.

Table 3 Quantitative screening of some phytochemicals in *Fagonia cretica*

		Extracts		
Phytochemicals	Plant parts	Water	Ethanol (70%)	Methanol (70%)
1. Flavonoids Test	Pods	++	+++	+++
	Leaf	+++	+++	+++
	Stem	++	+	++
	Root	-	-	+
2. Saponins Test	Pods	++	++	+
	Leaf	+	+	+
	Stem	+	-	+
	Root	+	++	++
3. Terpenoids Test	Pods	+	++	++
	Leaf	+++	+++	++
	Stem	+	++	++
	Root	++	++	+
4. Coumarin Test	Pods	++	+	++
	Leaf	+++	+++	+++
	Stem	++	++	+++
	Root	+	+	+
5. Quinones Test	Pods	++	+++	++
	Leaf	+++	+++	+++
	Stem	+	++	+++
	Root	++	++	+++
6. Cardiac Glycosides Test	Pods	++	++	++
	Leaf	+++	+++	+++
	Stem	++	+	++
	Root	++	+++	+++
7. Steroids Test	Pods	+	+	++
	Leaf	++	++	++
	Stem	++	+++	+++
	Root	++	++	+
8. Alkaloids Test	Pods	–	–	–
	Leaf	–	–	–
	Stem	–	–	–
	Root	–	–	–
9. Tannin Test	Pods	+	++	+++
	Leaf	++	+++	+++
	Stem	+	+	++
	Root	+	+	+
10. Phlobatannin Test	Pods	–	–	–
	Leaf	–	–	–
	Stem	–	–	–
	Root	–	–	–

+, ++, +++ respectively, representing the different amounts of the specific phytochemicals present in the extracts.
– means the absence of particular chemical in extracts.

3.4 Quantification of Total Phenolics, Total Flavonoids, Total Flavonol and Total Tannins

The quantitative analysis of total phenolic acid contents, total flavonoid contents, total flavanols, total tannins, reducing power, and total antioxidant capacity from solvent extracts of *Fagonia cretica* were estimated. The obtained results show the presence of a variable amount of these biochemicals found in all the extracts like the highest amount of total phenolic contents of 3.94±0.2 mg/ml found in the ethanolic extract of the pod. The highest total flavonoid contents of 1.4 mg/ml were found in the methanolic extract of the pod. The present results show the ethanolic extract of the pod of *Fagonia cretica* possesses the most value of total flavonols about 0.69 mg/ml, and the ethanolic extract of pods and methanolic extract of the leaf contained the maximum value of total tannins about 1.25 mg/ml. Figure 2. elaborate results of quantitative evaluation of these biochemicals.

4.5. Allelopathic Effects on Germination of Radish and Spinach

The allelopathy of the *Fagonia cretica* plant was observed in the germination of radish and spinach from water extracts of leaves and found the effect of inhibition on the germination of both vegetable plants. A schematic diagram of the practice is presented in Figure 4. The sample D1 showed growth inhibition as 39% and 52% of radish and spinach respectively and all the seedlings treated with

D1 were dried within one week of germination. as compared to D4 (control) where 100% survival of seedlings was noted in both vegetables. The detailed result of allelopathy is mentioned in Table 4.

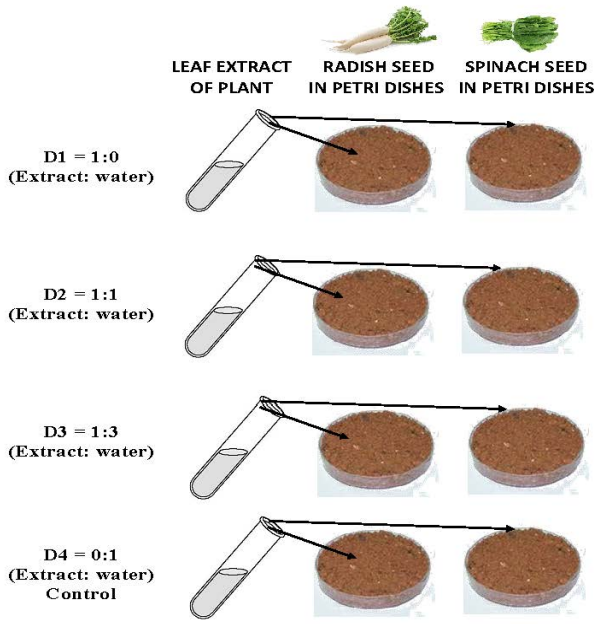

Figure 4. Schematic illustration of allelopathy: water extract of the leaf with various concentrations along with the water was added to the soil at the time of germination of Spinach and Radish, and effects were noted on the germination and growth of plants.

Table 4 Allelopathic effect of water extract of leaves of *Fagonia cretica* against germination of radish and spinach

		% Germination*		Survival**	
S. No.	Treatment	Radish	Spinach	Radish	Spinach
1	**D1 = 1:0 (Extract : water)**	39 ± 1.7	52 ± 2.6	0	0
2	**D2 = 1:1 (Extract : water)**	52 ± 2	58 ± 1	28 ± 1.7	36 ± 3.45
3	**D3 = 1:3 (Extract : water)**	74 ± 2	69 ± 3	63 ± 2.6	67 ± 2.6
4	**D4 = 0:1 (Extract : water) Control**	95 ± 3.45	97 ± 3.45	100	100

* Percentage of germination was obtained by the equation (1).
** Frequency of survived plants was obtained by the equation (2).

Conclusion

The present study confirms the significance and importance of *Fagonia cretica* L. It is commonly used as a traditional medicinal plant for the treatment of various diseases. This study was intended to evaluate the potential of *Fagonia cretica* L. belonging to district Jamshoro, Pakistan. The results of this study highlight that the various parts of the plant develop antimicrobial activity, against various bacteria and fungi. All the selected parts of the plant produce the potential power of antioxidant activity, and its body contains various phytochemicals and biochemicals. The qualitative study of biochemicals confirmed the highest number of total sugars, reducing sugar and total proteins. Quantitative analysis of the phytochemicals of this plant revealed the highest number of total flavonoids, and total phenolics. Thus, we conclude through all the present results that *Fagonia cretica* L. collected from district Jamshoro, Pakistan is a very valuable and significant plant for pharmaceutical sciences and in various biological fields.

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Research Article

Identification and Characterization of Fluorescent Pseudomonas Producing Active Compounds Controlling Plant Pathogens

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Abstract: Fluorescent pseudomonad is one of the biocontrol agents against plant pathogens. Various compounds reportedly can be produced by fluorescent pseudomonad, including chitinase, β-1,3-glucanase, HCN, siderophore, antibiotics, Indole Acetic Acid (IAA), phosphate solvent compounds, and 2,4-diacetylphloroglucinol (DAPG). In this study, it was identified and characterized six isolates of fluorescent pseudomonad (PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2). All isolates were isolated from the rhizosphere of various types of plants. The results showed that six isolates were identical to *Pseudomonas aeruginosa* (93-94%). All bacterial isolates tested were able to produce siderophore, HCN, and solubilize phosphates. The highest siderophore was produced by isolate PfPj2. Whereas isolate PfKd7 had the highest at HCN production and the ability to dissolve phosphates.

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1. Introduction

Bacteria are the most commonly found organisms and their existence is not evenly distributed. The number of bacteria in the rhizosphere is much higher than in the soil. This could be related to the plant's roots' secreting exudate as a food source for bacteria. Certain bacteria have the ability to control plant pathogens, and can also affect plant growth (Olanrewaju et al., 2017; Sakci et al., 2021; Kaba and Bektas, 2022; Yerli and Sahin, 2022). Bacteria that affect plant growth directly or indirectly are referred to as Plant Growth Promoting Rhizobacteria (PGPR) (Alizadeh, 2011). Several genera of bacteria such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Bradyrhizobium,* and *Rhizobium* show PGPR activity in different plants (Ahemad and Kibret, 2014).

Chemical compounds are commonly used to control various plant pathogens. It is difficult to do since pathogens are able to live in diverse environments and very broad host ranges. Another alternative that can be used as a control for plant pathogens is biocontrol agents. Nguyen and Ranamukhaarachchi (2010) reported *Bacillus megaterium*, *Enterobacter cloacae*, *Pichia guillermondii*, and *Candida ethanolica* which were isolated from the soil and suppressed effectively growth of the *Ralstonia solanacearum*.

Various factors can affect the ability of biocontrol agents to reduce the intensity of plant diseases. The biocontrol agents are capable of producing several compounds involved in antagonistic mechanisms. According to Viveros et al. (2010), the ability of biocontrol agents to suppress plant diseases is caused by their colonizing plant roots, and the ability to produce antibiotics, siderophore, HCN, hydrolytic enzymes (chitinase, protease, lipase, etc.) or through activities plant defense mechanism. Yanti et al. (2017) reported that the biocontrol agent *Bacillus subtilis* CIFT-MFB-4158A can produce a siderophore, IAA, and it is able to dissolve phosphate.

Other biocontrol agents that can be used to control plant diseases are a group of fluorescent pseudomonad bacteria. The beneficial effect of the use of fluorescent pseudomonad is that it can produce chitinase, β-1,3-glucanase, HCN, siderophore, antibiotics, phytohormones, phosphate solvent compounds, induction of systemic resistance to various pathogens (Podile and Kishore, 2006), producing 2,4 -diacetylphloroglucinol (DAPG), phenazines, and pyrrolnitrin (Haas and Défago, 2005), Indole Acetic Acid (IAA), and show the activity of boosting plant growth (Deshwal and Kumar, 2013).

The different characteristics possessed by biocontrol agents make them good candidates for suppressing plant pathogens. Fluorescent pseudomonads are non-pathogenic rhizobacterial groups, and some of them are *Pseudomonas fluorescens*, *P. putida*, *P. aeruginosa*, and *P. aureofaciens* which have a wide ability to suppress soil-borne pathogens with different mechanisms of action (Karthikeyan et al., 2006). The isolates PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2 of fluorescent pseudomonad produced the HCN in the medium added by ZnSO4.7H2O (Advinda et al., 2018). IAA can be produced by *P. fluorescens* and *B. subtilis* (Reetha et al., 2014). Furthermore, Anhar et al. (2011) reported that fluorescent pseudomonad can increase plant height, number of tillers, and wet weight of rice plants. Whereas Qessaoui et al. (2019) reported that *Pseudomonas* sp. strain Q6B can increase seed germination, while *Pseudomonas* spp. (Q6B, Q14B, Q7B, Q1B, and Q13B) stimulated the increase in tomato seedling height. The Fluorescent pseudomonad can control root rot disease in black pepper plants caused by *Phytophthora capsici* (Paul and Sarma, 2006). *In vitro*, the growth of *Xanthomonas axonopodis* pv. *malvacearum* can be inhibited by various strains of *P. fluorescens* and *B. subtilis* (Salaheddin et al., 2010).

In the previous study, we reported that the effectiveness of fluorescent pseudomonad isolates of PfPj1, PfPj2, PfPj3, PfPb1, PfPb2, PfPb3, and PfPm1 to control the Blood Disease Bacteria (BDB) in banana seeds (Advinda, 2009). However, the detailed information about these isolates is still inadequate. Then in the present study, we report the identification and characterization of isolates PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2 of fluorescent pseudomonad.

2. Material and Methods

2.1. Condition of fluorescent pseudomonad isolates

The isolates PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2 of fluorescent pseudomonad were isolated from the rhizosphere of various types of plants and deposited in the L. Advinda collections of the Biology Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang, West Sumatra, Indonesia. Isolates were stored at room temperature in Eppendorf tubes containing sterile aquadest. Rejuvenation of all isolates using a solid King's B medium, and multiplication of inoculums in a liquid King's B medium.

2.2. 16s rRNA gene PCR and Sequencing

Isolation of bacterial genomes was done by using the boiling method (Dashti et al., 2009). As much as 2-3 Loopful of bacterial colonies were put into a 1.5 mL Eppendorf tube containing 200 µL 1/10 TE buffer pH 8. The suspension was vortexed until it was homogeneous. The boiling process is carried out in a water bath at a temperature of 95-100ºC, for 15 minutes. The suspension is then centrifuged at 12,000 rpm for 10 minutes to separate the supernatant and pellet. The supernatant is transferred into a new Eppendorf tube and stored at - 20ºC until it is about to be used.

The amplification process is carried out by the PCR method using thermal cycle tools with universal primers 27F (forward) and 1492R (reverse). The PCR reaction was carried out in 25 µL reactions, consisting of 6.5 µL ddH2O, 12.5 µL Dream Taq PCR (Thermo Scientific) Kit, 2 µL for each forward and reverse primer, 2 µL DNA template. The temperature used was an initial denaturation of

95ºC for 3 minutes, followed by 35 cycles consisting of denaturation of 95ºC for 45 seconds, primary attachment (annealing) at 55ºC for 30 seconds, and elongation at 72ºC for 2 minutes. The final extension step of the reaction was carried out at 72ºC for 7 minutes (Frank et al, 2008). PCR product analysis was performed by electrophoresis using 1% agarose gel. The electrophoresis results were then checked with Gel Documentation System. The amplification results are sent to Macrogen Singapore Sequencing for sequencing using an automated DNA sequencer (ABI Prism 3100 Analyzer, Applied Biosystem, USA).

2.3. Phylogenetic analysis

The sequences of 16S rRNA gene of the tested bacterial isolates were compared with the gene sequences listed in the NCBI database using BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Singh et al., 2014) and sequence alignment DNA between strains was carried out using the ClustalW program. The phylogenetic tree was constructed using the Maximum Likelihood statistical method and the Tamura-Nei model, 1000 pseudoreplicate bootstrap integrated in MEGA X software (Kumar et al., 2018). Reference to the 16S rRNA gene sequence was obtained from data in GenBank (Table 1).

Table 1. The 16S rRNA gene reference sequence that was obtained from the NCBI website

Reference sequence of *Trichoderma*		
1	*Pseudomonas aeruginosa*	NR117678
Reference sequence *outgroup*		
1	*Pseudomonas fluorescens*	NR043420
2	*Pseudomonas putida*	NR043424
3	*Rhizobacter gummiphilus*	NR132677
4	*Rugamonas rubra*	NR104915

2.4. Siderophore production

The level of siderophore of isolates is determined quantitatively. Siderophore production is known by growing fluorescent pseudomonad on low-iron synthetic medium containing 20 g sucrose, 2 g L-asparagine, 1 g K_2HPO_4, and 0.5 g $MgSO_4$ dissolved into distilled water up to 1 liter. Then the medium was sterilized in an autoclave at 121 ^oC and a pressure of 1 atm for 15 minutes (Elad and Baker, 1985).

To find out the siderophore production of each isolate was carried out by taking 1 mL of fluorescent pseudomonad suspension, then inoculating it in 25 mL of low-iron synthetic medium and incubating it for 24 hours on a shaker. The resulting suspension is centrifuged at 11,000 rpm for 30 minutes, then the supernatant is filtered with filter paper. Detection of siderophore is done by adding 1 mL of FeCl 0.01 M to 3 mL of the supernatant. Siderophore detection is measured using a spectrophotometer at a wavelength (λ) of 405 nm (Cody and Gross, 1987).

2.5. HCN production

The procedure of Vanitha and Ramjegathesh (2014) was modified and used to determine HCN production of fluorescent pseudomonad. Fluorescent pseudomonad was grown on TSA + glycine medium (10 g TSA + 4.2 g glycine), which was dissolved with distilled water up to 1 L volume. Indicators of HCN production were picric acid solution consisting of 2 g picric acid and 8 g sodium carbonate dissolved into distilled water up to a volume of 200 mL.

To determine the production of cyanide acid, it was done by taking 0.1 mL of fluorescent pseudomonad suspension (population $3x10^8$ cfu mL^{-1}, 1 Mc Farland's scale), then inoculating the petri dish which contained glycine medium. On the lid of the petri dish is attached a piece of filter paper that has been dropped with 1 mL of picric acid solution. Bacterial cultures were incubated at room temperature for 2x24 hours. The color of filter paper which remained yellow showed that the tested isolates did not produce cyanide acid, whereas the light brown, dark brown, and brick red colors indicated increasing cyanide acid production.

2.6. Phosphate solubilization

The ability of fluorescent pseudomonad to dissolve phosphate can be determined by inoculating it on Pikovskayas agar medium. Sterile disc paper is placed in a petri dish and dropped with 0.1 mL of a pseudomonad fluorescent suspension (population $3x10^8$ cfu mL^{-1}, scale 1 Mc Farland's). Then the disc was placed in the middle of the medium so that Pikovskayas was in a petri dish, and incubated for 48 hours at room temperature. The ability to solubilize phosphate is indicated by the formation of a halo zone.

3. Results and Discussion

3.1. Molecular identification

A total of six isolates were isolated from the rhizosphere of various types of plants and were successfully identified by molecular methods. BLASTN results of 16s rRNA gene sequences with reference sequences in the NCBI Database can be seen in Table 2.

Table 2. The results of the identification of 16S rRNA gene of bacterial isolates using BLAST

Isolate	BLAST Results				Access no
	Description	Max Score	E Value	Ident (%)	
LAHf2 (901 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	992	0.0	93.97	SAMN29359229
PfCa5 (910 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	1007	0.0	94.05	SAMN29359230
PfCa53 (904 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	1013	0.0	94.19	SAMN29359231
PfPj1 (900 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	1033	0.0	94.17	SAMN29359232
PfPj2 (893 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	1000	0.0	94.13	SAMN29359233
PpKd7 (904 bp)	*Pseudomonas aeruginosa* strain DSM 50071, 1527 bp (NR117678)	1031	0.0	93.82	SAMN29359234

Based on the measurement of sequential kinship through the average nucleotide identity (ANI) value in the BLAST program, all isolates of the tested bacteria were suspected to be not identical to *Pseudomonas aeruginosa* because they were below the threshold of similarity between species for the bacterial and archae categories. According to Reller et al. (2007) if the percentage of homology is close to 100% or > 97%, it can be confirmed as the same species but conversely if homology is less than 97% this isolate might be a new species or unconfirmed species. Kim et al. (2014) through their research revealed that there were significant differences in the overall ANI distribution between intra- and interspecies relationships in ANI 95-96%. Then they determined which level of similarity in 16S rRNA gene sequences was within the ANI threshold that is currently accepted for species demarcation using more than one million comparisons. Two-fold cross-validation statistical tests reveal that the similarity in the 16S rRNA gene sequence of 98.65% can be used as a threshold to distinguish between two species. From the results of BLAST it can also be observed that there are differences in base length between isolates and reference sequences. According to Sukweenadhi et al. (2019) this could affect the accuracy of the prediction of test bacterial isolates.

3.2. Phylogenetic analysis

Figure 1 is a molecular phylogenetic tree arranged from the sequence of nucleotide genes encoding 16S rRNA regions from the tested bacterial isolates and *Pseudomonas aeruginosa* which have

the highest similarity values based on BLAST analysis (Figure 1). The chosen outgroups come from different species and genera, these outgroups help provide character polarization (apomorphy and plesiomorphy characters). Apomorphy characters are characters that change and are inherited in test bacterial isolates (ingroup), while plesiomorphy characters are primitive characters found in outgroups. Bootstrap values which are represented by numbers on tree branches show high tree reliability. Hillis and Bull (1993) in Lemoine et al. (2018) stated that phylogenetic trees with high bootstrap values above 70% are good phylogenetic trees.

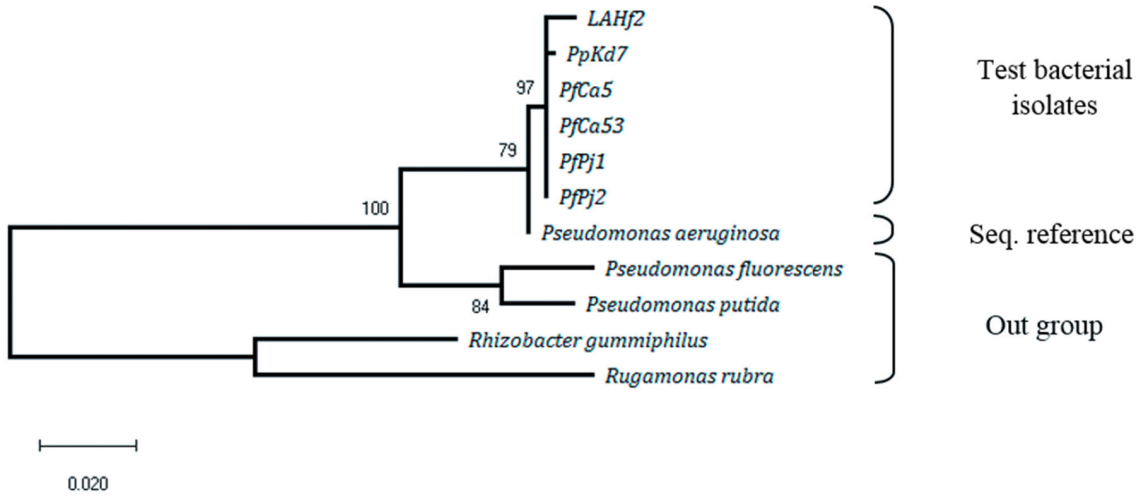

Figure 1. The phylogenetic tree (Neighbor-joining tree) of the 16S rRNA gene sequences of the *Pseudomonas* bacteria which was constructed using the Maximum Likelihood statistical method and bootstrap analysis (1,000 replicates) in MEGA X 10.0.5 software (Kumar et al., 2018).

Table 3. Percentage of homology of *P. aeruginosa* isolate nucleotide sequence against data at Genbank

Seq->	P.A	LAHf2	PfCa5	PfCa53	PfPj1	PfPj2	PpKd7
P.A	ID	0.93	0.934	0.935	0.935	0.933	0.934
LAHf2		ID	0.992	0.994	0.994	0.991	0.992
PfCa5			ID	0.998	0.998	0.998	0.997
PfCa53				ID	1	0.997	0.998
PfPj1					ID	0.997	0.998
PfPj2						ID	0.995
PpKd7							ID

Info: P.A = *Pseudomonas aeruginosa* DSM 50071.

From the results of phylogenetic tree construction, it is known that the tested bacterial isolates and *Pseudomonas aeruginosa* are from common ancestors and have a very close kinship, but then a separation of gene evolution lines occurs so that the tested bacterial isolates form new groups which are shown by short branches on the meeting point (node) between the test bacterial isolate and *P. aeruginosa*. Among the 6 bacterial isolates tested, LAHf2 and PpKd7 isolates had longer evolution than other isolates. It is assumed that the tested bacterial isolate is a derivative of *P. aeruginosa* that has undergone a change or evolution. Hillis and Bull (1993) stated that branch length shows the number of changed sequences that occurred before the level of separation. The greater the length of the branch, the more sequential changes occur.

Various developments regarding the observations of various PGPR traits have been widely reported. The production of diverse microbial metabolites such as antibiotics, siderophore, ammonia, HCN, pyrrolnitrin, phenazine, 2,4-diacetyl phloroglucinol, and lytic enzymes by *P.fluorescens* against several pathogenic bacteria has been reported (Maleki et al., 2010; Subramanian and Satyan, 2014). This

study reports the identification and characterization of the isolates PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2 of fluorescent pseudomonad. Based on the results of phylogenetic tree construction, these fluorescent pseudomonad isolates were closely related to *P. aeruginosa*, but with some genetic variations. Further specific studies and more analysis were needed to confirm these isolates.

From the observations of siderophore production, all isolates produced siderophores (Figure 2). The siderophore levels were determined using a spectrophotometer at a wavelength (λ) of 405 nm, and figures indicated Optical Density (OD) absorbance values related to results. The difference in OD produced showed that each isolate was able to produce a siderophore at different levels. Isolate PfPj2 produced the highest siderophore, 3.240 (OD_{405}), and the lowest was produced by isolate PfPj1, 2.185 (OD_{405}).

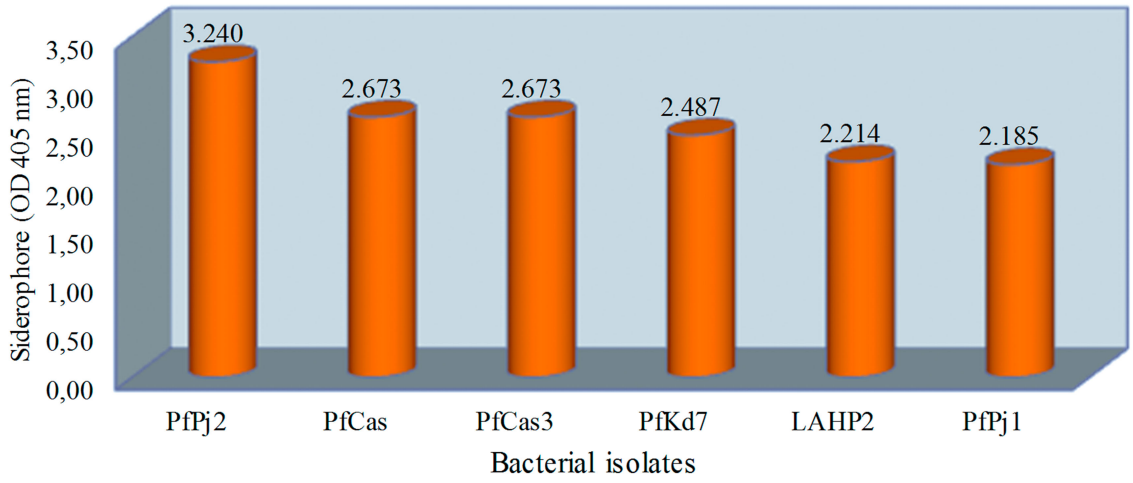

Figure 2. The ability of bacterial isolates to produce siderophores.

The ability of bacteria to produce siderophore is an important trait in Plant Growth Promoting Rhizobacteria (PGPR) because siderophore is able to bind iron ($Fe^{3+)}$ into siderophore-iron bonds that become available to plants. According to Ahmed and Holmström (2014), the siderophores produced by microorganisms can inhibit the growth of pathogens. This happens because $Fe^{3+}$is already bound to siderophore, so pathogens have Fe^{3+} deficiency.

The fluorescent pseudomonad isolates JUPF31 and JUPF37 of Anitha and Kumudini (2014) were able to produce a siderophore. Isolate JUPF37 were the highest in its ability to produce siderophores followed by isolate JUPF32 (Anitha and Kumudini, 2014). Advinda et al. (2019) stated that siderophore production can be influenced by the growth medium of fluorescent pseudomonad. Fluorescent pseudomonad PfCas3 isolates grown on glucose medium produce higher siderophore than fructose medium. According to Yeole and Dube (2001), the differences in the levels of siderophore produced by fluorescent pseudomonad can be caused by various things, such as the type of isolate, changes in time, space, nutrition, and the growth environment of these microorganisms.

In the present study, all bacterial isolates showed different abilities to produce HCN. The ability to produce HCN from isolates PfPj1, PfPj2, PfKd7, PfCas, PfCas3, and LAHp2 is characterized by the resulting color changes in filter paper pieces that have been dropped with 1 mL of cyanide acid detection solution. The color of the filter paper which remained yellow showed that the tested isolates did not produce cyanide acid, whereas the light brown, dark brown, and brick red colors indicated increasing cyanide acid production (Figure 3).

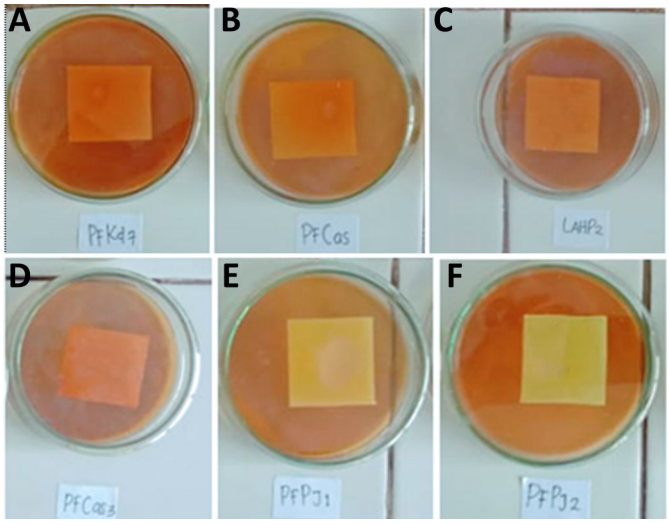

Figure 3. The ability of bacterial isolates to produce HCN: A, isolate PfKd7; B, isolate PfCas; C, isolate LAHp2; D, isolate PfCas3; E, isolate PfPj1; F, isolate PfPj2.

The PfKd7 isolate produces the highest HCN, characterized by filter paper which is brick red. While the one that did not produce HCN was isolated PfPj2 (Figure 3). This is supported by research results reported by Prasad et al. (2017) that there are differences in the ability to produce HCN by *P. fluorescens-10* and *B. subtilis-2* which are isolated from the tomato plant rhizosphere. Thus, these two bacteria are very useful as plant pathogen biocontrol agents.

HCN production by *P. fluorescens* CPF1 to CPF10 isolated from *Coleus rhizosphere* has been reported by Vanitha and Ramjegathesh (2014). Fluorescent pseudomonad growth medium can affect its ability to produce HCN. The growth medium of pseudomonad fluorescent isolates PfCas and PfCas3 added by $ZnSO_4.7H_2O$ produced the best HCN (Advinda et al., 2018). While Kumar et al., (2012) analyzed HCN produced by bacterial isolates FBJ6 and FBS4 can induce the resilience of *Pisum sativum* and *Zea mays* plants.

From observations of the ability to dissolve phosphates in Pikovskaya's medium, it was found that all bacterial isolates showed different abilities (Figure 4). Isolate PfPj1 has the highest ability to dissolve phosphates, indicated by the diameter of the halo zone formed is 1.38 cm (Figure 4). While the lowest at the ability of dissolving phosphates is isolate PfKd7. Similar observations have been made on *B. subtilis* and *B. cereus* derived from the groundnut rhizosphere (Maheswar and Sathiyavani, 2012). *P. fluorescens* isolates PSM1, PSM2, PSM3, PSM4, PSM5, and *B. megaterium* isolate MTCC 8755 isolated by Yadav et al. (2016) from wheat rhizosphere are able to dissolve phosphates in Pikovskaya's medium.

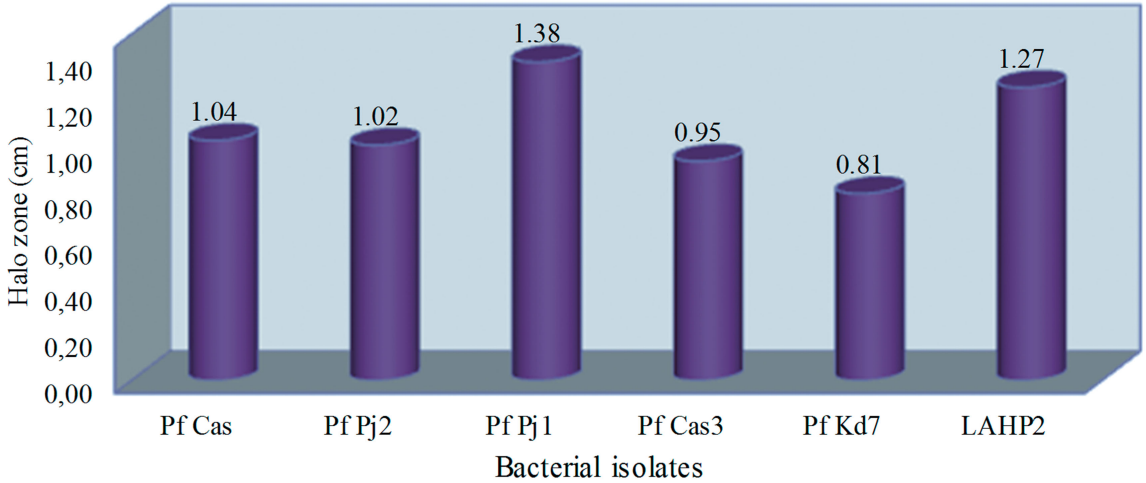

Figure 4. The ability of bacterial isolates to dissolve phosphate.

Phosphate solubilizing microorganisms are able to secrete organic acids such as gluconate acid, malic acid, succinic acid, lactic acid, formic acid, and citric acid which form a chelate with cations such as Al and Fe. These acids affect the dissolution of phosphates so that phosphorus becomes available and can be absorbed by plant roots (Vyas and Gulati, 2009). According to Widnyana and Javandira (2016), phosphate solubilizing microorganisms can also produce several enzymes including phosphatase. Phosphatase is an enzyme that is produced when the phosphate availability is low. In the process of mineralization of organic matter, organic phosphate compounds are converted into inorganic phosphates with the help of enzymes that are available to plants.

Conclusion

This research successfully identified *P. aeruginosa* which was isolated from the rhizosphere of various types of plants. All bacterial isolates tested were able to produce siderophore, HCN, and dissolve phosphates. The highest siderophore was produced by isolate PfPj2. Whereas isolate PfKd7 was the highest at producing HCN and at the ability to dissolve phosphates.

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Research Article

Assessment of Climate Variability and The Determinants of Rice Productivity in Southeastern Nigeria

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Rice productivity,
Southeastern Nigeria

Abstract: The study assessed the variabilities in climate and key factors of rice production in southeastern Nigeria. Trend analysis and spatial interpolation expressed the spatiotemporal variabilities in the climate and rice yield. Copies of the questionnaire were used to assess four hundred and eighty farm households from 12 local government areas. Other analyses included descriptive statistics, logistic regression, and Productivity Index. The farmers' socioeconomic characteristics show that the majority (62% of them are young males aged between 30-39 years. Over 80% of them were married while about 72% has household sizes between 5-9 persons. A greater proportion (54%) of them generate between N240,000.00 (578 USD) to N480,000.00 (1156 USD) annually. About 96 % have a farming experience above 10 years, while 50% have basic education (primary education). Logistic regression shows that sex (0.02), category of the farmer (0.00), age (0.03) (0.00), educational qualification (0.02) (0.00), membership of cooperative society (0.00), extension workers' visit (0.03) were the statistically significant determinants of rice productivity in the area. The area experiences significant rising temperatures and declining rainfall. This trend is more obvious in Ebonyi state. Study results acknowledge the necessity of an enabling environment for rice farming through adequate rural infrastructure, improved rice varieties, access to information, and improved government policies, programs, and interventions to accommodate non-ADP rural rice farmers in order to enhance rice production against the unwanted climate changes

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1. Introduction

Rice (*Oryza sativa)* is a major staple food across West Africa (Nosiru et al., 2014; Niang et al., 2017 and 2018). Its importance notwithstanding, local production often lags behind the demand (GATSBY, 2014; Niang et al., 2017 and 2018). Rice demand is increasing sharply in Nigeria due to

rising population, urbanization, relative ease of preparation, affordability, and ease of storage (Uga et al., 2013). Additionally, rice is widely consumed across all cultures, ethnic groups, or diverse geographical regions. It is highly priced and widely accepted as food during festivities (Basorun, 2013). Thus, sustainable production of rice is key to ensuring an adequate supply of rice to the teeming Nigerian population.

