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Correspondence Address

Van Yuzuncu Yil University, Faculty of Agriculture, Zeve Campus, 65080, Van-TÜRKİYE
(Van Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Zeve Yerleşkesi, 65080, Van-TÜRKİYE)

Phone	Fax	e-mail
+90 (432) 225 10 56; 225 10 24	+90 (432) 225 11 04	yyujagrsci@gmail.com

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Research Article

Investigating the Content and Bioaccessibility of Phenolic Compounds In Roots of *Rosa canina* L. and *Rosa pimpinellifolia* L.

Merve MACIT*[1], Aliye ARAS[2], Esra CAPANOGLU GUVEN[3], Sena BAKIR[4]

[1]Department of Crop and Animal Production, Food Agriculture and Livestock Vocational School, Bingol University, Bingol, Türkiye
[1,2]Department of Botany, Faculty of Science, Istanbul University, Istanbul, Türkiye
[3]Food Engineering Department, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Istanbul, Türkiye
[3,4]Department of Gastronomy and Culinary Arts, Tourism Faculty, Recep Tayyip Erdogan University, 53400 Ardesen, Rize, Türkiye

[1]https://orcid.org/0000-0001-9648-4799, [2]https://orcid.org/0000-0002-2188-4031, [3]https://orcid.org/0000-0003-0335-9433
[4]https://orcid.org/0000-0002-6258-1969

*Corresponding author e-mail: mmacit@bingol.edu.tr

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Abstract: Rosehip is among the most important plants with high economic value, mainly used in foods and beverages from ancient times to the present. In this study, *Rosa canina* L. and *Rosa pimpinellifolia* L. roots, consumed as tea in Aktoprak Village of Erzurum province, were collected together with the fruits. The main goal of the study was to investigate the *in vitro* bioaccessibility of phenolic compounds in the roots and fruits of *R. canina* and *R. pimpinellifolia* by a simulated gastrointestinal digestion procedure. Methanolic and aqueous extracts were prepared for the analysis of phenolic compounds in roots, whereas only methanolic extracts were used for the analyses of fruits. Total phenolic and total flavonoid contents were evaluated spectrophotometrically, while four different methods were used for antioxidant capacity measurements. The quantification of individual phenolic acids, flavonoids, and anthocyanins was performed with HPLC-PDA. Results demonstrated that *R. canina* and *R. pimpinellifolia* have high levels of total phenolics, total flavonoids, and antioxidant capacity. The roots of *R. pimpinellifolia* and *R. canina* were observed to contain higher amounts of phenolics compared to the fruits. Epicatechin, 4-hydroxybenzoic acid, gallic acid, syringic acid, p-coumaric acid, naringenin, and ellagic acid were not determined in the fruit extracts of *R. pimpinellifolia* and *R. canina*, while they were detected in aqueous extracts of roots. Bioaccessibility analyses carried out on aqueous root extracts showed total phenolic recovery was 12.73% in *R. canina*, 10.71% in *R. pimpinellifolia*, and total flavonoid recovery was 0% in both species.

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1. Introduction

Türkiye has one of the largest production areas of rosehips (Yıldız and Çelik, 2011). There are a hundred species in the world belonging to the genus *Rosa* of the Rosaceae family, and 26 species out of a hundred are found in Türkiye. Rosehip is a shrub-shaped plant that is usually 0.3-3 m (rarely 6 m) tall. It is widely grown in the Central and Western Black Sea regions such as Corum, Kastamonu, Amasya, Gumushane, Tokat, and in the Eastern Anatolian region, such as Erzincan and Erzurum, Hakkari, Bitlis, and Van, where continental climates are experienced (Dogan and Kazankaya, 2006). Rosa species fruits are generally rich in the bioactive compounds with antioxidant activity, especially in flavonoids, carotenoids, tannins, phenolic acids, mineral compounds and fatty and organic acids (Ayati et al., 2019). The medicinal properties of Rosaceae fruits can be partially attributed to their abundance of phenolics which possess a broad spectrum of biochemical activities; like antioxidant, antimutagenic effects, anticarcinogenic, and the ability to change gene expression (Tapiero et al., 2002; Nakamura et al., 2003, Sevket et al., 2016).

Many different factors including pollution in the environment, UV rays from the sun, industrial wastes, petrochemical products, infection, cosmic rays, chemicals, X-rays, viruses, extreme stress, and exhaust gases from automobiles, etc. which continuously increase the amount of exposure to free radicals in our body (Sies et al., 1991). Phenolic compounds help prevent chronic and oxidative stress-related disorders such as cardiovascular, cancer, and neurodegenerative diseases due to their free radical scavenging abilities (Bhuyan and Basu, 2017). Furthermore, phenolics with antioxidant properties have an imperative function in the human defense system against free radical species, carcinogenesis, the relative inducer of DNA damage, cardiovascular diseases, aging, lipid peroxidation, and diabetes. Besides, phenolics are connected with other biological functions like antimicrobial and antiinflammatory activities (Garavand et al., 2021).

Bioaccessibility is described as the amount of food product accessible for intestinal absorption after digestion (Hedren et al., 2002). Moreover, the bioaccessibility of a compound indicates the amount released from the food matrix and which is assumed to be available for absorption (Minekus et al., 2014). Bioaccessibility of a compound deal with various processes, such as the stages of release, absorption, distribution, metabolism, and elimination from a food matrix (Rain et al., 2013; Cosme et al., 2020). From a nutritional point of view, bioavailability accepts the part of a particular food that the body can use; therefore, it is considered as a nutritional activity (Benito and Miller, 1998; Fernàndez-Garcìa et al., 2009; Cosme et al., 2020). The current literature focuses mainly on the phenolics in the fruits of rosehip. Moreover, there are only a few studies about the *in vitro* bioaccessibility of *Rosa canina* L. and *Rosa pimpinellifolia* L. roots teas, in which the phenolic and flavonoid contents as well as antioxidant capacity were investigated before and after digestion.

This study aimed to determine the total phenolic and total flavonoid contents and antioxidant capacity of the roots and fruits of *R. canina* and *R. pimpinellifolia* rosehip species and to investigate the bioaccessibility of phenolic compounds in rosehip roots.

2. Material and Methods

2.1. Plant material

Rosa canina L. and *Rosa pimpinellifolia* L. roots and fruits were collected as three separate samples from the Aktoprak village of Erzurum in October 2016, which is the harvest time of the fruits.

2.2. Extraction procedure

According to Capanoglu et al. (2008), the methanolic extract was prepared in triplicate for roots and fruits belonging to two species. First, 1.00 ± 0.01 g from each sample was mixed with 5 mL of 80% methanol and sonicated in an ultrasonic bath (Azakli, Türkiye) for 15 min. Afterward, the extracts were centrifuged (Hettich Zentrifugen Universal 32R, UK) at 2700 × *g* for 10 min at +4 °C, and the supernatant was separated. Then 5 mL of 80% methanol was added to the residue to repeat the procedure. At the end of the extraction process, supernatants were kept at -20 °C until further analyses.

The method of Perk et al. (2016) was used to prepare aqueous extraction from root samples. Briefly, 2 g of root samples were boiled in 50 mL of distilled water. Then, 5 min after the starting point

of the boiling process, 50 mL of distilled water was added. This process was repeated four times until the total volume reached 200 mL, and the total mixture was allowed to cool down to room temperature. After cooling, the root parts were removed, and the mixture was filtered through filter paper and kept at -20 °C.

2.3. *In vitro* bioaccessibility

The methods of McDougall et al. (2005) and Minekus et al. (2014) were adapted for the determination of *in vitro* bioaccessibility. First, salivary fluid, gastric fluid, and intestinal fluid were prepared according to the protocol. For oral digestion, 2.5 mL of sample was taken and 3.5 mL of saliva, 0.5 mL of amylase solution, and 25 µL of 0.3 M $CaCl_2$ were added. After adjusting the pH to 7.0, the mixture was incubated in a shaker water bath for 2 min at 37°C. At the end of salivary digestion, 2 mL of sample was collected, and the residue was mixed with 6 mL of gastric fluid, 1.28 mL of pepsin solution, 4 µL of $CaCl_2$, and 1.6 µL of HCl, and the pH was adjusted to 3.0, and shaken at 37 °C for 2 h for gastric digestion. At the end of digestion, a 2 mL stomach sample was collected. Then, 7.7 mL intestinal fluid, 3.5 mL pancreatin solution, 1.75 mL bile solution, 28 µL of $CaCl_2$, and 972 µL of 1 M NaOH were added to the remaining part. Dialysis bags were prepared with 20 mL $NaHCO_3$ stock solution (10.5 g $NaHCO_3$ was dissolved in 250 mL distilled water). Prepared bags were placed in intestinal fluids media, then all beakers were kept in the shaker at 37 °C for 2 h, and the part remaining in the dialysis bag was separated as 'IN' and the rest as 'OUT'. These fractions were transferred into Eppendorf tubes and centrifuged at 23000 ×*g*, +4°C. The bioaccessibility (%) was calculated as follows;

Bioaccessibility (%) = Intestinal internal value / Initial value * 100

2.4. Total phenolic content

The total phenolic content of the samples was measured using the Folin-Ciocalteu method, according to the method of Turkmen et al., 2006. First, in the analysis tube, 100 µL of the sample and 900 µL of distilled water were added. After the addition of 1.5 mL of 0.2 N Folin-Ciocalteu reagent mixture was held for 5 min. Following the addition of 1.2 mL of saturated Na_2CO_3 solvent, the mixture was left for 90 min. Absorbance measurement of samples was performed at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1700; Shimadzu Corporation, Kyoto, Japan) against blank. For the generation of the standard calibration curve, gallic acid solution in 80% MeOH was used.

2.5. Total flavonoid content

The total flavonoid content was examined spectrophotometrically according to the method of Dewanto et al. (2002). After 250 µL of the sample was taken into the analysis tube, 1.25 mL of distilled water was added. Then, 75 µL of 5% $NaNO_2$ solvent was added, and the mixture was allowed to stand for 6 min. Afterward, 150 µL of 10% $AlCl_3.6H_20$ solution was mixed and held for 5 min, then 0.5 mL of 1 M NaOH solution was mixed, and the total volume was adjusted to 2.5 mL with distilled water. The absorbance was measured against a blank at a wavelength of 510 nm utilizing a UV–Vis spectrophotometer. For the preparation of the calibration curve catechin solution in 80% MeOH was used.

2.6. Antioxidant capacity

2.6.1. ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) radical scavenging method

The method was applied using the protocol of Miller and Rice-Evans (1997), 220 mg of ABTS radical was dissolved in 200 mL of distilled water, and 38 mg $K_2S_2O_8$ was dissolved in 2 mL of distilled water. These solutions were mixed and kept in the dark overnight to complete radicalization and obtain the ABTS+ solution. Before starting the analysis, until its absorbance achieved 0.9 ± 0.2 ABTS+ solution was diluted with 0.05 M KPi buffer (pH = 8). Then, approximately 100 µL of the sample was transferred into an analysis tube, and 1 mL of ABTS+ solution was added. The mixture was vortexed

(IKA Vortex Genius 3) for 25 sec. Using a UV–Vis spectrophotometer, the absorbance against the blank was measured at a wavelength of 734 nm.

2.6.2. CUPRAC (Cupric Reducing Antioxidant Capacity) method

According to Apak (2004), 0.4262 g of $CuCl_2H_2O_2$ was dissolved in 250 mL of distilled water, 0.039 g of Neocuproin was dissolved in 96% EtOH and diluted to 25 mL, and 19.27 g of NH_4Ac was diluted in 250 mL of distilled water. Nearly 100 µL of the sample was transferred into an analysis tube, and 1 mL of $CuCl_22H_20$ solution, 1 mL of NH_4Ac buffer, 1 mL of Neocuproin solution, and successively 1 mL of distilled water were added. Afterwards the mixture was waited for 30 minutes, and the absorbance using a UV–Vis spectrophotometer was evaluated at 450 nm against water.

2.6.3. DPPH (2,2-diphenyl-1-picrylhydrazyl) method

Shortly, 2 mL of 0.1 mM DPPH was mixed with 100 µL of sample in a test tube. Samples were stored in the dark at room temperature for 30 minutes. Using a UV–Vis spectrophotometer absorbance was measured against methanol at a wavelength of 517 nm. (Kumaran et al., 2006).

2.6.4. FRAP (Ferric Reducing Antioxidant Power) method

The method Benzie and Strain (1996) was used with slight modifications. In short, 3.1 g of $CH_3COONa·3H2O$ was dissolved in distilled water, 16 mL of 99.85% acetic acid was added and the total volume was put into 1 L with distilled water. 0.504 g of FeCl3·6H2O was dissolved in distilled water and mixed with 1 M HCl. The total volume of the mixture was put into 100 mL with distilled water. 0.156 g of tripyridyl triazine (TPTZ) was dissolved in 50 mL ethanol. FRAP reagent was made with a 10:1:1 volume rate with these solution sequences. Then, 100 µL of the sample was taken into a test tube and 900 µL of FRAP reagent was added. After keeping the mixture for 4 min at room temperature, absorbance was measured at 593 nm against distilled water.

2.7. HPLC analysis

HPLC analysis was performed based on the method described by Capanoglu et al. (2008). Standard calibration curves were created using solutions of catechin, gallic acid, *p*-hydroxy benzoic acid (PHBA), protocatechuic acid, vanillic acid, caffeic acid, p-coumaric acid, kaempferol, fumaric acid, naringenin, syringic acid, quercetin, quercetin di-hydrate, ellagic acid, cyaniding and rutin. These samples and their stock solutions mixture were filtered through a 0.45 µm membrane filter, and 1 mL of the filtered sample was accommodated in vials and analyzed in a Waters W600 HPLC system with a PDA (Waters 996) detector. Luna C18 column (Phenomenex, Utrecht, The Netherlands), was utilized as the stationary phase. The mobile phase consisted of solvent A, 0.1% (v / v) trifluoric acid (TFA) with distilled water, and solvent B, 0.1% (v / v) TFA with acetonitrile. A linear gradient was used: 95% solvent A and 5% solvent B at 0 min; At 45 minutes, 65% solvent A and 35% solvent B; At 47 minutes, 25% solvent A and 75% solvent B; Returns to primary conditions in 54 minutes. The flow rate was 1 mL/min. Detections were performed at wavelengths 280, 312, 360, and 520 nm.

2.8. Statistical analysis

The results were analyzed by Tukey's New Multiple Comparison post hoc test ($p<0.05$) using the One-Way Analysis of Variance (One–way ANOVA) with SPSS (21st version, IBM, New York, NY, USA) program. Each analysis was made in triplicate, and the results were reported as the mean ± standard deviation.

3. Results and Discussion

3.1. Total phenolic content and total flavonoid content of *Rosa canina* L. and *Rosa pimpinellifolia* L. roots and fruits

The total phenolics and flavonoid contents of the aqueous and methanolic extracts are presented in Table 1. Total phenolic content was measured as gallic acid equivalent (GAE). Total flavonoid

content measured as catechin equivalent (CA). When *R. canina* and *R. pimpinellifolia* are compared in terms of total phenolic content (TPC), it is observed that the *R. pimpinellifolia* root aqueous extracts exhibited the highest value with 52.81±6.81 mg GAE/g. Similarly, in terms of total flavonoid content (TFC), the highest value was observed in *R. pimpinellifolia* root aqueous extractions with 25.34±4.00 mg CA g^{-1}. The TFC of fruit methanolic extracts constituted the lowest level, while the lowest TFC was observed to be 3.43±1.44 mg CA g^{-1} in the fruit methanolic extracts of *R. canina*. The TFC of *R. pimpinellifolia* root aqueous extracts was observed to be higher than that of *R. canina*. Previous studies indicated that the TPC was 929.27 mg GAE 100 g^{-1} in the aqueous extract, higher than the value observed in the methanol extract of *R. pimpinellifolia* fruit (Çakır and Ergen, 2021). In another study, the total phenolic content levels of *R. canina* fruits were determined as 247.60 ± 0.95 µg EAG mg^{-1} and total flavonoid content as quercetin equivalents 187.66 ± 5.25 µg EQ mg^{-1}. (Fetni et al., 2020). Ozdogan and Coruh (2015) studied *R. heckeliana* roots by comparing methanolic, petroleum ether, ethyl acetate, chloroform, and n-butanol extracts. Accordingly, the TFC was determined as 0.184±0.0047 µg CA mg^{-1} in the methanolic extract. TPC of the six *R. pimpinellifolia* genotypes ranged from 1018-1407 mg GAE per 100 g fresh weight (Karatas, 2021). Encapsulation extract into lipid vesicular systems and biological activity of *Rosa canina* L. bioactive compounds in dermocosmetic use, TPC value was found to be 88.71 ± 0.95 µg GAE mg^{-1} and TFC value was 25.38 ± 0.49 µg QE mg^{-1} (Sallustio et al., 2022). In particular, *R. pimpinellifolia* root aqueous extracts were observed to have a significantly higher TFC compared to the other samples analyzed within the scope of this study. Compared with the values reported in the previous studies, the values observed for *R. pimpinellifolia* aqueous extractions were remarkably higher.