However, in recent years, rice production is declining. Accordingly, the price increased beyond what the average Nigerian can afford (Ajala and Gana, 2015). Due to the shortfall in supply, the country has depended heavily on rice importation (Okeowo, 2016; Obisesan, 2019). The import gulps up to $800 million of scarce foreign exchange (Ojogho and Alufohai, 2013). To boost local rice production and shore up the foreign reserve, a 75% hike in tariff was slammed on rice importation by the Nigerian Government. The policy reinvigorated interest in domestic rice production and research with the attendant rise in demand for local rice (Onyekwena, 2016; Ugalahi et al., 2016). However, rice supply still lags behind its demand (Talpur et al., 2011; GATSBY, 2014; FAOSTAT, 2015; Okeowo, 2016; Obisesan, 2019).

Additionally, to boost rice production and other food crops, the current administration launched the Agricultural Promotion Policy (APP) with an emphasis on boosting food production to ensure a food-secure Nigeria (FMARD, 2016; Ojong and Anam, 2018). This initiative also called the Green Alternative (Hendriks, 2018), is to leverage the successes of the Agricultural Transformation Agenda (ATA) that preceded the National Economic Empowerment and Development Strategy (NEEDS) (Smith, 2018). It aims to reposition agriculture as a business, food as a human right, and agriculture as critical to long-term economic growth and security (FMARD, 2016; Ojong and Anam, 2018). This is in line with the ECOWAS Agricultural Policy and the Comprehensive Africa Agricultural Development Program (ECOWAP/CAADP) that aims to increase yield including making the agricultural sector more attractive and competitive (Nwozor and Olanrewaju, 2020). The initiatives aim to create jobs and stem the tide of alarming food insecurity in Africa according to FAO (FAO et al., 2019). It will fast-track the attainment of SDGs target of eradicating hunger by 2030. Thus, to complement the government's action, research is key to finding solutions (Bennett, 2014; Mogga et al., 2019).

Thus, the above issues amongst others peculiar to rice farmers reinforce the need to assess the determinants of rice production from a geosocial perspective. It has been reported that labour hours, urea fertilizers, and irrigation significantly bolster rice yield in Pakistan (Ali et al., 2022) Some authors have cited factors that militate against rice productivity to include poor infrastructure, insufficient or unavailability of irrigation facilities, unavailability of high yielding varieties, unreliable rainfall, importation as well as difficulty in sourcing machinery and funding (Ita et al., 2013; Kadiri et al., 2014; Koirala et al., 2014; Onyekwena, 2016; Tanko et al., 2016; Adenuga et al., 2016; Ugalahi et al., 2016; Niang et al., 2017; Mogga et al., 2019; Hayat et al., 2020).

Further, curtailing farmer-herdsmen conflict and technological capacity building for rice farmers will boost productivity (Obi-Egbedi et al., 2012). There has been an appreciable increase in rice production from 2010 to 2019 but it is disproportionate to local demand (Okeowo, 2016; Onyekwena, 2016; Olasehinde et al., 2022). This is because most of rice farmers operate below their optimal potential (Obianefo et al., 2020 and 2021). The demand for rice has been rising at a fast rate of 10.3% annually (Maji et al., 2015) which is more than its supply. This increase in rice production has been at the expense of extensive land use rather than intensification of production. Yet agricultural intensification is advocated in recent years to curtail the impact of climate change (Foley et al., 2011; Talpur et al., 2011). Also, Nigeria has failed to attain self-sufficiency in rice production despite increased land size put into use (Kim et al., 2017).

Therefore, it has been suggested that an upgrade to/of irrigation will significantly boost rice production (Ugalahi et al., 2016). Diagne et al. (2013) highlighted the significance of improving extension services, weed, and pest control in addition to intensification. While water availability/irrigation, labour, capital, rice prices, fertilizer, and acreage are positively correlated with rice yield (Shaikh et al., 2016; Kamal Jan and Khan, 2019), the temperature was reported to have a negative correlation (Mech, 2017). Labour, irrigation, and hybrid seeds/quantity of seeds have a constructive impact on the technical efficiency of rice farmers (Balogun et al. 2021) but experience and tenure status have a negative impact on technical efficiency in a study carried out in the Indo-Gangetic plains(Chandel et al., 2022). Interannual variations in rainfall and spatial variations in soil moisture content affect rice yield (Niang et al., 2018). Rice farmers' technical efficiency is affected by irrigation,

production techniques, and the amount of agricultural supporting staff in Cambodia (Kea et al., 2016). However, Ara et al. (2016) find that irrigation has a stronger influence on rice yield than climatic conditions such that rainfall and temperature have negative impacts on yield. Nosiru et al. (2014) found farmers' age to have a negative influence on total productivity. Furthermore, climate change influences rice yield (Sarker et al., 2013; Akanbi et al., 2022; Heriansyah et al. 2022). The extent of per capita land area of rice harvested rather than yield per hectare is the key determinant of rice production in Southeast Asia (Dawe, 2013).

Furthermore, in Nigeria, Kadiri et al (2014) examined the determinants of sustainable rice growth and yield in the Niger Delta region of the country. Omofonmwan and Kadiri (2007) examined the problems and prospects of rice production in the Central District of Edo State, Nigeria, focusing mainly on farming systems and practices. Onyeneke (2017) assessed the determining factors for adopting improved technologies by rice farmers in Imo State which are contact with extension workers, age, income, cooperative membership, size of household, and education level. Cooperative marketing via membership of a cooperative society is boost to farmers' income. A similar study was done in southwestern Nigeria where farm size cultivated, frequency of extension contacts, and yield ratings of improved varieties are the key factors for adoption (Saka and Lawal, 2009). Climate change affects rice farmers and thus, it is advocated that capacity building at the farm level which is key to improving crop, soil, and water management be provided with an expansion of irrigation and effective extension service delivery (Akanbi et al., 2022). Pest infestation and difficulties of finance are the major constraints for rice farmers in Ekiti State, Nigeria (Osanyinlusi and Adenegan, 2016). However, only very little research exists that provides information on the determinants of rice production and the variabilities of climate in the southeastern region of Nigeria.

Yet, the region has notable floodplains for rice production. It has one of the fastest rates of urbanization and population growth in Nigeria which requires increased food availability. Hence, the research assessed the determinants of rice production on a regional scale via the following objectives: (i) to investigate the climate variabilities of the region (ii) to assess the socio-economic characteristics of rice farmers, and (iii) spatio-temporal appraisal of the key factors of rice production across the region. This permitted the advance of recommendations to boost production that will help in achieving food security in Nigeria and attainment of the SDG's goal of total eradication of hunger by 2030.

2. Materials and Methods

2.1. Study location

Southeastern Nigeria comprises five States; Abia, Anambra, Ebonyi, Enugu, and Imo. Nigeria has six geo-political zones which southeast is one of them. The geographical location of the region is shown in Figure 1. It shares a border to the north with Benue and Kogi States, to the south with Rivers State, to the east with Akwa-Ibom and Cross River States, and to the west, it is bounded by Delta State (Figure 1).

The relief of the study area can be broadly classified into lowlands regions and escarpments (Ofomata, 1975 and 2002). The lowlands comprise areas with heights less than 350 meters above mean sea level comprising the River Niger-Anambra plains and the undulating lowlands of the Bende-Ameke-Umuahia axis. The escarpments rise to heights above 350 meters above mean sea level and comprise the Nsukka-Okigwe and Awka-Orlu uplands (Ofomata, 2002).

The climate of the area is the tropical rainforest of the Koppen's classification (Koppen, 1936) with a long wet season and a short dry season. The rainy season lasts for about 8 to 9 months with a mean rainfall of 1800 to 2300mm per annum. The study area has a more humid climate in the south than in the northern part, which is almost a transition zone between the Rainforest and the Savanna climates. The northern part of the area has a derived savannah but the southern lowland area has rainforest in the south. The northern part has the derived Savanna due to human influence that has drastically altered its rainforest vegetation and now composed of a secondary forest/regrowth that resembles the Guinea Savanna region. The study area had a population of 16.4 million persons in 2006 (FGN, 2006), which was projected to be 30.61 million persons in 2022.

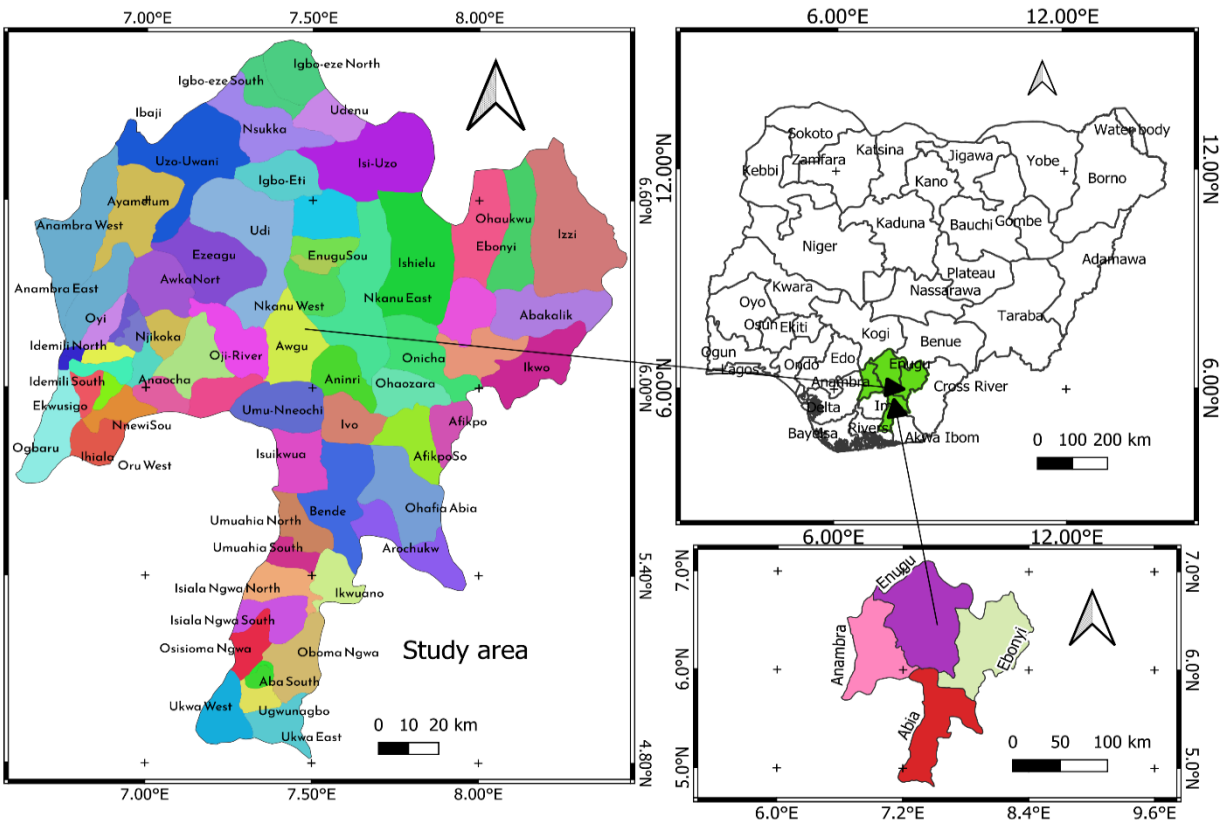

Figure 1. The study area.

2.2. The study and sample sizes

The rice farmers in the study area constitute the sampling frame. The study employed a multi-stage sampling to select the States, Local councils, and communities. The farmers' households were selected in the following ways: Four States comprising Abia, Anambra, Enugu, and Ebonyi. Purposive sampling was used to select those dominant rice-producing States in the region (Figure 4). Three Local council areas were purposively selected from each of the States because the pilot field study reveal they are the major rice growing areas in the zone. Twenty farmers' households from two communities were sampled from the selected 12 Local Government Areas, who are involved in rice production (i.e. 40 farmers' households per local council) totaling 480 households. The same number of respondents were selected from each of the sampled locations to achieve equity and fair representation from each local council.

2.3. Data collection

Two data sources were utilized in the study. The secondary data comprise historical rainfall and temperature data from 1991 to 2021 accessed from the National Aeronautics and Space Administration (NASA) (NASA, 2020) at https://power.larc.nasa.gov/data-access-viewer/. The data are freely acquired from the website that comprises high-resolution daily climatologic data on 0.5^{O} by 0.625^{O} horizontal resolution. The attributes of the data points were displayed in Table 1 and were used to assess climate variability in the region as a key determinant of rice production.

2.3.1. Household data collection

The data for the reliability test were collected by the authors and the internal consistency of the instrument was determined using Cronbach alpha (α) reliability co-efficient. The Cronbach alpha reliability coefficient was chosen because the questionnaire items were major of the multiple response types which gives a more suitable measure of homogeneity (Ezeh, 2005). The result showed that the instrument had an internal consistency of 0.79 which was considered high and reliable based on Emeka's (2009) decision, and could be useful in collecting data for the study.

The household data were principally from primary sources using copies of the questionnaire. The Agricultural Development Programme (ADP) is a program to assist farmers by the authority. Those who do not register with them are non-ADP farmers. They were specifically included in the research as a disadvantaged group and are also larger in number than the ADP farmers. It is an attempt to let their voices heard so that the concerned authority may make policy interventions and programs to accommodate them. Unlike the non-ADP farmers, the ADP farmers are registered rural farmers whose government has their database. They attend government programs for farmers and access all government interventions in agriculture. It also enabled us to find a distinction between the two groups in their output. ADP farmers are supported by the government in terms of soft loans and access to some farm inputs.

The data generated from the questionnaire were used in the analysis to determine the key factors of rice production in southeastern Nigeria. The studied variables were presented in Table 1.

Table 1. Variables studied

Determinants of rice production.	• Household size • Annual income • Sex • Category of farmer • Age • Educational attainment • Membership of cooperative society • Extension workers' visit

The data were standardized with the use of standard error estimate of the regression such as the standard deviation. The characteristics of the sample locations were presented in Table 2.

Table 2. The characteristic of the sample locations

Location	State	Latitude	Longitude	Height (m) asl
Abakaliki	Ebonyi	6.335	8.133	88.4
Awka	Anambra	6.220	7.061	103.94
Enugu	Enugu	6.444	7.490	151.33
Umuahia	Abia	5.542	7.506	92.75

2.4. Data analysis

2.4.1. Trend analysis

The study applied the Mann-Kendall test statistic to investigate the nature of the trend that exists in the data. It is a non-parametric test that is commonly applied for detecting monotonic trends in environmental and hydroclimatic data series (Hamed, 2011, 2009; Hamed and Rao, 1998; Önöz and Bayazit, 2012; Pohlert, 2020; Endale et al., 2021). The test requires the position of a null hypothesis which often is stated thus: "That the data are from a population that have independent realizations and are identically distributed which imply no trend exists within them". However, the alternate hypothesis posits the existence of a monotonic trend in the data. The test statistic is expressed as shown in equation 1 (Mann, 1945; Kendall, 1975; Pohlert, 2020)

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} \operatorname{sgn}\left(X_j - X_k\right) \quad (1)$$

$$\text{where } \operatorname{sgn} = \begin{cases} 1 & if \quad x > 0 \\ 0 & if \quad x = 0 \\ -1 & if \quad x < 0 \end{cases} \tag{2}$$

Hence, the mean of S is $E\left[S\right] = 0$ with the variance σ^2 as shown in eq. 3

$$\sigma^2 = \left\{ n\left(n-1\right)\left(2n+5\right) - \sum_{j=1}^{p} t_j \left(t_j - 1\right)\left(2t_j + 5\right) \right\} \Big/ 18 \tag{3}$$

where p represents the number of tied groups in the series, t_j stands for the number of data points in the j^{th} tied group (Pohlert, 2020). For a long data distribution, the S statistic is nearly normally distributed following the Z-transformation (eq. 4).

$$Z = \begin{cases} \dfrac{s-1}{\sigma} & if \quad S > 0 \\ 0 & if \quad S = 0 \\ \dfrac{s+1}{\sigma} & if \quad S < 0 \end{cases} \tag{4}$$

The S statistic has a close relationship with the Kendall's tau given as

$$\tau = \frac{S}{D} \tag{5}$$

where

$$D = \left[\frac{1}{2} n\left(n-1\right) - \frac{1}{2} \sum_{j=1}^{p} t_j \left(t_j - 1\right) \right]^{1/2} \left[\frac{1}{2} n\left(n-1\right) \right]^{1/2} \tag{6}$$

To obtain the trend following the above method, the study applied the trend package (Pohlert 2020) and due to the autocorrelation that exists in the data sets, the modified Mann-Kendall test was invoked utilizing the modified Mann-Kendall (modified mk) package (Hamed, 2011; Storch, 1999; Yue and Wang, 2004; Önöz and Bayazit, 2012; Patakamuri et al., 2021). Therefore, the pre-whitened Mann Kendall (pw mk) and (bbs mk) functions were used (Hirsch et al., 1982; Khaliq et al., 2009; Önöz and Bayazit, 2012; Patakamuri et al., 2021).

2.4.2. Descriptive statistics

The distribution of the rainfall and temperature in those selected stations was described with charts and graphs. The simple statistics also helped to reveal the spatiotemporal variations in their distribution. However, interpolation was employed to show the spatial variations in rainfall and temperature over the area in Quantum Geographic Information System (QGIS).

Furthermore, descriptive statistics were utilized to assess the socio-economic attributes of rice farmers in all the States in the region, and the obtained results were shown in bar charts. The productivity Index was used to determine rice yield in the previous growing seasons. They were analyzed using the STATA software version 12. All analyses were done at 0.05 level of significance.

2.4.3. Productivity index

The productivity index was employed to estimate rice productivity in the 2019 farming season. It was limited to 2019 due paucity of data on rice productivity and the inability of the farmers to recall distant past records. The productivity of the farmers was assessed using a productivity index (P1) (eq. 7). The index is vital for comparing and assessing outputs among farmers across different locations. This is because it considers not the total output but rather takes into consideration the output per hectare.

$$P_i = {}^{Y}/_{A} \quad (7)$$

where P1 is the productivity index, Y of the yield or output of the ith farmer in kilograms and A is the Area of farmland cultivated in hectares.

2.4.4. Logistic regression analysis

The binary logistic Regression Analysis model was used to find the key determinants of rice production in the study area. We recorded the productivity variable into high productivity (1) and low productivity (0) to become the dependent variable. Following Ekesiobi et al. (2018), we specify the binary logistic regression model to reflect the dichotomous of a rice farmer having high productivity or not as follows (eq 8):

$$\text{Logit}p_x = \log[\frac{P(Y=1)}{1-P(Y=1)}] = \sum_{k=1}^{k} \alpha_k X_k \quad \text{……...…… ……..…….. (8)}$$

Equation eight shows that there is a linear relationship between the logitp_x and the vectors of explanatory variables X. Therefore, the study can state the probability of a rice farmer having high output as thus;

$$\Pr(Y=1) = \frac{\sum_{ek=1}^{k} \alpha_k X_k}{\sum_{ek=1}^{k} \alpha_k X_k} \quad \text{……..…..……...……..... (9)}$$

Whereas the probability of not having high output (which is 1 minus the probability of having high output) is specified thus:

$$\Pr(Y = 0) = \frac{1}{\sum_{ek=1}^{k} \alpha_k X_k} \quad \text{……...……..…..…… …. (10)}$$

Equations 8 to 10 show the binary nature of the dependent variable which is the rice farmer recording high output categorized as 1 and not recording high output categorized as 0. The final model for the determinants of a rice farmer recording high yield or not is specified below as equation (11);

$$\text{Logit}(P) = \ln[\frac{P}{1-p}] = \propto_0 + x\beta_1 + x\beta_2 + x\beta_3 + \dots\dots\dots\dots + x\beta_n + \varepsilon \quad \text{…… ....…. (11)}$$

The choice of the method was based on the nature of the dependent and independent variables. Firstly, the dependent variable was binary in nature so can be best analyzed using a binary probability model. Secondly, the independent variables were majorly nominal which doesn't produce coherent and reliable results if regressed using OLS regression. The descriptive statistics of the variables in the model were represented in Table 3.

Table 3. Descriptive Statistics for the Variables in the model

Response Options	Frequencies	Percentages
Rice productivity by farmers		
Low	242	50.42
High	238	49.58
Household size of farmer		
0 to 4 household members	60	12.50
5 to 9 household members	320	66.67
Above 9 household members	100	20.83
Monthly income of farmer		
Below N20,000	48	10.00
N20,000 to N40,000	257	53.54
Above N40,000	175	36.46
Sex of farmer		
Male	297	61.88
Female	183	38.12
Category of farmer		
ADP farmer	98	20.42
Non ADP farmer	382	79.58
Age of farmer		
20 to 29 years	86	17.92
30 to 39 years	271	56.46
Above 39 years	123	25.62
Highest completed education of the farmer		
FSLC	241	50.21
SSCE	167	34.79
First Degree and above	28	5.83
Others	44	9.17
Membership of cooperative society		
No	182	37.92
Yes	298	62.08
Number of extension visits		
Once	69	14.38
Twice	182	37.92
More than twice	46	9.58
None	183	38.12

From the descriptive statistics in Table 3, rice farmers had approximately 50% low rice productivity and 50% high rice productivity. A majority (67%) of the respondents have a household size of 5-9 persons, and 54% make #20,000 to #40,000 Naira monthly income. A greater proportion (62%) of the farmers were males, 80% were non-ADP farmers, 56% were aged 30-39 years, 50% had FSLC and 62% were members of cooperative society, 38% had no extension visits and another 38% had extension visits twice.

3. Results and Discussion

3.1. Rainfall and temperature distribution

The rainfall and temperature distribution vary slightly across the study area. There is higher rainfall in the southern part of the study area comprising Abia, the southern part of Anambra, and some parts of Ebonyi State (Figure 2). The temperature distribution is nearly similar to that of the rainfall with higher temperatures in the southern and eastern parts of the study area. This concurs with the fact that rainfall decreases as one moves hinterland away from the southern coast of the country holds here (Odekunle and Adejuwon, 2007; Ezeh et al., 2016). Similarly, the lower temperatures in the north-central part of the study area could be attributed to the moderating effect of the higher plateau over there exemplified by the Udi-Okigwe-Awka-Orlu escarpment.

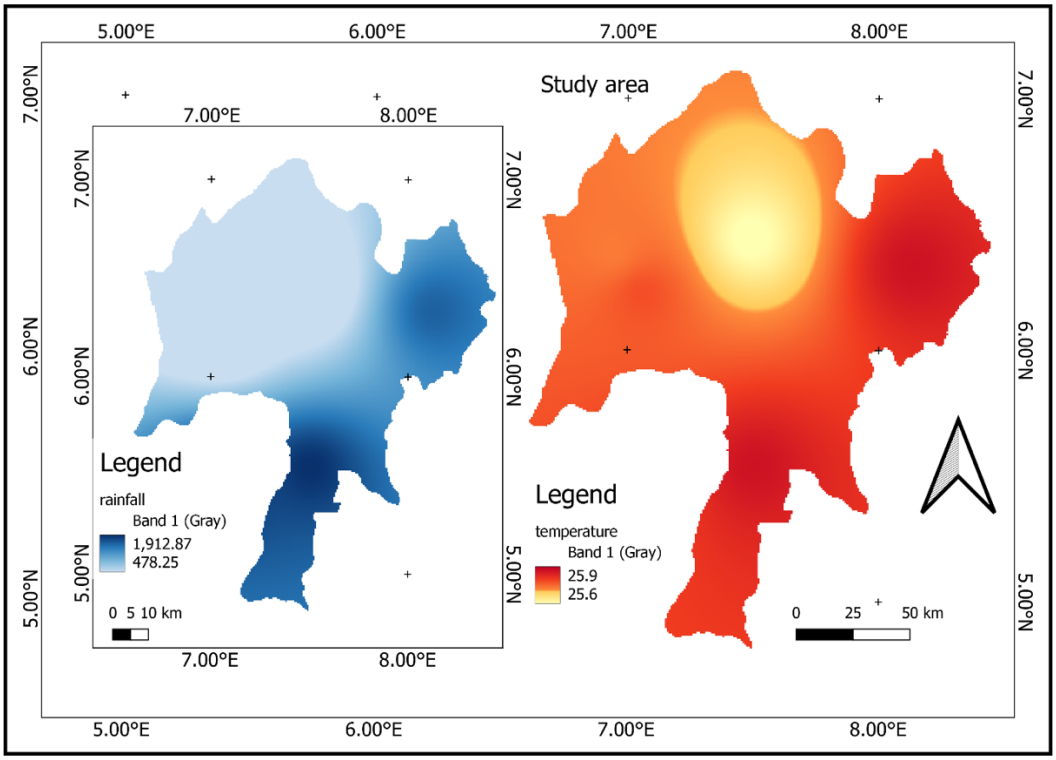

Figure 2. Spatial variations in rainfall and temperature over the region.

The mean monthly rainfall and temperature over the area show that rainfall maxima occur around July-September (Figures 3 and 4). The effect of the little dry season (Anyadike, 2002; Adejuwon and Odekunle, 2006; Ayadiuno et al., 2021) is very much more pronounced in Umuahia, Awka, and Enugu than it is in Abakaliki (Figure 3). The temperature distribution nearly follows the same pattern, increasing in November but dropping in December-January and again rising in February, peaking in March/April, and dropping gradually as the rainy seasons prevail from May to October (Figure 4). Thus, the lowest temperatures are obtained from July to September, the peak of the rainy season when the cloud cover is thicker than ever and thereby hinders the penetration of the insolation. Also, the temperature is low in December/January due to the effect of the harmattan wind that comes with much dust and haze that inhibit insolation.

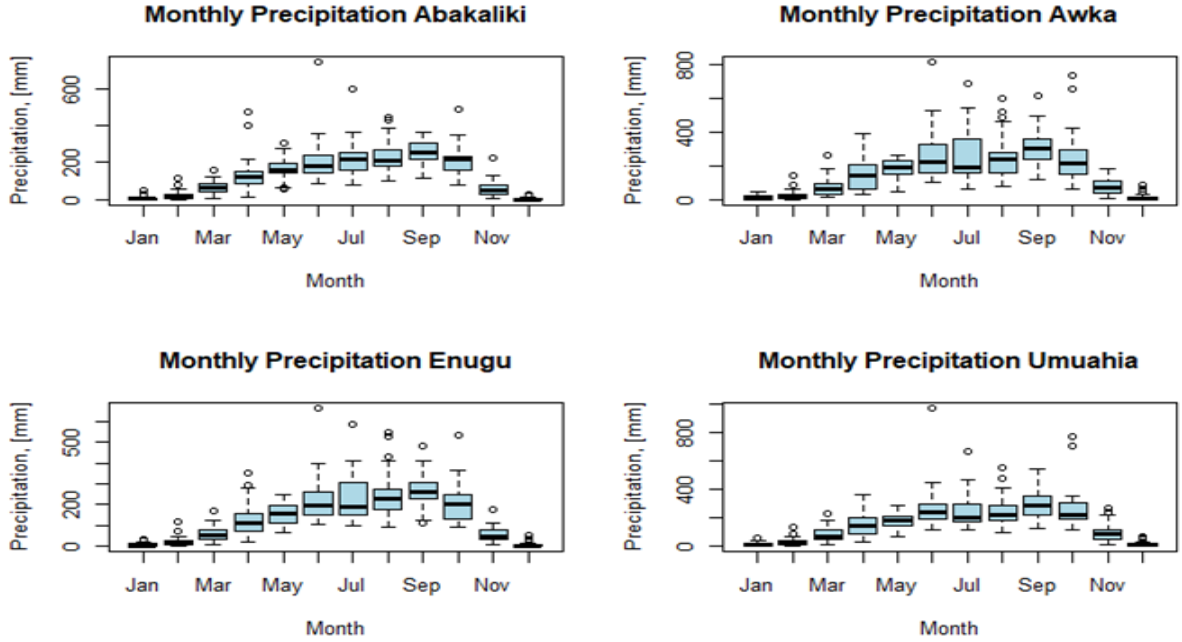

Figure 3. The rainfall distribution in the study area.

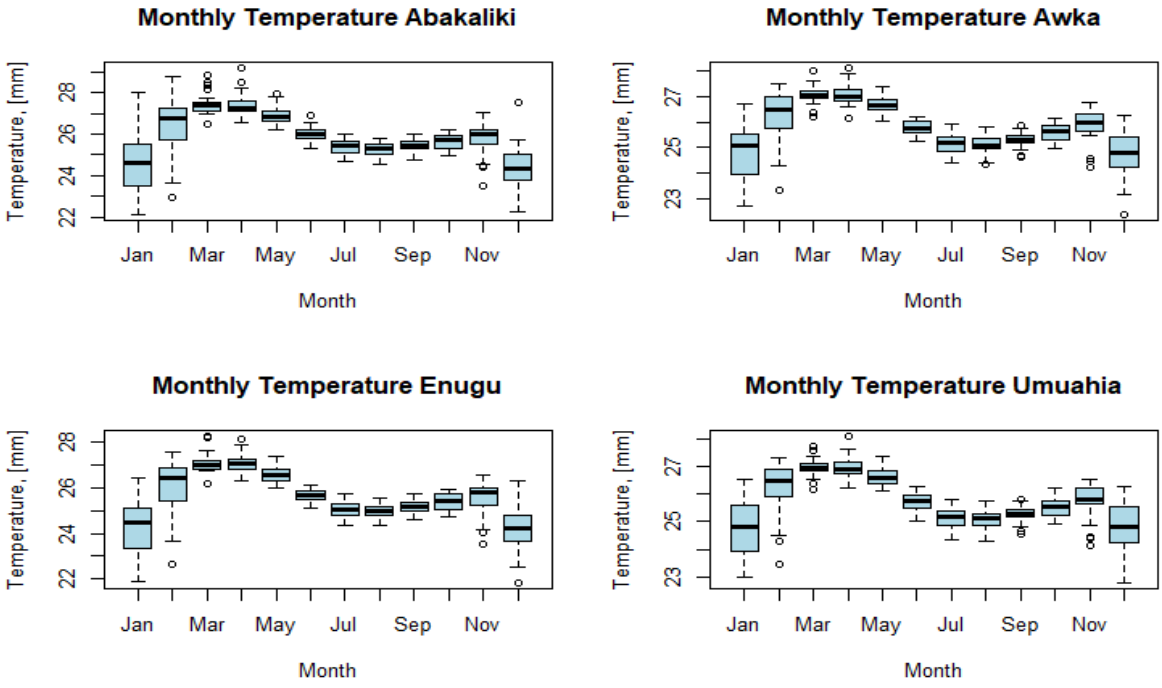

Figure 4. The temperature distribution in the area.

The coefficients of variation of the monthly rainfall and temperature are shown in Table 4.

Table 4. The Monthly CV for Rainfall

Rain	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
1	**1.58**	1.21	0.54	0.67	0.37	0.56	0.46	0.37	0.29	0.38	0.75	1.51
2	**1.12**	1.12	0.76	0.62	0.33	0.55	0.58	0.53	0.37	0.59	0.59	1.55
3	**1.29**	1.16	0.59	0.58	0.31	0.51	0.48	0.46	0.32	0.44	0.62	1.70
4	**1.06**	0.96	0.61	0.55	0.26	0.58	0.48	0.44	0.32	0.54	0.66	1.31

*1 is Abakaliki, 2 is Awka, 3 is Enugu and 4 is Umuahia.

Table 5. The Monthly CV for temperature

Temp.	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
1	**0.056**	0.050	0.019	0.02	0.015	0.013	0.014	0.013	0.012	0.014	0.032	0.045
2	**0.044**	0.037	0.012	0.015	0.012	0.011	0.015	0.014	0.011	0.014	0.025	0.038
3	**0.051**	0.044	0.016	0.016	0.013	0.011	0.014	0.013	0.011	0.015	0.030	0.040
4	**0.051**	0.044	0.016	0.016	0.013	0.011	0.014	0.013	0.011	0.015	0.030	0.040

Tables 4 and 5 shows that December to February has the highest coefficient of variations for the rainfall and temperature distribution while September has the least coefficient of variations in the rainfall and temperature. That shows that these climate variables are more variable during the dry season months than during the rainy season. Related findings were obtained by Ayanlade et al. (2018) except that their result has August with high variability which might be due to the severe little dry season in their area of study. The rainfall distribution in terms of the number of days with rainfall above 20 mm and above 90 mm was investigated (Table 6). It shows that Umuahia has a higher number of days with rainfall above 20 mm and 90 mm followed by Awka (Table 6). This shows that there is a sufficient supply of rainwater for rice production in the region, however such intensity of its occurrence can be detrimental for crops at the incipient stage.

Table 6. Number of days with rainfall above certain thresholds

20 mm	days	Rainfall amount	Mean amount
1	330	9940.8	30.1
2	547	17908.4	32.7
3	346	10931.3	31.6
4	479	15685.1	32.7
90 mm			
1	0	0	0
2	8	1060	132.5
3	3	345.5	115.2
4	8	966	120.8

3.2. Trend analysis

The initial diagnostics were done with the autocorrelation function (acf) in r. It was done with a lag of one and it revealed that there is significant autocorrelation in the monthly data (Figure 5). The rainfall data had significant seasonality and to visualize it, the number of lags was increased to fifteen (Figure 6). The results of the trend analysis indicate that temperature is increasing at all stations at a significant rate (Table 7). The monthly rainfall trend has a decreasing trend though none of the stations exhibits a significant trend. The rate of increase in temperature is higher at Abakaliki than at any of the other locations.

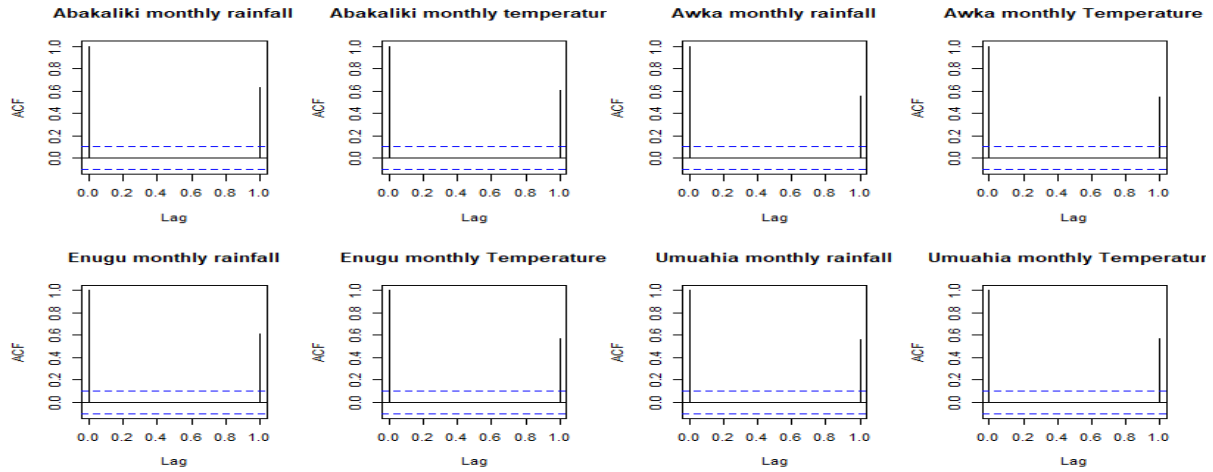

Figure 5. The autocorrelation results for the monthly data.

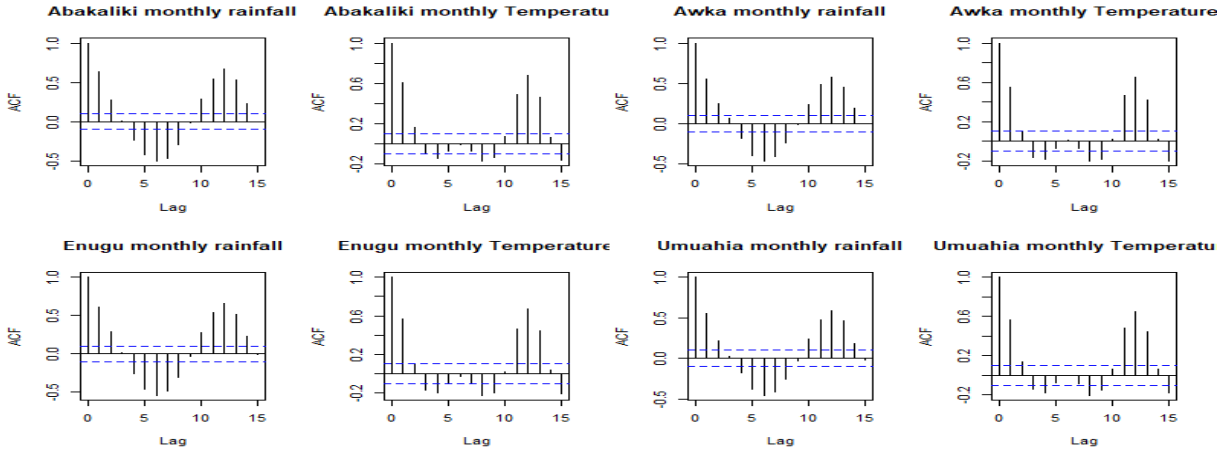

Figure 6. The seasonality plot for the annual rainfall and temperature data

Table 7. The trends of monthly rainfall and temperature

Location	Z	tau	Ss	Pw_Ss	p-va	L-T	U-T	Z-L	Z-U
			Rainfall						
Abakaliki	-1.393	-0.05	-0.04		0.16	-0.093	0.095	-2.763	2.866
Awka	-0.118	-0.004	-0.003		0.91	-0.102	0.100	-2.870	3.037
Enugu	-0.927	-0.032	-0.03		0.35	-0.098	0.103	-2.858	2.918
Umuahia	-0.267	-0.009	-0.01		0.79	-0.098	0.102	-2.787	2.93
			Temperature						
Abakaliki	2.97	0.103	0.0011	0.003	0.003				
Awka	2.24	0.078	0.0008	0.002	0.025				
Enugu	2.34	0.082	0.0009	0.002	0.019				
Umuahia	2.59	0.09	0.0009	0.002	0.009				

*L-T is Kendall's Tau Bootstrapped confidence interval lower bound, U-T is the upper bound, Z-L is the Z-value Bootstrapped confidence interval lower bound, Z-U is the upper bound. *Ss is Sen's slope, pw Ss is pre-whitened Sen's slope, tau is Kendall correlation, Z is the test statistic.

Additionally, the initial diagnostics performed on the annual data indicate that the temperature data have serial correlation while the rainfall data do not have (Figure 7). The annual trend analysis shows that there is a significant rising trend in temperature but not in rainfall (Table 7). Also, it shows that the rate of change is higher in the Abakaliki data than in any of the other locations (Table 7). The increasing warming in the area portends an increase in the rate of evapotranspiration in the area and could harm rice productivity due to reduced moisture during periods of low rainfall like August. This is in line with the studies that unreliable rainfall and temperature negatively affect rice yield (Ita et al. 2013; Tanko et al. 2016; Mech 2017). This could be a contributory factor to favorable productivity in Abakaliki as it is the only state with the least effect of the little dry season in August amongst the states (Figure 3)

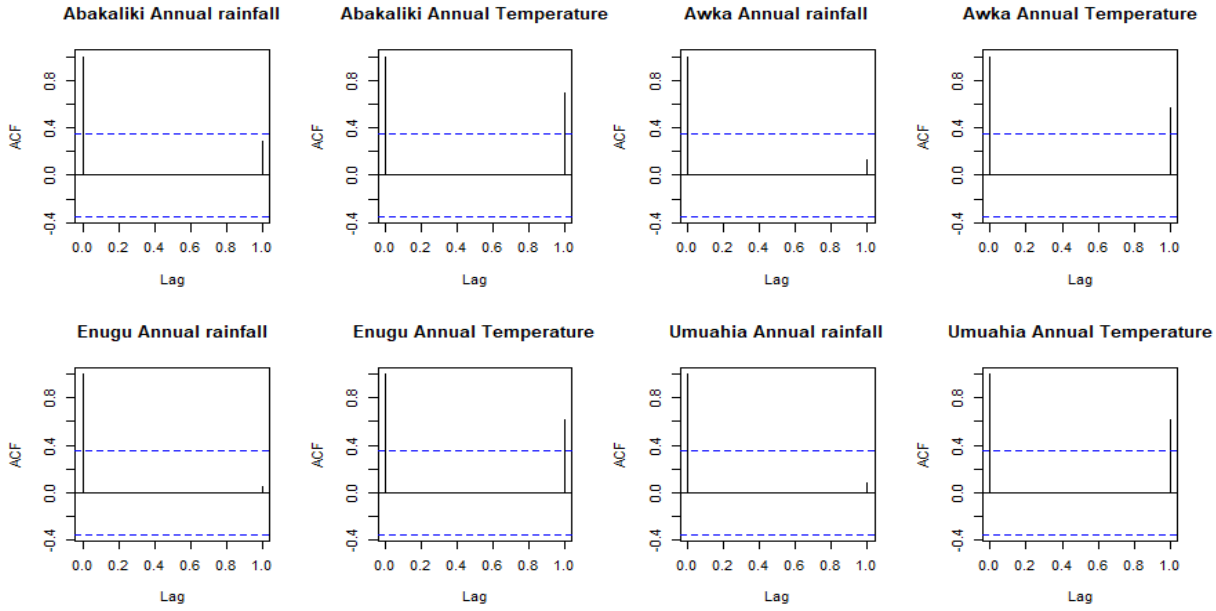

Figure 7. The autocorrelation results for the annual data.

Table 8. The trends of annual rainfall and temperature

Location	Z	tau	Ss	Pw_Ss	p-value
	Rainfall				
Abakaliki	**-2.355**	**-0.306**	**-15.06**	**-14.04**	0.019
Awka	**-0.428**	**-0.057**	**-1.01**	**-2.907**	0.670
Enugu	**-1.890**	**-0.246**	**-8.70**	**-10.46**	0.059
Umuahia	**-0.640**	**-0.085**	**-1.75**	**-4.97**	0.790
	Temperature				
Abakaliki	2.966	0.103	0.003	0.001	0.003
Awka	2.237	0.078	0.002	0.001	0.025
Enugu	2.344	0.082	0.002	0.001	0.019
Umuahia	2.594	0.090	0.002	0.001	0.009

*Ss is Sen's slope, pw Ss is pre-whitened Sen's slope, tau is Kendall correlation, Z is the test statistic.

3.3. Socioeconomic characteristics of rice farmers

Results on socioeconomic characteristics of rice farmers in the study area reveal that across Southeastern Nigeria, a majority (62%) of the rice farmers are males, while 38% are females (Figures 8 and 9). This implies that the sex distribution among rice farmers is skewed in favour of males.

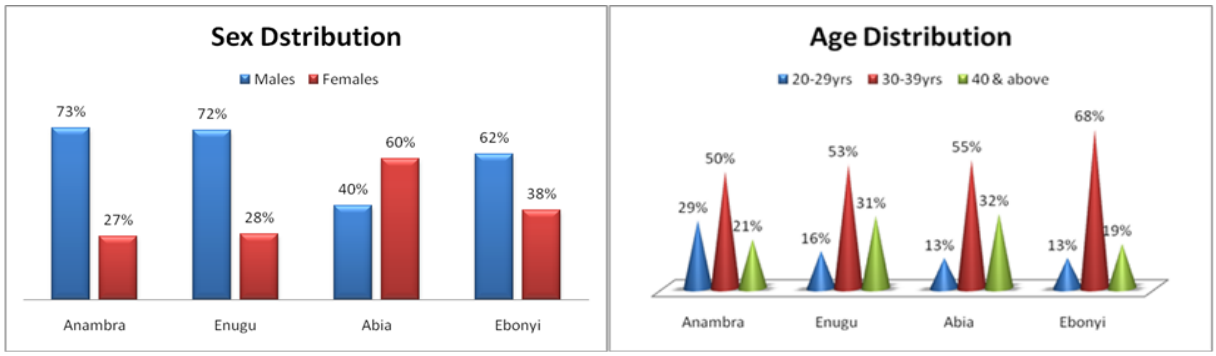

Figure 8. Sex and Age Distribution of Rice Farmers across the States.

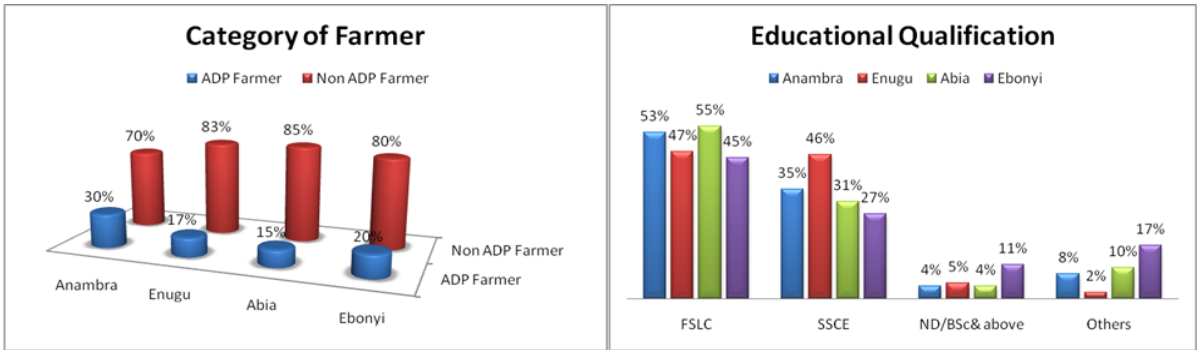

Figure 9. Categories of Rice Farmers and their Educational Qualification across the States.

The ages of the rice farmers in Southeastern Nigeria show that a greater percentage of the farmers' ages is between 30-39 years with variations among the States. More than (80%) of the rice farmers were married with a majority (72%) of the rice farmers' household size being between 5-9 persons. A greater proportion (80%) of the rice farmers were non-ADP farmers (Figure 9). A greater proportion (54%) of them generate between N240 000.00 (578 USD) to N480 000.00 (1156 USD) annually. Ninety-six percent (96%) had farming experiences above 10 years while 50% had First School Leaving Certificate (FSLC) as their highest educational qualification (Figure 9).

The mean productivity curve in Figure 8 displays the level of rice productivity across the states. The result indicates that the States in the region had an estimated mean rice productivity value of 28.117

kg ha^{-1} in the 2018/2019 growing season (Mba et al. 2021). The rice productivity per hectare shows that Enugu, Anambra, and the Abia States have low mean productivity values (6 839 kg ha^{-1}, 6 776 kg ha^{-1}, and 6 697 kg ha^{-1}) of rice productivity respectively, while Ebonyi State has the highest with an estimated mean productivity value of 7805kgha^{-1} (Figure 10). The States of Anambra and Ebonyi are endowed with notable floodplains and better drainages (Anambra River and Ebonyi Rivers). Yet productivity per hectare in Enugu surpasses that of Anambra state. This shows that factors other than suitable land size affect productivity like labour availability. However, a contrary result was reported by Dawe (2013) in southeast Asia that rice yield per capita is not the key determinant of rice production but the amount of per capita rice area harvested which is dependent on the proportion of well-suited land for rice-growing. Additionally, Anambra and Abia states are highly commercialized and the major employer of labour is commerce and trade. The result also indicates the existence of a negative effect on rice farmers in the study area of non-ADP farmers. The average yield is still low in Nigeria (Kamai et al., 2020).

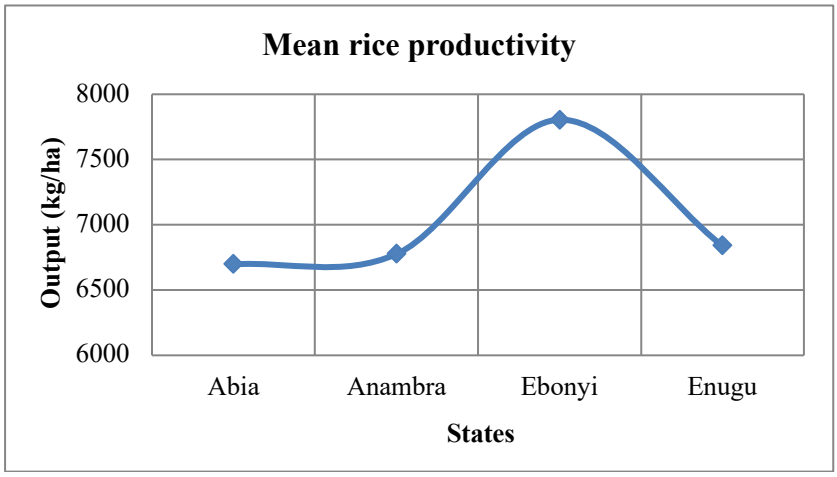

Figure 10. Rice Productivity of the States. Source: Mba et al. (2021).

The region was classified based on its level of productivity and a map of the mean productivity was produced to depict the spatial variations in productivity across the region (figure 11). It shows that Abakaliki is a very high productivity area, Enugu (high), Anambra (moderate), and Abia (low) productivity area (Figure 11).

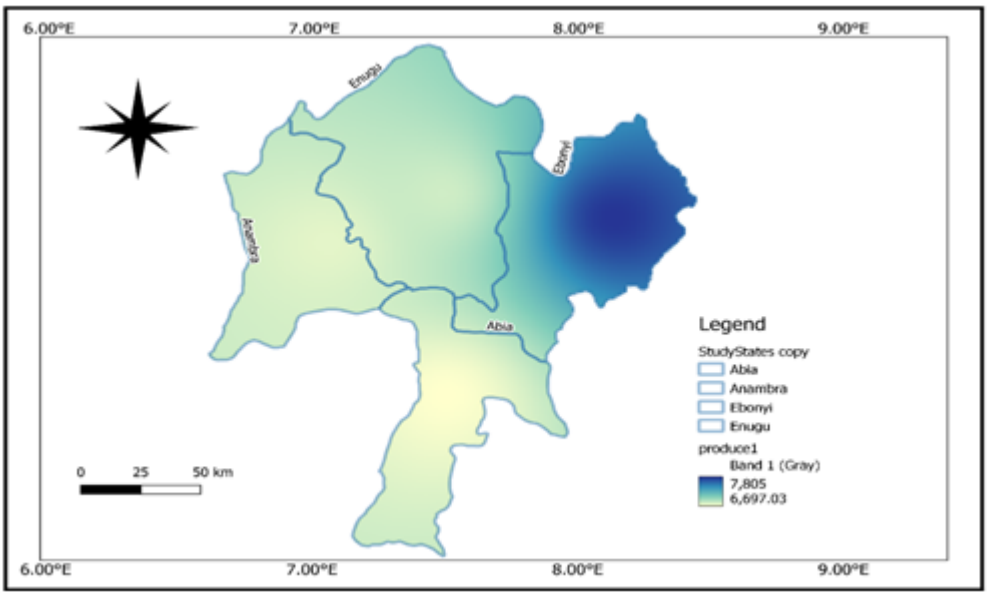

Figure 11. Spatial distribution of rice productivity in the area.

Figure 9 depicts the spatial differentiation in rice productivity in southeastern Nigeria. It shows that Ebonyi State followed by Enugu State recorded the highest levels of productivity based on the mean annual produce. However, modest to low yield was recorded in Anambra and Abia States. This spatial variation in rice productivity across the States is due to factors such as the category of the farmer, age, labour availability, level of education, infrastructural facilities, topography, capital, and climate. It shows that productivity increases from the southern to the northern part of the region.

3.4. Determinants of Rice yields in Southeastern Nigeria

Logistic regression was applied to determine the key factors of rice production across southeastern Nigeria as presented in Table 9.

Table 9 Logistic regression on determinants of rice production across Southeastern Nigeria

Determinants		Odds Ratio	Z	P>|z|
Household size	5 to 9 household members	2.18	1.73	0.08
	Above 9 household members	1.98	1.33	0.18
Monthly income	N20,000 to N40,000	0.66	-0.93	0.35
	Above N40,000	0.94	-0.14	0.89
Sex	Female	1.84	2.30	0.02*
Category of farmer	Non ADP Farmer	5.69	4.46	0.00*
Age	30 to 39 years	0.51	-2.23	0.03*
	Above 39 years	4.66	3.78	0.00*
Highest completed education	SSCE	0.80	-0.85	0.40
	First Degree and above	2.38	1.08	0.28
	Others	2.40	2.38	0.02*
Membership of cooperative society	Yes	0.40	-3.31	0.00*
Number of Extension visits	Twice	0.19	-4.24	0.00*
	More than twice	3.18	2.18	0.03*
	No Extension visit	0.56	-1.59	0.11
Constant		0.39	-0.88	0.38
Pseudo R^2			0.39	
Prob> chi square			0.00*	

Probability values with * denote significance at 95% confidence interval.

From Table 9, the Pseudo R-squared value of 0.39 explains 39% of the variation in rice productivity as accounted for by the combined effects of the 8 variables. This implies that the independent variables explained almost 40 % of the behaviour of the dependent variable. The calculated Chi-square probability value of 0.00 implies that there is a significant impact between the predicted variable and the predictor variables. For the probability effects of the independent variables on rice productivity; sex, category of the farmer, age of the farmer, educational qualification, membership of the cooperative society, and extension visits were the significant determinants of rice productivity in the study area (Table 9). This corroborates the findings of earlier studies (Ita et al., 2013; Osanyinlusi and Adenegan, 2016; Niang et al., 2017; Balogun et al., 2021; Chandel et al., 2022).

Specifically, male farmers are significantly ($p = 0.02$) more likely to produce a high quantity of rice (1.84) when compared to female rice farmers. This shows that male farmers who cultivate rice produce 84% more rice output than female rice farmers. In the same vein, the ADP farmers are significantly ($p = 0.00$) more likely to produce a high quantity of rice (5.69) when compared to non-ADP farmers. This implies that ADP farmers have 469% more output than the non-ADP farmers.

Again, rice farmers who are between 30 to 39 years compared to those who are above 39 years are significantly ($p = 0.03$) less likely to produce more rice by 0.51. This indicates that rice farmers who are 39 years and below produce 51% lower output than those that are aged 30 to 39 years. This is in line with Echebiri and Mbanasor's (2003) finding that rice production requires able-bodied farmers with a lot of experiences. That is, manpower and experience are key to increased rice production. Just as Balogun et al. (2021) showed that labour in addition to irrigation impacts rice production.

On the highest educational qualification of the sampled rice farmers, the study discovered that rice farmers with First School Leaving Certificate (FSLC) as the highest education qualification significantly ($p = 0.02$) increase rice output by 2.40. This indicates that rice farmers with FSLC are more likely to increase their rice output by 140% more than those with others as their highest completed education. More so, rice farmers who do not belong to any cooperative society compared to those who belong significantly ($p = 0.00$) reduce rice output by 0.40. This indicates that rice farmers who do not belong to any cooperative society produce 40% lower output than those who belong to a cooperative society. Therefore, membership in a cooperative society is a bolstering factor in rice production as there are several benefits from such membership which help to increase production.

Similarly, rice farmers who had only one extension visit compared to those with two visits significantly ($p = 0.00$) reduces rice output by 0.19. The implication of this is that rice farmers with only one extension worker visit produce 19% lower output than those with two extension visits. In the same way, rice farmers who had only one extension visit compared to those with more than two visits significantly ($p = 0.03$) reduce rice output by 0.56. This indicates that rice farmers with only one extension worker visit produce 56% lower output than those with more than two extension visits. This concurs with related findings that extension workers' visit influences rice production (Diagne et al. 2013).

Conclusion

The study assessed the climate variability and the determinants of rice production in the southeast of Nigeria. It reveals the existence of increasing warming in the area due to significant rising temperatures. It shows that rainfall is decreasing though not statistically significant. Significant warming with declining rainfall might lead to the depletion of soil moisture due to accelerated evapotranspiration. The study recommends that since an ADP farmer has a lot of perquisites such as getting enough government attention in terms of provision of improved farm inputs, improved technology, and attending state cooperative federation workshops, awareness should be created on the importance of being an ADP farmer to attract a greater number of farmers to belonging to ADP farmers. In other words, there should be increased access to information for the farmers while access to belonging should be open to all who want to belong at any point in time.

The results have emphasized the necessity of rainfall to increase rice productivity. However, with increasing warmth and declining rain, there should be a need of providing adequate irrigation facilities to assist farmers, especially during the dry season to ensure food production in all seasons rather than being rain-dependent. Therefore, there should be partnerships among the farmers, the state authorities, aid agencies, and other stakeholders in actualizing this in order to meet the 2030 SDGs of hunger reduction and achieving food security in Nigeria. Also, Anambra/Imo River Basin Development Authority projects should be revitalized to enhance and ensure adequate water supply all year round to rice farms in the area.

Finally, the findings of the study will aid policymakers and other stakeholders in devising policies and future scenarios to improve rice production in the southeast of Nigeria and the country at large. Therefore, more awareness should be created to enable more farmers to benefit from being ADP farmers in the region.

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Research Article

In-vitro and *In-vivo* Antimicrobial Properties of Pomegranate (*Punica granatum* L.) Peel Extract Genotypes Against Bacterial Vaginosis in Bovine

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Abstract: Drug resistance against bovine bacterial vaginosis (BV) in common treatments needs new therapeutic agents from other sources. Many plants demonstrate antimicrobial properties that could control pathogenic microorganisms. Uterus infections in bovine are associated with calving difficulty, retained placenta, and overgrowth of pathogenic microorganisms in the reproductive tract. This study examined the antibacterial effects of pomegranate peel extracts against various bacterial vaginosis in bovine. Methanolic and aqueous extracts of different pomegranate peels were prepared. An antibiogram test was performed against nine various bacterial vaginosis of bovine. Then, inhibitory concentration values were determined for pomegranate peel extracts (100, 200, and 400 mg/ml). In *in-vivo* observation, the greatest inhibition zone activated pink pomegranate peel extracts (8 mg/ml) prepared by methanolic extracts. The serial dilution tests indicated that the bactericidal effect of high-concentration methanolic extract was more than those of low-concentration types. Experimental treatments in *in-vitro* observation constituted including (treated, T) with 180 cows that encountered difficulty in birth and non-including (control, C) with 30 cows with no treatments after vaginal problems. The effect of various pomegranate peel extract (PPE) concentrations increased during the treatment days. All the treated Holstein cows with nearly 400mg/ml of PPE gel (both methanolic and aqueous extracts methods) were recovered after 4 days. The results of this study showed the PPE gel effectiveness in the pharmaceutical industry.

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1. Introduction

Drug resistance to animal pathogens has needed new therapeutic agents from other sources. The antimicrobial property of traditional plans based on medicines has been revisited over the last decade (Meléndez and Capriles, 2006). Many plants demonstrated antimicrobial properties, which could have pivotal activity against bacteria, allowing them to deal with pathogenic microorganisms (Meléndez and Capriles, 2006). Chemical compounds include flavonoids, antioxidants, and anthocyanin in many plants such as pomegranate (*Punica granatum* L.) which has an antibacterial role in bacterial diseases (Prashanth et al., 2001; Holetz et al., 2002).

Punica granatum L. is considered a member of the Punicaceae (Lythraceae) family, which consists of two species *Punica granatum* L. and *Punica protopunica* Balf. The *Punica granatum* L. species typically referred to as pomegranate and used in folklore medicine to cure various diseases across countries (Ricci et al., 2006; Lansky and Newman, 2007; Jurenka, 2008; Kahramanoglu, 2021). The known compounds of the *Punica* genus are Ellagitannins, Galoutanins, Anthocyanins, Flavonoids, Sterols, Terpenoids, Tannin, Polyphenols, Alkaloids, Organic Acids, B1, B2, and C vitamins (Dong et al., 2011). It is used in traditional medicine due to its anti-inflammatory and antimicrobial effects in Asia and Mediterranean Europe (Heber et al., 2006).

Various parts of pomegranate, such as fruit peel and seed, demonstrated the broadest antibacterial activity (Prashanth et al., 2001, Dahham et al., 2010; Ismail et al., 2012). Pomegranate peels are important in traditional medicine due to containing strong phenolic, flavonoid, and anthocyanin compounds. Essential oils in pomegranate peels contain a wide range of tannins (Reddy et al., 2007), anthocyanins, and flavonols (Naz et al., 2007). The latest scientific research studies among different *P. granatum* L. varieties (Black, White, and Red skin fruit) showed that black skin fruit has the highest amount of phenolic compounds, total anthocyanin, and antioxidant properties (Bayati and Asadi-Gharneh, 2019).

The effect of pomegranate extract feeding on animal health has been reported in previous studies. Those effects included modifying the microbial ecology of broiler chickens (Perricone et al., 2020) and the microbiome in mice (George et al., 2019). There are also studies on the effects of concentrated pomegranate extract on lactating cow rations (Jami et al., 2012) and pomegranate extract feeding on health, growth, and nutrient digestion in calves (Oliveira et al., 2010). However, the antimicrobial effect of pomegranate peel extract has been scarcely investigated in animal diseases.

Reproductive potentials, milk production, treatment expenses, and culls in infertile animals are notably affected by uterine infection (Lewis, 1997; Ross, 2002). In dairy cows, calving problems, retained placenta, and an abundance of pathogenic microorganisms in the reproductive tract lead to uterus infections (Coleman et al., 1985). Uterine pathogens, which cause clinical endometritis and severe endometrial inflammation, include *Escherichia coli*, *Staphylococcus aureus, and Klebsiella pneumoniae* sub. *pneumonia*, *Pseudomonas aeruginosa*, *Corynebacterium glutamicum*, *Salmonella enterica* subsp. *Enterica*, *Proteus mirabilis*, *Bacillus licheniformis*, and *Streptococcus agalactiae* (Sheldon et al., 2002; Williams et al., 2005; Carneiro et al., 2016).

To the best of our knowledge, the effect of pomegranate peel extraction on vaginosis diseases has not been adequately tested in animals. This study comprehensively reports the antibacterial effect of pomegranate peel extraction on vaginosis bacteria. Therefore, we aimed to find a vaginal gel that can be used against vaginosis bacteria in Holstein cows.

2. Material and Methods

2.1. Sample collection and extract preparation

Three pomegranates (*Punica granatum* L.) genotypes with different peel colors: black skin varieties of Yazd, white skin varieties of Abrandabad Yazd, and pink skin varieties of Shahreza were collected from Isfahan Agricultural and Natural Resources Research Center, Iran (Figure 1). Peels were first prepared and dried for a week at room temperature in the dark. Then, the dried peels were separately blended for 2 min.

Each powder was divided into two groups. The first group (5 g of pomegranate peel powder) was dissolved in 30 ml of distilled water. The second group was dissolved in 30 ml methanol (99.9%), shacked for 4 h, and then centrifuged (Universal 320 R, Iran) at 12000rpm for 10 min at 5°C. Each solution was filtered, followed by reduced concentration by an otary evaporation method. The concentrates were dried in an oven (FGiran, Iran), and the dried extract was stored at −20°C.

Figure 1. The pomegranate (*Punica granatum* L.) genotypes with different peel colors: Black peel (A), White peel (B), and Pink peel (C).

2.2. Microorganisms and growth conditions

Table 1 lists microorganisms used to evaluate the antimicrobial activity of aqueous extract (AE) and methanolic extract (ME). The reference microorganisms were obtained from the Iranian Research Organization for Science and Technology (IROST). The nine animal vaginal bacterial strains were used as the test organisms.

2.3. Formulating the topical herbal gel

Pomegranate herbal gel was made using the affected pomegranate peel extract. The gel base was prepared by applying lubricant gel (Jardines and Alcoba, 2020). The affected pomegranate peel extracts were added to the gel base and mixed well.

2.4. Antimicrobial activity (*in-vitro*) of extracts

The antimicrobial tests were implemented based on Kirby- Bauer standard method (Bauer, 1966) using a modified agar well diffusion method (Okunji et al., 1990). Various concentrations (50, 100, 200, and 400 mg/ml) of pomegranate peel extract were used in the current study. Moreover, 20 µl of each concentration was added to each well. Therefore, each well contained 1, 2, 4, and 8 mg of pomegranate peel extract. *Escherichia coli*, *klebsiella pneumoniae* subsp. *pneumonia*, *Salmonella enterica* subsp. *Enterica*, *Proteus mirabilis*, *Bacillus licheniformis*, and *Staphylococcus aureus* were grown at 35 ºC for 18-24 h by inoculating in Nutrient Agar (NA, Merck, Germany). In addition, *Streptococcus agalactiae*, and *Corynebacterium glutamicum* were inoculated in Brain heart infusion (BHI, Merck, Germany). *Pseudomonas aeruginosa* was inoculated in Tryptic soy agar (TSA, Merck, Germany). One hundred µl of each 1/100 dilution of McFarland 0.5 bacterial culture was added to the plate and was uniformly spread on the surface. Petri dishes with 10 ml of each bacterial culture were prepared. The wells (for each extract and antibiotic concentrations in triplicate) were planted, and then the plates were kept in an incubator at 37°C for 24 h, while *Bacillus licheniformis* was kept at 30°C.

2.5. Antimicrobial activity (*in-vivo*) of extracts

Two hundred and ten Holstein cows (2 or 3 years) were housed at the Alian husbandry in a shaded corral with free access to water in the spring of 2020. The experiment site was a semi-open indoor springboard that was in contact with the open air from all sides. Holstein cows were divided at the onset of the experiment into two groups, which were individually fed with a typical total mixed ration (TMR). Experimental treatments consisted of the including group (treated, T) with 180 cows that had problems at birth and the non-including group (control, C) with 30 cows with no treatments after vaginal problems. The treated cows received methanolic and aqueous PPE vaginal gel from 1 to 10 days after parturition. Vaginal gel performance was evaluated during the trial period from the beginning to the end of the experiment.

2.6. Data analysis

Statistical analysis was done by SPSS computer software (version 25; SPSS, USA, 2017). The data were evaluated in terms of mean ± SEM for each pomegranate peel extract. Analysis of variance (ANOVA) followed by Duncan's multiple range test was used to evaluate the impact significance of factors such as peel color, extraction method, and extract concentration. Differences considered significant in a p-value were less than 0.05. The homogeneity of variance test was used to examine factor effectiveness in each case (2 and 3 factors) and determine group homogeneity

3. Results

3.1. *In-vitro* observation

The results of *in-vitro* antibacterial activity of aqueous and methanolic extracts determined by the mean of inhibition zone diameters are presented in Table 1. The table summarizes the inhibition zone of various bacteria by different pomegranate peel extracts. Among the extraction methods (aqueous and methanolic), the methanolic extract was the most active with 25 mm of mean inhibition diameter. In our study, the type of pomegranate peel (white, pink, black) did not significantly increase the inhibition halos. Comparing inhibition zone means in extractions indicates notable effects of pomegranate peel extract with discs containing 8 mg pomegranate peel extract. *Corynebacterium glutamicum*, *klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae,* and *Bacillus licheniformis* showed the highest sensitivity (250.5 ± mm, Figure 2). The lowest sensitivity was illustrated by *Salmonella enterica* subsp. *enterica* (9± 0.0 mm). There was a significant difference between high- and low-concentration pomegranate peel extract ($P<0.05$). Furthermore, 8 mg of extraction was generally higher than those of other concentrations. There was no significant difference between aqueous and methanolic peel extract, though the efficacy of methanol peel extraction was more ($P<0.05$).