Table 1. Total phenolic content and total flavonoid content of *R. canina* and *R. pimpinellifolia* roots and fruits

***R. canina* and *R. pimpinellifolia* Extracts**	**Total Phenolic Content (mg GAE g^{-1})**	**Total Flavonoid Content (mg CA g^{-1})**
R. canina root methanolic extract	27.22±0.71^{c}	11.16±4.29^{b}
R. canina root aqueous extract	48.01±7.69^{b}	24.06±1.63^{a}
R. canina fruit methanolic extract	10.74±3.09^{d}	3.43±1.44^{c}
R. pimpinellifolia root methanolic extract	28.22±5.10^{c}	12.88±4.99^{b}
R. pimpinellifolia root aqueous extract	52.81±6.81^{a}	25.34±4.00^{a}
R. pimpinellifolia fruit methanolic extract	14.35±2.62^{d}	7.32±2.48^{c}

3.2. Phenolic compounds in *R. canina* and *R. pimpinellifolia* root and fruit extracts

The phenolic compounds and their amounts in *R. canina* and *R. pimpinellifolia* root and fruit extracts are presented in Table 2. Mainly 4- hydroxybenzoic acid was found in *R. canina* root methanolic extract and *also in both R. pimpinellifolia* root and fruit methanolic extracts. Cyanidin, an anthocyanin, was detected only in *R. pimpinellifolia* fruit methanolic extract. Rutin was also present in fruit extracts of both species; however, its amount was observed to be higher in *R. canina*. On the other hand, *p*-coumaric acid was not found in *R. canina* fruit methanolic extracts. The extract with the highest value among the catechin derivatives was observed to be *R. pimpinellifolia* root aqueous extract. Moreover, catechin and its derivatives were detected in large amounts in both species. Among the phenolic components in *R. pimpinellifolia* fruits, the most dominant ones are chlorogenic acid, gallic acid, and vanillic acid (Çakır and Ergen, 2021). In a previous study, phenolic compounds including procyanidin B1, epicatechin, chlorogenic acid (trans-5-O-caffeoylquinic acid) procyanidin B2, salicylic acid, gallic acid, catechin, etc. were found in rosehip (Ghendov-Mosanu et al., 2020). On the other hand, the root of *R. canina* was reported to show the highest content of epigallocatechin (almost 3% of the total catechins) among the determined catechins (Oproshanska et al., 2021). Catechin and catechin derivatives observed in the present study were in accordance with the previous reports. The highest amount of catechin was determined in *R. canina* fruit methanolic extract. The diversity of phenolic substances in *R. pimpinellifolia* fruit methanolic extraction, especially in terms of catechin derivatives, is variable compared to other studies.

Table 2. Phenolic compounds in *R. canina* and *R. pimpinellifolia* root and fruit extracts

Phenolic Substance	*R. canina* Root Ext.		*R. canina* Fruit Ext.	*R. pimpinellifolia* Root Ext.		*R. pimpinellifolia* Fruit Ext.
	Aqueous ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Aqueous ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})
4-Hydroxybenzoic acid	-	0.94±0.02	-	-	0.90±0.3	0.13±0.01
Catechin	4.58±0.07	3.3±0.14	8.40±0.96	2.08±0.14	2.21±0.08	4.27±0.23
Epicatechin	0.69±0.08	0.44±0.04	-	2.30±0.10	1.26±0.11	-
Gallic acid	0.20±0.04	0.12±0.02	-	0.40±0.02	2.21±0.08	-
Syringic acid	0.56±0.04	0.49±0.03	-	-	1.26±0.11	-
P-coumaric acid	0.30±0.05	0.11±0.02	-	0.23±0.02	0.06±0.01	0.01±0.0
Naringenin	-	0.11±0.01	-	-	-	-
Ellagic acid	0.39±0.06	0.55±0.07	-	1.53±0.15	0.34±0.02	0.21±0.03
Quercetin	1.33±0.07	0.14±0.02	0.03±0.00	0.60±0.16	0.18±0.01	0.10±0.01
Rutin	-	-	0.13±0.01	-	-	0.06±0.01
Quercetin di hydrate	0.12±0.03	0.05±0.01	-	0.15±0.01	0.09±0.01	-
Catechin derivative 3 minutes	-	-	2.56±0.15	-	-	1.86±0.14
Catechin derivative 5 minutes	-	-	-	-	-	1.01±0.06
Catechin derivative 7 minutes	-	-	-	-	-	0.48±0.10
Catechin derivative 10 minutes	3.50±0.36	2.69±0.15	-	5.86±0.41	2.75±0.13	-
Catechin derivative 11 minutes	-	-	-	3.45±0.18	1.51±0.08	-
Catechin derivative 14 minutes	-	-	-	0.95±0.14	1.31±0.06	-
Anthocyanin cyanidin	-	-	-	-	-	0.48±0.05

3.3. Total antioxidant capacities of *R. canina* and *R. pimpinellifolia* root and fruit

The total antioxidant capacities of *R. canina* and *R. pimpinellifolia* root and fruit are presented in Table 3. Antioxidant capacity methods DPPH, CUPRAC, FRAP, and ABTS were applied to the root and fruit extracts of *R. canina* and *R. pimpinellifolia*. The highest values among fruit and root parts were observed in aqueous root extractions. The highest values were determined to be 191.86 ± 17.95 mg TEAC g^{-1} and 171.47 ±25.32 mg TEAC g^{-1} in aqueous root extractions of *R. canina* and *R. pimpinellifolia* according to the CUPRAC method, respectively. The aqueous extracts of these two Rosa species and especially *R. canina* and *R. pimpinellifolia* roots were observed to show a much stronger antioxidant effect compared to the fruit (Table 3). In a study conducted on *R. canina* fruit powders, the result obtained with the DPPH method was 1793 mg TE 100 g^{-1} (Igual et al., 2022). Genome size, iPBS profiles, antimicrobial and antioxidant activities, characterization of *Rosa canina* L. fruits in a study that examined collected in urban areas of Slovakia, results on the total antioxidant activity was detected with DPPH method ranged from 6.99 to 7.73 mg TEAC g^{-1} (Rovna et al., 2020). In another recent study examining the functional compounds and antioxidant activities of rosa species cultivated in Türkiye, the antioxidant capacity of *R. canina* species was determined as 1932.50 ± 47.31 mg Trolox/100 g according to the CUPRAC method (Kayahan et al., 2022).

Table 3. *R. canina* and *R. pimpinellifolia* root and fruit total antioxidant capacities

***R. canina* and *R. pimpinellifolia* Extraction**	**DPPH (mg TEAC g^{-1})**	**CUPRAC (mg TEAC g^{-1})**	**ABTS (mg TEAC g^{-1})**	**FRAP (mg TEAC g^{-1})**
R. canina root methanolic extract	69.19±3.75^{b}	77.33±6.64^{b}	48.22±5.00^{cd}	49.05±1.40^{b}
R. canina root aqueous extract	157.68±4.81^{a}	191.86±17.95^{a}	66.56±13.63^{b}	84.44±8.69^{a}
R. canina fruit methanolic extract	25.03±4.91^{c}	64.93±12.41^{b}	35.93±3.23^{d}	13.14±1.82^{c}
R. pimpinellifolia root methanolic extract	67.31±7.62^{b}	77.39±15.26^{b}	59.84±11.50^{bc}	47.62±4.89^{b}
R. pimpinellifolia root aqueous extract	148.15±18.78^{a}	171.47±25.32^{a}	81.64±13.07^{a}	66.41±9.64^{a}
R. pimpinellifolia fruit methanolic extract	30.11±2.26^{c}	71.81±7.66^{b}	39.15±5.44^{d}	15.21±2.31^{bc}

3.4. Total phenolic and total flavonoid content in *in vitro* bioaccessibility analysis of *R. canina* and *R. pimpinellifolia* roots

The starting point of the study is that the local people have consumed the roots of rose hips since ancient times, as well as the fruits. In particular, the fact that black rose hips do not have a widespread distribution and show high values in terms of content enriched our study. Since the benefits of rosehip fruits are widely known, content analyzes of the fruits were also applied in order to make a comparison. According to the *in vitro* bioaccessibility result, the TPC of *R. canina* was 6.11±1.49 mg GAE/g, and the recovery was 12.73%. The TPC and TFC obtained from the mouth, stomach, and intestinal fractions and recovery values are presented in Table 4. In *R. canina,* the highest TPC was observed in the stomach fraction whereas the highest TFC was in the mouth fraction. The lowest phenolic and flavonoid content was found in the intestinal IN fraction. The recovery rate of the TPC was 12.73%, while the recovery of the TFC was 0%. In *R. pimpinellifolia*, the highest phenolic and flavonoid contents were observed in the gastric fractions (Gibis et al., 2012; Toktas et al., 2018). *R. pimpinellifolia* root aqueous extract the recovery rate of the TPC was 10.71%, while the recovery of the TFC was 0%. Reduction of TFC is attributed to the possible interaction between flavonoids, anthocyanins, phenolic compounds, and dissolved proteins, as well as the polymerization of phenolics which may result in the deterioration of their structure. The results of the bioaccessibility analysis show that there was no recovery in the flavonoid content of both *Rosa* species and the highest recovery rate in terms of TPC (12.73%) was found in *R. canina*. In the *in vitro* bioaccessibility study on sour cherry, the initial value of 147.6% was obtained as 10.1% in the inner part (Oksuz et al., 2019). In another recent study examining the *in vitro* bioaccessibility after thermal treatment applied to rosehip fruits, the TPC was determined as 74.9 ± 4.5%, and the TFC was 30.4 ± 0.4% (Ozkan et al., 2022). In the present study, significantly lower bioaccessibility was observed for the bioactives in the root parts compared to the fruits. Since there is limited data on the bioaccessibility of bioactives in rosehip roots, our findings were found to be consistent with the bioaccessibility studies with other plant species belonging to the Rosaceae family and rosehip fruits.

Table 4. Total phenolic and total flavonoid content in *in vitro* bioaccessibility of *R. canina* and *R. pimpinellifolia* plants root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**Total Flavonoid Content (mg CA g^{-1})**		**Total Phenolic Content (mg GAE g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	22.42±1.26^{bc}	26.43±5.76^{bc}	48.01±7.69^{a}	52.81±6.81^{a}
Mouth	41.38±5.36^{a}	21.98±4.49^{bc}	20.84±1.37^{de}	22.24±5.47^{cd}
Stomach	31.85±3.86^{bc}	32.98±6.19^{ab}	30.85±3.12^{b}	27.98±5.33^{bc}
Intestinal external	-	-	15.61±3.69^{de}	13.93±2.21^{ef}
Intestinal internal	2.16±1.01^{c}	7.75±13.93^{bc}	6.11±1.49^{fg}	5.66±1.1^{g}
Recovery %	0	0	12.73	10.71

3.5. Antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts after *in vitro* digestion

Results of DPPH, CUPRAC, ABTS, and FRAP antioxidant capacity methods in aqueous extractions of *R. canina* and *R. pimpinellifolia* root after *in vitro* digestion are presented in Tables 5 and 6. The highest antioxidant capacity was found in *R. pimpinellifolia* in the gastric fraction according to the CUPRAC method. The highest recovery rate (52.16%) was observed in *R. pimpinellifolia* with the CUPRAC method. Antioxidant capacity values measured with CUPRAC and ABTS tests, after *in vitro* digestion, of *R. canina* and *R. pimpinellifolia* root aqueous extractions were observed to be higher than the values measured with DPPH and FRAP methods. The highest antioxidant capacity was found in *R. pimpinellifolia* with the gastric fraction according to the CUPRAC method, and the recovery rate was 52.16%. In the *in vitro* bioaccessibility study with sour cherry (*Prunus cerasus* L.), the gain was determined as 6.5% as a result of the DPPH method, which is one of the antioxidant capacity determination methods (Oksuz et al., 2019).

Table 5. *In vitro* bioaccessibility, antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**DPPH (mg TEAC g^{-1})**		**CUPRAC (mg TEAC g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	157.68±4.81^{a}	148.15±18.78^{a}	191.86±17.95^{bc}	171.47±25.32^{cd}
Mouth	35.91±3.64^{d}	43.68±8.58^{d}	185.68±20.57^{cd}	144.5±26.76^{d}
Stomach	75.83±5.19^{b}	62.93±11.92^{c}	253.68±36.03^{a}	230.13±38.46^{ab}
Intestinal external	13.79±1.84^{e}	16.12±3.97^{e}	152.28±29.05^{cd}	69.49±13.17^{e}
Intestinal internal	10.92±1.5^{e}	10.51±3.51^{e}	45.63±9.34^{e}	89.43±13.46^{e}
Recovery %	6.93	7.09	23.78	52.16

Table 6. *In vitro* bioaccessibility, antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**ABTS (mg TEAC g^{-1})**		**FRAP (mg TEAC g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	66.56±13.63^{b}	81.64±13.07^{a}	84.44±8.69^{a}	66.41±9.64^{b}
Mouth	27.42±0.28^{d}	27.06±0.23^{d}	32.81±5.06^{c}	13.23±2.99^{e}
Stomach	51.82±0.29^{c}	47.01±5.02^{c}	65.54±10.04^{b}	52.46±9.72^{b}
Intestinal external	10.97±1.84^{e}	11.37±2.05^{e}	8.58±2.01^{d}	10.08±1.14^{d}
Intestinal internal	13.65±2.65^{e}	14.89±2.99^{e}	4.75±1.88^{d}	2.73±0.68^{d}
Recovery %	20.51	18.24	5.62	4.1

4. Conclusion

The aim of this study was to investigate the phenolic content, flavonoid content, and antioxidant capacity of the root parts of 2 species of rose hips, which were known to show high values in terms of

phenolic compounds and antioxidant capacity. In addition, the *in vitro* bioaccessibility of root aqueous extractions was investigated. The literature review showed that there were limited studies on rosehip roots. Our findings revealed that the content of rosehip root parts generally showed higher values compared to the previous reports on commonly known fruits. Moreover, the existing studies are mostly framed around the fruit of rosehip. In the black rosehip, *Rosa pimpinellifolia* L. cyanidin was detected, which is one of the valuable findings of this study as an essential component in the anthocyanin class of flavonoids. Unlike the fruit parts, phenolic compounds such as epicatechin, gallic acid, syringic acid, quercetin dihydrate, and naringenin were detected in the roots of *R. canina* and *R. pimpinellifolia*. On the other hand, rutin was observed only in *R. canina* and *R. pimpinellifolia* fruits. Catechin and its derivatives were observed to be intense in both fruit and root parts of both species. In the *in vitro* bioaccessibility analyzes applied to root aqueous extractions of 2 rosehip species, the CUPRAC method showed the highest values in both species in the results of total antioxidant capacity methods DPPH, CUPRAC, ABTS, and FRAP. In addition, in the *in vitro* bioaccessibility of root aqueous extractions of the two species, the total phenolic content was found to be the highest in *R.canina* with 12.73%, while the total flavonoid content was found to be 0% in both species.

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Research Article

A Research on The Milk Yield Traits of Brown Swiss, Simmental, Holstein Friesian Cattle and Their Crossbreeds Registered in The Herdbook System in Türkiye

Onur ŞAHİN*[1], İsa YILMAZ[2], Ömer AKBULUT[3]

[1,2]Mus Alparslan University, Applied Sciences Faculty, Department of Animal Production and Technologies, 49250, Güzeltepe-Muş, Türkiye
[3]Giresun University, Faculty of Engineering, Department of Genetics and Bioengineering, 28200, Giresun, Türkiye

[1]https://orcid.org/0000-0002-3801-3881, [2]https://orcid.org/0000-0001-6796-577X, [3]https://orcid.org/0000-0002-8860-3513

*Corresponding author e-mail: o.sahin@alparslan.edu.tr

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Abstract: This study was conducted to examine the milk yield traits of Holstein Friesian (HF), Brown Swiss (BS), and Simmental (SM) breeds, as well as their crossbreeds raised in enterprises registered in the herdbook system in Türkiye. With this study, the performances of cattle breeds and their crossbreeds widely raised in Türkiye were compared in terms of milk yield characteristics based on agricultural regions of Türkiye. The data used in the study included the completed lactation records of 22 331 heads of cattle for the years 2000-2014. The GLM ANOVA method was used in the statistical analysis of the effects of breed, genotype, and region on traits related to milk yield. Across the herd, the mean and standard deviation values were determined as 7551±2771 kg for lactation actual milk yield (AMY), 4.2±1.8 for total lactation number (TLN), and 382.9±9.2 days for lactation period (LP). The effects of genotypes and regions were found to be significant or very significant ($p<0.05$ or $p<0.001$) for all three studied traits, region x genotype interaction was found to be very significant for AMY and TLN ($p<0.001$), and not significant for LP ($p=0.184$). As a result, it was revealed that the interaction effects of genotype, region, and genotype x region should be taken into account in determining the milk yield traits of cultured breed and crossbreed cows that are widely raised in Türkiye. Accordingly, it was concluded that the interaction findings should be evaluated in determining the breed preferences by the regions.