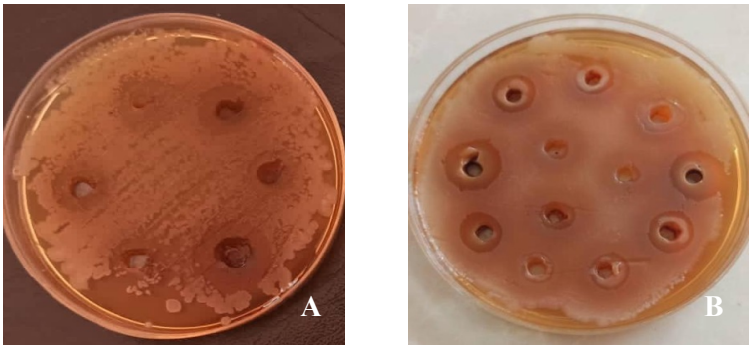

Figure 2. The antimicrobial inhibitory zone diameters (mm) of aqueous (A) and methanolic (B) extraction in different pomegranate peel extracts against *Streptococcus agalactiae.*

Table 1. Comparison of the inhibitory zone diameters (mm) in different pomegranate (*Punica granatum* L.) peel extract concentrations against vaginosis bacteria in bovine

				Inhibition zone (mm)					
				Aqueous extract			Methanolic extract		
No	Microorganism	PTCC*	Sample Concentration (mg/disc)	Black Peel	White Peel	Pink Peel	Black Peel	White Peel	Pink Peel
1	***Escherichia coli***	1860	1	6.5±0.5	1±0.5	0±0	13±2	8.5±0.5	5±0
			2	10.5±0.5	7.5±0.5	0±0	12.5±0.5	12±0	10±0
			4	15.5±0.5	10±0	5.5±0.5	10±0	15±0	11±1
			8	21±1	15.5±0.5	12.5±0.5	12±0	16±1	16±1
2	***Staphylococcus aureus***	1431	1	5.5±0.5	0±0	0±0	6.5±1.5	4±0.9	10±0
			2	9.5±0.5	0±0	9.5±0.5	7.5±0.5	7.5±0.5	14±1
			4	14.5±0.5	5.5±0.5	15±0	13.5±1.5	12.5±2.5	16±1
			8	17.5±0.5	9.5±0.5	17.5±0.5	14±1	14.5±2.5	22.5±0.5
3	***klebsiella pneumoniae***	1290	1	0±0	0±0	0±0	0±0	0±0	0±0
			2	0±0	5±0	0±0	0±0	0±0	9±0
			4	5±0	7±0	11±1	5.5±0.5	7.5±0.5	14±1
			8	7.5±0.5	10±0	20.5±0.5	9.5±0.5	9.5±0.5	25±0
4	***Pseudomonas aeruginosa***	1310	1	0±0	0±0	0±0	0±0	0±0	5±0
			2	0±0	0±0	0±0	7±0	6±1	10±0
			4	6±1	8.5±0.5	6±1	10±0	10.5±1.5	11.5±0.5
			8	17.5±2.5	12.5±2.5	15±0	10±0	12.5±2.5	24.5±0.5
5	***Corynebacterium glutamicum***	1532	1	0±0	0±0	0±0	0±0	0±0	0±0
			2	5±0	5±0	0±0	5.5±0.5	0±0	5±0
			4	8±0.9	9.5±0.5	15±0	10±0	0±0	13±1
			8	11±1	13±2	21±1	13.5±1.5	17.5±0.5	25±0
6	***Salmonella enterica subsp. enterica***	1787	1	0±0	0±0	0±0	0±0	0±0	0±0
			2	0±0	0±0	0±0	0±0	0±0	0±0
			4	0±0	0±0	0±0	0±0	5.5±0.5	0±0
			8	7.5±0.5	5.5±0.5	6±1	10±0	9.5±0.5	9±0
7	***Proteus mirabilis***	1776	1	6.5±1.5	0±0	0±0	0±0	5±0	5.5±0.5
			2	9±0	9±1	0±0	6±0	6.5±0.5	11±1
			4	8.5±1.5	9±1	5±0	11±1	10.5±4.5	15±0
			8	10.5±0.5	13.5±1.5	14±1	14.5±0.5	19.5±0.5	20±0
8	***Bacillus licheniformis***	1331	1	5±0	0±0	0±0	0±0	0±0	5.5±0.5
			2	5±0	0±0	10±0	0±0	0±0	10±0
			4	6±0	5.5±0.5	11.5±0.5	5.5±0.5	6±0	13.5±0.5
			8	13±1	10±0	20.5±0.5	12.5±0.5	10±0	25±0
9	***Streptococcus agalactiae***	1768	1	0±0	0±0	0±0	0±0	0±0	5±0
			2	0±0	0±0	0±0	5±0	0±0	10.5±0.5
			4	13.5±1.5	7.5±0.5	15±0	8±0	6±1	14±1
			8	19±1	14±1	20.5±0.5	11±1	18.5±1.5	25±0

*PTCC: Persian Type Culture Collection (PTCC is a member of the World Federation for Culture Collections and the UNESCO Microbial Resources Centers Network).

Means and standard deviations are considered for n = 3. For inhibition bacteria, the experimental values of pomegranate peel extract concentrations within each row are shown, which show significant differences ($p < 0.05$).

3.2. *In-vivo* observation

The PPE vaginal gel results are shown in Table 2. The post-treatment with pomegranate peel extraction gel in both treatment groups (Aqueous and Methanolic extract) produced similar results. In the current study, slightly more and less than the effective concentration in *in-vitro* results were evaluated in the *in-vivo* experiment. The recovery of various concentrations (6, 8, and 10 mg/ml) in PPE gel increased during the days. The results *in-vivo* confirmed those of *in-vitro*. In these experiments, the untreated Holstein cows remained infected until the end.

As a result, the 10mg/ml concentration showed the best inhibitory activity (Figure 3). All the treated Holstein cows with 500mg/ml concentration of PPE gel, which included methanolic and aqueous extract pomegranate peel, recovered after 3±1 and 4±1 days, respectively. There was no notable difference between 6 and 8 mg/ml concentrations in terms of their actions. All the treated Holstein cows with 8 mg/ml concentration of methanol PPE gel recovered after 6±1, while those treated with aqueous PPE gel recovered after 7±1 days. More than 95% of Holstein cows with 6 mg/ml concentration of methanolic and aqueous

PPE gel recovered after 7±1 and 8±1 days, respectively. The inhibitory activity of all concentrations was excellent. Furthermore, taking PPE gel in Holstein cows had no recurrence after the treatment.

Table 2. Recovery time of different pomegranate peel extract concentrations in *in-vivo* conditions

	Methanolic extraction	**Aqueous extraction**
The concentration of PPE gel (mg/ml)	**Number of days of use until recovery**	**Number of days of use until recovery**
6	7±1	8±1
8	6±1	7±1
10	3±1	4±1

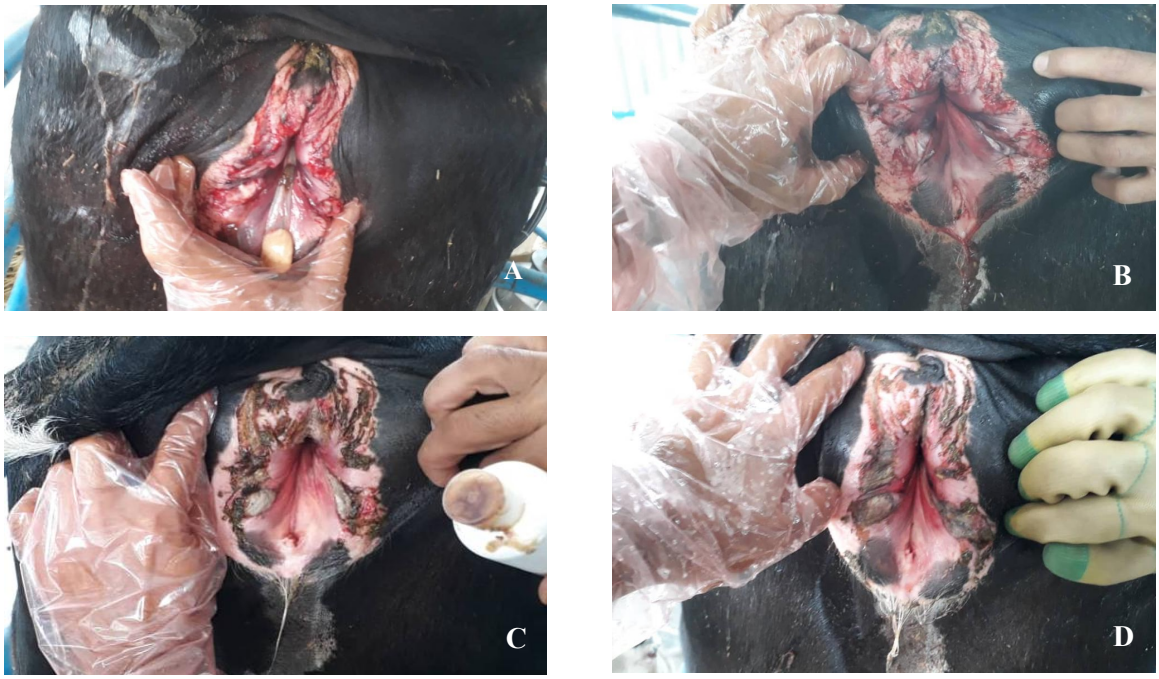

Figure 3. The effect of methanol PPE gel treatment on Holstein cow which passed difficulty in birth. The recovery increased by the gel used during the days. (A: The first day of using the gel; B: The second day of using the gel; C: The third day of using the gel; D: The fourth day of using the gel).

4. Discussion

4.1. The effect of PPE treatment in *in-vitro* condition

The current study showed the antibacterial activity of pomegranate peel extracts against vaginosis bacteria in animals. Our experiments confirmed the antibacterial activities in reaction to bacterial strains. Mean inhibition zone values indicate that pomegranate peel extracts (aqueous and methanolic) exert a more powerful effect on Gram-positive than Gram-negative bacteria ($P<0.05$). Similar results were obtained by Ismail et al. (2016), Casquete et al. (2015), and Naziri et al. (2012). The substantial antimicrobial effect of the pomegranate peel extracts is possibly due to the presence of polyphenols, flavonoids, and tannins (Al-Zoreky, 2009; Miguel et al., 2010; Alexandre et al., 2019). The antimicrobial impacts may be related to the chemical structure of the phenolic compounds. Phenolic compounds contain lipophilic natures that enter the pathogen cell layer, interfere with the enzymes responsible for energy and protein generation within the microorganism's cells, and subsequently cause cell death (Kantachumpoo and Chirapart, 2010). In our

study, varying amounts of antibacterial activity were observed depending on the pomegranate peel extract types, which could be, in turn, attributed to the compounds' chemical compositions. Among different compounds, only a few may have antibacterial activity (Elshafie et al., 2021). Medical centers can employ pomegranate wastes, including peel, which show great antioxidant capacities.

4.2. The effect of PPE treatment in *in-vivo* condition

The results showed that vaginal gel consisting of pomegranate peel extracts was effective against various bacterial vaginosis in Holstein cows without any serious side effects or relapse. The present study is a pioneering attempt in cattle domains that examines the antibacterial activity in *P. granatum* peel extract in reaction to bacterial vaginosis. We ascribe the antibacterial activity to metabolic toxins or broad-spectrum antibiotics in animals. Interestingly, previous research has attributed metabolites of herbs, such as alkaloids, tannins, and sterols, to antimicrobial activity (Machado et al., 2002; Voravuthikunchai et al., 2005). Moreover, the increase in oxygen-free radicals and lipid peroxidation could enhance antibacterial activities, destructing microorganisms' walls (Al-Saimary et al., 2002).

Despite the improvements in medical technologies, the strength of diseases is currently high. Many studies showed that medical drugs extracted from natural products are more successful than those manufactured in equal portions. These chemicals are used in the chemical-medical drug industry from low to heavy intensity (Nisar et al., 2018). In addition, despite the effectiveness of metronidazole and clindamycin treatments in infected animals, there still appear to be certain shortcomings in this respect (Joesoef et al., 1999; Yudin et al., 2003; Klebanoff et al., 2004).

In the present study, we focused on *P. granatum* peel extract effects, which modify the plasma membrane permeability, raise protein concentrations in a cell, and eventually destroy the cell (Kantachumpoo and Chirapart, 2010). Previous studies have mainly examined the antibacterial activity in *P. granatum* derivatives (Dahham et al., 2010; Ismail et al., 2012), and such effects on the *in-vivo* conditions have remained rather unclear.

The findings presented in this paper could help develop fresh and research-supported antibacterial therapies in cattle. Further research is also warranted investigating how pomegranate peel extracts and the affected compounds prevent bacteria growth.

Conclusion

In general, the results of this study show that vaginal gel consisting of pomegranate peel extract is effective in treating vaginosis diseases in cattle and could be used as an alternative option to chemical drugs. As noted, the pomegranate peel extract indicates antibacterial, antifungal, and anti-inflammatory capacities. Thus, further studies should probe into peel compound effects on bacterial vaginosis treatments.

Abbreviation

BV: bovine bacterial vaginosis, T: treated, C: control, PPE: pomegranate peel extract, AE: aqueous extract, ME: methanolic extract, IROST: Iranian Research Organization for Science and Technology, NA: Nutrient Agar, BHI: Brain heart infusion, TSA: Tryptic soy agar, TMR: total mixed ration, SPSS: Statistical Package for the Social Sciences, ANOVA: Analysis of variance, PTCC: Persian Type Culture Collection.

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Research Article

Study on Correlation of Agromorphologic Properties in Some Camelina (*Camelina sativa* (L.) CRANTZ.) Genotypes

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Abstract: The correlation of agromorphological traits can be useful for breeders in the selection of plant genotypes. In this study, the rosette period (days), days of maturity, plant height (cm), 1000-seed weight (g), and seed yield (kg ha^{-1}) characteristics of 42 different Camelina genotypes that grown in Ankara (middle Anatolia) and Şanlıurfa (southeastern Anatolia), and their correlations with each other, were investigated. The accessions showed different results depending on the location in terms of the studied characters in both locations under rainfed conditions. The results showed that the highest seed yield was obtained from the PI 311735 accession (3151.8 kg ha^{-1}) in Ankara and the PI 650142 accession (3056.0 kg ha^{-1}) in Şanlıurfa. While the rosette period (days), days of maturity, plant height (cm), and 1000-seed weight (g), in Ankara were between 152.3 and 132.3 days, 274 and 247 days, 103.8 and 59.5 cm, and 1.50 and 0.84 g, while there were between 108.8 and 88.8 days, 202.1 and 180.1 days, 115.4 and 59.2 cm, and 1.40 and 0.50 g, in Şanlıurfa, respectively. Results showed significant differences among the genotypes in all of the studied parameters. Correlation analysis of the genotypes in both locations on the mentioned parameters was also performed. Since climate and environment affect each agromorphological parameter differently, it was observed that a genotypic correlation independent of the climate and environment could not be explained in the *Camelina sativa* genotypes.

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1. Introduction

The *Camelina sativa*, known as fake flax, German sesame, and Siberian oilseed, is distributed in the natural flora of the Mediterranean Basin and the Central Asian region (Kınay et al., 2019; Mcvay, 2008). The cultivation of false flax began in the Neolithic age and it was used as an oilseed crop during the Iron Age (Katar and Katar, 2017; Knorzer, 1978; Yilmaz et al., 2019). It was reported that it had

been cultivated in a wide area up to southeast Europe and southwest Asia steppes during the Roman Empire (Knorzer, 1978). Today, in Canada (Downey, 1971; Robınson, 1987), Germany, Poland, and the former Soviet Union, it is cultivated in small quantities (Seehuber and Ambroth, 1984). False flax is known as an annual crop that can be sown both in winter and spring (Frohlıc and Rıce, 2005; Straton et al., 2007). Recently, its use as a biofuel raw material has been increasing steadily (Katar and Katar, 2017; Vollmann et al., 2007). *C. sativa* (L.) Crantz., *C. laxa* C. A. Mey, *C. rumelica*, *C. microcarpa* Andrz. ex DC., *C. hispida* Boiss., *C. anomala* Boiss. & Hausskn., and *C. Alpkoyensis* Yield are the 7 most widely known species of the genus Camelina Crantz (Mutlu, 2012). Of these species, only the *Camelina sativa* species has been cultured (Kurt and Seyis, 2008).

Although the cultivated form of the false flax (*C. sativa*) is annual, its wild forms are perennial. The plant height generally varies between 25 and 100 cm. Single stem development prevails in the plant, and the stem is round and covered with hairs. The leaves are lanceolate 5–8 cm long and the edges are straight. The flowers consist of 4 green leaves, 4 yellow or yellowish-white petals, 6 male organs, and one female organ. The camelina plant is mostly self-pollinated. The fruit is in the form of capsules with a diameter of 0.7–2.5 mm, and the color ranges from orange to brown. Each capsule contains 8–16 seeds. The 1000-seed weight varies between 0.8 and 1.8 g. While the seeds of the summer cultivars of the plant contain 42% oil, this rate is 45% for the winter cultivars (Katar and Katar, 2017; Kurt and Seyis, 2008; Yılmaz et al., 2019).

Camelina is highly adaptable to adverse extreme climatic conditions and marginal land sites, and both its synthetic chemical input and agricultural energy demand are low. In other words, the plant has a high tolerance to environmental stress factors. In addition, the use of synthetic chemical inputs in cultivation is limited due to the high competitiveness of the plant against weeds, and the tolerance of the plant to diseases and pests, which is an important feature (Kurt and Seyis, 2008).

Despite Camelina's many uses, there is a lack of deep scientific research on this plant in the world as well as in Turkey. Due to the importance of its oil for human health in recent years, the characterization and adaptation studies on this plant have been considered in many countries, especially in Germany. In this study, some morphological characteristics of 42 different Camelina genotypes were examined and their correlations were determined.

2. Material and Methods

2.1. Materials

The seeds of 42 camelina genotypes (*Camelina sativa* (L.) Crantz) in the study were obtained from the Seed Bank of the Agricultural Research Service of the United States Department of Agriculture (Table 1). Additionally, 3 standard genotypes [Line-1 (C1), PI 650149 (C2), and PI 650151 (C3)] were used in the trial. Field experiments were conducted during the 2014–2015 cropping season at the Research and Implementation Area of the Field Crops Agricultural Research Institute, in Ankara, and the GAP Agricultural Research Institute, in Şanlıurfa, Turkey.

The soil characteristics at the Şanlıurfa location were clayey and slightly alkaline, and there were no problems with regard to salinity. It was determined that the soil was very calcareous, the content of the organic matter was moderate, the available phosphorus content was low, and the available potassium content was high (sufficient). On the other hand, the soil structure at the Ankara location was clayey-loamy, high in potassium, poor in organic matter content, alkali with no problem of salinity, and enough phosphorus content.

In both locations, the precipitations were above the average for several years in March. Moreover, at the Ankara location, the minimum temperature decreased to –27,5 °C in January, which was below the average for the long-term mean (Table 2). Some of the plants were damaged from this cold temperature, but later vegetatively self-recovered in the following months.

Table 1. *Camelina sativa* (L.) Crantz. genotypes used in the research and their origins

Accession ID	Accession No.	Origin	Accession No.	Accession No.	Origin
Ames 31219	1	Georgia	PI 650147	22	Sweden
Ames 31220	2	Georgia	PI 650148	23	Germany
Ames 31224	3	Georgia	PI 650150	24	Denmark
Ames 31231	4	Georgia	PI 650152	25	Germany
Ames 31232	5	Georgia	PI 650153	26	Russia
Einfact	6	Germany	PI 650154	27	Russia
PI 258366	7	Russia	PI 650155	28	Poland
PI 258367	8	Russia	PI 650156	29	Russia
PI 304269	9	Sweden	PI 650157	30	Russia
PI 304270	10	Sweden	PI 650158	31	Poland
PI 304271	11	Sweden	PI 650159	32	Poland
PI 311735	12	Poland	PI 650160	33	Russia
PI 311736	13	Poland	PI 650161	34	Russia
PI 597833	14	Denmark	PI 650162	35	Poland
PI 633192	15	Germany	PI 650164	36	Austria
PI 633193	16	Germany	PI 650165	37	Russia
PI 633194	17	Germany	PI 650166	38	Russia
PI 650141	18	America	PI 650167	39	Poland
PI 650142	19	Denmark	PI 650168	40	America
PI 650144	20	Denmark	PI 652885	41	Slovenia
PI 650145	21	Germany	PI 652886	42	Slovenia

Table 2. Monthly average of meteorological data of the experimental farm during growing season

Location	Month	Temperature (°C)		Precipitation (mm)	
		Long-term mean	2015–2016	Long-term mean	2015–2016
Ankara	Oct	13.8	11.3	31.8	19.2
	Nov	7.8	4.4	34.2	20.0
	Dec	3.6	–2.9	42.0	33.8
	Jan	–1.4	–1.7	42.2	66.4
	Feb	0.8	5.0	4.5	18.6
	Mar	4.7	5.9	16.3	67.0
	April	9.5	11.9	12.8	11.9
	May	14.3	12.7	45.3	58.0
	June	22.8	19.0	1.2	8.4
Ş. Urfa	Oct	19.4	21.7	19.3	13.6
	Nov	12.0	14.0	18.2	28.1
	Dec	7.5	8.7	44.5	16.4
	Jan	5.6	4.7	46.7	22.0
	Feb	7.2	11.5	40.5	54.0
	Mar	11.2	13.6	39.7	65.4
	April	16.5	20.4	25.8	14.8
	May	22.5	23.2	18.7	1.0
	June	28.4	29.8	1.3	0.2

2.2. Methods

The materials were examined in augmented trial designs in 6 blocks. The materials were sown with 15- and 10-cm inter- and intra-row spacings, respectively. The row length was 5 m. All of the genotypes were examined for their rosette period (days), days to maturity, plant height (cm), 1000-seed weight (g), and seed yield (g) (Katar et al., 2012). No irrigation or fertilization was applied during the vegetation period. Weeds were controlled mechanically by hand-hoeing.

The materials were sown in early October 2015 with 15 cm between the rows, 10 cm spacing between plants of intra-row, and a row length of 5 m. Harvesting took place in Ankara in mid-June and in Şanlıurfa in mid-May 2016.

2.3. Observations and measurements

Rosette period (days): This is the number of days between the date when half of the plants in the plot completed emerging from the soil surface and the date when half of the plants in the same plot exhibited a shift in their stem elongation from horizontal to vertical.

Plant height (cm): In 20 randomly selected plants in each plot, the distance from the ground to the top point of the plant was measured with a ruler. The average of the values obtained from the 20 plants was considered as the plant height value of the genotype.

Days to maturity (days): This was determined as the number of days from the date when half of the plants in each plot emerged until the plants reached harvesting maturity.

Seed yield (kg ha^{-1}): The seeds in the plot were harvested and weighed, and then calculated as kg ha^{-1}.

The 1000-seed weight: 100 seeds with 4 replications from each plot were collected and weighed. The average values obtained were multiplied by 10 and the 1000-seed weights were determined.

2.3. Statistical analyses

Analysis of variance (ANOVA) was performed with JMP Pro11 (SAS Institute Inc., Cary, NC), while correlation analysis was performed using the XLSTAT program (Add in soft, Paris, France). The mean values of the properties were compared using the Duncan multiple range test ($P<0.05$).

3. Results and Discussion

Variance analysis, correlation analysis, and grouping of the values obtained in the study were examined (Tables 3 and 4). At the Ankara and Şanlıurfa locations, statistically significant differences were observed between the rosette periods, days to maturity, plant heights, 1000-seed weights, and seed yields. It was concluded that the rosette period in Ankara varied between 132.3 and 152.3 days, while, in Şanlıurfa varied between 88.8 and 108.8 days. Similarly, significant differences were determined between the locations in the number of days to maturity. These values were recorded between 274-247 days in Ankara, and 202.1-180.1 days in Şanlıurfa, respectively. Depending on the location, significant differences were observed in the plant heights. These values varied between 103.8-59.5 cm in Ankara, and 59.2-115.4 cm in Şanlıurfa. The variation in the plant heights was determined in different genotypes in both locations. These findings were coherent with those reported by Katar et al. (2012) (85.29 cm) and Arslan et al. (2014) (52.7 cm and 116.4 cm).

Table 3. Analysis of variance of augmented block design for five quantitative traits in 45 genotypes of *Camelina sativa*

Location	Variation Source	D.F.	Mean square				
			Rosette period	Days to maturity	Plant height (cm)	1000-seed weight (g)	Seed yield (kg ha^{-1})
Ankara	Block	5	19.193**	20.305**	12.460**	0.012**	38130.6**
	Genotype	44	15.428**	14.609**	119.298**	0.010**	460170.1**
	Error	10	0.861	0.805	0.130	0.001	1332.0
	C. V. (%)		0.640	1.900	4.600	2.420	2.2
Şanlıurfa	Block	5	2.305**	2.305**	2.074	0.010	22520.4**
	Genotype	44	17.822**	25.378**	198.716**	0.052**	443080.1**
	Error	10	0.222	0.222	13.139	0.009	3020.0
	C.V. (%)		0.490	0.250	4.430	8.430	4.1

**Indicate significance at the 1% levels.

The 1000-seed weights ranged between 0.84-1.50 g in Ankara, and 0.50-1.40 g in Şanlıurfa. These findings were similar to the findings of Vollmann et al. (2007) (0.96–1.21 g). The seed yields ranged from 168.4 kg ha^{-1} (PI 650141) to 3151.8 kg ha^{-1} (PI 311735) in Ankara, and from 205.6 (PI 650160) to 3056.0 (PI 650142) kg ha^{-1} in Şanlıurfa (Table 4). Kurt and Gore (2020) reported that the PI 650142 accession was highly tolerant against environmental stresses. The seed yield findings showed a wide variation, which was similar to the findings by Arslan et al. (2014) (1066.1–4198.2 kg ha^{-1}) and Katar et al. (1845.4 kg ha^{-1}). As is known, many factors are effective on the growth and development of plants (Katar and Katar, 2017). These factors are generally divided into two groups, intrinsic and

extrinsic factors (Janına, 2003). Intrinsic factors are controlled by the genetic makeup of the plants (Franz, 1993). Therefore, the growth and development performances of plants with various inherited characteristics differ due to the effect of their genes. On the other hand, extrinsic factors are defined as environmental factors that affect the growth and development vigor of plants (Smykal et al., 2011). This shows that the genetic makeup of the plants, as well as the environmental conditions, are also effective on the growth and development of plants (Reddy et al., 2013). In addition, changing the response of varieties/genotypes from one environment to another, especially in the yield and yield components defined as genotype by environment interactions (GEIs) (Goksoy et al., 2019). Considering the studied characters, the accessions were included in different groups at different locations. It was indicated that the GEIs played a significant role in the studied characters. This may have been attributed to the fact that the accessions responded differently to various environmental conditions. As with other agromorphological features, the accession data showed large differences in the traits between both locations. The rosette period lasted longer in all of the genotypes since Ankara has a cold climate. While genotype 13 had the longest rosette period in Ankara (152.3 days), genotype 38 showed the longest rosette duration in Şanlıurfa (108.8 days). The days to maturity were longer in the Ankara location due to its colder climate. While genotype 39 showed the longest days to maturity in Ankara at 274 days, genotype 38 showed 202.1 days in Şanlıurfa (Table 4).

Table 4. Agromorphologic values and groups of the *Camelina sativa* genotypes created from the Ankara and Şanlıurfa corrected data

Genotype No.	Rosette period		Days to maturity		Plant height (cm)		1000-seed weight (g)		Seed yield (kg ha^{-1})	
	Ankara	Şanlıurfa	Ankara	Şanlıurfa	Ankara	Şanlıurfa	Ankara	Şanlıurfa	Ankara	Şanlıurfa
1	144.3de	101.8d	253f-ı	195.1d	65.3z	82.4m-q	1.03r-u	0.5h	366.6v	2700.4b
2	138.3gh	101.8d	247ı	195.1d	90.6h	69.4t-v	0.99uv	0.5h	808.6s	1339.2k-m
3	135.3jk	100.8e	252g-ı	194.1e	60.8e	59.2y	0.87y	0.5h	2881.2b	568.8v-y
4	136.3ıj	99.8f	268a-c	193.1f	77.1t	89ı-m	1.07n-s	0.6gh	1249.8n-p	872.1p-t
5	134.3kl	92.8m	260c-h	184.1o	77.3t	71.4s-u	1.5a	0.9de	906.6s	1250.1l-n
6	138.3gh	92.8m	264a-e	184.1o	78.1rs	92.4e-j	1.19c-g	1cd	2754.6b	1331.7k-m
7	137.3hı	90.8n	263b-f	182.1p	89.1ı	84.7k-o	1.13h-m	0.7fg	1302.6m-o	695.7t-w
8	138.3gh	92.8m	264a-e	184.1o	85.5j	90.4h-l	1.1k-p	0.7fg	2094.6ef	992.5op
9	134.3kl	102.8c	260c-h	196.1c	83.4k	83l-p	1.23b-d	1cd	1396.2lm	1545.2g-j
10	139.3g	98.8g	248ı	192.1g	75.1u	102.4cd	1.06o-t	0.6gh	2109ef	937.2o-s
11	143.3ef	94.8k	252g-ı	186.1m	78.6r	92.4e-j	1.06o-t	0.9de	1152p-r	1392.4ı-l
12	144.3de	88.8o	253f-ı	180.1q	74.3v	77.8o-s	1.14g-l	1cd	3151.8a	1241.2l-n
13	152.3a	94.8k	261b-g	188.1k	99.5d	99.4c-e	1.02s-v	0.5h	1105.8qr	688.4t-w
14	146.3c	94.8k	255d-ı	186.1m	81.4lm	69.8t-v	1.08m-r	0.9de	1356.6l-n	608.4u-x
15	145.3cd	94.8k	254e-ı	186.1m	78.7qr	74.8r-u	1.14g-l	1cd	373v	687.2t-w
16	143.3ef	101.8d	252g-ı	195.1d	59.5f	72.8s-u	1.01t-v	0.6gh	518.2tu	1103.2no
17	142.3f	94.8k	251g-ı	188.1k	73.2y	68.8uv	1.15f-k	1cd	425.8uv	408y
18	142.3f	94.8k	251g-ı	186.1m	65.5a	80.8n-r	1.16e-j	1.2b	168.4w	1503.2g-k
19	133.3lm	100.8e	250hı	194.1e	76.8t	60.6y	1.14g-l	1.2b	1409.2lm	3056a
20	134.3kl	93.8l	251ghı	185.1n	80.1op	91.6g-k	0.97v	1cd	564.4t	767.2s-v
21	133.3lm	100.8e	250hı	194.1e	63.2bc	60.6y	1.01t-v	1cd	1369.8l-n	1872.8ef
22	137.3hı	100.8e	254e-ı	194.1e	75.2u	111.9ab	1.19c-g	1.4a	795.6s	885.6p-t
23	136.3ıj	104.8b	253f-ı	198.1b	63.6b	94.6e-ı	1.06o-t	1.1bc	2214.6de	1928.8de
24	133.3lm	100.8e	250hı	194.1e	79.4pq	96.9d-h	1.21c-e	1.4a	2214.6de	469.6xy
25	133.3lm	93.8l	250hı	187.1l	102.6b	99.2c-f	0.84y	0.5h	2082.6fg	1376.8j-l
26	136.3ıj	98.8g	253f-ı	192.1g	62.3d	91.9f-k	1.06o-t	1cd	2478.6c	981.6o-r
27	138.3gh	96.8ı	255d-ı	190.1ı	73.7vy	93.6e-ı	1.04q-u	1cd	2143ef	2088.8d
28	134.3kl	102.8c	266a-c	196.1c	101c	86j-n	1.19c-g	0.8ef	776.8s	1375.2j-l
29	135.3jk	100.8e	267a-c	194.1e	76.7t	98.4c-g	1.21c-e	0.8ef	244c	1368.8j-l
30	139.3g	97.8h	271ab	191.1h	96.3e	95de-ı	1.09l-q	0.7fg	2321.2d	789.6q-u
31	139.3g	95.8j	271ab	187.1l	100.2d	72s-u	1.19c-g	1cd	1384lm	1591.2g-ı
32	134.3kl	93.8l	266a-c	185.1n	77.4st	105.4bc	1.28b	1.1bc	1958.2gh	565.6w-y
33	133.3lm	90.8n	265a-d	182.1p	88.9ı	90.4h-l	1.05p-t	0.6gh	2816.2b	205.6z
34	136.3ıj	95.8j	268a-c	189.1j	91.8g	74.4r-u	1.18d-h	1cd	1702.6jk	972.9o-q
35	135.3jk	95.8j	267a-c	189.1j	78.2r	75.4q-u	1.24bc	0.9de	1815ıj	784.1r-u
36	135.3jk	95.8j	267a-c	189.1j	82.1l	76.7p-t	1.11j-o	0.8ef	2123ef	1678.5fg
37	132.3m	92.8m	264a-e	184.1o	80.5no	115.4a	1.21cde	1cd	1065r	896.1p-s
38	135.3jk	108.8a	261b-g	202.1a	81.1mn	63.7vy	1.2c-f	0.9de	1216.8o-q	1656.1gh
39	148.3b	99.8f	274a	193.1f	103.8a	90h-l	1.09l-q	0.8ef	2470.8c	1316.9k-m
40	138.3gh	102.8c	264a-e	196.1c	93.1f	70t-v	1.12ı-n	0.8ef	1870.2hı	1474.1h-k
41	139.3g	90.8n	265a-d	182.1p	72z	96.4d-ı	1.16e-j	0.9de	1025.4r	1594.1gh
42	137.3hı	92.8m	263b-f	184.1o	62.8cd	92.4e-j	1.17e-ı	0.9de	1467.6l	1650.1gh
C1	142.50c-f	97.0cd	263.17e-g	190.0bc	83.80fı	85.53d-g	1.111ı-l	1.077a-d	1679.5k	1269.2lm
C2	143.83cd	96.0de	265.00cd	189.0c	78.03k-n	86.75d-f	1.140e-k	1.117a-c	1824.4ı	1215.3mn
C3	144.33cd	93.5fh	260..33hı	184.5f	79.05j-m	73.40k-p	1.188c-k	1.150a-c	2136.5ef	2243.8c

When the correlations of the plant properties with each other were studied, significant differences were observed among the locations. Although there was a positive correlation between the rosette period and the days to maturity in Şanlıurfa, there was no correlation between these parameters in Ankara. A positive correlation was observed between the days to maturity with plant height and 1000-seed weight in Ankara. Additionally, there was a positive correlation between the seed yield and the rosette period in Şanlıurfa (Table 5).

Table 5. Pearson's correlation coefficient analysis for the different plant characteristics of the 42 Camelina genotypes

Location		**Rosette period**	**Days to maturity**	**Plant height (cm)**	**1000-seed weight (g)**	**Seed yield (kg ha^{-1})**
Ankara	Rosette period	**1**				
	Days to maturity	–0.002	**1**			
	Plant height (cm)	0.090	**0.487****	**1**		
	1000-seed weight (g)	–0.154	**0.404****	0.018	**1**	
	Seed yield (kg ha^{-1})	–0.161	0.253	0.093	–0.118	**1**
Şanlıurfa	Rosette period	**1**				
	Days to maturity	**0.992****	**1**			
	Plant height (cm)	–0.235	–0.220	**1**		
	1000–seed weight (g)	–0.030	–0.061	0.079	**1**	
	Seed yield (kg ha^{-1})	**0.304***	0.285	–0.259	0.131	**1**

Values in bold are different than 0 with a significance level of 5% (*P < 0.05) and 1% (**P < 0.01).

The first two PCAs were used to draw a graph in order to see the pattern of variation between the locations The PCA-based correlation matrix for the first two principal components accounted for 57.237% and 67.098% of the variation in Ankara and Şanlıurfa, respectively (Table 6).

Table 6. Eigenvectors, eigenvalues, and individual and cumulative percentages of variation explained by the first five principal components (PC) after assessing morphological properties in *Camelina sativa* accessions

	PCA1		**PCA2**		**PCA3**		**PCA4**		**PCA5**	
	Ankara	**Ş. Urfa**	**Ankara**	**Ş. Urfa**	**Ankara**	**Ş. Urfa**	**Ankara**	**Ş. Urfa**	**Ankara**	**Ş. Urfa**
Rosette period	–0.085	0.630	0.742	–0.114	–0.315	0.283	–0.572	–0.087	–0.123	–0.709
Days to maturity	0.697	0.625	0.046	–0.148	–0.064	0.292	–0.158	–0.076	0.694	0.705
Plant height (cm)	0.526	–0.299	0.451	–0.040	0.022	0.787	0.584	0.539	–0.422	–0.011
1000–seed weight (g)	0.391	–0.028	–0.492	0.844	–0.537	0.324	–0.297	–0.425	–0.478	0.022
Seed yield (kg ha^{-1})	0.277	0.350	–0.047	0.500	0.780	–0.333	–0.466	0.718	–0.310	0.009
Eigenvalue	1.695	2.266	1.167	1.089	1.128	0.977	0.697	0.661	0.313	0.007
Variability (%)	33.898	45.327	23.339	21.770	22.561	19.532	13.938	13.221	6.264	0.149
Cumulative (%)	33.898	45.327	57.237	67.098	79.798	86.629	93.736	99.851	100.000	100.000

When the correlations of the plant properties of the same genotypes were examined for both locations together via biplot analysis, large differences were observed between the locations (Figure 1).

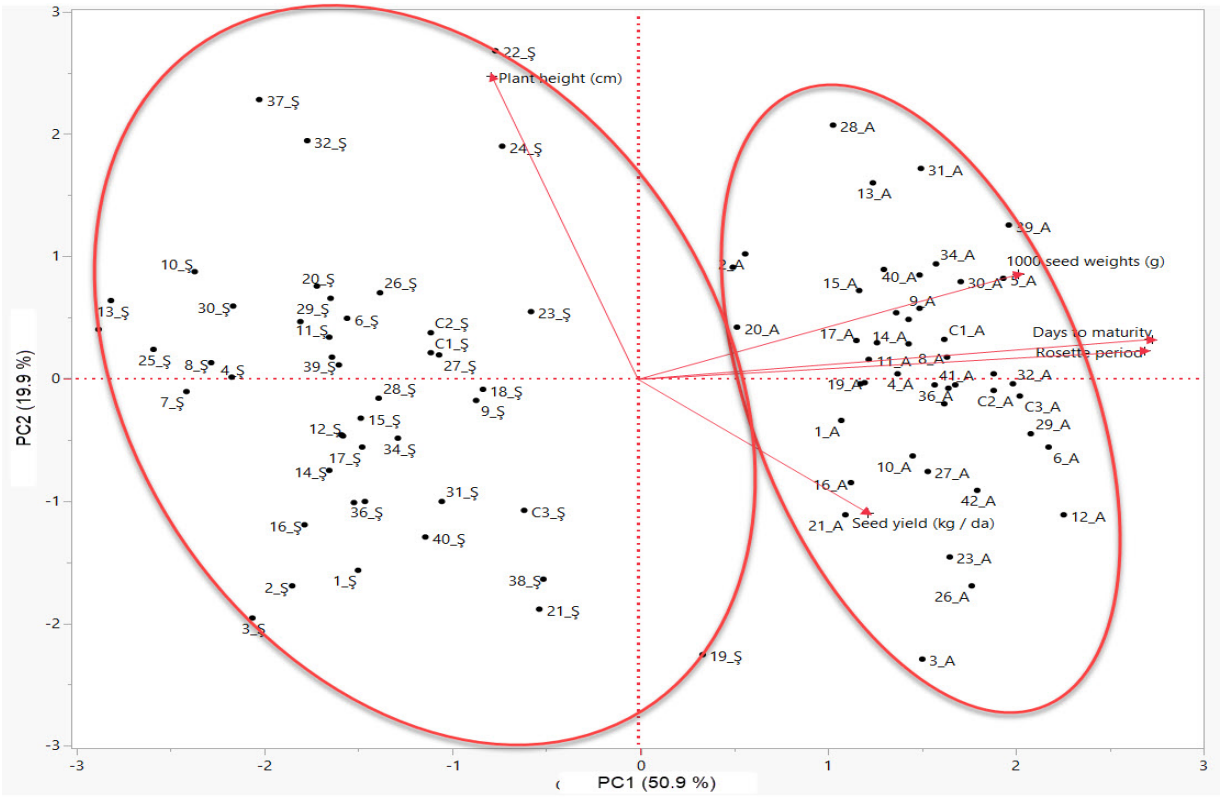

Figure 1. Biplot diagram of the agromorphological properties of the *C. sativa* genotypes in Ankara and Şanlıurfa.

Conclusion

It was observed that plant parameters were significantly affected by climate. Therefore, it can be suggested that a relationship cannot be established between genotypes based on agromorphological characters.

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Research Article

Storage and *In-vitro* Germination of Some Olive Pollens

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Pollen preservation

Abstract: In this study, the effect of storage at different times and temperatures on *in vitro* olive pollen germination was investigated. Pollens of Gemlik and Domat cultivars and GE119 and GK138 genotypes were stored at +4 °C, -20 °C, and -80 °C for 7, 35, 200, and 365 days, respectively. *In vitro* germination status of pollen was determined by the petri agar method, by choosing the most suitable nutrient medium for each olive. The most suitable germination media for Gemlik, Domat, GE119, and GK138 pollens were 50 ml water + 15% sucrose + 0.7% agar + 75 ppm boric acid, 50 ml water + 15% sucrose + 0.7% agar, 50 ml water + 25% sucrose + 0.5% agar, 50 ml water respectively. The interaction effect between storage time, temperatures, and cultivar on pollen germination and diameter was determined. At the end of the storage period, the highest pollen germination and diameter were observed in the Gemlik cultivar. Additionally, -80 °C temperature for Gemlik and Domat cultivars and -20 °C temperature for GE119 and GK138 genotypes were suitable for 35 days of storage. All olive pollens in the current study had germination rates below 9% in the following storage periods. The results show that storing olive pollens at sub-zero temperatures will reduce the need for daily fresh pollen collection required for important scientific studies such as breeding and artificial pollination.

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1. Introduction

The olive tree (*Olea europaea* L.) is known as the most iconic tree of the world, originating about six thousand years ago, emerging in connection with the emergence of many civilizations in Upper Mesopotamia and Southern Asia Minor (Zohary et al., 2012; Besnard et al., 2018). Semites carried out the first olive cultivation, and this situation spread to the Aegean Sea Islands, Anatolia, Egypt, Greece, Italy, Morocco, Spain, and Tunisia (Özkaya et al., 2008). It has great socio-economic importance in the Mediterranean basin (Topaklı and Hepaksoy, 2019). Olive and olive oil consumption is increasing due to health components and many high-quality olive cultivars are grown in olive growing regions (Rallo et al., 2018; Shavakhi et al., 2021). However, as in every fruit species, some problems arise in olive cultivation. Since parthenocarpic fruits have no commercial value in olives, pollination is needed for proper yield (Koubouris et al., 2009). In addition, olives show absolute periodicity and yield decreases

over the years. Similar issues are seen in olive orchards that are not established with the appropriate pollinator due to low self-productivity (Alagna et al., 2019; Dölek Gencer and Özkaya, 2020). Furthermore, scientists working with olives emphasize the importance of cross-pollination to increase fruit set, especially in partially self-fertile and self-sterile cultivars (Sanchez-Estrada and Cuevas, 2018; Sanchez-Estrada and Cuevas, 2019). Cross-pollination is therefore a mandatory practice for completely self-infertile cultivars. For all these reasons, biological or mechanical artificial pollination with pre-collected and stored pollen together with the selection of suitable pollinators for high fruit set is considered an important cultural process in olive production (Sanchez-Estrada and Cuevas, 2020).

Pollen is always needed to pollinate olives due to the lack of sample flowering caused by structural in the cultivars and the fact that the flowering periods of the cultivars with very different flowering dates do not coincide. For this, pollen retention is of particular importance. Only at low temperatures can high pollen viability and germination ratings be protected for an extended period of time. Hechmi et al. (2015) found that the pollens of Koroneik, Frantoio, Manzanille, and Nabali olive cultivars all have a great tolerance to low temperatures (-20 and 10 °C). These pollens showed decreasing germination during storage periods and this situation was clearly evident even after 1 month of storage. And also, pollen deaths were observed after 1 month in pollens stored at 25 °C, while deaths occurred after 180 days in pollen stored at -20 and +10 °C.

Pollen storage and germination are the subjects of studies for plant breeding, conservation of gene resources, and examining the physiological conditions of different species. Artificial pollination is a necessity in fruit growing, especially in hybridization breeding and the selection of suitable pollinators. For this reason, pollen storage has a special importance in terms of providing the pollen needed at different times. Besides, pollen storage is considered necessary for the preservation of olive genetic resources and their use in olive improvement programs. In the studies conducted with different fruit species, it is stated that pollen can be stored for 1-3 months at 4 °C, but temperatures below 0 °C are more suitable for longer storage (El Kadri and Ben Mimoun, 2020; Özcan, 2020; Özcan and Bükücü, 2020). Moreover, pollen germination after cold storage is reported to differ even among cultivars of the same species (Kuroki et al., 2017; Quinet and Jacquemart, 2020), while germination levels can vary according to factors such as the nutrient content of the germination medium, humidity, temperature and pH (Mertoğlu et al., 2018; Kılıç et al., 2020; Güçlü et al., 2021; Fagundes et al., 2021; Đorđević et al., 2022).

It was intended to find the best germination medium for pollen belonging to different olive cultivars and genotypes under *in vitro* conditions and to determine the germination levels with pollen swelling changes after storing these pollens at different temperatures.

2. Material and Methods

2.1. Material

Pollen of Gemlik and Domat olive cultivars and GE119 (Gemlik × Edincik Su) and GK138 (Gemlik × Karamürsel Su) olive genotypes were grown in Atatürk Horticultural Central Research Institute, were used as materials.

2.2. Method

Laboratory studies were carried out together with Ankara University, Faculty of Agriculture, Department of Horticulture, and Bülent Ecevit University in 2015-2016. Pollen was collected early in the morning when the flowers reached the balloon stage and brought to the laboratory under appropriate conditions. After the petals were removed, the filaments were separated with forceps and kept at 20 °C for 24 hours. Due to its practicality, the petri dish agar method was used to determine in-vitro pollen germination rates (Koyuncu, 2006). In order to find the most suitable nutrient medium for each cultivar and genotype, 23 different combinations of water, sucrose, boric acid, and calcium chloride were studied (Table 1). The sown pollens were taken into ovens at a temperature of 24 °C determined as a result of the preliminary trials, and *in vitro* germination rates were checked after 3 hours. Pollen diameters were observed after 24 hours under a Leica light microscope at 40 magnification using an ocular micrometer. Pollens were stored at +4 °C, -20 °C, and -80 °C for 7, 35, 200, and 365 days.

Table 1. Nutrient media combinations used in *in vitro* germination

Nutrient No	Nurtient Name	Nurtient Concentrations
1	**Z1**	50 ml water
2	**Z2**	50 ml water + %10 sucrose + %0.7 agar
3	**Z3**	50 ml water + %10 sucrose + %0.5 agar
4	**Z4**	50 ml water + %10 sucrose + %0.3 agar
5	**Z5**	50 ml water + %10 sucrose + %0.1 agar
6	**Z6**	50 ml water + %15 sucrose + %0.7 agar
7	**Z6A**	50 ml water + %15 sucrose + %0.7 agar + 75 ppm boric acid
8	**Z6B**	50 ml water + %15 sucrose + %0.7 agar + 100 ppm boric acid
9	**Z6C**	50 ml water + %15 sucrose + %0.7 agar + 125 ppm boric acid
10	**Z6D**	50 ml water + %15 sucrose + %0.7 agar + %0.01 calcium chloride
11	**Z6E**	50 ml water + %15 sucrose + %0.7 agar + %0.05 calcium chloride
12	**Z6F**	50 ml water + %15 sucrose + %0.7 agar + %0.1 calcium chloride
13	**Z7**	50 ml water + %15 sucrose + %0.5 agar
14	**Z8**	50 ml water + %15 sucrose + %0.3 agar
15	**Z9**	50 ml water + %15 sucrose + %0.1 agar
16	**Z10**	50 ml water + %20 sucrose + %0.7 agar
17	**Z11**	50 ml water + %20 sucrose + %0.5 agar
18	**Z12**	50 ml water + %20 sucrose + %0.3 agar
19	**Z13**	50 ml water + %20 sucrose + %0.1 agar
20	**Z14**	50 ml water + %25 sucrose + %0.7 agar
21	**Z15**	50 ml water + %25 sucrose + %0.5 agar
22	**Z16**	50 ml water + %25 sucrose + %0.3 agar
23	**Z17**	50 ml water + %25 sucrose + %0.1 agar

2.3. Statistical analysis

The study was conducted according to the randomized plot design with 3 replications and 3 petri dishes in each replication. Each petri dish was divided into 3 zones and 250 pollens were counted in these zones. The data obtained in the study were analyzed with separate analyses. Firstly, a two-way ANOVA was applied according to the model: $Yijk = \mu + OCi + NCj + (OC \times NC)ij + eijk$, where:

Yijk is the dependent variable,
μ is the overall mean,
OCi is the olive cultivar or genotype (i = Gemlik, Domat, GE119, or GK138)
NCj is the nutrient medium (j = Z1, Z2,….., Z16, or Z17)
(OC × NC)ij is the interaction between the olive cultivar or genotype and the nutrient medium and eijk is the residual error term.

After that, the nutrient medium was selected for each cultivar or genotype and pollens to determine the best storage temperature and duration and the study was a 4 × 3 × 4 factorial design with 4 olive cultivars or genotypes, 3 storage temperatures, and 4 storage durations. Data were analyzed via a GLM procedure in the Minitab Version 17 package program (Minitab, Inc., State College, PA). Significant differences that emerged at the end of the variance analysis were compared with Tukey's test ($P \leq 0.05$) with MSTAT-C statistical software. The results were expressed as mean values with standard error means (SEM). Cultivar and germination medium factors in determining the best germination medium; cultivar or genotype, storage time, and storage temperature factors and their interactions in the determination of pollen germination rate and diameter during storage were taken into account as variables.

3. Results and Discussion

Various chemicals are needed for optimal pollen germination and tube growth. It is important to determine the most suitable germination environment in different species and even between different genotypes of the same species, usually by modifying the concentrations of these chemicals. In our study, pollen planted in different nutrient media swelled in the form of beads, and germination was observed with pollen tubes within 1 hour in Gemlik pollen as seen in pear pollen (Vasilakakis and Porlingis, 1985) and within 3 hours in other cultivars and genotypes (Figure 1 and 2).

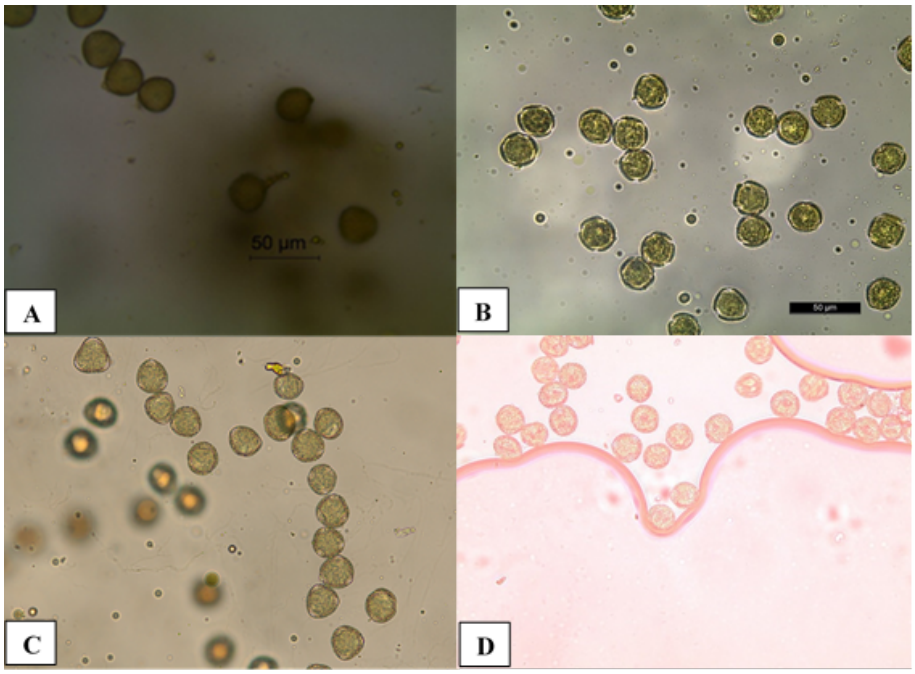

Figure 1. Swelling of pollens Gemlik (A), Domat (B), GE 119 (C), GK 138 (D).

The interaction of cultivar and medium appears to be important in the germination of olive pollen on the 10th day in different nutrient media (P = 0.001). Z6A (50 ml water + 15% sucrose + 0.7% agar + 75 ppm boric acid) for Gemlik, Z6 (50 ml water + 15% sucrose + 0.7% agar) for Domat, Z15 (50 ml water + 25% sucrose + 0.5% agar) for GE119 and Z1 (50 ml water) for GK138 were determined to be the best combinations for pollen germination (Table 2). In these nutrient media, a germination rate of 45% in the pollen of the Gemlik cultivar, 44% in the Domat cultivar, 42% in the GE119 genotype, and 27% in the GK138 genotype was observed. In addition, the germination levels of olive pollen differed for each medium. In this context, it is known that the effect of different nutrient media on pollen germination differs according to plant species and cultivars (Altunbaş and Engin, 2016).

The interaction of cultivar × storage time × storage temperature is important for *in vitro* germination of olive pollen stored at different temperatures and storage times (P = 0.000) (Table 3).

When the differences between the cultivars were examined in terms of pollen germination rates for each storage period and storage temperature, it was seen that the pollen of the Gemlik cultivar germinated more than other pollens at all storage temperatures during the 7th day of storage, and the GK138 genotype showed similar germination at only +4 °C for the same period. While the Gemlik cultivar and GE 119 genotype showed a high germination rate at both +4 °C and -20 °C temperatures on the 35th day, only the Gemlik cultivar achieved a high germination rate at -80 °C in the same period. All olive pollens had similar germination after 200 days of storage at -20 °C, but Gemlik showed the highest germination rate among the cultivars at -80 °C for the same period. Decay was observed in all olive pollens stored at +4 °C on the 365th day of storage. No germination was seen in GE 119 and GK138 genotypes stored at -20 °C for 365 days and in GE 119 genotypes stored at -80 °C for the same time. Gemlik pollens had higher germination rates than other olive pollens, and GK138 pollens had lower germination rates. Similarly, it is stated in many studies that different cultivars and genotypes of the same species have different pollen germination rates (Ferri et al., 2008; Bhat et al., 2012; Mesnoua et

al., 2018; Aldahadha et al., 2019). Considering cultivars and genotypes, it is thought that there is a strong genetic influence on pollen germination.

Table 2. *In vitro* germination percentages of olive pollen on day 0th in different nutrient media

Nutrient Media	Gemlik	Domat	GE119	GK138
Z1	17 ± 2.31 C, cdef*	32 ± 2.31 A, b*	20 ± 2.31 BC, bcde*	27 ± 2.31 AB, a*
Z2	3 ± 1.73 C, gh	25 ± 2.31 A, bcd	14 ± 2.31 B, defg	5 ± 2.31 C, cd
Z3	8 ± 2.31 A, fgh	11 ± 2.31 A, efgh	6 ± 2.31 A, gh	12 ± 2.31 A, bc
Z4	1 ± 0.57 C, h	17 ± 2.31 B, cdef	26 ± 2.31 A, bc	20 ± 2.31 AB, ab
Z5	9 ± 2.31 B, fgh	16 ± 2.31 AB, cdefg	20 ± 2.31 A, bcde	9 ± 2.31 B, bcd
Z6	17 ± 2.31 B, cdef	44 ± 2.31 A, a	18 ± 2.31 B, bcdef	12 ± 2.31 B, bc
Z6A	45 ± 2.31 A, a	6 ± 2.31 C, fgh	20 ± 2.31 B, bcde	0 ± 0.00 C, d
Z6B	12 ± 2.31 A, efgh	0 ± 0.00 B, h	9 ± 2.31 A, efgh	4 ± 2.31 AB, cd
Z6C	26 ± 2.31 A, bc	17 ± 2.31 B, cdef	8 ± 2.31 C, fgh	8 ± 2.31 C, cd
Z6D	17 ± 2.31 A, cdef	13 ± 2.31 A, efg	10 ± 2.31 A, efgh	9 ± 2.31 A, bcd
Z6E	17 ± 2.31 AB, cdef	19 ± 2.31 A, cde	5 ± 2.31 C, gh	10 ± 2.31 BC, bcd
Z6F	24 ± 2.31 A, bcd	14 ± 2.31 B, defg	7 ± 2.31 B, egh	12 ± 2.31 B, bc
Z7	15 ± 2.31 B, cdef	14 ± 2.31 B, defg	28 ± 2.31 A, b	0 ± 0.00 C, d
Z8	32 ± 2.31 A, b	18 ± 2.31 B, cde	16 ± 2.31 B, cdefg	20 ± 2.31 B, ab
Z9	12 ± 2.31 B, efgh	26 ± 2.31 A, bc	10 ± 2.31 B, efgh	0 ± 0.00 C, d
Z10	9 ± 2.31 A, fgh	5 ± 2.31 A, gh	2 ± 1.15 A, h	9 ± 2.31 A, bcd
Z11	22 ± 2.31 A, bcde	10 ± 2.31 B, efgh	16 ± 2.31 AB, cdefg	9 ± 2.31 B, bcd
Z12	25 ± 2.31 B, bc	36 ± 2.31 A, ab	14 ± 2.31 C, defg	8 ± 2.31 C, cd
Z13	9 ± 2.31 C, fgh	33 ± 2.31 A, ab	23 ± 2.31 B, bcd	12 ± 2.31 C, bc
Z14	10 ± 2.31 B, fgh	18 ± 2.31 B, cde	28 ± 2.31 A, b	0 ± 2.31 C, d
Z15	13 ± 2.31 B, defg	15 ± 2.31 B, cdefg	42 ± 2.31 A, a	15 ± 2.31 B, bc
Z16	15 ± 2.31 A, cdef	12 ± 2.31 A, efg	9 ± 2.31 A, efgh	0 ± 0.00 B, d
Z17	24 ± 2.31 A, bcd	0 ± 0.00 C, h	9 ± 2.31 B, efgh	0 ± 0.00 C, d

*: mean ± standard error. The capital letters in the first row represent the differences between cultivars in each nutrient medium, and the lower letters in the second row represent the differences between the nutrient media for each cultivar at $P \leq 0.05$.

When the differences between temperatures for each cultivar and storage period are examined, pollen germination rates at all temperatures on the 7th day for the Gemlik cultivar were in the same statistical group, while the positive effects of -20 °C and -80 °C temperatures in other storage processes were observed. It was determined that only pollens stored at -80 °C on the 35th day showed higher germination in the Domat cultivar, and all temperatures had similar effects on the other days. Seven days of storage at +4 °C and 35 days of storage at -20 °C were found to be effective for the GE 119 and the GK138 genotypes, while the other days were ineffective. Similar to our study, Özcan (2020) stated that storing pollens of different cherry cultivars at temperatures below -20 °C had a positive effect on germination compared to higher temperatures. In general, germination rates of pollens stored at temperatures above zero are lower than those stored below zero (Pham et al., 2015). However, it is specified that +4 °C temperature is suitable for the preservation of some pollens under suitable conditions (Martínez-Gómez et al., 2000). This situation is thought to be caused by the differences in genetic characteristics of species and cultivars. In our study, the ineffectiveness of -20 °C and -80 °C temperatures in the GK138 genotype can be ascribed to the unfavorable effects of cell injuries that occur when some pollens freeze and thaw at sub-zero temperatures (Luza and Polito, 1988).

When the differences between storage times for each cultivar and storage temperature were examined, decreases in in-vitro pollen germination rates were determined after 200 days at the same temperatures for all cultivars. Especially in the 200th and 365th days analyses, low germination rates below 9% were observed in all pollens. Similar to our study, Mortazavi et al. (2010) reported that palm pollens did not germinate after 200 days of cold storage, but Martinez-Gomez et al. (2002) stated that different almond cultivars' pollens had germination rates of over 40% in 1-year storage period at subzero temperatures. In our study, the highest germination rates were observed in Gemlik (59%) and Domat (24%) cultivars stored at -80 °C for 35 days, in the GE 119 (40%) genotype stored at -20 °C for 35 days, and in the GK 138 genotype stored at +4 °C for 7 days. Especially after the 35th day of storage, pollen germination rates, which decrease with the progress of storage, are compatible with other studies (Bolat

and Güleryüz, 1994). In a study conducted with the Manzanillo olive cultivar, the germination of pollens stored at -20 °C decreased by 40% after 365 days (Pinney and Polito, 1990). Besides that, it has been reported that the pollens of the Arbequina olive cultivar can be stored for 60 days at -10 °C (Zambon et al., 2018).

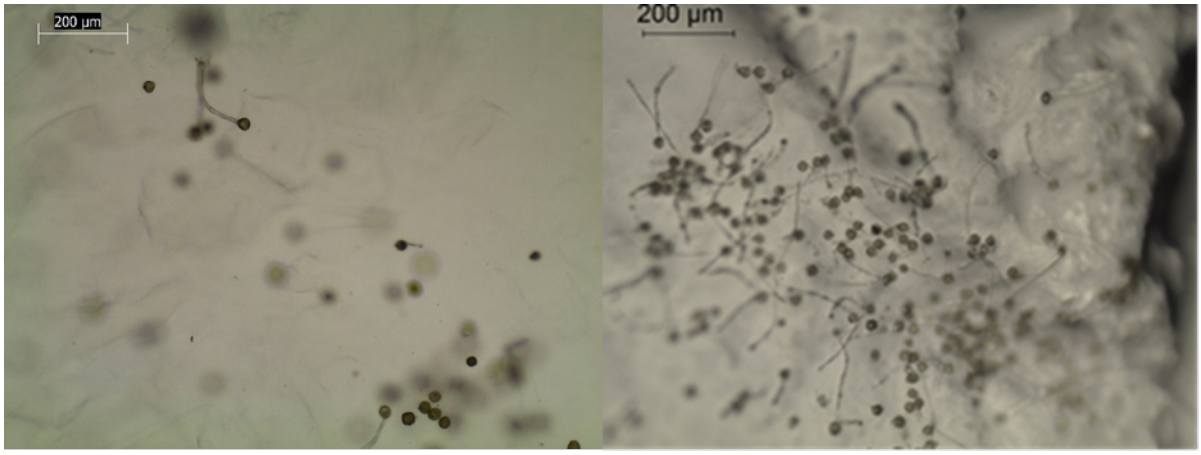

Figure 2. Pollen tube formation and germination in Gemlik cultivar.

The interaction of cultivar × storage time × storage temperature was important for the effect of the storage of olive pollens at different temperatures and storage times on pollen diameters ($P <= 0.001$) (Table 3). During the study, pollen diameters were determined between 18.14 - 25.80 µm. These values are seen as close values for Italian (17-20 µm) (Isocrono and Vallania, 2009), Greek (14-22 µm) (Koubouris et al., 2012), and Iranian olive (15-29 µm) (Javady and Arzani, 2001) cultivars.

When the differences between cultivars were examined in terms of pollen diameters for each storage period and storage temperature, it was observed that the Gemlik cultivar had higher pollen diameters in 7 days of storage at +4 °C and -20 °C, and the GK 138 pollen diameters in 7 days of storage at -80 °C. While GK 138 genotype had lower pollen diameters at +4 °C and -20 °C temperatures in 35th day samples, low pollen diameter values were determined in the GK 138 and Domat pollen at -80 °C in the same storage period. The highest pollen diameter was observed in the pollen of the GE 119 genotype in the samples stored on the 200th day at all temperatures. Among the pollens stored at -20 °C and -80 °C for 365 days, GK 138 had the lowest pollen diameter.

When the differences between temperatures for each cultivar and storage period were examined, it was discovered that +4 °C temperature for Gemlik and GE 119 pollens, as well as +4 °C and -80 °C temperatures for Domat and GK 138 pollens, were effective on pollen diameter values on the 7th day of storage. While Gemlik, GE 119, and GK 138 pollen diameters were higher after 35 days of storage at -80 °C, higher pollen diameters were determined for Domat at +4 °C during the same storage period. During storage periods of 200 and 365 days, temperatures of -20 °C and -80 °C for Gemlik and Domat cultivars, -80 °C for the GE 119 genotype, and +4 °C and -20 °C for the GK 138 genotype were effective in terms of pollen diameter.

When the differences between the storage times for each cultivar and storage temperature were analyzed, it was observed that the diameter of the pollen decreased with the progression of the storage period for all pollens stored at +4 °C. The changes in the pollen diameters after the 35th day at -20 °C temperature in the Gemlik cultivar were statistically insignificant, and the values were in the same statistical group in all storage processes at -80 °C. The highest pollen diameter in the Domat cultivar was determined on the 365th day of storage at -20 °C and on the 7th day of storage at -80 °C. For the GE 119 genotype, the diameters of the pollen stored at -20 °C and -80 °C were found to be high on the 200th and 365th days. In the GK 138 genotype, high pollen diameters were determined on the 200th day of storage at -20 °C and on the 7th day at -80 °C.

Table 3. *In vitro* germination rate and diameters of olive pollens during storage at different temperatures

Cultivar × Storage Time × Storage Temperature	Pollen Germination Rate (%)	Pollen Diameter (µm)
Gemlik × 7. days × +4 °C	32 ± 2.31 AB,a,A*	25.80 ± 0.00 A,a,A*
Domat × 7. days × +4 °C	11 ± 2.31 C,b,A	23.62 ± 0.04 C,a,A
GE119 × 7. days × +4 °C	27 ± 2.31 B,a,A	25.42 ± 0.25 AB,a,A
GK138 × 7. days × +4 °C	37 ± 2.31 A,a,A	25.00 ± 0.00 B,a,A
Gemlik × 7. days × -20 °C	35 ± 2.31 A,a,A	22.72 ± 0.10 A,b,A
Domat × 7. days × -20 °C	18 ± 2.31 B,a,A	20.47 ± 0.47 B,b,B
GE119 × 7. days × -20 °C	10 ± 2.31 C,b,B	19.45 ± 0.02 C,b,C
GK138 × 7. days × -20 °C	16 ± 2.31 BC,b,A	18.89 ± 0.21 C,b,C
Gemlik × 7. days × -80 °C	38 ± 2.31 A,a,B	21.53 ± 0.50 C,c,A
Domat × 7. days × -80 °C	8 ± 2.31 C,b,B	23.92 ± 0.24 B,a,A
GE119 × 7. days × -80 °C	16 ± 2.31 B,b,A	18.75 ± 0.00 D,c,C
GK138 × 7. days × -80 °C	11 ± 2.31 BC,b,A	25.00 ± 0.00 A,a,A
Gemlik × 35. days × +4 °C	33 ± 2.31 A,c,A	21.42 ± 0.13 A,a,B
Domat × 35. days × +4 °C	16 ± 2.31 B,b,A	21.58 ± 0.19 A,a,B
GE119 × 35. days × +4 °C	27 ± 2.31 A,b,A	21.14 ± 0.13 A,a,C
GK138 × 35. days × +4 °C	0 ± 0.00 C,b,B	0.00 ± 0.00 B,b,B
Gemlik × 35. days × -20 °C	41 ± 2.31 A,b,A	21.50 ± 0.25 A,a,B
Domat × 35. days × -20 °C	14 ± 2.31 B,b,A	20.93 ± 0.09 A,b,AB
GE119 × 35. days × -20 °C	40 ± 2.31 A,a,A	20.91 ± 0.06 A,a,B
GK138 × 35. days × -20 °C	10 ± 2.31 B,a,A	18.84 ± 0.13 B,a,C
Gemlik × 35. days × -80 °C	59 ± 2.31 A,a,A	21.08 ± 0.12 A,a,A
Domat × 35. days × -80 °C	24 ± 2.31 B,a,A	17.64 ± 0.00 B,c,C
GE119 × 35. days × -80 °C	16 ± 2.31 C,c,A	20.57 ± 0.25 A,a,B
GK138 × 35. days × -80 °C	6 ± 2.31 D,ab,AB	17.64 ± 0.00 B,a,C
Gemlik × 200. days × +4 °C	0 ± 0.00 A,b,B	21.39 ± 0.20 B,a,B
Domat × 200. days × +4 °C	0 ± 0.00 A,a,B	20.15 ± 0.16 C,b,C
GE119 × 200. days × +4 °C	0 ± 0.00 A,a,B	22.21 ± 0.18 A,b,B
GK138 × 200. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 D,c,B
Gemlik × 200. days × -20 °C	5 ± 2.31 A,ab,B	21.18 ± 0.31 B,a,B
Domat × 200. days × -20 °C	4 ± 2.31 A,a,B	21.07 ± 0.14 B,a,AB
GE119 × 200. days × -20 °C	5 ± 2.31 A,a,BC	22.03 ± 0.18 A,b,A
GK138 × 200. days × -20 °C	3 ± 1.73 A,a,B	21.25 ± 0.03 B,a,A
Gemlik × 200. days × -80 °C	9 ± 2.31 A,a,C	21.08 ± 0.10 B,a,A
Domat × 200. days × -80 °C	1 ± 0.57 B,a,C	20.78 ± 0.15 B,a,B
GE119 × 200. days × -80 °C	3 ± 1.73 AB,a,B	23.83 ± 0.40 A,a,A
GK138 × 200. days × -80 °C	1 ± 0.57 B,a,B	19.32 ± 0.12 C,b,B
Gemlik × 365. days × +4 °C	0 ± 0.00 A,b,B	0.00 ± 0.00 A,b,C
Domat × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,b,D
GE119 × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,c,D
GK138 × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,c,B
Gemlik × 365. days × -20 °C	8 ± 2.31 A,a,B	21.71 ± 0.07 A,a,B
Domat × 365. days × -20 °C	2 ± 1.15 AB,a,B	21.41 ± 0.25 A,a,A
GE119 × 365. days × -20 °C	0 ± 0.00 B,a,C	21.78 ± 0.07 A,b,A
GK138 × 365. days × -20 °C	0 ± 0.00 B,a,B	19.88 ± 0.24 B,a,B
Gemlik × 365. days × -80 °C	5 ± 2.31 A,ab,C	21.52 ± 0.05 B,a,A
Domat × 365. days × -80 °C	3 ± 1.73 A,a,BC	21.39 ± 0.30 B,a,B
GE119 × 365. days × -80 °C	0 ± 0.00 A,a,B	24.35 ± 0.07 A,a,A
GK138 × 365. days × -80 °C	3 ± 1.73 A,a,B	18.14 ± 0.07 C,b,C
Significant Effects		
Cultivar (C)	< 0.001	< 0.001
Temperature (T)	< 0.001	< 0.001
Storage Time (ST)	< 0.001	< 0.001
C × T	< 0.001	< 0.001
C × ST	< 0.001	< 0.001
T × ST	< 0.001	< 0.001
C × T × ST	< 0.001	< 0.001

*: mean ± standard error. Capital letters in the first row show the differences between cultivars for each storage period and storage temperature, lower letters in the second row show the differences between storage temperatures for each storage period and cultivar, and capital letters in the third row show the differences between storage times for each cultivar and storage temperature according to the Tukey test. ≤ 0.05 refers to the error level.