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1. Introduction

The animal production sector has strategic importance in terms of economic and social aspects, such as adequate and balanced nutrition of the population, successful rural development, reducing unemployment in the agricultural field, and preventing migration from villages to cities (Ekmen, 2017; Alev, 2018). In this respect, it is important to ensure the sustainability of agricultural production and animal production (Kaygısız et al., 2022). Cattle breeding is a production area where human communities on a small or large scale carry out activities for milk, meat, and their derivatives. The main

source of these products is cattle (Akbulut and Yılmaz, 2013). Globally, the milk consumed by humans is largely (83.1%) obtained from cattle (Faye and Konukpayeva, 2012).

Approximately 90% of red meat production and 91% of milk production in Türkiye are met from cattle (TİGEM, 2019). In the livestock sector, between 2002 and 2019, the presence of cultured cattle increased by 360%, the presence of cultured crossbreed cattle increased by 73%, and the presence of native cattle decreased by 56% (ESK, 2019).

Today, the Holstein Friesian (HF) cattle breed ranks first in the world cattle presence and is intensively raised. This breed is also preferred in breeding and selection studies due to its high milk yield (Jasirowski et al., 1987; Akbulut et al., 2001; Genç and Karaağaç, 2019). After the HF breed, Simmental (SM) is in second place, and Brown Swiss (BS) breed is in third place (Yavuz and Yeniyurt, 2022).

The main aim in cattle breeding is to obtain the highest yield most economically, as in the breeding of other species. To achieve this, it is necessary to ensure the sustainability of the positive contribution of the genetic structure of the animal and the environmental conditions (Erdem et al., 2007). In other words, first of all, it is necessary to determine the cattle genotype suitable for the macro-environmental conditions (climate, altitude, fauna, etc.) and improve the necessary breeding conditions (shelter, feeding, etc.) for this breed. In addition, genetic breeding studies should be carried out simultaneously (Bıyıkoğlu, 2009).

In Türkiye, priority is HF, BS, and SM breeds in breeders' preference of cattle genotype. In the enterprises registered in the Herdbook system, the ratio of HF breed is 72.46%, while the ratio of Simmental (SM) and Brown Swiss (BS) breeds is 19.94% (CBAT, 2020). However, in the study conducted by Şahin et al, (2022), breeders declared that they preferred the SM breed primarily (31.2%). The preference rates of breeders for other breeds were 27.9% for HF, 14.8% for BS, and 5.0% for Jersey.

HF, SM, and BS breeds, which are of European origin, are raised on almost all continents of the world. In countries with advanced animal husbandry, sub-genotypes of these breeds (line, breed) have been developed depending on the breeding program of that country. The yield performances of the mentioned breeds and lines differ based on the conditions of the country and region where they are raised (Jasirowsski et al., 1987; Akbulut et al., 2001). Therefore, the countries that import these breeds have to make breed preferences by taking into account region and market conditions. The most important guiding information in this preference is to know the yield performance of the breed or genotype whose breeding is being considered in that region and, more specifically, in enterprises. In this context, a large number of studies examining the milk and meat yield traits of HF, SM, and BS breeds and crossbreeds have been conducted in Türkiye. The results of 73 studies conducted with HF and BS breeds were evaluated analytically by Akbulut (1998a and 1998b). In addition, the original research results examining the milk yield and fertility traits of these breeds in Türkiye were compiled by Şahin (2021).

Studies examining some yield traits of HF cattle population registered in herdbook system (or in CBA herd) in Türkiye have been conducted (Kumlu and Akman, 1999; Özkök and Uğur 2007; Genç and Soysal, 2018). In this context, a study covering all breeds was carried out by Özyürek and Tüzemen (2015) in the province of Erzurum.

In this study, the comparison of milk yield traits of HF, SM, and BS cattle breeds, as well as their crossbreeds registered in the herdbook system in Türkiye on the basis of breed and genotype, was made and the change in their performance by region was discussed. It is expected that the obtained results will guide breeders and possible policies. In addition, this study will be useful in terms of determining the level reached by the current yield performances of cattle breeds and crossbreeds that are widely raised in Türkiye.

2. Material and Methods

2.1. Material

In this research, herd records between the years 2000-2014 were used for the milk yield traits of herds registered in the herdbook system in Türkiye. The cattle whose yield records were evaluated were born in Türkiye and completed their development in Türkiye. The data of Holstein Friesian (HF), Brown Swiss (BS), and Simmental (SM) cattle breeds, as well as Holstein Friesian crossbreed (HFX),

Brown Swiss crossbreed (BSX), and Simmental crossbreed (SMX) cattle, were evaluated in the study. Abbreviations of breeds and crossbreeds were made based on the BCSM (2022) coding standard.

In the analysis of yield records, i) animals with first calving age less than 700 days and longer than 1500 days, ii) animals with lifetime milk yield less than 1000 kg, and iii) animals whose milking time or lactation period was less than 100 days and longer than 660 days were excluded from the evaluation.

In the measurement of milk yield, as the main milk yield traits, i) actual milk yield (AMY), which is an indicator of milk yield during lactation, ii) lifetime number of lactations or total lactation number (TLN) and mean lactation period (LP) traits were analyzed.

2.2. Methods

Statistical analyzes of the data were made by the SPSS package program (SPSS, 2013). The GLM ANOVA method was used to analyze the effects of race or genotype and region on the traits related to milk yield. The linear statistical model with interaction used in data analysis is as follows:

$Y_{ijk}=\mu+g_i+b_j+(gb)_{ij}+e_{ijk}$

where;

Y_{ijk}: The observation value of the studied trait ($Y_{ijk}\sim N(\mu, \sigma^2)$)

μ: The general mean of the examined trait,

g_i: The effect of breed or genotype

b_j: Effect of the geographical region,

$(gb)_{ij}$: The effect of breed/genotype and region interaction,

e_{ijk}: Random error ($e_{ijk}\sim N(0, \sigma_e^2)$).

As descriptive statistical measures, the mean of the central location measure and the standard deviations of the variability measures were used. Since it was aimed to determine the magnitude of the variability in the studied traits, the standard deviation was presented. To determine the different subgroups of the factor (genotype or region) found to be significant for any trait, Duncan's Multiple Range test (DMRT) was used (Yıldız and Bircan, 1994).

3. Results

The results of the analysis of variance for the effects of genotype and geographical region on the studied traits are given in Table 1. Descriptive statistics and Duncan's Multiple Range test results for AMY, TLN, and LP traits by genotypes and regions are given in Table 2, Table 3, and Table 4.

As seen in Table 1, significant ($P<0.01$) and very significant ($p<0.001$) differences were detected between the genotypes in terms of AMY and LP traits, while differences were not found significant in terms of TLN. It was found that the effect of the region factor on all the examined traits was significant ($P<0.01$ or $P<0.001$). On the other hand, whereas the genotype-region interaction was not significant in the LS trait, it was significant in the other two traits (Table 1).

Table 1. Summary table of statistical significance test for milk yield traits

Variation Source[1]	Df	AMY		TLN		LP	
		F	P	F	P	F	P
G	5	23.047	<0.001	2.202	0.051	3.178	<0.007
B	6	40.225	<0.001	6.463	<0.001	3.623	<0.001
Interaction (G x B)	30	2.935	<0.001	2.038	<0.001	1.225	0.184
Error	22289						
A-R^2		0.891		0.850		0.952	

[1] AMY: Actual milk yield; TLN: total lactation number; LP: Lactation period; Df: Degrees of freedom; G: Genotype; B: Geographic region; A-R^2: Adjusted coefficient of determination.

Table 2. Descriptive statistics for AMY by race and region, and Duncan's Multiple Range test results

		MR	AG	CA	MD	BLS	EA	SEA	BRD
HF	n	8029	3551	7141	584	445	28	107	19885
	x̄±Sd	8165±3070	7706±2623	7428±2326	7305±2238	6021±2732	5970±2113	7138±2130	7737±2739^{a}
BS	n	73	128	222	6	123	22	7	581
	x̄±Sd	6520±2388	7216±3010	6372±2235	7737±2406	3816±1435	3368±2030	5416±896	5924±2637^{c}
SM	n	97	238	274	48	144	25	14	840
	x̄±Sd	7252±2601	6116±2090	6079±1785	7532±1904	4191±1805	4401±1453	6456±2078	5941±2211^{c}
HFX	n	127	104	276	14	23	2	20	566
	x̄±Sd	8276±3190	7299±2544	6716±2266	7159±2237	4612±1683	4966±1799	5508±1427	7049±2644^{b}
BSX	n	7	20	44	1	15	37	2	126
	x̄±Sd	6707±1383	6711±3109	5918±2102	3265± -	2977±1004	1826±953	6036±709	4517±2768^{e}
SMX	n	42	56	123	9	52	44	7	333
	x̄±Sd	7210±2431	6289±2360	5795±1794	6099±1407	3750±1390	3034±2134	4325±4680	5350±2362^{d}
REG	n	8375	4097	8080	662	802	158	157	22331
	x̄±Sd	8136±3063^{k}	7564±2636^{l}	7296±2327^{l}	7300±2211^{l}	5110±2517^{n}	3558±2255^{o}	6653±2094^{m}	7551±2771

HF: Holstein Friesian (Black and White), HFX: Holstein Friesian Crossbreeds, BS: Brown Swiss, BSX: Brown Swiss Crossbreeds, SM: Simmental, SMX: Simmental Crossbreeds; a, b, c, d: Use to geographic region ($p<0.05$); k, l, m, n, o : Use to breed-genotype ($p<0.05$); BRD: Breed; REG: Region; MR: Marmara region; AG: Aegean region; CA: Central Anatolia region; MD: Mediterranean region; BLS: Black sea region; EA: East region; SEA: Southeast region.

Table 3. Descriptive statistics for TLN by race and region, and Duncan's Multiple Range test results

		MR	AG	CA	MD	BLS	EA	SEA	BRD
HF	n	8029	3551	7141	584	445	28	107	19885
	x̄ ±Sd	4.0±1.8	4.4±1.8	4.3±1.7	4.5±1.8	4.0±1.8	3.7±1.9	3.3±1.4	4.2±1.7^{cd}
BS	n	73	128	222	6	123	22	7	581
	x̄±Sd	3.8±1.9	4.6±2.1	4.5±1.8	4.3±2.3	4.5±2.4	3.2±1.5	2.9±1.4	4.4±2.0b^{c}
SM	n	97	238	274	48	144	25	14	840
	x̄±Sd	4.2±2.2	5.2±2.1	4.6±2.0	3.9±1.8	4.7±2.1	3.6±1.8	4.4±1.8	4.7±2.1^{a}
HFX	n	127	104	276	14	23	2	20	566
	x̄±Sd	3.5±1.6	4.3±1.7	4.2±1.7	5.1±2.0	4.2±1.9	3.5±0.7	3.3±1.7	4.1±1.8^{d}
BSX	n	7	20	44	1	15	37	2	126
	x̄±Sd	4.3±1.8	4.7±2.2	4.5±1.9	3.00± -	5.5±2.6	4.0±1.7	5.0±4.2	4.5±2.0^{ab}
SMX	n	42	56	123	9	52	44	7	333
	x̄±Sd	4.1±2.0	4.7±2.1	4.7±2.0	4.6±2.8	4.8±2.1	4.0±1.9	3.0±1.4	4.5±2.0^{ab}
REG	n	8375	4097	8080	662	802	158	157	22331
	x̄±Sd	4.0±1.8^{l}	4.5±1.9^{k}	4.3±1.7^{k}	4.5±1.9^{k}	4.3±2.0k	3.8±1.8^{l}	3.4±1.5^{m}	4.2±1.8

HF: Holstein Friesian (Black and White), HFX: Holstein Friesian Crossbreeds, BS: Brown Swiss, BSX: Brown Swiss Crossbreeds, SM: Simmental, SMX: Simmental Crossbreeds; a, b, c, d: Use to geographic region ($p<0.05$); k, l, m : Use to breed-genotype ($p<0.05$); BRD: Breed; REG: Region; MR: Marmara region; AG: Aegean region; CA: Central Anatolia region; MD: Mediterranean region; BLS: Black sea region; EA: East region; SEA: Southeast region.

Differences between geographical regions and races for AMY and TLN treatments were found to be statistically significant ($p<0.05$).

If the interaction finding is interpreted in a more explicit way, it can be said that a genotype with a high yield in a region in terms of AMY and TLN traits may not be highly efficient in all regions. The same can be expressed for a genotype that has a low yield in a region. The fact that genotype-region interaction is found to be significant in terms of any trait requires the evaluation of interaction findings in determining breed preference by region.

Differences between geographical regions and races for LP treatment were found to be statistically significant ($p<0.05$).

Table 4. Descriptive statistics for LP (Day) by race and region, and Duncan's Multiple Range test results

		MR	AG	CA	MD	BLS	EA	SEA	BRD
HF	n	8029	3551	7141	584	445	28	107	19885
	x̄ ±Sd	375.4±8.4	387.9±9.5	392.8±9.3	380.4±8.4	370.0±8.3	387.6±9.1	379.9±9.9	383.9±9.2^{c}
BS	n	73	128	222	6	123	22	7	581
	x̄±Sd	389.7±99.2	396.8±96.6	391.0±83.1	342.3±80.5	351.6±65.0	339.9±90.5	366.6±78.7	381.0±87.0^{bc}
SM	n	97	238	274	48	144	25	14	840
	x̄±Sd	363.7±84.5	374.4±84.9	365.2±79.6	364.8±71.9	359.3±80.7	341.2±50.1	383.9±82.0	366.2±80.9^{a}
HFX	n	127	104	276	14	23	2	20	566
	x̄±Sd	366.6±95.5	394.0±79.5	390.9±91.9	348.7±63.9	383.4±79.6	329.0±45.3	365.6±100.	383.5±90.3^{c}
BSX	n	7	20	44	1	15	37	2	126
	x̄±Sd	389.9±43.0	394.5±64.5	377.4±89.1	317.0±-	351.5±60.2	337.1±89.6	366.0±73.5	365.2±81.9^{a}
SMX	n	42	56	123	9	52	44	7	333
	x̄±Sd	366.7±90.5	370.4±73.8	375.9±80.5	306.8±42.2	380.7±95.6	351.0±91.6	387.4±56.6	369.7±85.7^{ab}
REG	n	8375	4097	8080	662	802	158	157	22331
	x̄±Sd	375.2±84.8^{lm}	387.3±85.6^{kl}	391.4±88.0^{k}	377.2±82.3^{l}	366.0±77.5^{lm}	350.8±85.4^{n}	378.0±85.1^{lm}	382.9±9.2

HF: Holstein Friesian (Black and White), HFX: Holstein Friesian Crossbreeds, BS: Brown Swiss, BSX: Brown Swiss Crossbreeds, SM: Simmental, SMX: Simmental Crossbreeds; a, b, c, d: Use to geographic region ($p<0.05$); k, l, m, n, o : Use to breed-genotype ($p<0.05$); BRD: Breed; REG: Region; MR: Marmara region; AG: Aegean region; CA: Central Anatolia region; MD: Mediterranean region; BLS: Black sea region; EA: East region; SEA: Southeast region.

4. Discussion

Lactation Actual Milk Yield (AMY)

In the study, the general mean and standard deviation of the AMY trait for the examined genotypes (HF, BS, SM, HFX, BSX, and SMX) were found to be 7 551±2 771 kg. The general mean values of AMY for the same breeds and genotypes were reported as 3 834 liters for herds registered to the Cattle Breeders' Association (CBA) of the Erzurum province (Özyürek and Tüzemen, 2015).

According to the results of the Duncan's Multiple Range test, the highest AMY was determined as 7 737±2 739 kg in the HF breed. This breed was followed by the HFX genotype with a yield of 7 049±2 644 kg. Among these six genotypes, the lowest AMY was determined as 4 517±2 768 kg in the BSX genotype.

In terms of AMY, the highest yield was found in the Marmara region (8 136±3 063) and the lowest yield was found in the Eastern Anatolia region (3 558±2 255). In the Aegean, Central Anatolia, and Mediterranean regions, the mean yield in question was similar, and they were followed by the Southeastern Anatolia region, which had a lower yield level (Table 2).

When the genotype-region subgroups were examined, it was observed that the highest yield was obtained in the HFX in the Marmara region and it was followed by the HF breed in the same region (8 276 and 8 165 kg, respectively). The lowest yield, on the other hand, was determined in BSX animals in Eastern Anatolia and the Black Sea regions (1 826 and 2 977 kg). The yield of the HFX genotype in the Marmara region was 4.5 times the yield of the BSX genotype in the Eastern Anatolia region and approximately 2.8 times the yield of the same genotype in the Black Sea region. Hundreds of studies have been conducted in Türkiye on the milk yield traits of HF, BS, and SM breeds as well as their crossbreeds raised in different regions and enterprises. In this context, the milk yield of HF and BS breeds by agricultural regions was evaluated in a study conducted by Akbulut (1998a). The findings of this study revealed that AMYs of both breeds showed significant differences in agricultural regions.

According to the results of 23 studies, the mean AMY for the HF breed varied between 2 657 kg and 8 264 kg, and the highest yield was reported as 7 340 kg in the Middle North agricultural region. For the BS breed, this yield varied between 1 571 and 6 045 kg, and the lowest mean value was found in the Northeastern agricultural region (2 318 kg) (Akbulut, 1998a). In this context, another review study was conducted by Şahin (2021). In this review study, milk yield was reported in the range of 4 398-7 892 kg in HF breed cattle, 2 243-8115 kg in BS breed, and 3 412-7 693 kg in SM breed.

The findings obtained in this current study were higher than the values reported by Akbulut (1998a) and similar to the reports of Şahin (2021). In addition, the milk yield values found by Tüzemen and Özyörük (2015) in herds registered to Erzurum CBA were compatible with the results found in this study for the eastern Anatolia region.

When examining the mean lactation milk yield reported by some studies conducted in other countries, it was observed that Mellado (2011) reported this mean value between 10071 ± 281 and 14680

± 642 kg for HF cattle breed with lactation between <2 and >6 in northern Mexico, respectively. In a study where the mean number of lifetime lactations of animals in SM-breed cattle was found to be 3.61±0.04 in Serbia, Petrović et al. (2015) reported the amount of milk given by cows for life as an average of 14 604 kg. This means that an average of 4 045 kg (14 604 kg/3.61 pieces) of milk was obtained per lactation. In another study conducted in Bulgaria, the mean actual lactation milk yield of SM breed cattle was reported as 5478±112 kg (Karamfilov and Nikolov, 2019).

Total Lactation Number (TLN)

In this study, the mean total lactation number (TLN) and its standard deviation were found as 4.2±1.8 for all examined genotypes (HF, BS, SM, HFX, BSX, and SMX) (Table 3).

According to the results of the Duncan's Multiple Range test, the highest TLN was found to be 4.7±2.1 in the SM breed. This breed was followed by BSX and SMX genotypes with a yield of 4.5±2.0. Among these six genotypes, the lowest TLN was found in the HFX genotype as 4.1±1.8.

In terms of TLN, the highest value was found in the Aegean and Mediterranean regions with the same value (4.5±1.9), while the lowest value was found in the Southeastern Anatolia region (3.4±1.5). The mean TLN was similar in the Central Anatolia and Black Sea regions, and these regions were followed by the Eastern Anatolia region, which had a lower TLN (Table 3).

When the Genotype-Region subgroups were examined, it was seen that the highest TLN was obtained in the BSX genotype in the Black Sea region, and it was followed by the SM and HFX genotypes in the Aegean and Mediterranean regions (5.5±2.6, 5.2±2.1 and 5.1±2.0, respectively). The lowest TLN was found in the BS breed in the Southeastern Anatolia region (2.9±1.4). The TLN of the BSX genotype in the Black Sea region was 1.9 times higher than the TLN of the BS breed in the Southeastern Anatolia region and about 1.8 times higher than its TLN in the Eastern Anatolia region.

Many studies have been conducted on the milk yield and fertility traits of HF, BS, and SM breeds as well as their crossbreeds raised in different regions and enterprises in Türkiye. However, there was no study in Türkiye that examined these animals' total lactation numbers (TLN) that they give during the time they are used in production.

Moreover, in studies conducted in some countries other than Türkiye, the mean TLN in HF breed was reported as 5.5±1.4 in Egypt by Afifi et al. (2004), 2.88±1.8 in South Africa by Theron and Mostert (2009), 2.94 in the United States (USA) by Hare et al. (2006), 2.3±1.3 in Sweden by Hagnestam-Nielsen et al. (2009), 4.35 in Ethiopia by Goshu and Sing (2013), and 3.6 in Ireland by McNamara et al. (2003). Hare et al. (2006) reported the mean TLN in BS cattle as 2.89 in the USA. As for Simmental cattle, Petrović et al (2015) reported the mean lactation number of animals from three different farms in Serbia as 3.38±0.06, 3.72±0.09, and 3.76±0.07 for Dobričevo, Zlatiborski Suvati, and Kotraža, respectively. The mean of these numbers was 3.61±0.04. Martens and Bange (2013) found that the mean lactation number in the HF breed varies between 3.5 and 4.5 in Germany.

The TLN values obtained in this current study in HF breed were 1.3 times lower than the values reported by Afifi et al. (2004), while they were 1.2 times higher than the value reported by McNamara et al (2003), and 1.5 times higher than the values determined by Theron and Mostert (2009) and Hare et al. (2006).

Lactation Period (LP)

In the studied genotypes (HF, BS, SM, HZX, BSX, and SMS), the overall mean and standard deviation of the LP trait was found to be 382.9±9.2 days. The overall mean LP values for the same breeds and genotypes were reported as 304.12±1.5 days in cattle herds registered in the CBA of the Erzurum province (Özyürek and Tüzemen, 2015).

As a result of Duncan's Multiple Range test, the highest LP was found to be 383.9±9.2 days in HF breed. This breed was followed by the HFX genotype with 383.5±90.3 days and the BS breed with 381.0±87.0 days. Among these six genotypes, the lowest LP was detected as 365.2±81.9 days in the BSX genotype.

In terms of regions, the highest LP value was found in the Central Anatolia region (391.4±88.1 days) and the lowest value was found in the Eastern Anatolia region (350.8±85.4 days). In the Marmara, Aegean, Mediterranean, and Southeastern Anatolia regions, the mean lactation period in question was obtained close to each other (Table 4).

When the Genotype-Region subgroups were examined, it was observed that the highest LP values were obtained in BS and BSX breeds in the Aegean region (396.8±96.6 and 394.5±64.5 days, respectively). On the other hand, the lowest LP values were obtained in SM and BSX animals in the Mediterranean region (306.8±42.2 and 317.0±0.0 days, respectively). In addition, when the regions other than the Mediterranean region were compared, the lowest values for all breeds and genotypes, except for the SA breed, were found in animals bred in the Eastern Anatolian regions.

Numerous studies have been conducted on the lactation period traits of HF, BS, and SM breeds and their crossbreeds raised in different regions and enterprises in Türkiye. In this context, the lactation period of HF and BS breeds by agricultural regions was examined in a study conducted by Akbulut (1998a). According to the findings of this study, the LP values of both breeds showed significant differences in agricultural regions. According to the results of 23 studies, LP for the HF breed varied between 240 days and 355 days, and the highest period was reported as 334 days in the Northeastern agricultural region.

For the BS breed, the LP varied between 214 and 376 days, and the lowest mean was determined in the Northeastern agricultural region (258 days). In this context, in a study conducted by Özyürek and Tüzemen (2015), the lactation period of HF, BS, and SM breeds for herds registered in the CBA of the Erzurum province was reported as 308.1±7.6 days, 298.2±5.6 days, and 288.2±8.7 days, respectively. In the same study, LPs for HFX, BSX, and SMX cattle were reported as 315.2±11.8 days, 291.9±4.4 days, and 303.2±8.0 days, respectively. In a review study conducted by Şahin (2021), LP values were reported in the range of 299-358 days for the Holstein Friesian, 266-356 days for the BS breed, and 300-337 days for the SM breed. Tankal and Tüzemen (2022) reported that the mean lactation period in HF cows raised at Gökkale Agricultural Enterprise in Türkiye was 357.5±1.3 days.

The LP value obtained in this research was determined to be higher than the values obtained for the same breeds and genotypes in the cattle herd registered to the Erzurum CBA by Özyürek and Tüzemen (2015) and the LP value reported by Şahin (2021).

When the mean lactation periods reported in some studies conducted in other countries were examined, it was seen that in HF cattle breed with lactation orders between <2, 2-4, 4-6, and >6 in Northern Mexico, the LP values were reported as 414±9, 502±11, 479±9 and 483±19 days (Mellado, 2011). In Bulgaria, on the other hand, Karamfilov and Nikolov (2019) reported the mean lactation period in SM-breed cattle as 342.60±6.18 days.

Conclusion

In this study, it was determined that the milk yield traits had the desired values in cattle breeding enterprises. The obtained mean milk yield is higher than the mean of Türkiye and meets the expectations. However, it is possible to say that the fact that the mean milk yield is high is due to the length of the lactation period. The fact that the lactation period is higher than the value of 305 days reduces the number of highly productive days and also does not meet the expectation of "one offspring per animal per year" in cattle breeding enterprises.

The obtained results showed that the effect of genotype x region interaction was significant on milk yield traits. As a result, it was concluded that in determining the breed preference by region, it is necessary to evaluate the interaction findings and take these findings into account in decision-making.

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Ethical statement

Milk yield records registered in the Herdbook system were used.

Conflict of Interest

As authors, we declare that there is no conflict of interest between us.

Contributions of authors

OŞ, IY and ÖA designed the study. OŞ collected the data. ÖA made the statistical analysis. The article was written by OŞ, IY, and ÖA. All authors contributed to the critical revision of the article.

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Research Article

Investigation of the Antifungal Activity of *Bacillus megaterium* Against *Fusarium* Species

Esra Nur KELEŞ[1], Ayşe Feyza TUFAN DÜLGER*[2], Emre YÖRÜK[3]

[1]Haliç University, Graduate Institute of Education, Program of Molecular Biology and Genetics, 34445, İstanbul, Türkiye
[2]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun, Türkiye
[3]İstanbul Yeni Yüzyıl University University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, 34010, İstanbul, Türkiye

[1]https://orcid.org/0000-0002-6665-4723, [2]https://orcid.org/0000-0003-4779-6811, [3]https://orcid.org/0000-0003-2770-0157

*Corresponding author e-mail: feyzadulger@omu.edu.tr

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Bacillus megaterium, Biocontrol, *Fusarium culmorum*, *Fusarium graminearum*, Gene expression

Abstract: Several *Fusarium* species are emerging as serious pathogens on small grain cereals worldwide. The use of fungicides is a short-term strategy in the fight against *Fusarium* diseases. The use of biocontrol agents is an attractive alternative strategy by reducing the chemical input to the environment as well as being economical. *Bacillus* species have received attention as biocontrol agents. In this study, the antagonistic activities of *Bacillus megaterium* CTBmeg1 and HMA5 strains on *Fusarium culmorum* UK99 and *F. graminearum* PH-1 isolates were investigated *in vitro* and at molecular level. On the 7th day of the dual culture assay, both of *B. megaterium* strains significantly reduced the mycelial growth of *Fusarium* isolates, with very high antifungal activity with the inhibition rate between 72.7% and 77.7%, respectively. Similarly, both strains caused high antifungal activity in the volatile organic compound (VOC) analysis between 52.1% and 62.4%, respectively. At the molecular level, in all tested groups, transcript levels of the *tri5* gene, which is associated with trichothecene production, decreased, while the transcript levels of *cat*, an antioxidant gene, and *mst20*, a gene related to apoptosis, increased. Findings from this study showed that *B. megaterium* CTBmeg1 and HMA5 strains could be accepted as highly effective biocontrol agents against worldwide phytopathogens *F. culmorum* and *F. graminearum*.

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1. Introduction

F. culmorum and *F. graminearum* are the major causal agents of the *Fusarium* head blight and crown rot diseases of small grain cereals worldwide. Epidemics of these diseases result in yield losses reaching up to billions of dollars in economic damages and mycotoxin contamination e.g., deoxynivalenol, nivalenol, and zearalenone (Pasquali and Migheli, 2014; Matny, 2015). Several strategies including fungicide usage, biocontrol agents and the development of disease resistant cultivars have been used in order to combat *Fusarium* diseases (Moya-Elizondo and Jacobsen, 2016; Tufan et al., 2017; Uluhan et al., 2019). The use of fungicides is a short-term strategy in the fight against diseases

and is not effective because pathogenic fungi can easily become resistant to these fungicides and cause environmental pollution. The use of biocontrol agents is the more friendly strategy by reducing the chemical input to the environment as well as being economical. Among various bacterial genera, *Bacillus*, *Pseudomonas,* and *Streptomyces* species are widely used as biocontrol agents (Legrand et al., 2017). However, the *Bacillus* genus has an advantage over other microorganisms by endospore production that is tolerant to stress conditions such as heat and high concentrations of chemicals. They produce a broad spectrum of antibiotics, a wide range of antifungal lipopeptides (iturine, fengycin, surfactins, etc.), and hydrolytic enzymes and act as bacterial and fungal antagonists against phytopathogens. They promote plant growth through mechanisms such as siderophore production, potassium solubility, and phytohormone synthesis (Khan et al., 2017; Nayak et al., 2017; Bolivar-Anillo et al., 2021).

The aim of this study was to examine the antagonistic effects of *B. megaterium* CTBmeg1 and HMA5 on *F. culmorum* and *F. graminearum*. In this context, first, the antagonistic effects of *B. megaterium* on *Fusarium* species were tested *in vitro* by dual culture assay and VOC analysis. Then, the effects of *B. megaterium* strains on oxidative stress, trichothecene biosynthesis, and apoptosis in Fusarium spp. were analyzed by qPCR at the transcript level. Finally, the toxigenic effects of *B. megaterium* strains on *Fusarium* spp. were determined by WST-1- assay.

2. Material and Methods

2.1. Culture conditions of *Fusarium* isolates and *Bacillus megaterium* strains

The reference strains of *F. graminearum* (PH-1) and *F. culmorum* (UK99) isolates were used for experiments. Carboxymethylcellulose (CMC) liquid medium (1.0 % CMC, 0.3 % NaCl, 0.1 % KH_2PO_4) was used for *Fusarium* spore production. *Fusarium* isolates were grown in ½ potato dextrose agar (PDA) medium for 5 days at 25 °C. Then, fungal discs were added to a CMC medium and incubated at 28°C, 100 rpm for 5 days. The harvested fungal spores were standardized to 1 x 10^5 macroconidia/mL (Nalam et al., 2016). Then, 20 µL of *Fusarium* spores were placed in the center of the PDA medium and grown for 7 days at 25°C, 50 % humidity. Fungal discs with a diameter of ~3mm were used for the following experiments.

Bacillus megaterium CTBmeg1 (Akcay and Kaya, 2019) and HMA5 (Aksoy et al., 2018) strains were obtained from Ondokuz Mayıs University, Agricultural Biotechnology, and Plant Protection Departments, respectively. The strains were grown in Luria Bertani (LB) medium at 30°C, 200 rpm overnight, and diluted to an OD_{600} of 0.4. Strains were stored as 50% glycerol stock at -80°C.

2.2. Dual culture assay

Fusarium discs with a diameter of ~3mm were placed in the centers of new PDA media and bacterial strains were swab inoculated ~2.5 cm away from the *Fusarium* disc and ~1 cm away from the petri dish. Radial growth diameter from *Fusarium* disc center to bacteria was measured after 7 days and the percentage of inhibition of radial growth (PIRG) was determined according to the following formula: PIRG (%) = $[(R1-R2) / R1] \times 100$ % where R1 is the radial growth of *Fusarium* in the control plate, R2 is the radial growth of *Fusarium* towards antagonist bacteria. This study used a scale described by Bivi et al. (2010) as follows; PIRG <30 % indicates low antifungal activity, 30 - <50 % indicates moderate antifungal activity, 50 - <70 % indicates high antifungal activity, ≥70 % indicates very high antifungal activity. A Petri dish containing only *Fusarium* isolate was used as a negative control. Experiments were performed with 5 technical and 3 biological replicates.