In the current study, the cultivar, storage temperature, and duration were effective in terms of pollen diameters. However, it is stated that these variables were ineffective on Olivo della Strega olive pollen diameters (Petruccelli et al., 2021).

Conclusion

It is thought that 35 days of storage for Gemlik, Domat, and GE119 pollens, and 7 days of storage for GK138 pollens, were considered appropriate. All cultivars and genotypes had very low germination ability after 200 and 365 days of storage. In terms of storage temperature, the pollen of Gemlik and Domat cultivars had higher germination ability with storage at -80 °C, and 20 °C for GE119 genotype pollens, and +4 °C for GK138 genotype pollens were found to be effective. In terms of pollen diameters, while the pollen of the Gemlik cultivar had a higher diameter than the others, the GK138 genotype had a low pollen diameter. However, pollen diameters were higher in olive pollens stored at -80 °C for 35 days. The study showed that Gemlik, Domat, and GE119 pollens could be stored acceptably at zero temperatures for 35 days. Aside from their contribution to artificial pollination studies, the obtained results are critical in terms of incompatibility evaluations and producing a solution to the pollination problem of genotypes that did not bloom at the same time.

In cross-pollination of cultivars, the effective pollination period and the amount of time pollen can stay alive can be very different from one another. In addition, thanks to pollen retention, pollen from cultivars that are not already present in the region can provide great convenience to pollination efforts by preserving their viability and germination abilities. It also helps to save time during studies.

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Research Article

Investigations of Some Morphological, Pomological, and Physiological Parameters with Mineral Content of Different *Rosa* L. Taxa Grown under Greenhouse Condition

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Abstract: Horticulture is a discipline mainly concerned with the cultivation of plant material for food supply, medicinal use, or functional and aesthetic purposes by humans, they are a genetically diverse group and play an important role in the economy of modern society, as well as at the center of the healthy diet of the urban population. In this respect, *Rosa* L. are important plants for traditional pharmacological practices and landscape studies. In this context, within the scope of the research, some morphological, pomological, physiological, and mineral contents of important taxa such as *Rosa alba* L.'Semiplena', *R. banksiae* R.Br. cv '*Alba*', *R. canina* L.'Yildiz', *R. centifolia* L., *R. chinensis* Jacq. 'Old Blush', *R. foetida* Herrm., *R. heckeliana* Tratt. subsp. *vanheurckiana* (Boiss. Ö. Nilsson), *R. hemispharica* J.Herrm., *R.* x *odorata* (hort ex. Andrews) Sweet 'Louis XVI', *R. pisiformis* (Christ) Sosn., *R.* x *damascena* Mill., and *R.* x *damascena* Herrm. 'Semperflorens'(Loisel. & Michel) Rowley for landscape design and horticulture were determined. Within the scope of the research, the morphological, physiological, and pomological characteristics and nutrient contents of taxa adapted to semi-arid conditions and different *Rosa* taxa spreading in Anatolia were determined. Principal component analysis and cluster analysis were also used to determine the similarities and differences of these parameters measured in different *Rosa* L. taxa.

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1. Introduction

Rosa species are in the *Rosales* order. There are approximately 8000 species of economic and ecological importance, nine families of this order are divided into three main subgroups. Members of the *Rosaceae* family, which is one of these three subgroups, are generally distributed in the area from the temperate regions of the Northern Hemisphere to the subtropical region and contain nearly 100 genera and more than 2000 species throughout the world. There are 58 endemic *Rosa* species in Anatolia and there is an endemism rate of 24% in Turkiye (Nilsson, 1972; Campbell, 2002; Evans, 2002; Heywood et al., 2007; Hurkul and Koroglu, 2019; Altun et al., 2021).

Horticulture is a discipline primarily concerned with the cultivation of plant material by humans for food supply, medicinal use, or functional and aesthetic purposes. They are a genetically diverse group and play an important role in the economy of modern society. They are also central to the healthy diet of the urban population. From this point of view, roses and rosehip plants are extremely important garden plants because they contain these features. Although there is a wide variety of rose taxa in the world today, studies are continuing to determine some characteristics of roses due to the abundance of roses grown and the increasing number of newly bred varieties.

The first systematic description of roses was made by Aristotle's student Theophrastos, and he also included roses in his work known as Historia Plantarum. Theophrastos mentions three types of roses in his work and it is assumed that these are *Rosa canina*, *R. sempervirens,* and *R. centifolia* (Ozcan, 2012). Some roses that were used in ancient times and still exist today; *R.* x *damascena*, *R. moschata*, *R. hemisphaerrica,* and *R.centifolia* species (Baktir, 2015). In addition, natural roses that are sectorally important are *R. canina*, *R. dumalis*, *R. foetida,* and *R. hemisphaerica* (Sahin, 2011; Korkmaz and Ozcelik, 2015). In this context, within the scope of the research, some morphological, pomological, physiological, and mineral contents of important taxa such as *Rosa alba* L.'Semiplena' *R. banksiae, R.* Br. Cv '*Alba*', *R. canina* L.'Yildiz', *R. centifolia* L., *R. chinensis* Jacq. 'Old Blush', *R. foetida* Herrm., *R. heckeliana* Tratt. subsp. *vanheurckiana* (Boiss. Ö. Nilsson) *R. hemispharica* J.Herrm., R.x *odorata* (hort ex.Andrews) Sweet 'Louis XVI', *R. pisiformis* (Christ) Sosn., *R.* x *damascena* Mill. and *R.* x *damascena* Herrm. 'Semperflorens' (Loisel. & Michel) Rowley, for landscape design and horticulture were determined. Within the scope of the research, the morphological, physiological, and pomological characteristics and nutrient contents of taxa adapted to semi-arid conditions and different *Rosa* taxa spreading in Anatolia were determined. These parameters were classified by different statistical evaluations.

2. Material and Methods

2.1. Material

Some of the natural rose species distributed in the Iran-Turanian Phytogeographic Region are the widely used Bengal rose (*R. chinensis* cv. 'Old Blush'), Halfeti rose (*R.* x *odorata* cv. 'Louis XIV'), Rosehip (*R. canina*), white rose (*R. alba* 'Semi Plena'), Van yellow rose (*R. heckeliana* subsp. *vanheurckiana*), Damask rose (*R.* x *damascena*), hedge rose (*R. hemisphaerica*), Banks rose (R. *banksiae* cv. '*Alba*'), *Rosa* de Mai (*R.* x *centifolia*), yellow rose (*R. foetida*), Hosap rose [*R. pisiformis* (Christ) D.] and evergreen rose (*R.* x *damascena* 'Semperflorens') rose species and subspecies of some morphological, pomological, physiological, and mineral contents were determined. Taxa names are written in full at this stage, and in other parts of the manuscript, they are given in a short form without authorization.

2.1.1. Climatic characteristics of the greenhouse

Natural rose species are generally spread at high altitudes. Within the scope of the research, changes in stomatal parameters were determined in relatively warm conditions. In the Southeastern Anatolia Region, very high temperatures are observed in June and July. In this context, leaf samples were collected in these periods in 2020 and 2021. In this process, cooling units were not operated in the greenhouses where the plants were grown. Temperature values were also measured with Onset Computer H21-002 HOBO® Micro station and the averages were transferred to the data compiler. At the time the leaf samples were taken, the average temperature of the greenhouse was 39.8°C and the RH (%) value was determined as 59.8.

2.1.2. Physical and chemical properties of the growing medium

The findings obtained in the analyzes made; the pH of the growing medium in which the study was carried out was 7.14, clayey/loamy structure, and low in organic matter. Lime content was determined as 6.14% and salt content as 0.04%. In Japan, Yamane (1990) reported that the pH of the topsoil in natural stands of *R. rugosa* varied between 5.1 and 7.6. According to unpublished data from the University of Copenhagen Biology Institute; in the regions where *R. rugosa* grows naturally in Denmark, pH values were determined as 4.66-7.74, lime rate 6.26%, and organic matter content 1.20%

(Bruun, 2005). The results of the soil analysis carried out within the scope of the thesis and the aforementioned literature were found to be close to each other.

The chemical properties of the soil used in the greenhouse where the research was carried out, were analyzed. According to the results, the P value was high, the K value was critical, and the Fe, Cu, Zn, Mn, and B values were sufficient. It was determined that the growing environment was generally at sufficient levels in terms of the content of plant nutrients required for rose cultivation, fertilization was not done and the research was carried out in this way.

2.2. Methods

2.2.1. Determination of plant growth characteristics

Plants of different *Rosa* taxa propagated by cuttings were transplanted into 5 L pots in 2019, plant length (cm), plant crown width (cm), and plant crown/height ratio measurements were made in 2021. Growth patterns of 2-year-old plants were determined according to height and crown width. The codes of these parameters were denoted as; PL:plant height, PW:plant grown, PR:the ratio of the crown to the height of the plant.

2.2.2. Morphological analysis

Leaf area was calculated in cm^2 with the help of ImageJ program and determined according to Klamkowski and Treder (2008). Leaflets were classified as the bottom (1), middle (2), and tip (3), and leaf areas were determined by the same method. The leaves and leaflets of the plants belonging to each taxon were counted one by one, and the average leaf and leaflet measurements per plant were made in cm. In the same way, pedicel lengths were determined. The codes of these parameters were denoted as; LL: leaf length, LWH: leaf width, LA: leaf area, PW: pedicel width, LR: the ratio of the length to the width of the leaf, L1L: tip leaflet length, L1WH: tip leaflet width, L1A: tip leaflet area, L2A: middle leaflet area, L3A: bottom leaflet area.

2.2.3. Pomological analysis

Pomological analyzes were carried out according to the mentioned literature (Ercisli, 1996; Gunes, 2010; Kazankaya et al., 2005; Najda and Buczwoska, 2013; Encu, 2015; Hatipoglu and Ak, 2021; Guler et al., 2021). After the harvest of rosehip/rose fruits that reached harvest maturity, the yields of the samples brought to the laboratory were determined, and the fruits of each taxon were counted and recorded. 10 replications and 30 fruits in each replication were taken from the related taxa and weighed individually with a scale sensitive to 0.01 g and the average values were recorded. 30 fruits were selected from the *Rosa* L. taxa included in the study, and the width and height values of each fruit were determined using a 0.05 mm precision caliper. A vertical line was drawn between the point where the fruit connects to the sepal and the mark of breaking off from the branch, and the horizontal part was recorded as width and the vertical part as height, making an angle of 90 degrees with this line.

From the *Rosa* L. taxa examined in the study, 30 fruits were selected randomly, and after removing the seeds from each fruit, the weight of the fruit was checked. The fruit flesh ratio of taxa was determined by the ratio of the fruit flesh weight to the total fruit weight. Fruit shapes in *Rosa* L. taxa were calculated according to the average aspect ratio of 30 randomly selected fruits in line with the index determined by Ercisli (1996).

The absorbic acid (Vitamin C) content of *Rosa* L. taxa examined within the scope of the research was determined by spectrophotometric method (Cemeroglu, 1992; Karasakal, 2007; Sanlidere Aloglu, 2018). The separated fruit fleshes of the fruits collected in November 2020 from different locations were ground using a blender and sieved. After the fruits were pureed, 5 g of fruit juice was taken into the flasks in 3 repetitions, the fruit sample was weighed, 45 ml of 0.4% oxalic acid was added and filtered with filter paper. 1 ml of the obtained filtrate was taken and 9 ml of dye solution ($C_{12}H_6Cl_2NNaO_2$-H_2O) was added to it and readings were made at 520 nm wavelength. As a standard, a solution in which 9 ml of distilled water was added to 1 ml of filtrate was used (Ozdemir and Dundar, 1998; Aksoy, 2019).

The codes of pomological parameters were denoted as; FW: fruit weight, FL: fruit length, FWH: fruit width, FFR: fruit flesh ratio, FSI: fruit shape index, CV: C vitamin content.

2.2.4. Physiological analysis

The wet weights of the leaf samples taken from the pots in the experiment were determined, and the turgor weight was determined as a result of keeping them in petri dishes containing 100 ml of water for 24 hours. The samples were dried in an oven at 65-70ºC and their dry weights were determined by weighing them on a precision balance. Leaf relative water content was calculated according to Sanchez et al. (2004) (Dogan, 2018; Hatipoglu and Ak, 2021). This parameter is encoded as LRWC.

The amount of chlorophyll in the leaf was determined by the SPAD-502 Plus device with 10 separate measurements made from the bottom, middle, and tip parts of the compound leaflets in 2020 and 2021 (Khan et al., 2004). This parameter is encoded Ch.

In *Rosa* L. species, the leaves are in the form of leaflets; the differences in stomatal characteristics between the tip (A), middle (B), and lower (C) leaflets of the leaves were also investigated. Leaf samples were taken at noon for stomatal counts and measurements. The lower surfaces of the leaves were completely painted with transparent nail polish and left to dry. The tape was attached to the dried nail polished leaves and then removed and attached to the slides. Thus, stomata were examined under the microscope. The number of stomata per unit area (pcs/mm-2) value of the slide molds was photographed under the microscope; the number of stomata displayed in the 0.776 mm² field of view was determined by adapting to 1 mm2 area. (Kara and Ozeker, 1999; Bekisli, 2014; Dikmetas, 2019). For stomata/pore length and width (µm) values, the length and width of 10 stomata in the photographs of stomata/pore patterns were measured in MShot-1.3.10 computer program and measured in µm (Bekisli, 2014). For the stomata width/stomata length ratio, it was obtained by dividing the stomatal width by the stomatal length. The codes of stomata parameters were denoted as; STL: stomata length, SWH: stomata width, SD: stomata density, PRL: pore length, PWH: pore width.

2.2.5. Mineral content analysis

Leaf samples were taken from the middle parts of annual shoots together with their stems, representing the bottom, middle, and end leaflets of each plant. This process was performed with 10 replications for the samples taken. The samples brought to the laboratory were first thoroughly washed with running water and then washed with distilled water. The samples were left to dry for 7 days at room temperature and were mixed 3 times a day to prevent moisture. After these processes, it was dried in an oven and then ground. Nitrogen amount was calculated as % by the Khejdal method (Kacar, 1972; Ozdemir, 2005). Phosphorus in the leaves was determined by the colorimetric determination of the color in the spectrophotometer as a result of the dry burning process (Olsen, 1954). The determination of calcium and potassium in leaves was read in a flame photometer (Kacar, 1972). The determination of magnesium in leaves; after dilution of plant samples prepared by the dry burning method, it was determined by reading with an atomic absorption instrument (Kacar, 1972). 1 ml of sulfuric acid and then 19 ml of ethyl alcohol were added to the leaf samples weighing 1 g. The ash was first burned in the furnace at 250˚C for 2 hours, then at 650˚C for 4 hours, and left to cool. Then 5 ml of HCL (Hydrochloric Acid) has been added to the burnt leaves. Leaf samples were filtered using sterile filter paper, crucibles were filled with distilled water, and the process was repeated 2-3 times. Mn, Cu, Zn, and Fe contents were calculated in ppm units in atomic absorption spectrophotometer (Kacar, 1972; Ryan et al., 2001).

2.2.6. Statistical analysis

Statistical analysis of data for all variables was performed using Minitab 18. program. The difference between applications was determined by the LSD multiple analysis test. Principal component analysis (PCA) and clustering analysis (Dendogram) were performed using the R 4.1.1 package program.

3. Results

3.1. Plant growth characteristics of some *Rosa* L. taxa

In order to determine the differences in the growth characteristics of the plants, the findings related to the plant height (cm), plant crown width (cm), and plant crown/height ratio of 12 taxa are given in Table 1, and the numerical values were found to be statistically significant at the 5% level. In the study, the PLs of the taxa varied between 38.00 cm (*R.* x *damascena* 'Semperflorens') to 138.33 cm

(*R. chinensis* 'Old Blush'). PW values were determined between 46.32 cm (*R. foetida*) – 112.14 cm (R. *heckeliana* subsp. vanheurckiona). *R. hemisphearica* taxa had the most superior PR value (1.47), while *R. alba* 'Semiplena' had the least value (0.70) in terms of this feature (Table 1). In line with this information, a shape index was created for the growth characteristics of rose species. According to this shape index, if the crown/height ratio of plants grown (PR) under the same conditions and at the same age is between 0.70 and 0.95, the plant is erect; 0.96-1.24, the plant is broad/erect; bigger than 1.24, the plant is broad. According to PR; *R. alba* 'Semiplena', *R. canina* 'Yildiz', *R. centifolia*, *R. foetida*, *R. odorata* 'Louis XIV' and *R. pisiformis* taxa are 'erect'; *R. banksiae* '*Alba*', *R. chinensis* 'Old Blush', *R. chinensis* 'Viridflora' and *R.* x *damascena*taxa are 'broad/erect'; *R. heckellana* subsp. vanheurckiona, *R. hemispharica,* and *R.* x *damascena* 'Semperflorens' taxa are 'broad'. This classification is similar to the evaluations of Koca (2014).

Table 1. Growth characteristics of some *Rosa* L. taxa

Taxa	**Plant growth characteristics**		
	PL	**PW**	**PR**
***R. alba* 'Semiplena'**	91.33±3.50cd	64.62±3.04f	0.70±0.01g
***R. banksiae* '*Alba*'**	96.33±2.66bc	95.32±3.42c	0.98±0.01d
***R. canina* 'Yildiz'**	83.67±2.07e	68.81±4.09e	0.81±0.01f
R. centifolia	100.04±3.53ab	94.02±4.19c	0.94±0.01de
***R. chinensis* 'Old Blush'**	103.33±3.32a	107.33±4.20b	1.05±0.01c
R. foetida	57.66±3.78f	46.32±3.21i	0.80±0.01f
R. heckeliana* subsp. *vanheurckiona	89.30±3.01de	112.14±4.51a	1.25±0.01b
R. hemispharica	62.60±2.64f	91.64±3.05d	1.47±0.01a
***R. odorata* 'Louis XIV'**	61.33±4.33f	52.34±2.51g	0.77±0.01f
R. pisiformis	84.31 ±2.51e	68.63±3.51e	0.81±0.01f
R.* x *damascena	56.67±4.72f	50.31±5.50h	0.89±0.01e
***R.* x *damascena* 'Semperflorens'**	38.00±3.00g	49.00±3.78h	1.29±0.01b
Average	**77.05**	**75.04**	**0.98**
LSD (%5)	**6.626**	**1.731**	**0.060**

*: The differences between the averages with different letters in the same column are statistically significant. (P <0.05). **: It is the average of 10 repetitions. PL:plant height, PW:plant grown, PR:the ratio of the crown to the height of the plant.

3.2. Morphological parameters of some *Rosa* L. taxa

Morphological parameters of some *Rosa* L. taxa are given in Table 2. *R. foetidataxa* had the lowest values in LWH, LL, LA, and PW parameters. The highest values were obtained by R. *alba* 'Semiplena' (7.50 cm) in LWH, *R. pisiformis* (7.15 cm) in LL, *R. centifolia* (43.31 cm2) in LA, *R. centifolia* (2.41 cm) in PW, and *R. heckeliana* (1.13 cm) in LR. In the leaflet measurement parameters, *R. centifolia* taxa had the highest values. Similarly, *R. foetida* taxa generally had the lowest values in leaflet measurement parameters (Table 2 and 3.).

Table 2. Morphological parameters of leaves

Taxa	Leaf Characteristic				
	LWH	LL	LA	PW	LR
***R. alba* 'Semiplena'**	7.50±0.90a	6.82±1.19a	33.85±1.17b	1.53±0.51d	1.10±0.06e
R. banksiae'Alba'	5.72±0.09f	6.16±0.61 cd	12.83±0.53g	1.46±0.10e	0.93±0.08e
***R. canina* 'Yildiz'**	6.06±0.37d	5.57±0.36f	18.67±1.58d	1.53±0.25d	1.08±0.02b
R. centifolia	7.30±0.20b	6.47±0.28b	43.31±0.71a	2.41±0.10a	1.12±0.05a
***R. chinensis* 'Old Blush'**	6.32±0.88c	6.02±0.47de	13.05±1.47g	1.29±0.22f	1.04±0.07c
R. foetida	2.89±0.09h	2.77±0.18h	4.35±0.40h	1.01±1.10g	1.04±0.09c
***R. heckeliana* subsp. vanheurckiona**	6.27±0.57c	5.54±0.51f	17.42±1.07e	1.28±0.29f	1.13±0.01a
R. hemispharica	6.29±1.19c	6.22±1.16c	20.72±1.05c	1.76±0.07b	1.01±0.01d
***R. odorata* 'Louis XIV'**	5.69±0.34f	6.40±0.35b	18.61±0.96d	0.96±0.28g	0.88±0.05f
R. pisiformis	5.94±0.86e	7.15±0.97a	36.98±0.81de	2.20±0.52e	0.85±0.03g
R.* x *damascena	5.48±0.35g	5.06±0.28g	14.26±1.41f	1.46±0.46e	1.08±0.05b
***R.* x *damascena* 'Semperflorens'**	6.10±0.43d	5.87±0.25e	18.53±0.57d	1.68±0.24c	1.03±0.04cd
Average	**5.96**	**5.82**	**19.47**	**1.48**	**1.01**
LSD (%5)	**0.119**	**0.152**	**0.713**	**0.062**	**0.098**

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05). LL: leaf length, LWH: leaf width, LA: leaf area, PW: pedicel width, LR: the ratio of the length to the width of the leaf,

Table 3. Morphological parameters of leaflets

Taxa	Leaflet Characteristic				
	L1L	L1WH	L1A	L2A	L3A
***R. alba* 'Semiplena'**	4.02±0.32a	2.25±0.24ab	7.39±1.41b	6.38±10.29bc	2.26±0.76bc
R. banksiae'Alba'	3.46±0.25abc	1.18±0.10de	2.80±0.32ef	1.92±0.14ef	1.16±0.38cd
***R. canina* 'Yildiz'**	3.22±0.24bc	2.23±0.11ab	5.39±0.48cd	4.22±0.15c	2.13±0.41bc
R. centifolia	4.14±0.17a	2.60±0.26a	11.92±0.16a	8.68±1.00a	5.80±1.18a
***R. chinensis* 'Old Blush'**	4.03±0.39a	1.65±0.10cd	4.41±0.78de	2.63±0.65de	1.35±0.69cd
R. foetida	1.27±.0.19d	1.01±0.07e	1.14±0.02f	0.90±0.14f	0.66±0.08d
***R. heckeliana* subsp. vanheurckiona**	3.23±0.40bc	1.87±0.29bc	3.94±1.01de	3.69±0.68cd	1.30±0.53cd
R. hemispharica	3.46±0.58abc	2.14±0.65ab	5.23±2.46cd	3.87±2.04cd	1.72±0.58bcd
***R. odorata* 'Louis XIV'**	4.13±0.90a	2.41±0.53a	6.83±2.20bc	4.00±0.31cd	0.70±0.23d
R. pisiformis	3.95±0.18a	2.50±0.10a	6.96±0.61bc	6.04±0.37b	2.73±1.18b
R.* x *damascena	2.88±0.34c	1.63±0.11cd	3.46±0.51de	3.00±0.17 cde	1.14±0.77cd
***R.* x *damascena* 'Semperflorens'**	3.67±0.15ab	2.18±0.22ab	5.38±0.97cd	3.90±0.83cd	1.57±0.67 bcd
Average	**3.46**	**1.98**	**5.40**	**4.09**	**1.88**
LSD (%5)	**0.683**	**0.495**	**1.983**	**1.497**	**1.187**

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05). L1L: tip leaflet length, L1WH: tip leaflet width, L1A: tip leaflet area, L2A: middle leaflet area, L3A: bottom leaflet area.

3.3. Pomological parameters of some *Rosa* L. taxa

Pomological parameters were determined as a result of fruit measurements and ascorbic acid analysis in *Rosa* L. taxa. In the study, FWs of taxa varied between 0.19 g (*R. banksiae* '*Alba*') to 4.44 g (*R. centifolia*), and FL values were between 8.82 – 23.18 mm (Table 4.). FWH values were determined between 6.65–21.24 mm in *R. banksiae* '*Alba*' and *R. centifolia*, respectively. *R. pisiformis* had the least FWH (0.06 mm), while *R. centifolia* had the most superior value (3.33 mm) in terms of this feature. FFRs varied by 58.64% (*R. pisiformis*) to 89.32% (*R. chinensis* 'Old Blush'), and FSI values were recorded between 0.95-1.70 (Table 3). It can be said that the fruit characteristics of rose hips vary according to the species, growing region, climate, and ecological conditions. In this context, the aforementioned data of the fruits collected from the regions to which they are adapted show significant differences. The findings are similar to the pomological data determined in the literature (Kovacs et al., 2005; Celik et al., 2005; Kizılci, 2005; Savir, 2008; Gunes, 2010; Ozcelik, 2013; Akkus, 2016; Ipek and Balta, 2020; Tomlijenovic et al., 2021; Guler et al.,2021).

However, vitamin C values vary widely in studies. As a result of the analyzes made to determine the amount of vitamin C; 12.04-43.77 mg/100 g (Turkben et al., 2010), 1074 mg/100 g, and 2962 mg/100 g (Ercisli et al. 2001), 282.70-1173.40 mg/100 g (Gunes and Sen, 2001), 73-987 mg/100 g (Kazankaya et al., 2001), 1074-2557 mg/100 g (Ercisli and Esitken, 2004), 301-1183 mg/100 g (Kazankaya et al.

2005), 65.75 to 136.14 mg/100 g (Celik et al., 2006), 575.48-1369.89 mg/100 g (Savir, 2008), 2200 mg/ 100 g (Kazaz et al., 2009), 517-1032 mg/100 mL (Celik et al., 2009), 108.57- 908.57 mg/100 g (Gunes and Dolek, 2010), 575.48-1369.89 mg/100 g (Ekincialp and Kazankaya, 2012), values such as 332.47-1603.53 mg/100 g (Ozen, 2013). In the study, the CVs of the taxa varied between 36.34 to 1101.36 mg/100 g. As a result of the vitamin C analysis; *R. pisiformis* (1101.36 mg/100 g), *R. alba* 'Semiplena' (1041.85 mg/100 g) had high values.

Table 4. Pomological parameters of some *Rosa* L. taxa

Taxa	Pomological Parameters						
	FW	**FL**	**FWH**	**FSI**	**FFW**	**FFR**	**CV**
***R. alba* 'Semiplena'**	3.48±0.54b	23.18±1.68a	18.72±1.54b	R(1.27e)	2.62±0.75b	77.82±1.60bcd	1041.85±2.05a
***R. banksiae* '*Alba*'**	0.19±0.05j	8.82±0.62f	6.65±0.70g	R(1.32c)	0.12±0.03i	61.81±1.78f	506.56±1.50d
***R. canina* 'Yildiz'**	1.65±0.34d	22.17±1.59ab	15.09±1.59c	O(1.58b)	1.14±0.41cde	72.05±1.76e	99.85±2.13f
R. centifolia	4.44±0.75a	20.31±1.25b	21.24±1.80a	Fr(0.95i)	3.33±0.68a	72.40±1.10e	401.06±1.75e
***R. chinensis* 'Old Blush'**	1.60±0.18de	17.49±1.25c	13.45±0.80e	R(1.30d)	1.43±0.16cd	89.32±0.58a	30.79±1.58g
R. foetida	0.61±0.14hi	13.73±1.20e	12.38±1.41ef	Fr(1.11g)	0.49±0.13ghi	81.09±1.95b	40.17±0.94g
R. heckeliana	0.35±0.08ij	13.89±1.18de	8.18±1.21g	C(1.70a)	0.25±0.08hi	80.21±1.92bc	592.61±2.39bc
R. hemispharica	0.74±0.19gh	13.94±1.00de	13.42±1.32e	Fr(1.04h)	0.62±0.18fgh	82.11±2.01b	75.77±1.17fg
***R. odorata* 'Louis XVI'**	1.00±0.09fg	12.80±1.40e	11.65±0.62f	Fr(1.10g)	0.85±0.10efg	87.99±1.87a	36.34±1.39g
R. pisiformis	0.11±0.03j	7.35±0.71f	7.14±0.66g	Fr(1.03h)	0.06±0.01i	58.64±1.79f	1101.36±1.45a
R.* x *damascena	1.31±0.39ef	16.16±2.11c	13.66±1.47cd	Fr(1.18f)	1.01±0.29def	75.37±2.23cde	632.87±0.23b
***R.* x *damascena* 'Semperflorens'**	1.99±0.55c	15.70±1.50cd	15.20±1.69c	Fr(1.03h)	1.55±0.50c	74.55±1.76de	577.34±1.55c
Average	**1.46**	**15.47**	**13.07**	**1.21**	**1.12**	**76.11**	**420.55**
LSD (%5)	**0.331**	**1.878**	**1.580**	**0.017**	**0.450**	**5.402**	**50.962**

*Fr: Flat Round, R: Round, O: Oval, C: Conical**: The difference between the averages with different letters on the same column is statistically significant. (P <0.05). ***: It is the average of 30 repetitions. FW: fruit weight, FL: fruit length, FWH: fruit width, FFR: fruit flesh ratio, FSI: fruit shape index, CV: C vitamin content.

3.4. Physiological parameters of some *Rosa* L. taxa

Physiological parameters of some *Rosa* L. taxa are given in Table 5. The values determined on the change of the LRWC according to the taxa were found to be statistically significant ($p<0.05$). As a result of the research; the highest LRWC were *R. chinensis* 'Viridflora', *R. canina* 'Yildiz, *R. foetida* and R. *chinensis* 'Old Blush; the lowest LRWC were determined in R. *heckeliana* and R. pisiformis taxa. Bucsa and Zamfirce (2017) found the highest LRWC values in R. nitidula, R. rubiginosa and R. *canina* taxa in their study, and they stated that there were statistically significant differences in LRWC rates between different taxa. In the study, it was concluded that these values varied during the vegetative and flowering periods in *Rosa* L. taxa. In studies conducted on different plant species, it has been stated that stress applications reduce the proportional water content of the leaves (Cekic, 2004; Irfan et al., 2014; Semida et al., 2015; Dogan, 2018). In woody landscape and ornamental plants; It is stated that determining physiological data such as leaf proportional water content will provide sustainable plant use in many application areas such as determining the tolerance of the plant against stress conditions such as water, temperature and drought, and determining ozone damage.

Ch values in leaves of different taxa were found to be statistically significant. According to the results of the research, the lowest chlorophyll content was obtained from *R. banksiae* '*Alba*' (28.13), and the highest chlorophyll content was obtained from plants belonging to R. *chinensis* 'Old Blush' (44.39) and *R. odorata* 'Louis XIV' (42.99) taxa. On the other hand, it was determined that the chlorophyll content of the taxa commonly used in semi-arid conditions had higher leaf chlorophyll content than the taxa brought from different ecologies. In studies conducted on different plants, it has been stated that the amount of chlorophyll in the leaves of plants that are under stress conditions and have adaptation problems to the ecology it is in, decreases, and it has been reported that this low value causes problems in photosynthesis and carbon fixation (Cekic, 2004; Hassan et al., 2005; Krantev et al., 2008; Liu et al., 2015; Semida et al., 2015; Piršelová et al., 2016).

The stomatal parameters of the leaflets at the tip (A), middle (B), and lower (C) ends of the leaves taken from 2 different directions of the rose taxa used as plant material in the study were determined. When the mean stomatal length values of the taxa were examined, the highest value was *R. odorata* 'Louis XIV' (36.99 µm); the lowest values were found in the R.*canina* 'Yildiz' taxa (23.81 µm). In all taxa, the stomatal length parameter was found to be statistically significant in all four groups.

Descriptive statistics and comparison results for stomatal width in six taxa are given in Table 2. As seen in Table 2; The highest stomatal width value was determined as 25.65µm in R.*odorata* 'Louis XIV'. The lowest stomatal width value was determined in *R.* x *damascena* (17.55 µm) and R. *canina* 'Yildiz' (17.82 µm). While the variation in A-B-C leaflet values was five in stoma width values, four variations were detected in the two-year general average values. As a result of its adaptation to semi-arid conditions, *R. odorata* 'Louis XIV' received a geographical indication in 2021 in order to contribute to rural tourism and the rural population to turn to different business areas (TURKPATENT, 2022). It is important to compare the parameters of these two taxa with rose species grown in Anatolia.