2.3. Effects of VOCS on radial mycelial growth and hyphae morphology

A ~3mm diameter fungal disc was inoculated into the center of a new PDA medium, and the bacterial strain was inoculated into another PDA medium with the help of a swab. The two petri dishes were placed on top of each other with only common air flow between them and incubated at 25°C, 50 % humidity for 7 days. The percentage inhibition of diameter growth (PIDG) was determined by measuring the difference in diameter of the fungal culture after the two petri dishes were brought together, according to the formula: $PIDG\ (\%) = [(D1-D2) / D1] \times 100$ % where D1 is the diameter of the *Fusarium* in the control plate, D2 is the diameter of *Fusarium* treated with antagonistic bacteria (Toh

et al., 2016). PIDG <30 % indicates low antifungal activity, 30 - <50 % indicates moderate antifungal activity, 50 - <70 % indicates high antifungal activity, and ≥70 % indicates very high antifungal activity. A Petri dish containing only *Fusarium* isolate was used as a negative control. Experiments were performed with 5 technical and 3 biological replicates.

2.4. qPCR for expression of oxidative stress, trichothecene biosynthesis, and apoptosis related genes

Total RNA extraction from *Fusarium* isolates on day 7 of the dual culture assay was performed according to the manufacturer's protocol (NucleoSpin RNA Mini Kit, Macherey Nagel). 30 mg of fungal hyphae was taken and homogenized for 1 min (Retsch MM 400, Germany). The quality and quantity of the total RNAs were determined by agarose gel electrophoresis and spectrophotometer (A260, A280, A230).

Synthesis of cDNA from total RNA was performed according to the manufacturer's protocol (ProtoScript® First Strand cDNA Synthesis BioLABs). 1 µg of total RNA was used and the synthesized cDNAs were diluted to 20 ng μl^{-1}.

qPCR was performed with 1X Sybr Green I mix, 5 pmoL primers, and 40 ng μL^{-1} cDNA in a total volume of 10 µL. The qPCR conditions were 2 min at 95°C, followed by 40 cycles of 5 s at 95 °C, 10 s at 58 °C, and 10 s at 72 °C. At the end of the cycle, melting curve analysis was performed between 65 ºC - 95 ºC by increasing 0.5 ºC at 2-5 sec/step (Bio-Rad, CFX Connect RealTime System). β-tubulin was used as the housekeeping gene while *cat*, *mst20,* and *tri5* were the target genes (Gazdağlı et al., 2018). Gene expression profiles were calculated according to $2^{-\Delta\Delta CT}$ normalization values (Livak and Schmittgen, 2001). Experiments were performed in 3 technical and 2 biological replicates.

2.5. WST-1 cell viability assay

WST-1 assay was performed from *Fusarium* isolates on day 7 of the dual culture assay by optimizing the manufacturer's protocol (Cell Proliferation Reagent WST-1, Roche). 1X PBS (1 L DHSO, 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4) was added onto ~1cm^2 mycelium and homogenized for 40 sec. 1:10 (v/v) WST-1 was added to 100 µL of cell suspension and incubated at 28 °C, 100 rpm for 3 h. Cell viabilities % were calculated by 450/600 nm wavelengths (Teker et al., 2021). The experiments were performed with 2 technical and 2 biological replicates.

2.6. Statistical analysis

Cell viability % assays were performed with a two-way analysis of variance (ANOVA) Bonferroni post-test using GraphPad Prism (Version 5.01) (*P < 0.05, ** P < 0.01, *** P < 0.001). qPCR analyses were performed using one-way ANOVA with LSD test in R statistical software with RStudio (Version 1.3.1093) and the package agricolae.

3. Results

3.1. Effects of *B. megaterium* strains against *Fusarium* spp.

Antifungal activities of *B. megaterium* CTBmeg1 and HMA5 strains against *F. graminearum* PH-1 and *F. culmorum* UK99 isolates were evaluated by *in vitro* dual culture assay. After 7 days of the assay, morphologically significant differences were observed in the experimental groups compared with the control groups in terms of hyphae color and structure of PH-1 and UK99. The hyphae of the PH-1 control group were looser and cottony, and the color of the hyphae was dark pink. As a result of the antagonistic effect of CTBmeg1 and HMA5 strains, the PH-1 hyphae structure was found to be like the control group, but the color of the hyphae turned white. PH-1 covered the entire petri dish on the 7th day in the control group but did not grow in the experimental groups (Figure 1. a-c). In the UK99 control group, the hyphae structure was looser and cottony, and the color was dark pink. As a result of the antagonistic effect of CTBmeg1 and HMA5 strains in the experimental groups, it was observed that the hyphae structure was more frequent and felt-like, and the color of the hyphae was white (Figure 1. d-f). When the antagonistic effects of *B. megaterium* strains against PH-1 and UK99 were analyzed in terms of the radial growth, it was found that the bacteria showed high *in vitro* antifungal activity against UK99

(76.4 % and 72.7 % in CTBmeg1 and HMA5, respectively) and PH-1 (77.7 % and 73.7 %, in CTBmeg1 and HMA5, respectively) (Figure 2. a).

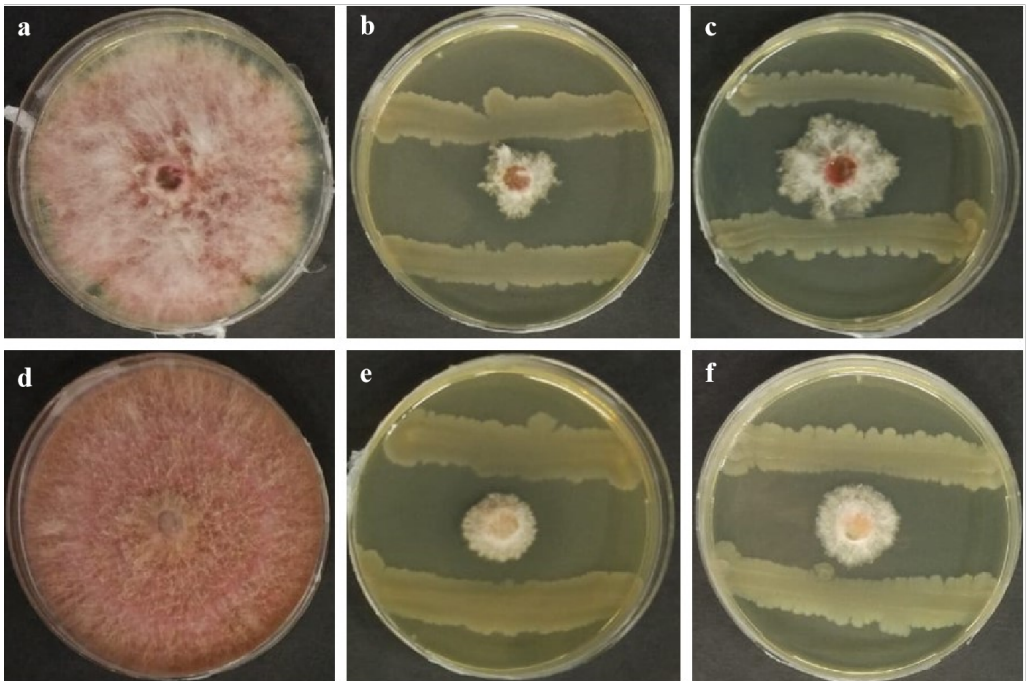

Figure 1. Dual culture assay for the screening of *B. megaterium* CTBmeg1 and HMA5 against *F. graminerarum* PH-1 and *F. culmorum* UK99. a) PH-1 (control), b) PH-1+CTBmeg1, c) PH-1+HMA5, d) UK99 (control), e UK99+CTBmeg1 and f UK99+HMA5.

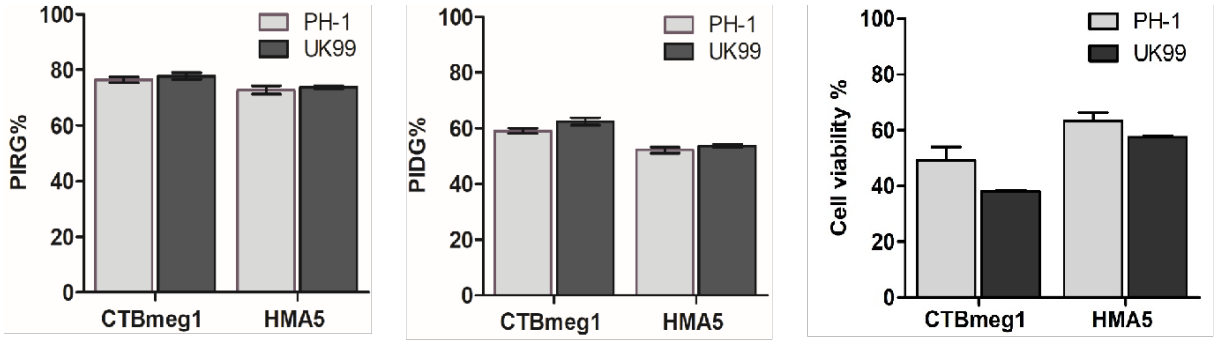

Figure 2. (a) PIRG %, (b) PIDG %, and (c) cell viability % of F. graminerarum PH-1 and F. culmorum UK99 isolates obtained from dual culture assays. Error bars represent ± standard errors (SE) of three replicates.

3.2. VOC analysis

The antifungal activities of CTBmeg1 and HMA5 strains against PH-1 and UK99 isolates were evaluated by *in vitro* VOC analysis. As a result of the analysis, morphologically significant differences were observed in the experimental groups compared with the control groups in terms of hyphae color and structure of both *Fusarium* isolates. In the PH-1 control group, the hyphae structure was looser and cottony, and the color is dark pink, while in the experimental group, the hyphae structure was loose but more fragmented than in the control group. Hyphae growths were longer in all experimental groups compared with the control group and no significant color changes were observed. While the control group grew to cover almost the entire petri dish, it was observed that the diameter growth was

significantly suppressed in the experimental group (Figure 3. a-c). Compared with the control group, it was observed that CTBmeg1 and HMA5 strains also caused morphological differences in the hyphae structure and diameter growth of UK99 isolate. While the hyphae structure was loose and cottony and the color was dark pink in the control group, the group exposed to VOC had a looser hyphae structure than the control group, and the hyphae color was burgundy in the center and the surrounding mycelial growth was white. Although the control group covered the entire petri dish on the 7th day, it was observed that diameter growths were suppressed in the experimental groups (Figure 3. d-f). In terms of PIDG %, bacteria showed high *in vitro* antifungal activity against PH-1 (59.0 % and 52.1 % in CTBmeg1 and HMA5, respectively) and UK99 (62.4 % and 53.6 % in CTBmeg1 and HMA5, respectively). (Figure 2. b).

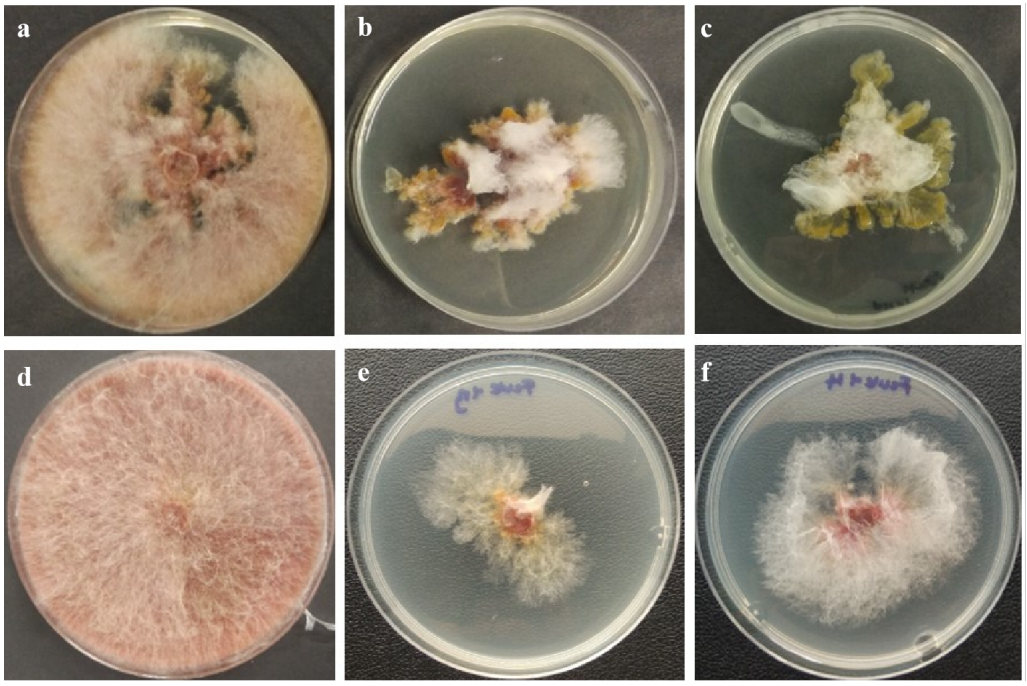

Figure 3. The effects of VOCs produced by CTBmeg1 and HMA5 on the growth of PH-1 and UK99. a) PH-1 (control), b) PH-1+CTBmeg1, c) PH-1+HMA5, d) UK99 (control), e) UK99+CTBmeg1 and f) UK99+HMA5.

3.3. Gene expression analysis

Both in the control and experimental groups, expression levels of *mst20*, *cat*, and *tri5* genes were examined. In each experimental set *tri5* gene decreased, while the decrease in PH-1 + CTBmeg1 (0.15 fold) was significant. *cat* gene increased significantly in all experimental sets (between 3.6 to 4.7 fold). Although there was a significant increase in the *mst20* gene in all experimental sets, these increases were significant in the groups treated with CTBmeg1 strain (2.3 and 3.7 fold in PH-1 and UK99, respectively) (Figure 4).

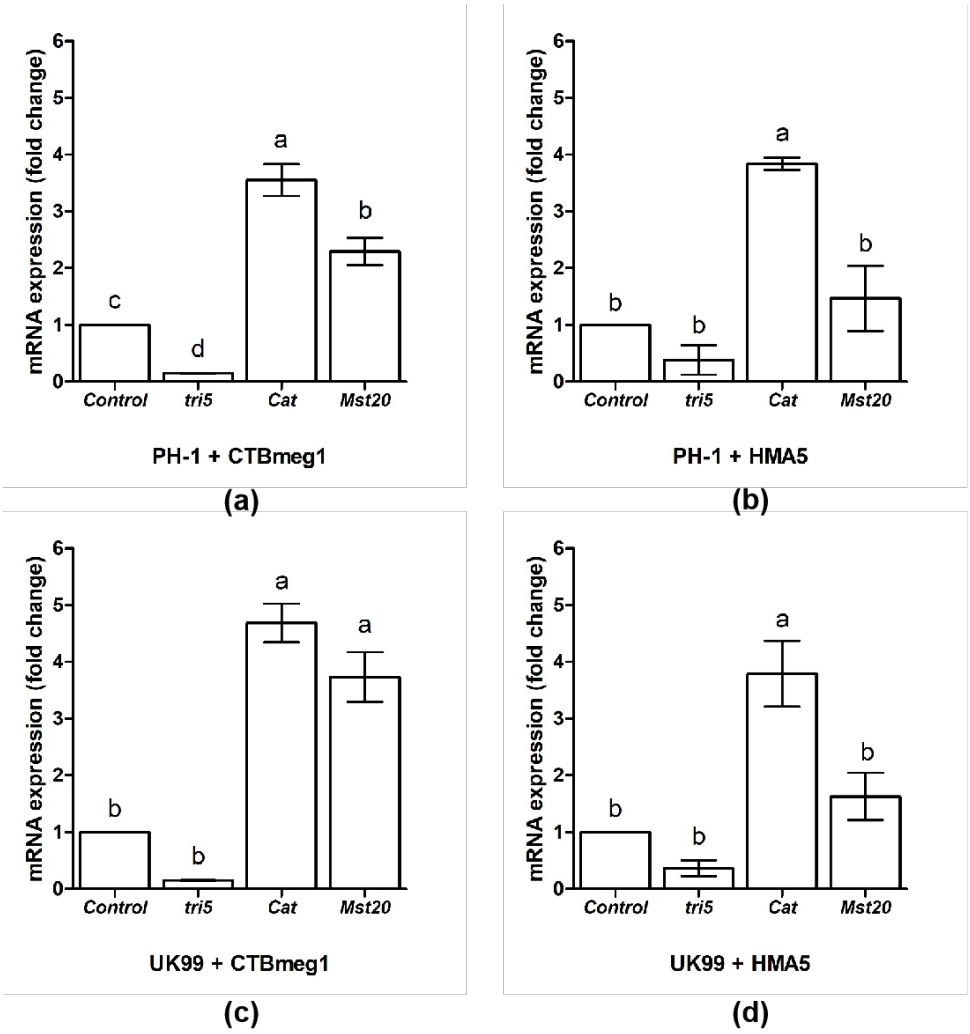

Figure 4. Gene expression analysis of *tri5*, *cat,* and *mst20* genes by means of qPCR. a) PH-1+CTBmeg1, b) PH-1+HMA5, c) UK99+CTBmeg1 and d) UK99+HMA5. Error bars represent ± standard errors (SE) of three replicates.

3.4. Cell viability

Bacillus strains were compared in terms of the cell viability of *Fusarium* isolates. It was observed that both bacteria significantly reduced cell viability (***). Besides, CTBmeg1 suppressed cell viability more than HMA5 in both *Fusarium* species. However, no significant difference was observed between CTBmeg1 and HMA5 in PH-1, while a significant difference was observed between both strains in UK99 (***) (Figure 2. c).

4. Discussion

F. culmorum and *F. graminearum* are the main causative agents of the most destructive *Fusarium* diseases, *Fusarium* head blight and *Fusarium* crown rot. Struggle with these diseases includes several different strategies such as fungicide treatment, disease resistant cultivar development, or plant-derived essential oil usage (Jones, 2000; Bernardo et al., 2007; Özsoy et al., 2020). Each strategy has several disadvantageous. From past to present, common fungicides treatment, in particular triazole group-demethylation inhibiting fungicides, has been the most powerful strategy to manage *Fusarium* diseases (Yörük, 2018). However, fungicide resistance development and ecotoxicological characteristics of fungicides led researchers to find out novel strategies in order to control *Fusarium* diseases. The use of antagonistic microorganisms as biological control agents provide a promising strategy for the management of plant pathogens. *Bacillus* spp. offer several advantages over other

biocontrol microorganisms due to their endospore-forming, antibiotic producing ability, and resistance to extreme conditions (Aksoy et al., 2018; Akcay and Kaya, 2019). There are several reports on the *Bacillus* spp. for biological control of *Fusarium* diseases (Zhao et al., 2014; Grosu et al., 2015; Zalila-Kolsi et al., 2016; Wu et al., 2019; Cantoro et al., 2021). However, there are only limited reports related to the effects of *B. megaterium* on fungal pathogens (Pan et al., 2015; El-Gremi et al., 2017). Also, *B. megaterium* against *F. culmorum* was not used in these studies. Instead, various pieces of research have been studied on the nematicidal and insecticidal effects of *B. megaterium* (Aksoy et al., 2018; Zhou et al., 2020). Studies by Pan et al. (2015) showed that *B. megaterium* BM1 significantly reduced *F. graminearum* growth. Similarly, in our study, both *B. megaterium* CTBmeg1 and HMA5 strains could effectively inhibit both growths of UK99 and PH-1.

Volatile organic compounds produced by *Bacillus* spp. also play an important role in antagonistic activities toward plant pathogens by suppressing the growth and spore germination (Raza et al., 2016; Tahir et al., 2017; Wu et al., 2019). Similarly, in our study, both strains caused high antifungal activity in the VOC analysis. Wu et al. (2014) showed that phenol, toluene, phenol, and benzothiazole VOCs released from the *B. amyloliquefaciens* strain have antifungal effects against *Sclerotinia sclerotiorum*. Gao et al. (2017) found that *B. velezensis* ZSY-1 strain synthesized pyrazine (2,5-dimethyl), benzothiazole, 4-chloro-3-methyl, and phenol-2,4-bis (1,1-dimethylethyl) VOCs with antifungal activity against *Alternaria solani* and *Botrytis cinerea*. Li et al. (2020) showed that *Bacillus velezensis* CT32 synthesized decanal, benzothiazole, 3-undecanone, 2-undecanone, 2-undecanol, undecanal and 2,4-dimethyl-6-tert-butylphenol VOCs with high antifungal activity against *Verticillium dahliae* and *F. oxysporum*.

In the next step, we aimed to detect potential alterations in *Fusarium* spp. in response to *B. megaterium* treatment. Apoptosis related (*mst20*) and oxidative stress related (*cat*) genes were increased while the deoxynivalenol biosynthesis related gene (*tri5*) was decreased. Similarly, significant differences were reported by previous studies Gazdağlı et al., 2018; Yörük, 2018; Cantoro et al. 2021; Teker et al., 2021). Moreover, both *B. megaterium* strains reduced significantly the cell viability of *Fusarium* isolates.

Conclusion

In this study, *B. megaterium* CTBmeg1 and HMA5 strains significantly reduced the mycelial growth of *F. culmorum* UK99 and *F. graminearum* PH-1 isolates. VOC could affect the inhibition of mycelial growth. At the molecular level, a decrease in the gene associated with toxin production and increases in genes associated with antioxidants and apoptosis were detected in *Fusarium* isolates treated with *Bacillus* strains. To our knowledge, this is the first report to reveal transcripts analysis in *B. megaterium* strains against *Fusarium* spp. However, detailed and comprehensive investigations such as in planta tests and more *Fusarium* species would be useful in providing more detailed data in disease management.

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Research Article

Effects of Shading and Nitrogen Fertilizer on Growth and Physiology of Gandarusa (*Justicia gendarussa* Burm. F.)

Syaefudin SUMINTO*[1], Syahrul RAMADHAN[2], Uswatun HASANAH[3], Waras NURCHOLIS[4]

[1,2,4]IPB University, Faculty of Mathematics and Natural Sciences, Department of Biochemistry, 16680, Bogor, Indonesia
[1,4]IPB University, Tropical Biopharmaca Research Center, 16128, Bogor, Indonesia
[3]IPB University, Faculty of Agricultural Engineering and Technology, Department of Food Science and Technology, 16680, Bogor, Indonesia
[3]IPB University, South-East Asia Food and Agricultural Science and Technology Center, 16680, Bogor, Indonesia

[1]https://orcid.org/0000-0002-4246-9048, [2]https://orcid.org/0000-0002-6477-3702, [3]https://orcid.org/0000-0002-2202-1924
[4]https://orcid.org/0000-0001-7047-5093

*Corresponding author e-mail: wnurcholis@apps.ipb.ac.id

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Abstract: Gandarusa (*Justicia gendarussa* Burm. F.) is a shrub used in herbal medicine, but knowledge of optimal cultivation methods for enhancing plant growth and metabolite yield is limited. This research aimed to evaluate the effect of shading and nitrogen fertilizer on the growth, photosynthetic parameters, and total sugar content of gandarusa. A split-plot experimental design was used with shading (S) (0% (S_0), 25% (S_{25}), and 50% (S_{50})) as the main plots and nitrogen fertilizer (N) (0 (N_0), 90 (N_{90}), 180 (N_{180}), and 270 (N_{270}) kg ha^{-1}) as the subplots. The results showed that the combination of S_0 and N_{270} was the most effective treatment for plant growth, indicated by the highest values of plant height and the number of leaves and branches. It also yielded high sugar content, with a value range of 72-76 mg g^{-1} leaves wet weight. The combination of S_0 and N_0 produced the highest photosynthetic rate (Pn) in the plant at 23.91 mol CO_2 m^{-2} s^{-1}, and total chlorophyll content was highest with S_{25} and N_{270}. Based on the results, shading decreased Pn, sugar production, and growth of gandarusa, while nitrogen fertilizer enhanced them. However, there was no interaction between shading and fertilizer on sugar production and growth of gandarusa, except for Pn.

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1. Introduction

Gandarusa (*Justicia gendarussa* Burm. F.) is a shrub found in forests and along river embankments, thriving in areas with sufficient water. It belongs to the class Magnolipsida, order Scrophulariales, family Acanthaceae, genus *Justicia*, and species *gendarussa* (Kavitha et al., 2014). Empirically, gandarusa has been widely used in herbal medicine for various conditions, including rheumatism, eczema, bronchitis, jaundice, and the common cold by Indians. In Papua, males use gandarusa as a contraceptive herb (Ratih et al., 2019). It reportedly has bioactive compounds, such as phenols, flavonoids, tannins, alkaloids, steroids, glycosides, saponins, stigmasterol, lupeol, 16-

hidroxilupeol, triterpenoids, and justicin (Hesturini et al., 2017). Previous research also revealed that gandarusa exhibited anticancer, antidiabetic, and anti-inflammatory activities (Nirmalraj et al., 2015; Adelina, 2020; Subbiah et al., 2021).

Identifying the best cultivation method to increase the yield of bioactive compounds is crucial for maximizing the potential of gandarusa as a medicinal plant. According to Moo-Young (2011), the growth and development of plants are dominantly affected by light intensity, nutrients, soil moisture, and substrate. Although sufficient light intensity is essential for photosynthesis, certain plants require proper cultivation methods to enhance their growth, such as using shading treatment. Shading was shown to reduce microclimate, radiation, temperature, and absorption of water and nutrients (Semchenko et al., 2012; Masabni et al., 2016; Arevalo-Gardini et al., 2021). Fan et al. (2018) reported that under the full intensity of light, corn showed higher biomass productivity than with shading treatment. In contrast, Khalid et al. (2019) reported enhanced bell pepper yield with shading treatment.

Another factor involved in improving plant productivity is nitrogen (N). Nitrogen fertilizer use on crops has increased in the past three centuries. Along with phosphorus (P) and potassium (K), nitrogen is classified as an essential macronutrient (Xu et al., 2012). Tian et al. (2020) reported that nitrogen fertilizer could increase biomass productivity, antioxidant enzyme activity, and photosynthetic rate (Pn) of corn. However, Zhang et al. (2016) found only a slight improvement in crop production after its application. Rahmah et al. (2021) reported that the application of manure and NPK fertilizer could improve growth, stomatal conductivity (Sc), intercellular CO_2 concentration, transpiration rate (Tr), leaves yield, and sugar content in gandarusa. However, the cultivation method to enhance plant growth and metabolite yield of gandarusa has not been thoroughly researched. There is also no research available on the influence of shading and nitrogen fertilizer treatments on its growth and physiology. Therefore, this research evaluated the effect of shading and nitrogen fertilizer treatments on the growth, Pn, total chlorophyll content, and carbohydrate production of gandarusa. This research will be vital for the development of gandarusa as a medicinal plant.

2. Material and Methods

2.1. Instrument and material

The instruments used in this research included Li-Cor portable photosynthesis system (L1-6400XT, Li-Cor Inc., Lincoln, NE), UV-Visible spectrophotometer (T60UV, PG Instruments, UK), and microcentrifuge (KITMAN-T24, Tomy Kogyo Co., Ltd., Tokyo, JP). Several materials were used in this research, including gandarusa (*Justicia gendarussa* Burm. F. local variety), urea (nitrogen fertilizer), dimethylsulfoxide (DMSO), EtOH, H_2SO_4, phenol, and glucose. All chemical materials used were commercially available. This research was conducted for five months, from November 2021 to April 2022, at the Biopharmaca Cultivation Conservation Unit Garden, Tropical Biopharmaca Research Center of the Institute for Research and Community Service (LPPM), IPB University, Cikabayan Garden Block C, Dramaga IPB Campus, Bogor (6o3'49"S and 106o42'57"T) at an elevation of 141 m above sea level. It was also conducted in the Biochemistry Department Laboratory at the IPB University, West Java, Indonesia.

2.2. Experimental design

A split-plot design was used, where seedlings obtained from 15 cm stem cuttings of gandarusa were planted into 10 × 15 cm polybags. After one month, the gandarusa seeds were transferred into 25 × 30 cm polybags consisting of soil, rice husk, and manure (1:1:1) for the treatments. The two parameters used for this research included shading as the main plot and nitrogen fertilizer (urea) as the subplot. Table 1 shows the treatment design for shading and nitrogen fertilizer. Furthermore, the experiment was carried out in triplicate, with each replicate consisting of six plants.

Table 1. Treatment design of shading and nitrogen fertilizer on gandarusa

Subplot (N Fertilizer (kg ha^{-1}))	Main Plot - Shading (%)		
	0	25	50
0	S_0N_0	$S_{25}N_0$	$S_{50}N_0$
90	S_0N_{90}	$S_{25}N_{90}$	$S_{50}N_{90}$
180	S_0N_{180}	$S_{25}N_{180}$	$S_{50}N_{180}$
270	S_0N_{270}	$S_{25}N_{270}$	$S_{50}N_{270}$

Note: "S" = shading (%), "N"= nitrogen fertilizer (kg ha^{-1}).

2.3. Photosynthetic parameters measurement

The photosynthetic parameters, including Pn, Sc, CO_2 intercellular (Ci), and Tr, were measured according to the method previously described by Zhang et al. (2016).

2.4. Chlorophyll content measurement

Chlorophyll was extracted from gandarusa following the method described by Parry et al. (2014). Leaves from each treatment were cut into small discs of 0.1 g and soaked with 7 mL DMSO solution before being boiled at 65°C for 25 minutes. The chlorophyll content was then measured using a spectrophotometer at λ_{649} and λ_{665} by a method developed by Arnon (1949). Furthermore, DMSO was used as a control in this experiment.

$$chlorophyll\ a\ (mg\ g^{-1}) = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \cdot \frac{V}{1000 \cdot W} \quad (1)$$

$$chlorophyll\ b\ (mg\ g^{-1}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \cdot \frac{V}{1000 \cdot W} \quad (2)$$

$$Total\ chlorophyll\ (mg\ g^{-1}) = chlorophyll\ a + chlorophyll\ b \quad (3)$$

Note: A : Absorbance
V : Volume of solution (mL)
W : Weight of sample (g)

2.5. Sugar content measurement

Sugar production in the leaves was evaluated using the phenol sulfuric acid method described by Pandey 2018. Fresh gandarusa leaves were crushed and ground using a mortar. A total of 0.1 g of the mashed leaves was dissolved in 1 mL of 80% EtOH and homogenized for one minute. The resulting suspension was centrifuged at 1000 RPM and 4°C for 15 minutes, and the obtained pellet was mixed with the 80% EtOH until the volume reached 10 mL. Subsequently, 1 mL of the sample solution was added to 1 mL of 5% phenol and 5 mL of H_2SO_4 before measuring the absorbance of the sample using a spectrophotometer at λ_{480}. Carbohydrate concentration was determined by comparing sample data with a standard curve. The standard curve (R^2 = 0.9945) was created using glucose solution with concentrations of 0, 5, 10, 15, 20, 25, and 50 µg mL^{-1}.

2.5. Data analysis

The effects of the treatment on the split-plot design were evaluated based on plant growth observation, photosynthetic parameters measurement, chlorophyll content measurement, and sugar production evaluation. The data were analyzed using ANOVA to determine the difference between each treatment. The significant differences ($p<0.05$) in the data were then subjected to the Duncan Multiple Range Test (DMRT) by SAS and SPSS software with a test level of 95%.

3. Results

3.1. Plant growth parameters

Figure 1 shows that shading treatment significantly decreased plant height, while nitrogen fertilizer did not. The greatest height was observed in S_0 at 39.8 cm. Each level of fertilizer treatment did not show any significant difference in enhancing plant height, except for N_{270} which produced the greatest height than those of lesser concentrations. There was no interaction between shading and nitrogen fertilizer treatments on plant height, as shown in Table 2.