Zarinkamar (2007), Orcen el. (2013) and Alp et al. (2016) stated in their study that the number of stomata per unit area (mm²) tends to increase as stoma sizes decrease. Kalariya et al.(2017) explain this by reducing the stomatal density and osmotic stress caused by drought, disrupting normal stomatal motility, and forming more adjacent stomatal clusters in the leaf epidermis. The research gives similar results to that study. These results confirm that the size of the stomata decreases as the water content in the leaf decreases (Xu and Zhou, 2008). However, the surface area parameter in the leaf did not change which provides a linear proportion with these two parameters. Due to anthropogenic effects, nature is adversely affected in many ways during the rapid change process on earth and deterioration in the ecological balance occurs. In this context, studies on the health of plants affected by these effects and their sustainable use in landscape planning studies have gained importance. Determination of data such as chlorophyll content, stomatal parameters, and leaf proportional water content in woody landscape and ornamental plants can be used in many application areas such as the determination of plant water stress, cold/drought tolerance, and ozone damage. For this reason, it is thought that sustainability in plant uses will be ensured as a result of studies aimed at revealing this situation and determining the different characteristics of plants. The research focused on the identification of stress resistant species in roses, an important species for landscape planning studies. It is thought that the research will contribute to the cultivation and promotion of high temperature resistant species and also to urban plant planning by determining the relationship between the relevant parameters.

Table 5. Physiological parameters of some *Rosa* L. taxa

Taxa	Physiological Parameters						
	LRWC	Ch	STL	SWH	SD	PRL	PRWH
***R. alba* 'Semiplena'**	41.19±1.65d	34.87±3.23e	30.57±2.33bc	15.34±2.31g	156.20±6.93i	19.57±3.01g	7.76±0.98i
***R. banksiae* '*Alba*'**	39.50±1.93e	28.13±1.40i	27.73±3.13c	19.73±2.53bc	169.40±7.54h	20.63±2.41e	10.99±1.21c
***R. canina* 'Yildiz'**	74.33±5.25a	38.85±1.64d	23.81±4.66g	15.51±3.03fg	202.76±8.11d	19.02±3.93h	8.53±1.02h
R. centifolia	30.44±0.71h	32.94±2.00g	31.14±6.06b	18.16±2.63d	148.04±6.43j	24.03±1.97c	10.70±1.31cd
***R. chinensis* 'Old Blush'**	73.41±5.69b	44.39±1.82a	26.90±5.08e	21.26±4.03a	199.47±6.21e	21.93±2.22d	14.90±1.10a
R. foetida	73.99±5.80ab	35.69±1.75e	27.51±5.01c	16.15±3.93ef	212.30±8.24b	20.20±3.05f	10.34±1.24de
R. heckeliana	24.06±0.93j	33.30±1.75fg	27.76±3.03c	18.77±3.33d	213.40±7.06a	17.42±1.98j	10.73±1.46cd
R. hemispharica	55.00±5.00c	29.79±3.49h	29.90±3.31c	19.60±2.33c	188.10±4.88f	22.24±1.46d	10.13±1.46ef
***R. odorata* 'Louis XVI'**	38.23±2.93f	42.99±3.53ab	36.99±6.60a	21.51±2.33a	133.20±3.44k	30.12±2.15a	13.46±1.05b
R. pisiformis	25.49±0.84i	34.69±2.55ef	26.59±5.01ef	15.45±2.33fg	173.80±7.83g	17.90±1.85i	9.42±1.96g
R.* x *damascena	31.30±5.78g	42.34±1.65a	25.62±5.09f	16.68±2.33b	203.50±3.95c	18.17±2.53i	9.67±1.46fg
***R.* x *damascena* 'Semperflorens'**	41.60±5.13g	40.99±3.23c	31.81±4.08b	17.79±2.33e	124.30±5.20l	26.14±2.16b	10.17±1.64ef
Average	**45.70**	**36.59**	**28.33**	**18.22**	**177.04**	**21.45**	**10.57**
LSD (%5)	**0.844**	**1.414**	**0.970**	**0.763**	**0.490**	**0.417**	**0.514**

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05). **: It is the average of 20 repetitions. LRWC: leaf relative water content Ch: chlorophyll content STL: stomata lenght, SWH: stomata width, SD: stomata density, PRL: pore lenght, PWH: pore width.

3.5. Mineral contents of some *Rosa* L. taxa

The macro and micro nutrient elements examined in the samples taken from *Rosa* L. taxa are given in Table 6. It was determined that nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca), magnesium (Mg) contents of macronutrients analyzed in the samples taken from *Rosa* L. taxa differed statistically from each other. Accordingly, the highest nitrogen content (1.63%) was R. x *damascena*; the lowest nitrogen content (1.00%) was determined in the leaves of the *R. pisiformis* taxa. The average phosphorus (P) content of the leaf samples of the *Rosa* L. taxa in the study was the highest in the R.

heckeliana (0.36%) taxa. According to Kacar and Katkat (1998), potassium, one of the mobile elements, passes from old leaves to young leaves in plants. For this reason, it is stated that the potassium content of young leaves is higher than that of old leaves. According to the analysis results of the leaf samples taken from *Rosa* L. taxa, the mean potassium (K) content was the lowest in *R. pisiformis* (0.68%), and the highest value was found in the leaves of the *R. banksiae* '*Alba*' (1.1.82%) taxa (Table 6.).

It was determined that the contents of iron (Fe), copper (Cu), zinc (Zn), and boron (B) examined in the samples taken from *Rosa* L. taxa differed statistically from each other (Table 7.). When the average micronutrient content of the leaf samples taken from the middle parts of the annual shoots of *Rosa* L. taxa were compared, it was determined that the accumulation of all the nutrients examined in the leaves differed according to the species and subspecies, except for the Mg element. Looking at the results of the analysis, *R.* x *damascena* (Damask rose), which has the highest nitrogen content, draws attention to its low copper and boron content. It has been concluded that the taxon *R. odorata* 'Louis XIV' has generally lower contents than other taxa in terms of both macro and micro nutrients.

Table 6. Macro mineral contents in leaves of *Rosa* L. taxa

Taxa	N (%)	P (%)	K (%)	Mg (%)
***R. alba* 'Semiplena'**	1.13±0.00g	0.17±0.00e	1.54±0.01e	0.27±0.01f
***R. banksiae* '*Alba*'**	1.16±0.01f	0.14±0.00f	1.82±0.02a	0.27±0.01f
***R. canina* 'Yildiz'**	1.09±0.00h	0.32±0.00b	1.75±0.01b	0.34±0.00d
R. centifolia	1.19±0.01f	0.13±0.00g	1.58±0.02d	0.27±0.00f
***R. chinensis* 'Old Blush'**	1.34±0.00d	0.23±0.00d	1.67±0.01c	0.27±0.01f
R. foetida	1.52±0.02b	0.10±0.00h	1.49±0.01f	0.36±0.01c
R. heckeliana* subsp. *vanheurckiana	1.36±0.05d	0.36±0.00a	1.03±0.01h	0.42±0.01b
R. hemispharica	1.24±0.01e	0.17±0.00e	1.59±0.02d	0.33±0.00d
***R. odorata* 'Louis XVI'**	1.47±0.01c	0.09±0.00i	1.68±0.02c	0.28±0.01f
R. pisiformis	1.00±0.00i	0.10±0.00h	0.68±0.01i	0.43±0.01a
R.* x *damascena	1.63±0.05a	0.25±0.00c	1.50±0.02f	0.29±0.01e
***R.* x *damascena* 'Semperflorens'**	1.03±0.01i	0.10±0.00 h	1.35±0.01 g	0.34±0.01d
Average	**1.26**	**0.18**	**1.48**	**0.32**
LSD (%5)	**0.030**	**0.009**	**0.030**	**0.013**

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05).

Table 7. Micro nutrient contents in leaves of *Rosa* L. taxa

Taxa	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	B (ppm)
***R. alba* 'Semiplena'**	120.51±1.44d	12.01±0.97d	27.32±1.30h	73.37±1.18d	114.72±1.34d
***R. banksiae* '*Alba*'**	92.91±1.35i	13.00±0.22c	25.50±0.21i	32.30±0.40j	91.84±1.84h
***R. canina* 'Yildiz'**	119.28±1.44d	13.54±0.32b	37.62±0.66b	43.18±0.49i	101.50±1.60f
R. centifolia	112.60±1.79e	13.02±0.18c	45.89±0.81a	60.98±0.92f	104.15±1.06e
***R. chinensis* 'Old Blush'**	125.77±1.70c	11.55±0.08e	28.49±0.28f	49.24±0.73g	164.99±1.88b
R. foetida	138.01±1.79b	10.58±0.41fg	27.47±0.17gh	76.89±1.08c	90.41±1.88h
R. heckeliana* subsp. *vanheurckiana	157.14±1.80a	11.58±0.18e	31.27±0.38d	64.94±1.06e	69.89±1.42j
R. hemispharica	106.39±1.37f	12.01±0.32d	28.31±0.51fg	78.13±1.38c	139.07±1.71c
***R. odorata* 'Louis XVI'**	78.68±1.01j	10.27±0.20g	25.10±0.24i	74.49±1.09d	94.36±1.10g
R. pisiformis	119.95±1.80d	10.76±0.19f	33.72±0.24c	92.77±0.95b	85.34±1.30i
R.* x *damascena	96.15±1.33h	14.24±0.31a	30.66±0.23de	45.08±0.99h	56.30±1.39k
***R.* x *damascena* 'Semperflorens'**	102.97±1.71g	10.40±0.31fg	29.86±0.38e	111.33±0.98a	168.22±0.38a
Average	**114.01**	**11.91**	**30.93**	**66.88**	**106.74**
LSD (%5)	**2.735**	**0.423**	**0.930**	**1.687**	**2.479**

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05).

3.6. Cluster and principal component analyses

Cluster analysis, which is one of the multivariate statistical analysis methods, is a method used to divide individuals into groups according to their similarities. As a result of cluster analysis, plants within the same group are more similar in terms of characteristics than plants between other groups (Polumackanycz et al., 2020; Guler et al., 2021). The purpose of using cluster analysis in our research is to distinguish plants that are similar in terms of morphological, physiological, pomological, and

biochemical characteristics from those that are different. The advantage of applying a dendogram for the interpretation of the results is that it divides the data into groups, taking into account all the data variability, without the need for any generalization.

In our study, a dendogram tree was formed as a result of 37 different parameters examined in 12 different rose taxa, and as a result, 4 different groups were formed in the dendogram graph (Figure 1). In group 1, *R. foetida* took place alone. In the taxa under the 2nd group in the dendogram tree, *R. canina* 'Yildiz' and R. *damascena* are more similar to each other, and *R. banksiae* '*Alba*' and *R. chinensis* 'Old Bush' are also similar to each other, and also in the 2nd group of *R. heckeliana*. *R. alba* 'Semiplena' and R. *centifolia* were under group 3 in the dendogram and were found to be similar in line with the parameters examined. *R.* pisiformis, *R. odorata* 'Louis XVI' clustered in group 4 in the dendogram graph. In *R. hemispharica* and *R. damascena* 'Semperflorens' were found to be more similar compared to the others.

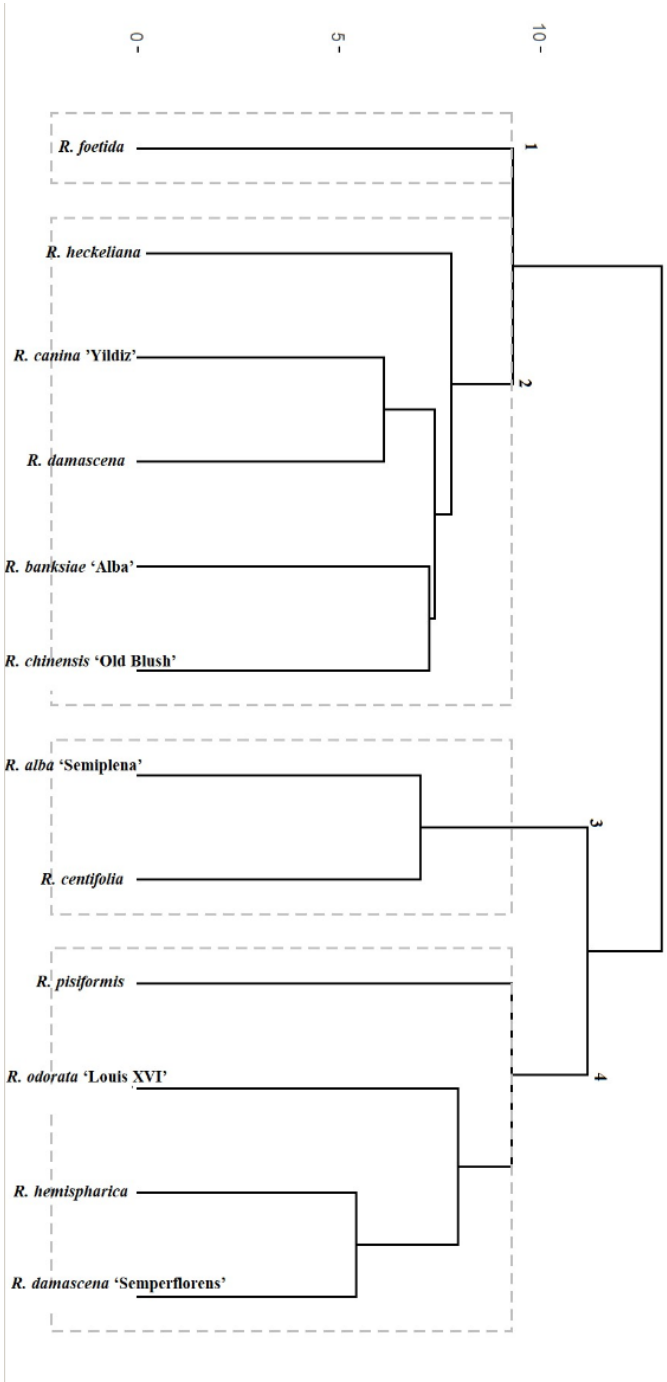

Figure 1. Comparative dendogram plot of some *Rosa* L. taxa.

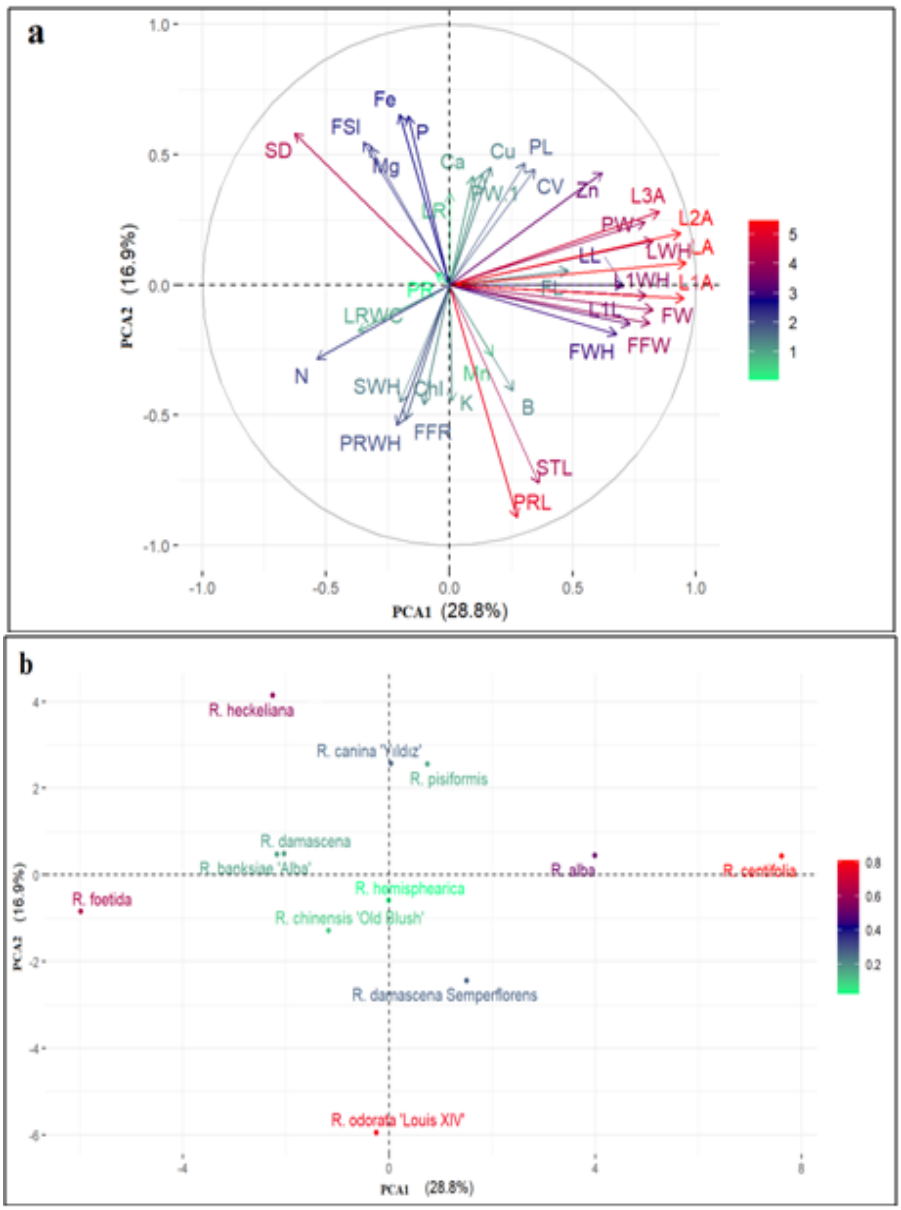

Figure 2. Comparative dendogram plot of some *Rosa* L. taxa.

PCA analysis is a statistical technique that is used to define the data in a smaller area by finding the general features in the multidimensional data, reducing the number of dimensions, and compressing the data. This method combines highly correlated variables to create a smaller set of artificial variables, called 'principal components', that generate the most variation in the data. The data obtained by PCA analyzes are reduced to smaller sizes and the differences and similarities between the applications are visually defined (Bozhuyuk et al., 2021; Guler et al., 2021). In our study, which we conducted to examine the morphological, physiological, pomological, and biochemical properties of different rose taxa, similarities and differences between 12 different rose taxa were determined. As seen in Figure 2 (a,b), the 12 rose taxa used in the study, which have similar characteristics, are located in the same place. The main logic of these groupings is shown in Figure 2 (a). The arrows in the graph show the values of the different parameters examined, that is, these values increase as you move toward the arrow direction.

4. Discussion

Within the scope of the research, 12 different *Rosa* L. taxa were studied. As a result of the research, in terms of plant growth parameters, *R. chinensis* 'Old Blush' and *R. centifolia*; in terms of

morphological features, *R. alba* 'Semiplena', *R. centifolia* and *R. pisiformis*; in terms of pomological properties, *R. centifolia*, *R. alba* 'Semiplena' and *R. odorata* 'Louis XIV' were found to have high values. It was concluded that *R. pisiformis* and *R. alba* 'Semiplena' species contain high levels of vitamin C. In addition, *R. rugosa*, *R.* x *damascena*, *R.* x *damascena* 'Semperflorens', *R. banksiae* '*Alba*', *R. heckeliana* subsp. *vanheurckiana* and *R. montana* subsp. *woronovii* 'Gercekcioglu' were determined to be promising species in terms of vitamin C content. The vitamin C contents of the fruits of *R. chinensis* and *R. odorata* species, which are used extensively in urban landscape studies, were found to be low. In addition, it is thought that the *R. alba* species, which has higher values compared to other species in parameters such as weight, width and height in fruit and starts to bear fruit early, can be considered as an alternative agricultural product apart from its landscape feature. *R. canina* 'Yildiz', *R. chinensis* 'Old Blush', *R. odorata* 'Louis XIV', *R. heckeliana* stand out in photosynthetic parameters. It was determined that the contents of macro and micro nutrients examined in the samples taken from *Rosa* L. taxa differed statistically from each other. Principal component analysis and cluster analysis were also used to determine the similarities and differences of these parameters measured in different *Rosa* L. taxa.

Conclusion

Roses that adapt to the ecological conditions they are in can be used with their aesthetic appearance and functional effects (erosion prevention, landscape restoration works, etc.). It can be used in slope areas, especially on intercity roads. If a visuality is provided with the fruits of the rose taxa studied and the value of the fruits is understood, the local people adopt these plants and a sustainable use can be achieved. The data obtained in the determination of physiological characteristics are the values reached under greenhouse conditions. In this respect, it is important to carry out pomological analyzes in the same ecology and to evaluate the plants in question as genetic materials.

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Review Article

Significant Natural Wound Healing Agents: Herbs and Single Bioactive Principles

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Abstract: Wounds are caused by skin injuries that damage the soft tissue. Wound healing is a dynamic process that involves a series of interconnected cellular and molecular processes that result in the restoration of anatomic continuity and function. Herbal therapy has been gaining popularity recently because of plants' usefulness in treating ailments with hardly any negative effects. Over the years, a variety of plant items have been developed and utilized to heal wounds. Throughvarious mechanisms, herbal extracts aid in blood clotting, infection prevention, and wound healing. This review provides a comprehensive look at herbal therapy's potential for hastening wound healing, as well as its antioxidant, anti-inflammatory, and anti-microbial properties that can be manifest in the treatment of injuries, as well as a first attempt at developing new wound-healing formulations with high potency for human use. Furthermore, we have highlighted medicinal plants as natural antioxidant resources in wound repair and healing.

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1. Introduction

In clinical practice, chronic wounds are among the most significant health issues and are considered with a high impact on social health, mainly in elderly people suffering from chronic diseases like diabetes, chronic motor deficit, nutritional abnormalities, cardiovascular diseases, and obesity. These ailments are accompanied by alterations in wound healing mechanisms and skin repair. An injury or wound is a disruption of tissue's cellular and anatomic continuity, or breaks in the skin's epithelial integrity, or a tear in the cellular, anatomic, or functional continuity of living tissue, with or without microbial infection. It can occur with the exploitation of tissues through physical, chemical, thermal, microbial, or immunological means (Lazarus et al., 1994). Chronic wounds affect nearly 60 lakh people worldwide, as per the latest

projections. When a wound does not heal properly, it causes inflammation, pain, and swelling in the wound area. Chronic wounds can often cause multiple organ failures in patients (Kumar et al., 2007). Wound recovery begins with a complicated series of interconnected events that are arbitrated by a diverse variety of chemically coordinated biological processes in addition to hormonal influences. These events are mediated through the inflammatory phase, proliferate phase, and finally, the remodeling phase by a wide range of chemically coordinated cellular functions as well as hormonal influences (Chan et al., 2008).

As per WHO reports, traditional remedies are used by more than 80% of the world's population to cure various diseases. Plants and their derivatives are now being used in 25 percent of medical drugs in developed countries (Priya et al. 2002; Steenkamp et al., 2004). Besides, chemical therapeutic agents have inadequate efficacy as well as serious adverse effects. Therefore, natural remedies have been used in medicine for a long period since ancient times for their abilities to encourage wound healing safely without fatal side effects. Natural treatments may be utilized to treat wounds as an alternate technique.
Considering that fatty acids, phytosterols, tocopherols, phenols, and flavonoids derived mainly from plant origin possess important antioxidant, nourishment, protection, soothing potentials, act as stimulators of cell metabolism for fibroblasts, enhance the ability of herbal preparations to promote and hasten the healing process in chronic skin wound (Ragno et al., 2016).

Hence, this systematic review highlights the herbs that can be used for wound healing complications, the phytoconstituents responsible for this activity, and their mechanism of action. Scientific databases like PubMed, Science Direct, and Google Scholar were searched using different keywords.

2. Classification of Wounds

Classification of wounds is characterized into two types such as open wounds or closed wounds based on the primary cause of wound formation and as acute or chronic wounds based on the physiology of wound healing.

2.1. Open wound

In this case, blood exits the body with prominent bleeding. It can also be classified as an incised wound, a laceration wound, or a tear wound. Penetration wounds, abrasions, puncture wounds, and gunshot wounds are all examples of superficial wounds (Mickelson et al., 2016).

2.2. Closed wound

Blood leaves the circulatory system and collects in closed wounds. Contusions or bruises, hematomas or blood tumors, and crush injuries are all examples (Menke et al., 2007).

2.3. Acute wound

These wounds are most caused by small cuts or surgical incisions, and they heal within the expected time frame. Tissue damage in an acute wound is usually caused by a systematic and timely process that leads to the tissue's structural and functional integrity being restored (Shaw and Martin, 2009).

2.4. Chronic wounds

These types of wounds have not finished the normal healing stages and have thus become inflamed for an extended period. Chronic wounds require a long healing time, or they will recur frequently. Trauma, Local infection, hypoxia, foreign bodies, and systemic problems such as diabetes, malnutrition, immunodeficiency, and medication are the most common causes (Roberts et al., 1998).

3. Phases and Mechanisms of Wound Healing

The body's reaction to injury, whether surgically or traumatically induced, is instant, and the harmed tissue goes through three phases: inflammation, proliferation, and remodeling. A description of each phase is given below:

3.1. Inflammatory phase

This begins instantly after the wound and lasts for 24 to 48 hours. In certain cases, it can last up to two weeks. The hemostatic mechanisms immediately stop bleeding from the wound site in this step. Clinically identifiable cardinal signs of inflammation, such as rubor, fire, tumor, and pain, result from this effect. Coagulation in the blood is caused by platelet aggregation and vasoconstriction, which is followed by vasodilation and phagocytosis, which promotes inflammation in the wound area. (Li et al., 2007).

3.2. Proliferative phase

The proliferative phase can last anywhere from 2 to 3 weeks. Granulation, contraction, and epithelialization are the three steps in this phase. During the granulation process, fibroblasts fill the gap with collagen and produce new capillaries. Wound edges are contracted, reducing the defect, and epithelial tissues form throughout the wound site. The proliferative phase, which is common in skeletal muscle injuries, involves the production of repair material (Guo and Dipietro, 2010).

3.3. Remodeling phase

This stage can last anywhere from 3 weeks to 2 years. During this phase, more collagen is produced. The tensile strength of tissues improves because of the intermolecular cross-linking of collagen caused by vitamin-C-dependent hydroxylation (Guo and Dipietro, 2010).

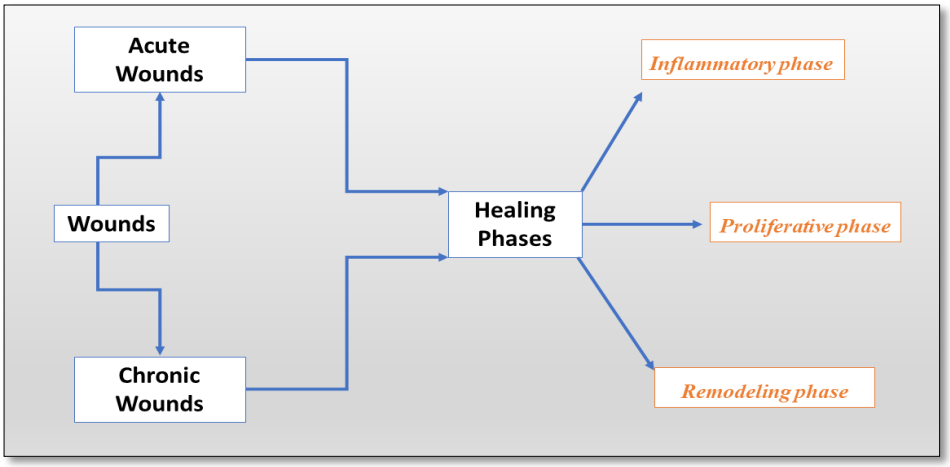


Figure 1. The Phases of wound repair.

4. Role of Natural agents in Wound Healing

Natural extracts were suggested to work with several mechanisms to possess the wound healing activity. Extracts are extensive mixes of many chemical classes that operate together through various methods to produce a safe and synergistic effect on the proposed activity. They have several effects that all help with the wound healing property as being antioxidants, anti-inflammatory, and anti-angiogenic, in addition to cell signaling factors which all lead to enhanced wound healing activity (Sivamani et al., 2012).

Natural compounds are one of the greatest sources of antioxidants, such as polyphenols and flavonoids, which have a radical scavenging effect and control oxygen levels at the injured area, resulting in improved and faster healing.

Natural extracts also control the inflammatory phase that is achieved by decreasing the inflammatory responses of the cells, inhibiting NF-kB, targeting the inflammation pathways, either intracellular transcription or transduction, and downregulating the levels of the proinflammatory cytokine.

4.1. Pharmacotherapy

Plants' wound-healing abilities have been studied in folklore since then. The curing of wounds is aided by a variety of herbal plants. At this point, plants with substantial wound-healing properties are highlighted.

4.1.1. Adhatoda vasica Nees

Adhatoda vasica Nees*,* known as Chue Mue (Family: Acanthaceae), grows in almost all parts of India as a vine. In an experimental wound healing model in Wistar rats, the breaking strength, tensile strength, absorption and extensibility, and the wound repair tissue was improved by *A. vasica* Nees. Moreover, in animal research, treatment with *A. vasica* showed an increase in levels of elastin, collagen, hydroxyproline, hexosamine, and zinc (Bhargava et al., 1988).

4.1.2. Aloe Vera

Aloe vera (L.) Burm. f. *(*Family: Liliaceae) is one of the eldest medicinal plants identified to humans. Topically used for wounds, burns, bruises, insect bites, acne, blemishes and infections, sores, eczema, skin lesions, and sunburns. A wound excision method was utilized to examine the impact of *A. vera* gel on the healing process, and histopathology was employed to study the influence on wound remedy. The consequence of *Aloe vera* gel concerning wound contraction wound closure, the surface area of the wound, tissue regeneration at the wound area, and histopathological characteristics were significantly improved in treated rats. The effect of *A. vera* gel on biochemical tests showed a significant increase in collagen and a decrease in hexosamine and malondialdehyde levels (Panahi et al., 2020). The active agents accountable for this potential include Acemannan, a mucopolysaccharide present in *A. vera*, which has a major role as a potent macrophage stimulator and activator for T-cell and induces proinflammatory mRNAs transcription (IL-1α, IL-1β, IL-6, TNF-α, PGE2, and nitrous oxide) (Shedoeva et al., 2019).

4.1.3. Andrographis paniculata

Andrographis paniculata (Family: Acanthaceae), known as green chiretta, is mainly used in China, India, and Southeast Asian countries. Experimentally, it significantly enhanced wound closure in rats after treatment with 10% of its aqueous leaf extract (Al-Bayaty et al., 2012). In addition, animals showed a reduction in inflammation, and scarring, while angiogenesis and collagen fibers in healed wounds increased (Al-Bayaty et al., 2012). In surgical open wounds, andrographolide, a bicyclic diterpenoid extracted from the leaves of *A.paniculata*, promoted the healing process. (Sridharan et al., 2021).

4.1.4. Anredera diffusa

Anredera diffusa (Family: Basellaceae) is known as “Lloto” (Moura-Letts et al., 2006). The ethanolic extract of the fresh stem and leaves possesses potent wound healing activity. Oleanolic acid is the main active component of *Anredera diffusa* responsible for its wound healing activity (Moura-Letts et al., 2006).

4.1.5. Azadirachta indica

Azadirachta indica (Family: Meliaceae) is commonly recognized as the Neem tree (Subapriya and Nagini, 2005). It contains various chemical compounds, namely alkaloids, triterpenoids, limonoids, flavonoids, fatty acids, and steroids and their glycosides (Maan, Yadav and Yadav, 2017). Several studies showed that *Azadirachta indica* different extracts possess wound curative potential. It was established that

applying a paste made from an aqueous extract of the root bark to a wound greatly increased re-epithelialization, hastened wound healing by elevating protein and hydroxyproline levels, and increased cell proliferation (Maan et al., 2017).

4.1.6. Camellia sinensis

Camellia sinensis (Family: Theaceae), as green tea, or aqueous extracts, are distributed all over Asia and are famous for their biological effects (Yang et al., 2014). The dynamic fundamentals responsible for biological activities along with wound healing properties are the presence of polyphenolic compounds known as catechins (Yang et al., 2014). Epigallocatechin-3-gallate (EGCG) is the most prominent compound which possesses *Camellia sinensis* activity as a proliferation stimulant and promotes keratinocytes differentiation (Hsu et al., 2003). EGCG suppresses TGF-β receptors by modifying TGF-β signaling, reducing MMP-1 and MMP-2 expression, and attenuating collagen type 1 in human dermal fibroblasts. These properties propose that EGCG is an active anti-scarring agent (B. R. Klass, 2010). Besides, EGCG was found to induce keloid shrinkage (Syed et al., 2013) and augment the growth and pathological features of keloids by suppressing STAT3 signaling (Park et al., 2008).

4.1.7. Carica papaya

Carica papaya (Family: Caricaceae) is commonly used to treat different hair disorders. It is frequently used throughout developing nations to efficiently heal several wounds, particularly burns, and it is freely available. The hydroxyproline content and epithelialization of laboratory animals have been substantially increased by *Carica papaya* latex in a preclinical test which also showed an increase in the contraction of wounds (Gurung and Skalko-Basnet, 2009). Different extracts of *Carica papaya* showed wound healing potential as the ethanolic extract of the seeds (Ramdeen et al., 2012), the aqueous leaf extract (Mahmood, 2005), different epicarp extracts (Anuar et al., 2008), the aqueous extract of the roots (Tiwari P., 2011), and the latex (Gurung and Škalko-Basnet, 2009).

4.1.8. Catharanthus roseus

Catharanthus roseus (Family: Apocynaceae) plant is a key well-spring of monoterpenoid indole alkaloid, vincristine, and vinblastine which were found valuable in the treatment of malignancy. In an incision wound model, the extract of *Catharanthus roseus* expanded the wound-breaking strength in rats. The extract-treated wounds showed re-epithelialization faster, and the wound constriction rate was additionally increased in contrast with control wounds (Anom-Dddd, 2013; Nayak and Pinto Pereira, 2006). The mechanism of wound repair takes place by increasing wound contraction and tensile strength (Nayak and Pinto Pereira, 2006).

4.1.9. Chamaemelum nobile

Chamaemelum nobile (Family: Asteraceae) is generally known as chamomile. The major active components of chamomile are chamazulene, alpha-bisabolol, bisabolol oxides present in its volatile oil, and flavonoids which are responsible for most of its biological effects. *Chamaemelum nobile* ointment used to treat wounds accelerates the healing progression by increasing the protein and hydroxyproline levels and increasing propagation of the cells. In addition, it acts as a potent antibacterial in infected wounds (Kazemian et al., 2018).

4.1.10. Centella asiatica

Centella asiatica (Family: Apiaceae) is commonly known as Gotu kola, kodavan, Indian pennywort, and Asiatic pennywort. It is used by several cultures as a wound-healing agent where a topical application on wounds led to a decreased in granulation and scar formation of the wound and increases the skin tensile strength, and prevents inflammatory responses (de Fátima et al., 2008). The main active agent found in *Centella asiatica* responsible for wound healing activity is asiaticoside which yields *in vivo* Asiatic acid by deglycosylation, which stimulates collagen synthesis (Lawrence, 1967).