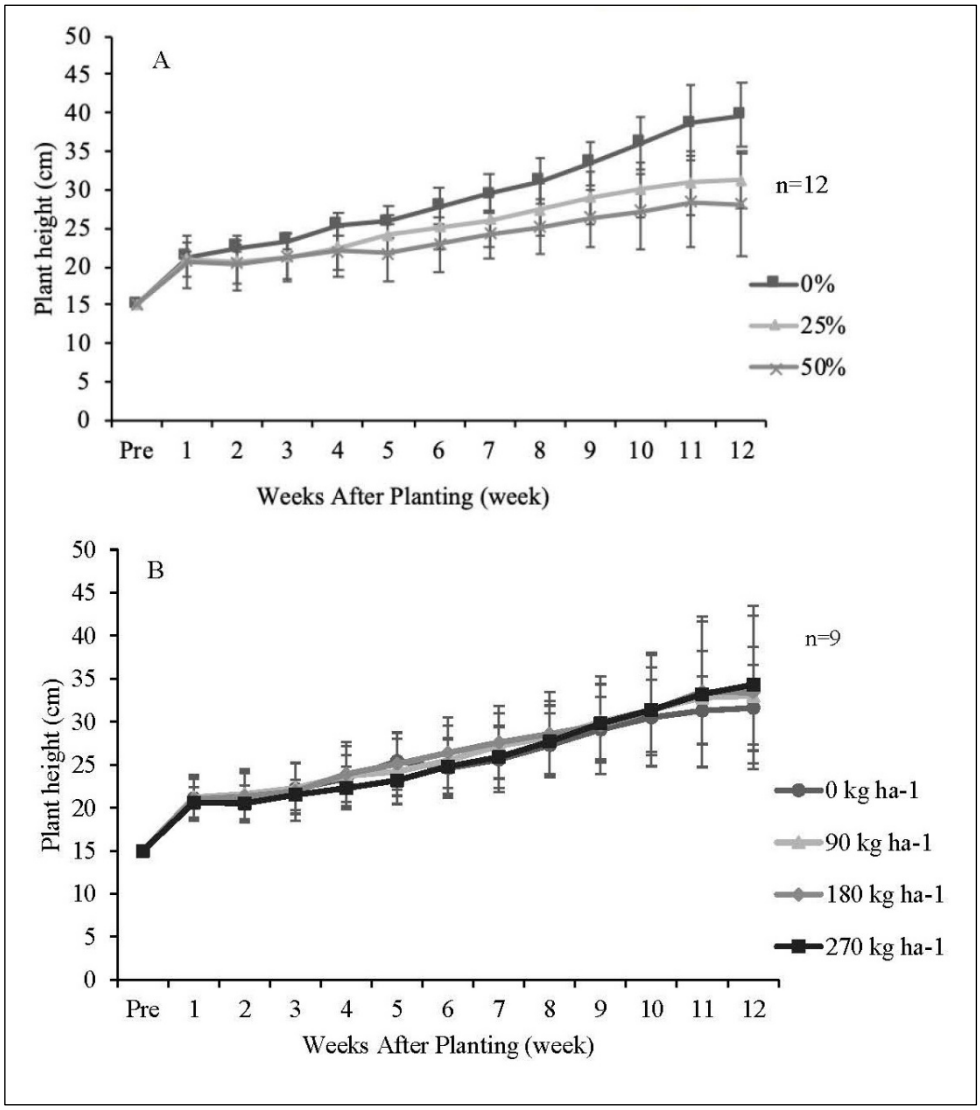

Figure 1. Effects of (A) shading and (B) nitrogen fertilizer on the plant height of gandarusa.

Table 2. The height of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	Height of Plant (cm)											
0	21.4^{a}	22.4^{a}	23.4^{a}	25.5^{b}	25.9^{b}	27.9^{b}	29.5^{b}	31.2^{b}	33.5^{b}	36.2	38.7^{b}	39.8^{b}
25	20.9^{a}	20.6^{a}	21.3^{a}	22.0^{a}	21.8^{a}	22.9^{a}	24.2^{a}	25.2^{a}	26.4^{a}	27.3^{a}	28.5^{a}	28.2^{a}
50	20.6^{a}	20.4^{a}	21.3^{a}	22.4^{a}	24.1^{ab}	25.2^{a}	26.0^{a}	27.4^{a}	29.0^{a}	30.1^{a}	30.9^{a}	31.3^{a}
Nitrogen fertilizer (kg/ha)	Height of Plant (cm)											
0	21.1^{a}	21.2^{a}	22.2^{a}	23.4^{a}	25.3^{a}	24.6^{a}	25.6^{a}	27.3^{a}	29.1^{a}	30.5^{a}	31.3^{a}	31.6^{a}
90	21.2^{a}	21.6^{a}	22.3^{a}	23.7^{a}	24.2^{a}	25.5^{a}	27.2^{a}	28.2^{a}	30.0^{a}	31.4^{a}	32.8^{a}	33.0^{a}
180	21.0^{a}	21.3^{a}	21.9^{a}	23.9^{a}	25.1^{a}	26.4^{a}	27.6^{a}	28.6^{a}	29.6^{a}	31.3^{a}	33.5^{a}	33.4^{a}
270	20.6^{a}	20.5^{a}	21.5^{a}	22.3^{a}	23.2^{a}	24.8^{a}	25.9^{a}	27.7^{a}	29.8^{a}	31.4^{a}	33.2^{a}	34.3^{a}
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the height of the plant.

Based on the result, shading treatment significantly reduced the number of leaves each week. As shown in Figure 2, the number of leaves increased following an increase in nitrogen fertilizer concentration. However, there was no difference between treatments at different concentrations. There was an interaction between shading and nitrogen fertilizer in the number of leaves, particularly within 2-5 weeks after treatment (WAT), no interaction was found at 6-12 WAT This can be attributed to the optimal growth conditions that led to faster leaves growth. The best treatment at 2-5 WAT was the combination S_0N_{90}. The interaction between shading and nitrogen fertilizer on the number of leaves is shown in Table 3.

Table 3. Number of leaves of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	leaf											
0	11.7	23.9^{b}	27.5^{b}	36.3^{c}	38.0^{c}	45.1^{b}	49.4^{b}	52.9^{b}	56.4^{b}	58.4^{b}	60.4^{b}	60.8^{b}
25		13.3^{a}	14.1^{a}	16.3^{a}	18.3^{a}	21.5^{a}	23.9^{a}	24.9^{a}	26.0^{a}	25.3^{a}	25.7^{a}	23.5^{a}
50	9.0	15.9^{a}	18.1^{a}	21.7^{b}	25.3^{b}	27.6^{a}	30.1^{a}	29.8^{a}	29.2^{a}	27.7^{a}	24.7^{a}	23.1^{a}
Nitrogen fertilizer (kg ha^{-1})	leaf											
0		16.0^{a}	17.4^{a}	21.8^{a}	22.9^{a}	26.2^{a}	29.3^{a}	31.6^{a}	33.1^{a}	31.8^{a}	29.6^{a}	28.6^{a}
90	10.1	18.3^{a}	20.1^{ab}	26.2^{ab}	28.4^{ab}	32.3^{a}	35.6^{a}	36.4^{a}	37.2^{a}	36.7^{a}	35.9^{ab}	33.7^{ab}
180	11.1	19.7^{a}	23.0^{b}	28.2^{b}	30.4^{b}	34.1^{a}	37.4^{a}	37.1^{a}	37.6^{a}	36.3^{a}	36.4^{ab}	35.2^{ab}
270	9.2^{a}	16.8^{a}	19.0^{ab}	22.9^{ab}	27.0^{ab}	32.9^{a}	35.6^{a}	38.4^{a}	40.9^{a}	43.7^{a}	45.9^{b}	45.8^{b}
Interaction	ns	s	s	s	s	ns	ns	ns	ns	ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the number of plant leaves, s = significant or there is an interaction between shading and nitrogen fertilizer on the number of plant leaves.

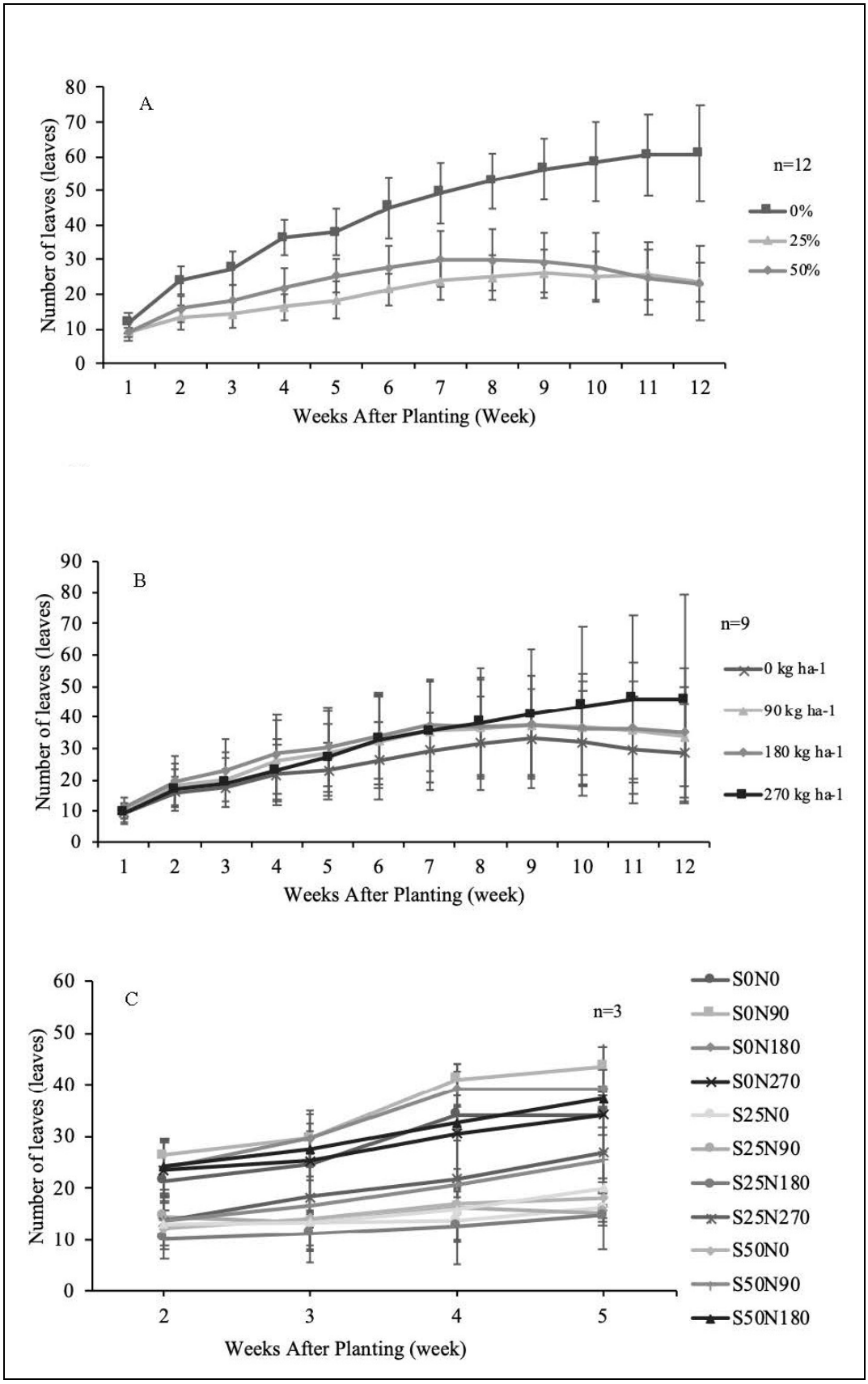

Figure 2. Effects of (A) shading, (B) nitrogen fertilizer, and (C) interaction between shading and nitrogen fertilizer on the number of leaves of gandarusa.

A further experiment on the number of branches revealed that both shading and nitrogen fertilizer treatments had significant effects, as shown in Figure 3. Shading treatment decreased the number of branches, indicated by a higher number of branches with the un-shading treatment (11.4 branches). This value was significantly different from S_{25} and S_{50}, with 4.3 and 4.2 branches, respectively. Based on nitrogen fertilizer treatment, N270 had the highest value of 10.8 branches, and the number decreased with concentration. As shown in Table 4, an interaction between the two treatments was observed at 2, 5, and 12 WAT. Additionally, at 5 WAT, the greatest treatment was observed in S_0N_{90}, while at 12 WAT, it was found in S_0N_{270}.

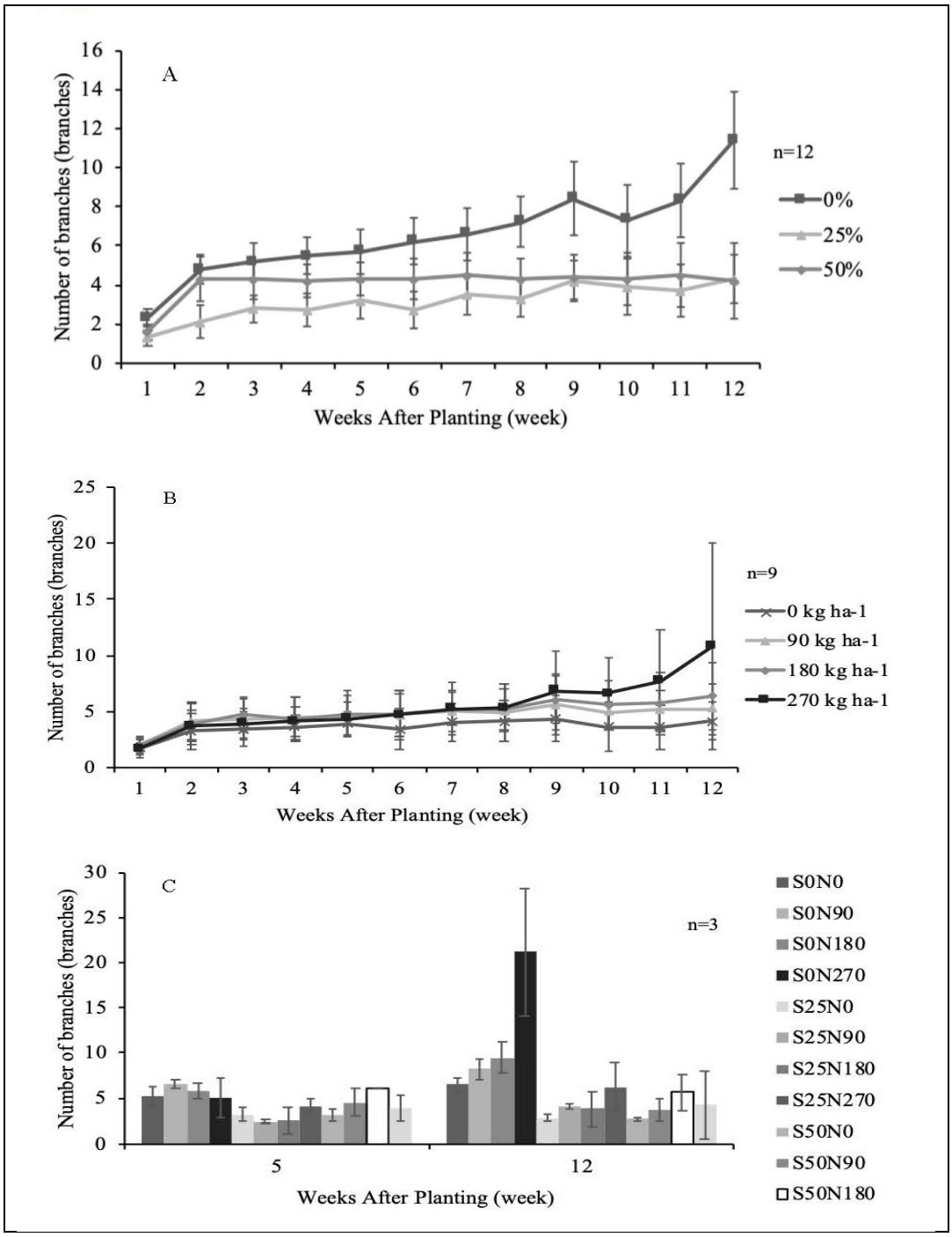

Figure 3. Effects of (A) shading, (B) nitrogen fertilizer, and (C) interaction between shading and nitrogen fertilizer on the number of branches of gandarusa.

Table 4. Number of branches of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	branch											
0	2.3^{b}	4.8^{b}	5.2^{b}	5.5^{c}	5.7^{c}	6.2^{c}	6.6^{b}	7.2^{b}	8.4^{b}	7.3^{b}	8.3^{b}	11.4^{a}
25	1.3^{a}	2.1^{a}	2.8^{a}	2.7^{a}	3.2^{a}	2.7^{a}	3.5^{a}	3.3^{a}	4.2^{a}	3.9^{a}	3.7^{a}	4.3^{a}
50	1.6^{a}	4.3^{b}	4.3^{b}	4.2^{b}	4.3^{b}	4.3^{b}	4.5^{a}	4.3^{a}	4.4^{a}	4.3^{a}	4.5^{a}	4.2^{a}
Nitrogen fertilizer (kg ha^{-1})	branch											
0	1.7^{a}	3.2^{a}	3.4^{a}	3.6^{a}	3.9^{a}	3.4^{a}	4.0^{a}	4.1^{a}	4.3^{a}	3.6^{a}	3.6^{a}	4.1^{a}
90	1.9^{a}	4.1^{a}	4.3^{ab}	4.4^{a}	4.6^{a}	4.7^{b}	5.0^{a}	4.9^{a}	5.6^{ab}	4.9^{ab}	5.1^{ab}	5.2^{a}
180	1.9^{a}	3.9^{a}	4.7^{b}	4.3^{a}	4.8^{a}	4.8^{b}	5.2^{a}	5.2^{a}	6.0^{ab}	5.6^{b}	5.7^{bc}	6.4^{a}
270	1.6^{a}	3.7^{a}	3.9^{ab}	4.1^{a}	4.3^{a}	4.7^{b}	5.2^{a}	5.3^{a}	6.8^{b}	6.6^{b}	7.7^{c}	10.8^{b}
Interaction	ns	s	ns	ns	s	ns	ns	ns	ns	ns	ns	s

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the number of branches, s = significant or there is an interaction between shading and nitrogen fertilizer on the number of branches.