4.1.11. Curcuma longa

Curcuma longa (Family: Zingiberaceae) is commonly stated as turmeric and haldi in Hindi. It has been documented that *Curcuma longa* possesses antibacterial, antifungal, and anti-inflammatory activity. In an investigation, an animal model tested the possible efficacy of fresh turmeric paste for healing wounds. In 18 rabbits, turmeric paste was compared with honey acting as a topical drug against the control of experimentally produced circular full-thickness wounds. Wound healing was measured on treatment days 0, 3, 7, and 14 based on physical, histo-morphological, and histochemical parameters. The tensile strength on day 14 was assessed. It was found that wound healing in both treatment groups was statistically significantly faster than in the control group (Rao SGV, 2003). Curcumin extracted from *Curcuma longa* showed noteworthy wound healing potential as it acts on different stages of the natural wound healing progression to fasten the process (Akbik et al., 2014). Studies confirmed that Curcumin reduces oxidative stress and lipid peroxidation, inhibits AGEs accumulation (Sajithlal et al., 1998), reduces the activation of TNF-α, IL-1β, and MMP-9, increases the levels of IL-10, SOD, catalase, and glutathione peroxidase (Vinay et al., 2014), increases the anti-inflammatory cytokine IL-10, upregulates the expression of VEGF, TGF-β1, HIF-1α, SDF-1α, and HO-1, as a result, increases new vascular formation (Kant et al., 2015).

4.1.12. Gymnema sylvestre

Gymnema sylvestre (Family: Asclepiadaceae). The leaf is commonly used as an antidiabetic, astringent, salty, digestive, acrid, anodyne, thermogenic, anti-inflammatory, and liver tonic in traditional Ayurvedic medication. Tannins and saponins are important chemical constituents of *Gymnema sylvestre* and possess the potential for wound healing. In a model named excision wound model, orally administered *Gymnema sylvestre* leaves ethanolic extract has been found to substantially raise the wound healing rate. The extract demonstrates high healing potential in granuloma models, excision, incision, and dead space model (Malik, 2009). Besides, the increased wound healing activity of the hydroalcoholic extract is related to the presence of phytoconstituents (flavonoids) which act as free radical scavenging candidates that may act individually or possess synergistic effects (Tiwari et al., 2014).

4.1.13. Heliotropium indicum

Heliotropium indicum (Family: Boraginaceae) is commonly known as Indian heliotrope, Indian Turnsole. The wound healing activity of the methanol, chloroform, petroleum ether, and aqueous isolates of *H. indicum* leaves was investigated in rats using incision, excision (infected and normal), and dead space wound models. In the incision wound infection model, the group of animals treated with methanol ex-tract showed significant healing efficacy with an epithelialization period of 16.23 ± 0.98 days, compared to the group of animals treated with regular nitrofurazone medication, which had an epithelialization period of 13.5 ± 1.54 days. In this model, the treated animals with aqueous and methanol extracts showed considerable improvements in wound breaking strength, reaching 378.63 ± 18.02 g and 478.55 ± 12.63 g, respectively, whereas the other extracts failed to provide meaningful effects (Fayed, 2021).

4.1.14. Hibiscus syriacus

Hibiscus syriacus (Family: Malvaceae) is an ornamental shrub distributed through Eastern and Southern Asia. It showed wound healing effect through the acceleration of wound closure possessing a potential decrease in TNF-α, besides an increase in TGF-β and VEGF levels resulting in the enhancement of re-epithelization, angiogenesis, and perfect epithelial remodeling (Bakr et al., 2021). Various phytochemical studies of different organs of *Hibiscus syriacus* revealed positive triterpenoids (Shi et al., 2014), naphthalene, and lignans (Yeon et al., 2019), coumarins (Yun et al., 2001), sterols and flavonoids, anthocyanidin malonyl glucosides which are suggested to contribute to the wound healing activity of the plant (Yun et al., 2001).

4.1.15. Hippophae rhamnoides

Hippophae rhamnoides (Family: Elaeagnaceae) is regularly known as sea buckthorn (SBT). In an experimental study in rodents, it was discovered that the topical utilization of SBT expanded collagen synthesis and healing at the injury site, as confirmed by an expansion in hydroxyproline, and hexosamine levels and a raise in the expression of collagen type-III. The histological assessments and matrix metalloproteinases (MMP-2 and - 9) expression likewise affirmed the property of wound healing of SBT leaf extract (Upadhyay et al., 2011). Omega-7 extracted from the oil of *Hippophae rhamnoides* increased the telomerase activity and keratinocyte growth factors levels which increase wound healing. As a result, the wound healing activity of *Hippophae rhamnoides* was due to the presence of Omega-7 as one of its constituents (Niimi et al., 2021).

4.1.16. Kigelia pinnata

Kigelia pinnata, known as Sausage of the Bignoniaceae family, is a small tree found in southern, central, and western Africa and India. In Wistar rats, the plant had a significant, positive effect on wound healing. It was found that the plant increases the epithelization speed, thus supporting the conventional claim (Sharma UK, 2010). The key components isolated from *Kigelia pinnata* are hentriacontane, *β*-tocopherol, 3-hydro-4,8-phytene, trans-phytol, (9Z,12Z)-methyl octadeca-9, 12-dienoate and 1,3,3,5,6,6-hexamethylcyclohexa-1,4-diene (Olubunmi Atolani, 2012).

4.1.17. Lawsonia inermis

Lawsonia inermis leaves (Family: Lythraceae) is generally known as henna. It is utilized for treating various complications such as wounds, burns, ulcers, and skin inflammations. Lawsone was also tested for wound repairing operations isolated from leaves. It was estimated that in both excision and incision wound models, the ethanolic extract of henna leaves and lawsone had a strong healing response. However, the topical route in the case of ethanolic extract, along with isolated lawsone, showed more active than the oral administration. Thus, the topical route of ethanol extract can be used for wound healing (Sakarkar DM, 2004). Studies showed that the fatty acids of the oil of *Lawsonia inermis* were responsible for full wound re-epithelialization with the reappearance of skin appendages and well-organized collagen fibers without any inflammatory cells (Rekik et al., 2019).

4.1.18. Lycopodium serratum

Lycopodium serratum (Family: Lycopodiaceae) is known as club moss. The wound healing potential of *Lycopodium serratum* reduces the epithelialization duration and raises wound contraction speed, skin brittle strength, granulation tissue brittle strength, granulation tissue dry weight, and elevated hydroxyproline concentration in an experimental study in rats. Histopathology studies of the granulation tissue of the ethanol extract-treated animals showed fewer macrophages with increased production of collagen, suggesting the efficacy of the ethanol extract in facilitating wound healing. The whole plant is ground in hot water, and the thick paste is thus obtained and applied to sores, cuts, wounds, and burns (Manjunatha BK, 2007).

4.1.19. Morinda citrifolia

Morindacitrifolia (Family: Rubiaceae) is a traditional medicinal plant in Polynesia. It is commonly called noni. The key native uses tend to be leaves as a topical treatment for wound healing. In the preclinical test on day 11, the extract-treated animals demonstrated a 71 percent decrease in the wound site relative to the normal control animals, with a 57 percent decrease in the wound site. Non-treated animals had considerably higher granulation tissue weight and hydroxyproline concentration in dead space wounds than controls. Enhanced wound contracting, decreased epithelial time, higher hydroxyproline content, and histological properties propose that leaf extract should have potential wound healing properties (Shivananda et al., 2009).

4.1.20. Moringa oleifera

Moringa oleifera (Family: Moringaceae) has been used in the Indian diet for centuries. The plant leaves have been documented for anti-tumor, antioxidant, radioprotective, hypotensive, anti-inflammatory, and diuretic properties. The aqueous extract investigated for wound healing activity in experimental animals showed a significant reduction in scar area and wound closure time and a rise in the granuloma breaking force, skin breaking strength, hydroxyproline size, and granuloma dry weight (Rathi et al., 2006).

4.1.21. Radix paeoniae

Radix paeoniae (Family: Ranunculaceae), known as Baishao in China, is a widely used medicinal herb in traditional Chinese medication (TCM). The aqueous extract of *Radix paeoniae* roots was estimated for wound healing properties by excision, incision, and dead space wound models on experimental rats. The parameters considered were tissue breaking strength, epithelialization, wound contraction, and granulation tissue dry weight for study. In comparison to nitrofurazone-treated control rats, the extract indicated potential wound healing characteristics (Malviya and Jain, 2009).

4.1.22. Rosemarinus officinalis

Rosemarinus officinalis (Family: Lamiaceae), known as Rosemary, is an evergreen herb that grows wild in most Mediterranean regions (Alizargar et al., 2012). The ethanolic extract of the leaves showed wound healing activity which was suggested to work through the following mechanisms: increase in the division of fibroblasts and the deposition of collagen, neo-vascularization, enhanced granulation tissue formation, reduction in the action of collagenase, and decrease in the bacterial contamination in wounds due to potent anti-bacterial effect (Alizargar et al., 2012). The main active constituents of the ethanolic extract are quercetin, carvacrol, and caffeic acid. Besides, the essential oil of *Rosemarinus officinalis* accelerates wound healing development in infected wounds (Nejati et al., 2015).

4.1.23. Rubia cordifolia

Rubia cordifolia (Family: Rubiaceae), known as Manjistha, Indian madder. This plant's roots comprise high therapeutic value and are officially recognized. *Rubia cordifolia* possesses broad series of uses such as blood purifiers, anti-inflammatory, immunomodulatory, and antioxidants. The alcoholic extract and hydrogel were investigated to evaluate their healing efficiency on the template of excision wounds in mice. A unique alcoholic extract formulation of the roots was applied topically as a single dose on the excision wound surface. To determine the effect on wound healing, wound area measurement and histopathology studies were performed. In treated mice, the effect created by gel was significant in terms of wound contracting capacity, wound closure, declining wound surface area, tissue regeneration at the wound site, and histopathological characteristics. As a result, this research offers a conceptual foundation for the plant's widespread usage in wound care (Thakur et al., 2011).

4.1.24. Sesamum indicum

Sesamum indicum (Family: Pedaliaceae) is a blossoming plant in the genus Sesamum. Various wild relatives of *Sesamum indicum* are present in Africa, and a more modest number in India. To treat the wounds in the experimental rats, a mixture of Sesamum indicum seeds and oil was made using carbopol at 2.5 percent and 5 percent. The formulation handled in this way showed a considerable decrease in the period of epithelization and a 50% reduction in wound contraction in the excision and burn wound models. The breaking strength also increased noticeably in the incision model. Delivered oils and seeds increased the granulation tissue's breaking strength, dry weight, and hydroxyproline content in the dead space wound model. According to the findings, *Sesamum indicum* seeds have wound-healing properties. (Kiran and Asad, 2008).

4.1.25. Solanum xanthocarpum

Solanum xanthocarpum is a very stably permanent herb, found in south-east Asia, Malaya, and tropical Australia. The methanolic fruit extracts showed notable promise for wound healing in a preclinical

investigation in rats. At the time of diagnosis, it was also discovered that the healing tissue's tensile strength was much higher than that of the control (37.5%) (More et al., 2013).

4.1.26. Tephrosia purpurea

Tephrosia purpurea (Family: Leguminosae) is also referred to as "Sarwa Wranvishapaka". The wound healing ability of the aerial part of *Tephrosia purpurea* ethanolic extract in the form of simple ointment was tested in experimental animals using three types of wound models in rats such as incision wound, excision wound, and dead space wound. In terms of wound contraction, tensile strength, histopathological and biochemical parameters such as hydroxyproline content and protein rate, the findings are comparable to generic medication (Fluticasone propionate ointment) (Lodhi et al., 2006).

4.1.27. Terminalia bellirica

Terminalia bellirica (Family: Combretaceae) is commonly called belliricmyrobalan. It's been noted that *Terminalia Chebula* treatment showed wound healing at a higher rate than suggested by increased contraction rates and decreased epithelialization duration. Biochemical tests have shown a substantial rise in total protein, collagen, and DNA content in the granulation tissues of the treated wounds. Hexosamine and uronic acid concentrations have also increased in these tissues (Singh et al., 2019).

4.1.28. Trigonella foenum-graecum

Trigonella foenum-graecum (Family: Leguminoseae) is commonly known as Fenugreek. It is an annual herb where its seeds are mostly used as a spice in many kitchens around the world. The seeds of *Trigonella foenum-graecum* are rich in polysaccharides and saponins, which are responsible for wound healing. A study reported the application of polysaccharides of the seeds of *Trigonella foenum-graecum* in the form of a hydrogel, when applied over the wound area, significantly enhanced the wound healing and lead to the acceleration of the wound closure after 14 days of induction of wound (Ktari et al., 2017).

In addition, many other potential herbs that have been assessed for their wound healing property are recapitulated in Tables (1 and 2). The image of medicinal plants with wound healing activity is prearranged in Figure 2.

Figure 2. Some medicinal plants with wound healing activity.

Table 1. Some common wound healing natural agents and their mechanism of action

Natural agent	Active principles	Suggested mechanism of action	Ref.
Azadirachta indica	paste of stem bark	accelerate the healing process through increasing the protein and hydroxyproline levels and increased proliferation of the cells potent antibacterial in infected wounds.	**(Maan et al., 2017)**
Bee pollen	about 250 substances including amino acids, lipids (triglycerides, phospholipids), vitamins, macro- and micronutrients, phenols, and flavonoids.	Burn wound healing	**(Ragno et al., 2016)**
Centella asiatica	topical application	decreased in granulation and scar formation of the wound and increased the skin tensile strength and prevents inflammatory responses	**(De Fátima et al., 2008)**
Chamaemelum nobile	*Chamaemelum nobile* ointment	significant acceleration and increase in wound healing,	**(Kazemian et al., 2018)**
Gymnema sylvestre	hydroalcoholic extract	presence of phytoconstituents (flavonoids) which act as free radical scavenging candidates that may act individually or possess synergistic effects.	**(Tiwari et al., 2014)**
Hibiscus syriacus	mucilage and petroleum ether extract.	In addition to vascular endothelial growth factor, tumor necrosis factor is accelerated, histopathologically examined, and linked inflammatory parameters are modulated.	**(Bakr et al., 2021)**
Honey	hydrogen peroxide, high osmolarity, acidity, non-peroxide factors, nitric oxide, phenols, and flavonoids.	produces enzymes that contain hydrogen peroxide (a known antimicrobial agent). antimicrobial, debriding, deodorizing, and anti-inflammatory properties and it stimulates the growth of new tissue.	**(Ragno et al, 2016; Rajendran, 2018)**
Lawsonia inermis	Oil	full re-epithelialization of wounds with the reappearance of skin appendages and well-organized collagen fibers without any inflammatory cells.	**(Rekik et al., 2019)**
Lycopodium serratum	thick paste	decrease in the epithelialization duration and increase in wound contraction speed, skin brittle strength, granulation tissue brittle strength, dry weight of granulation tissue, and elevated hydroxyproline concentration	**(Manjunatha, 2007)**
Morindacitrifolia	extract of the plant	enhanced wound contracting, decreased epithelial time, raised hydroxyproline content	**(Shivananda et al., 2009)**
Moringa oleifera	aqueous extract of the leaves	The aqueous extract was tested for wound healing potential in experimental animals, and it was found that there was a significant decrease in wound closure time and scar area and an increase in the skin breaking strength, granuloma breaking force, hydroxyproline size, and granuloma dry weight.	**(Rathi et al., 2006)**
Radix paeoniae	aqueous extract of roots	potential wound healing properties of the extract as compared to nitrofurazone-treated control rats.	**(Malviya and Jain, 2009)**
Rosemarinus officinalis	ethanolic extract of the leaves	An increase in the proliferation of fibroblasts • An increase in the deposition of collagen. • Neovascularisation • An enhanced granulation tissue formation • A decrease in the action of collagenase • A decrease in bacterial contamination in wounds.	**(Alizargar et al., 2012)**
Rubia cordifolia	alcoholic extract formulation of the roots	wound contracting capacity, wound closure, declining wound surface area, tissue regeneration at the wound site, and histopathological characteristics.	**(Thakur et al., 2011)**
Sesamum indicum	Seeds and oil	significant decrease in the time of epithelization and 50 % wound contraction.	**(Kiran and Asad, 2008)**
Solanum xanthocarpum	methanolic fruit extract	significant wound healing potential, a high tensile strength of the healing tissue.	**(More et al., 2013)**
Tephrosia purpurea	the aerial parts	wound contraction, tensile strength	**(Lodhi et al., 2006)**
Terminalia bellirica	Extracts	increased contraction rates and decreased epithelialization duration.	**(Singh et al., 2019)**
Trigonellafoenum-graecum	Polysaccharides from the seeds	enhanced the wound healing process and lead to the acceleration of the wound closure after 14 days of induction of wound.	**(Ktari et al., 2017)**

Table 2. Phytochemicals with wound healing activity

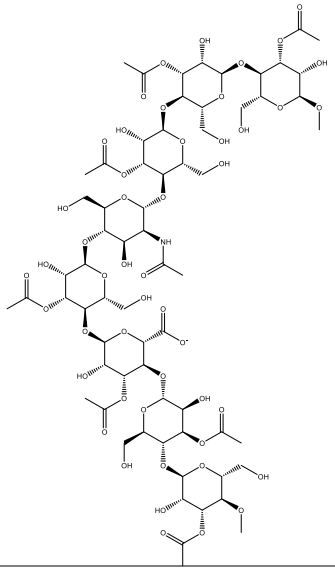
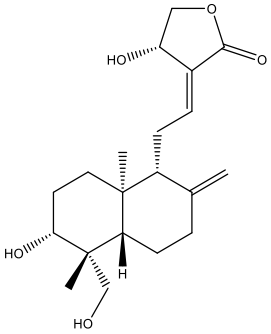
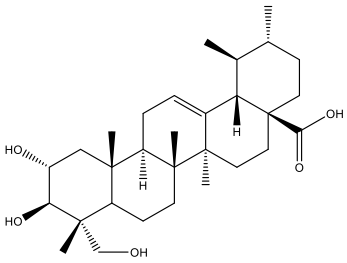
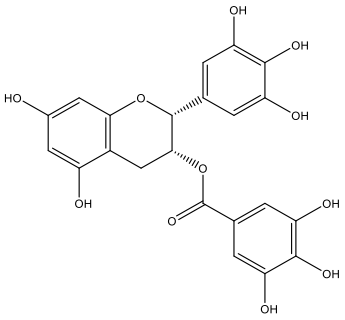
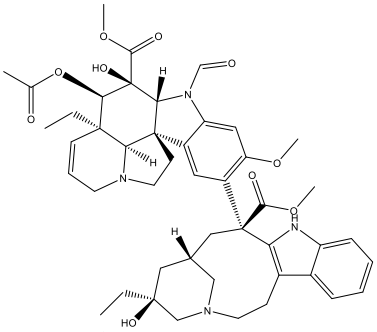
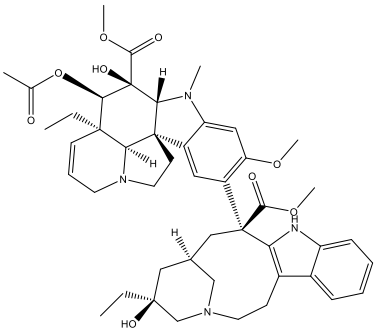
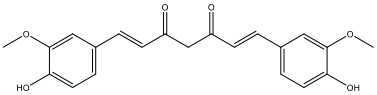
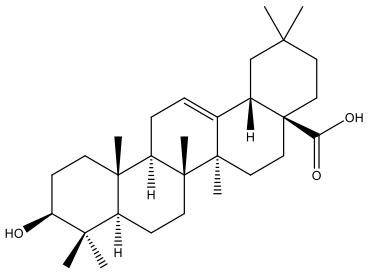
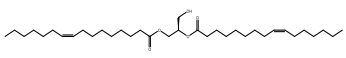
Wound healing agents	Phytochemical class	Structure	Source/ Mechanism of activity	Ref.
Acemannan	mucopolysaccharide		***Aloe vera*** / a potent macrophage stimulator and activator for T-cell and induces proinflammatory mRNAs transcription (IL-1α, IL-1β, IL-6, TNF-α, PGE2, and nitrous oxide).	**(Shedoeva et al., 2019)**
Andrographolide	diterpene lactone		***Andrographis paniculate***/ improved wound healing in surgical open wounds.	**(Al-Bayaty et al., 2012; Sridharan et al., 2021**)
Asiatic acid	Terpenes		***Centella asiaticai***/ stimulates collagen synthesis.	**(Lawrence, 1967)**
Epigallocatechin-3-gallate	Catechins		***Camelia sinensis***/EGCG inhibits TGF receptors in human dermal fibroblasts by changing TGF signaling, lowering MMP-1 and MMP-2 expression, and reducing collagen type 1 synthesis. These characteristics suggest that EGCG could be used as an anti-scarring agent. Furthermore, EGCG has been shown to cause keloid shrinking [124] as well as prevent keloids' growth and degenerative characteristics by decreasing STAT3 signaling.	**(Hsu et al., 2003)**

Table 2. Phytochemicals with wound healing activity (continued)

Wound healing agents	Phytochemical class	Structure	Source/ Mechanism of activity	Ref.
Vincristine	monoterpenoid indole alkaloid		***Catharanthus roseus*** **/** re-epithelialization faster, and the rate of wound constriction was additionally increased.	**(Anom-Dddd, 2013)**
Vinblastine	monoterpenoid indole alkaloid			**(Anom-Dddd, 2013)**
Curcumin	Phenolics		***Curcuma longa*****/** acts on different stages of the natural wound healing process to fasten the healing process, in addition reduces the oxidative stress and lipid peroxidation, inhibits AGEs accumulation, reduces the expression of TNF-α, IL-1β, and MMP-9, increases the levels of IL-10, SOD, catalase and glutathione peroxidase, increases the anti-inflammatory cytokine IL-10, upregulates the expression of VEGF, TGF-β1, HIF-1α, SDF-1α, and HO-1, as a result increases new vascular formation.	**(Akbik et al., 2014; V. Kant et al., 2015; Vinay Kant et al., 2014; Sajithlal et al., 1998)**
Oleanolic acid	terpenes		***Anrederadiffusa*** **/** Oleanolic acid is the main active component of *Anredera diffusa* responsible for its wound healing activity.	**(Moura-Letts et al., 2006)**
Omega-7	fatty acids		***Hippophaerhamnoides*** **/** increase telomerase activity and keratinocyte growth factors levels which accelerate wound healing.	**(Niimi et al., 2021)**

4.2. Medicinal plants as a resource of natural antioxidants in wound mending

A great number of bioactive natural compounds with substantially lowering as well as free radicals scavenging qualities can be found in medicinal plants and foods. Phenolic compounds, flavonoids, essential oils, carbohydrates, their derivatives, and other natural chemical constituents are just a few of the compounds that can help prevent oxidative assaults. According to investigations, naturally occurring

antioxidative molecules added to food products or pet food have been demonstrated to lessen oxidation, enhance as a whole product value, and extend the shelf life. Natural active molecules have also, in recent times, been investigated against harmful microbes such as fungus, viruses, and bacteria that are attacking and resistant to conventional drugs and posing a major threat to human wellness and health (Cardoso, 2019).

Numerous research has suggested that plant compounds, especially flavonoids have several bioactivities such as anti-hypersensitivity, active against the virus, potent anti-inflammatory, vasodilating, and many more biological properties. The antioxidant capacity of flavones or flavonoids and polyphenols, through their responsibility to mitigate free radicals' production, has obtained the most attention in recent days. The majority of consumed flavonoids are broken down into different phenolic acids, some of which show similar properties against free radicals as that of flavonoids (Pietta, 2000).

Reactive Oxygen Species (ROS) are tiny O_2 derived molecules that are made primarily in the cell's powerhouse by the respiratory chain; examples include hydrogen peroxide, superoxide anion, and peroxide (Scialò et al., 2017). They are oxidizing species and mainly contribute to cell membrane damage. Further, they serve a useful purpose, particularly in the case of usual tissue repair. As a result, achieving a balance between higher and lower ROS levels is crucial. Low concentrations of ROS protect body organs or tissues from pathogenic infections and stimulate the impactful healing process of wounds by producing cell survival signaling, however when concentrations exceed, they lead to oxidative assaults, which results in cellular damage and a pro-inflammatory state (Ponugoti et al., 2013). Under usual conditions, the cell's endogenous system, i.e. the body's natural antioxidant defense processes, can minimize the adverse effect of such free radicals or ROS. ROS detoxifying enzymes like catalase, superoxide dismutase, and glutathione peroxidases are among the antioxidant defenses in the body's organs or tissue (Aruoma, 1994). This becomes completely obvious that the only way to fruitfully lower the damaging effect of free radicals is to use an external system. As a result, nutritional supplement with antioxidant properties is required to maintain a continual supply of antioxidants to the physiological system. Antioxidants are thought to aid in reducing wound free radicals and thus speed tissue repair. They play a substantial role in controlling the possible injury that reactive species can cause to cellular molecules like protein, lipids, and nucleic acids.

4.3. Evidence of natural antioxidants in wound healing

Numerous researches conducted on natural herbs have addressed the emergence of new resources with the ability to help in a wide variety of wounds with negligible toxicity, ease of usage, increased efficacy, and economical therapeutic interventions for patients in the latest times. According to the published research works, flavonoids seem to be the greatest important and promising group of plant molecules for wound mending.

Flavonoids were found to have valuable anti-inflammatory characteristics across several research findings, as they lowered the levels of inflammatory facilitators such as prostaglandin, leukotrienes, inflammatory cytokines, and cyclooxygenase while increasing anti-inflammatory mediators, especially IL-10 (Ahmed et al., 2018; Du et al., 2021). NO and ROS are produced by macrophages of the M1 type. Quercetin, a flavonoid, lessens M1 cellular function while increasing M2 macrophage activity; but even so, it does not mean that almost all flavonoids control M1 or M2 macrophages function (Fu et al., 2020), and an inability to control in the regulation of these phenotypes is connected to the progression of the normal wound to chronic state (Anower, 2014; Benoit et al, 2008).

Besides this, flavonoids were demonstrated to function in the down-regulation of NF-κB expression, which is a crucial component in the inflammatory signaling reaction that adds to collapse in the process of wound healing (Dinda et al., 2015). All immune and non-immune cells adversely affected by chronic inflammation show NF-κB induction. NF-κB stimulation promotes the activation of several pro-inflammatory genes, which help to manage and organize the inflammatory activities that trigger tissue injury. However, NF-κB was shown to have a favorable impact on epithelial cells. In epithelial cells, NF-κB signaling is important for maintaining immune homeostasis (Wullaert et al., 2011). Plant extracts have been shown to activate NF-κB, which promotes wound healing. In human immortalized keratinocytes and

dermal fibroblast cells, the flavonoid/phenol-rich n-hexane extract of *Calendula officinalis* has been shown to boost the function of NF-κB (Nicolaus et al., 2017).

MMP-2 is involved in angiogenesis matrix remodeling, whereas MMP-9 is a sign of re-epithelization during the initial stages of wound recovery (Baker and Leaper, 2003; Manuel and Gawronska-Kozak, 2006). In the healing process, MMP-8 (collagenase-2) cleaves collagens, and MMP-13 (collagenase-3) indirectly promotes re-epithelialization by influencing wound closure (Caley et al., 2015). It was conceivable to demonstrate the response of flavonoids on MMPs in this review. Flavones (Mohammadi et al., 2019) and flavanols (Jaiswal et al., 2013) caused up-regulation of MMPs 2, 8, 9, and 13, demonstrating their ability to aid tissue repair or wound mending.

Smad is required for TGF cell signaling, according to new research. These markers, particularly Smad 2 and 3, function as latent nuclear transcriptional activators and governs the cellular functions in tissue repair (Ashcroft and Roberts, 2000). TGF also induces Smad 7, but negative feedback prevents Smad 2 and 3 from being phosphorylated and translocating to the nucleus (Shi et al., 2003). Molecules like flavanone, flavonol, and isoflavone have been demonstrated to boost TGF-β expression, resulting in higher Smad 2/3 expression and lowered Smad 7 regulation. Further, TGFβ1 is involved in angiogenesis by up-regulating VEGF, a well-known factor for angiogenesis (Penn et al., 2012). These growth factors bind to their specific receptor in keratinocytes and macrophages, allowing them to perform critical functions throughout the tissue repair. Chronic and nonhealing wounds frequently have woefully inadequate vascularization. Delay wound healing has been observed in hyperglycemia conditions in diabetic animals, with impoverished vascularization being the cause of disrupted wound contraction, epithelialization, and granuloma tissue regeneration (Stallmeyer et al., 2001). *H. rosa-sinensis* extract rich in phenolic or flavonoids compounds has been shown to promote wound healing by raising these growth factors (Shen et al., 2017).

The PI3K pathway promotes cell growth, which is important for wound healing. Akt (serine/threonine-specific protein kinase) is phosphorylated at serine 473 residues when the PI3K pathway is activated, which has been discovered to be critical for the directional immigration of skin and corneal epithelial cells in response to injury or wound (Yu et al., 2014). The PI3K pathway has been linked to the induction of wound healing by medicinal plants. The PI3K pathway is activated by natural herbs like *Calendula officinalis*, which promotes wounded tissue healing (Dinda et al., 2015).

Although ROS generation is required to introduce the healing process of tissue, too much of it is harmful to wound healing, leading to delays in wound closure, keratinocyte migration, and epithelialization (Zhang et al., 2019). Superoxide dismutase, catalase, and glutathione peroxidase are antioxidative enzymes that behave as shielding factors, ensuring that tolerable levels of ROS are maintained for correct body function, with the homeostatic balance among antioxidant enzymes and ROS becoming critical in the tissue healing process (Abdulaziz, 2019). Several flavonoids have been shown to boost endogenous antioxidant capacity, implying a shielding effect and aiding tissue repair.

The several strands of data suggested that wounds are subjected to oxidative assault as a result of increased neutrophil activity due to oxidants and MPO activity. In a chronic wound, enhanced neutrophil activity tends to cause tissue injury due to oxidants and MPO activity (Song et al., 2008). In chronic wounds, the production of ROS causes cytotoxicity and delays wound healing (Mikhal'chik et al., 2006). The availability of multiple phytochemicals in medicinal herbs contributes to their antioxidant activity (Pawar et al., 2007). This has been revealed that using a bioadhesive gel containing an ethanolic extract of Leea macrophylla (5 percent w/v) raised endogenous antioxidant capacity while decreasing MPO action (Joshi et al., 2016).

Flavonoids are, therefore, essential factors in tissue repair, according to the data collected from the articles published. Medicinal herbs and plant-derived antioxidants, on the other hand, are gaining in popularity, and we've highlighted the evidence for their use in wound healing. The use of antioxidants appears to be a great promise for wound healing, but there are few animal investigations, and even fewer clinical researches are available. The use of antioxidants for wound healing as a field of science is still in its early stages, and prospective research will help to better understand this same.

4.4. Herbal combinations in several pharmaceutical dosage forms for wound healing

Antimicrobial dressings, including natural antimicrobial agents, can be used for wound healing (Rajendran, 2018). Honey, Aloe vera, and Neem tree extracts are just a few of the antibacterial agents that are utilized solely in wound dressings (Rajendran, 2018). These are considered potential antibacterial agents for modern wound dressings. Honey dressings help to maintain wound moisture, which facilitates faster healing and decreases the bacterial load by releasing hydrogen peroxide. Honey's osmatic action prevents the development of bacteria and encourages speedy wound healing (Archer et al., 1990). It also promotes autolytic debridement. Aloe vera is extensively utilized for wound healing dressings owing to its active principles of the Aloe vera gel, which has moisturizing, anti-inflammatory, antibacterial, antifungal, antiviral agent, and anti-odor properties.

4.5. Nanomedicine and wound healing activity

Amongst the operative strategies for potent wound healing potential is the formulation of medicinal plant extracts in biocompatible nanoparticles in a green and cost-effective method, producing nanostructures with excellent wound healing effects (Hajialyani, 2018). Loading herbal-based principles in nanoparticles, nano-emulsions, nanoliposomes, and hydrogels, enhance their availability, controls their release like sustained release dosage forms to stay for a longer time on the wound, in addition, the enhancement of the permeability of herbal preparations constituents to the deep skin that is vital for the progression of wound healing to take place properly.

Several topical herbal-based nanostructures mainly contain curcumin which contributed to the increase of healing being most potent in the inflammatory phase through regulating the levels of TNF-α, IL-10, and TGF-β1. Besides, biosynthesized herbal-based silver nanoparticles also have a major potential in wound inflammation control.

5. Need of Innovative Research

The knowledge of phytochemicals and other naturally derived drugs is still in its early stages, with more discoveries to come. Many herbs and plants have still to be researched for their therapeutic efficacy in clinical systems that emphasize phytochemicals, such as traditional Chinese medicine, Ayurveda, and naturopathy. Increased research and use of phytochemicals and other biologically derived compounds suggest that wound care is becoming more complex and difficult, with complex physiology and pathophysiology.

6. Future Developments

Wound healing with phytochemicals and naturally derived substances is a promising development. More prospective, well-controlled studies are needed to assess the importance of these naturally derived products in wound treatment. The impact of the extraction method on the extract's final composition needs to be better defined, and the value of this information is currently undervalued. Naturopathic medicine, Herbal remedies, and homeopathy, unlike allopathic medicine, make substantial use of plant/herbal extracts and naturally derived compounds. Collaboration would be required for future systematic and thoughtful studies into the role of naturally derived products in wound healing.

Conclusion

Plants are excellent wound healers because they innately heal wounds. Throughout different wound models, the rate of wound closure, epithelialization, tensile strength, histopathology, and granuloma weight can all be measured clinically to control the healing process. This study showed that traditional medicines play an important role in wound healing. Herbal medicines are becoming increasingly popular in various countries as they are healthier and more widely accepted than allopathic drugs. As a result, combining

traditional and modern expertise can provide better or faster wound healing with minimal toxicity. In addition, the use of nanotechnology and preparation of topical herbal-based nanostructures may contribute to the enhancement of the effect of natural extracts as wound healing agents due to the enhancement of their bioavailability, increasing their absorption at the site of administration in addition to their stability for longer periods. In conclusion, although natural sources showed significant results concerning wound healing activity, still more clinical trials must be carried out to confirm their complete safety and efficacy.

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