3.2. Photosynthetic parameters

The investigation of the photosynthetic parameters showed an interaction between shading and nitrogen fertilizer (S*N) in Pn and intercellular CO_2 (Ci), but none in Sc and Tr, as shown in Tables 5 and 6, respectively. As depicted in Figure 4, S_0N_0 had a high Pn of 23.91 µmol CO_2 m^{-2} s^{-1}, while $S_{50}N_0$ resulted in a high Ci with a value of 174.45 µmol CO_2 µmol air^{-1}. It was also noted that the Ci increased as shading concentration increased.

Sc was significantly different in the fertilizer treatment (N) alone but not significantly different in shading treatment alone (S). There was also no treatment interaction between the treatments (S*N). According to the results, N_{90} and S_0 had a high Sc with values of 0.1516 H_2O m^{-2} s^{-1} and 0.1504 H_2O m^{-2} s^{-1}, respectively. On the contrary, Tr was significantly different in both shading and nitrogen treatments. The result revealed that the highest Tr based on shading was in S_0 (0.0050 mol H_2O m^{-2} s^{-1}), while based on nitrogen fertilizer, it was in N_{90} (0.0048 mol H_2O m^{-2} s^{-1}). This research demonstrated that Tr decreased as shading level increased.

Table 5. Photosynthetic rate (Pn) and intercellular CO_2 (Ci) of gandarusa under various levels of shading and nitrogen fertilizer

Treatment		Photosynthetic rate (Pn) (µmol CO_2 $m^{-2}s^{-1}$)	Intercellular CO_2 (Ci) (µmol CO_2 mol air^{-1})
S_0	**N_0**	23.91^{a}	94.48^{d}
	N_{90}	21.55^{bc}	151.08^{abc}
	N_{180}	22.29^{b}	116.94^{cd}
	N_{270}	23.05^{ab}	138.91^{abc}
S_{25}	**N_0**	21.47^{bc}	133.16^{abcd}
	N_{90}	22.08^{b}	138.78^{abc}
	N_{180}	19.59^{d}	115.71^{cd}
	N_{270}	18.76^{d}	133.79^{abcd}
S_{50}	**N_0**	18.71^{d}	174.45^{a}
	N_{90}	20.08^{cd}	160.68^{ab}
	N_{180}	19.72^{d}	134.65^{abcd}
	N_{270}	20.28^{cd}	126.89^{bcd}

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. S = shading (%) and N = nitrogen fertilizer (kg ha^{-1}).

Table 6. Stomatal conductivity (Sc) and transpiration rate (Tr) of gandarusa under various levels of shading and nitrogen fertilizer

Treatment	Stomatal conductivity (Sc) (mol H_2O $m^{-2}s^{-1}$)	Transpiration rate (Tr) (mol H_2O $m^{-2}s^{-1}$)
Shading (%)		
0	0.1504[a]	0.0050[a]
25	0.1361[b]	0.0045[b]
50	0.1389[b]	0.0038[c]
Nitrogen fertilizer (kg ha^{-1})		
0	0.1454[a]	0.0046[ab]
90	0.1516[a]	0.0048[a]
180	0.1309[b]	0.0041[c]
270	0.1392[ab]	0.0043[bc]
Interaction	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on stomatal conductivity or transpiration rate.

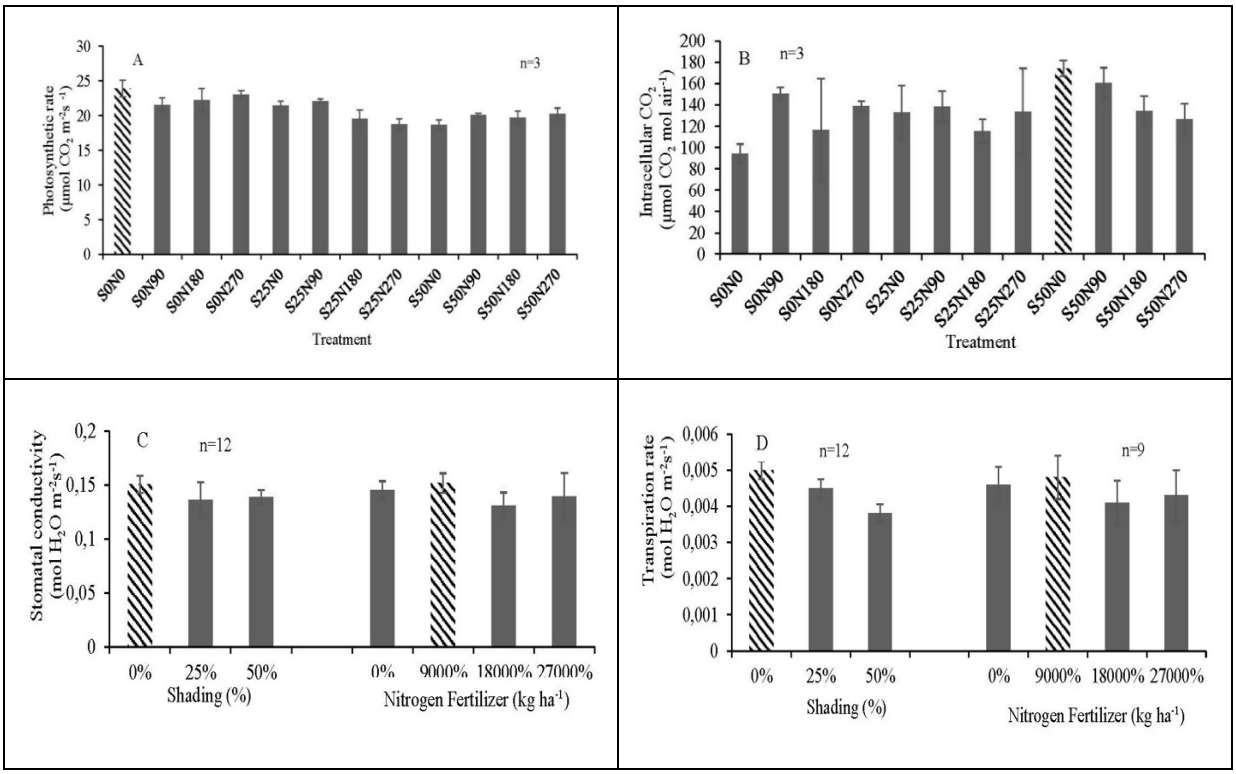

Figure 4. Effects of shading and nitrogen fertilizer on photosynthetic rate, intracellular CO_2, stomatal conductivity, and transpiration rate of gandarusa.

3.3. Chlorophyll content

Figure 5 shows the chlorophyll content of gandarusa leaves, including chlorophyll a, b, and total chlorophyll. The results indicated that shading treatment increased chlorophyll content, while nitrogen fertilizer treatment had no effect. Chlorophyll a in gandarusa leaves was also observed to be higher than chlorophyll b. As shown in Table 7, there was no interaction between the two treatments on the levels of chlorophyll a, b, and total chlorophyll. However, there was a significant difference in the levels of chlorophyll a in shading alone ($p<0.05$), and no significant difference with the fertilizer treatment ($p>0.05$).

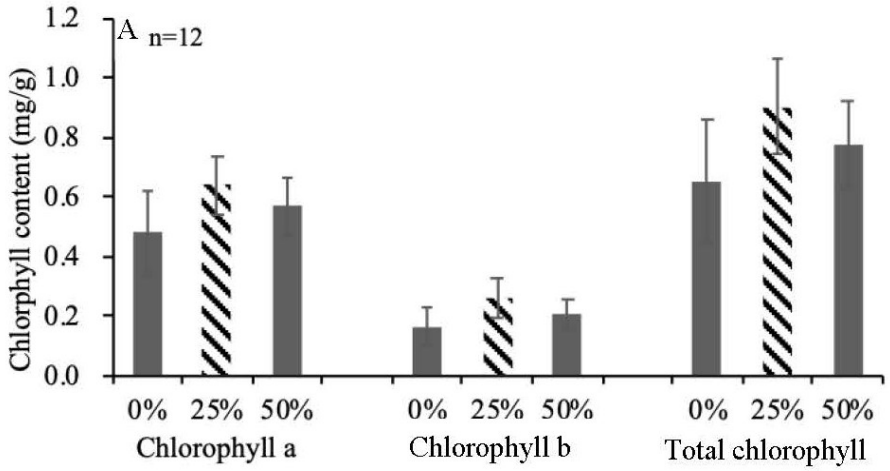

Figure 5. Effects of (A) shading and (B) nitrogen fertilizer on the chlorophyll a, chlorophyll b, and total chlorophyll contents of gandarusa.

Table 7. Chlorophyll content of gandarusa at various levels of shading and nitrogen fertilizer

Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll
Shading (%)			(mg/g)	
	0	0.481^{b}	0.167^{b}	0.648^{b}
	25	0.639^{a}	0.263^{a}	0.902^{a}
	50	0.568^{ab}	0.207^{ab}	0.776^{ab}
Nitrogen fertilizer (kg ha^{-1})			(mg/g)	
	0	0.585^{a}	0.209^{a}	0.794^{a}
	90	0.532^{a}	0.181^{a}	0.712^{a}
	180	0.551^{a}	0.213^{a}	0.763^{a}
	270	0.584^{a}	0.247^{a}	0.832^{a}
Interaction		ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on chlorophyll content.

3.4. Total sugar content

The determination of the total sugar content in gandarusa leaves revealed that there was no significant interaction between shading and nitrogen fertilizer (S*N), as shown in Figure 6 and Table 8. There was also no significant difference in sugar content in shading-only treatment (S). However, a significant difference was found in nitrogen fertilizer treatment alone (N). The highest sugar content in nitrogen and shading treatments was observed in N_{270} and S_0 with values of 76.25 mg g^{-1} and 72.88 mg g^{-1}, respectively. These results suggested a decrease in leaves sugar content due to higher levels of shading.

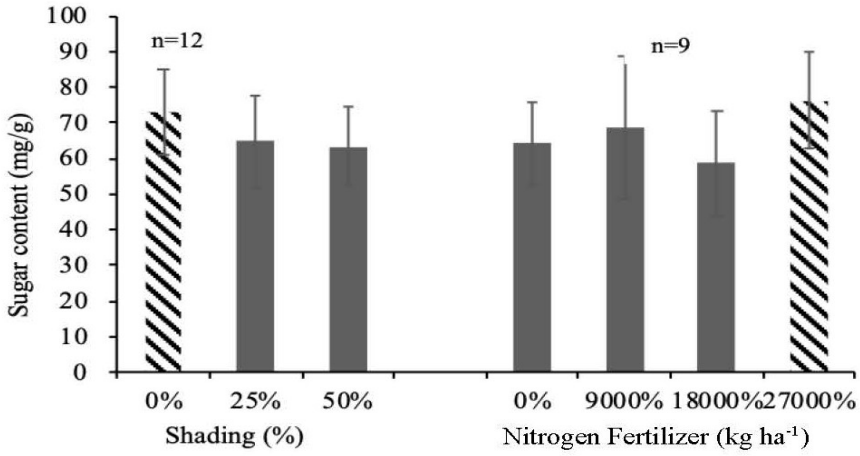

Figure 6. Effects of shading and nitrogen fertilizer on the total sugar contents of gandarusa leaves.

Table 8. Sugar content of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Sugar content (mg g^{-1})
Shading (%)	
0	72.88[a]
25	64.69[a]
50	63.19[a]
Nitrogen fertilizer (kg ha^{-1})	
0	64.08[ab]
90	68.70[ab]
180	58.64[b]
270	76.25[a]
Interaction	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on sugar content.

4. Discussion

4.1. Plant growth

Shading treatment decreases photosynthetic capacity, which is essential for plant growth and energy production (Wang et al., 2020). The evaluation of gandarusa growth also showed a decrease in plant height, number of leaves, and number of branches. Raai et al. (2020) reported a decrease in the height and number of leaves and branches of *Psophocarpus tetragonolobus* due to shading and nitrogen fertilizer treatment. Lu et al. (2021) also reported that light deficiency inhibited plant growth by directly affecting photosynthesis, phytohormone transduction, stress-related transcription factor, and R genes that are responsible for the immune system in *Magnolia sinostellata*. On the contrary, Yasoda et al. (2018) found an increase in the number of cauliflower leaves due to 50% shading. These findings implied that the use of shading to increase plant growth would depend on the type of plant.

It has been known that nitrogen fertilizer contributes to plant growth. In this research, many branches were affected significantly by nitrogen fertilizer, while plant height and the number of leaves were not. Research on Egyptian cotton (Ibrahim et al., 2022), eggplant (Tanko et al., 2015), and maize (Tian et al., 2020) demonstrated the essential role of nitrogen in vegetative growth, number of tillers, biosynthesis of chlorophyll, amino acid, and protein synthesis. Further investigation revealed an interaction between shading and nitrogen fertilizer, indicating that both were required to achieve specific plant growth parameters and could not be separated (Vargas et al., 2015). Previous research explained that shading could decrease the absorption of water and nutrients, leading to a decrease in the concentration of nitrogen fertilizer required for optimal productivity (Semchenko et al., 2012; Arevalo-

Gardini et al., 2021). This interaction was most prominent in the middle of the observation, indicating an optimum growth phase of gandarusa.

4.2. Photosynthetic parameters

Photosynthetic parameters are necessary to understand plant physiology during treatment (Manjarrez-Sanchez et al., 2020). In this research, shading significantly decreased some photosynthetic parameters, such as Pn, Sc, and Tr, while Ci increased as shading levels increased. Lu et al. (2021) stated that shading for an extended period could reduce rubisco levels, limit the light intensity absorbed by photosynthetic antennae, and inhibit photo-system II (PSII) and photo-system I (PSI) expressing genes. Meanwhile, nitrogen fertilizer treatment increased Pn. Zhang et al. (2021) reported that high nitrogen application on crabapple plants could increase the net photosynthesis rate and growth rate of shoot tips.

Several photosynthetic parameters have been reported to contribute to plant growth (Moo-Young, 2011; Kirschbaum et al., 2011). For example, Sc measured the ability of stomate to regulate gas (CO_2) and water exchange in and out of leaves and was significantly affected by nitrogen fertilizer in this research. Additionally, a sufficient amount of nitrogen fertilizer could optimize the leaves' anatomy and physiology by activating the aquaporin enzyme (AQP) and carbonic anhydrase (CA). AQP was responsible for mediating genes in plasma membrane intrinsic protein and increasing the permeability of CO_2 through the membrane, and CA regulated mesophyll conductivity by converting CO_2 into HCO_3^- . Zhu et al. (2020) revealed that a high concentration of nitrogen fertilizer decreased rubisco activity, leading to Sc reduction.

Tr is another parameter which represented how much water transpired per unit area of leaves in a given time. In this research, increased shading levels decreased Tr, while nitrogen fertilizer (N90) produced the highest Tr value. The light intensity increased temperature and air drought, which removed water from leaves and increased Tr value. This result is consistent with the research by Zhu et al. (2020), which reported a decrease in transpiration and Sc in leaves with excessive nitrogen fertilizer (>90 kg ha^{-1}) use. Meanwhile, Ma et al. (2022) found that the addition of 90 kg ha^{-1} nitrogen fertilizer to the sunflower plant increased Sc and Tr values.

4.3. Chlorophyll content

Based on the results, the chlorophyll content of gandarusa increased by applying shading treatment. This is consistent with a result obtained by Juhaeti et al. (2021) in millet crops. Chen et al. (2021) proposed that there was a gene responsible for chlorophyll biosynthesis, known as CsPOR, which can be stimulated by decreasing light intensity. This research found no significant difference in chlorophyll content enhancement with nitrogen fertilizer treatment Similarly, Saparso et al. (2020) reported that nitrogen fertilizer had no significant effect on the chlorophyll content in red onion.

4.4. Total sugar content

The total sugar content was observed to be higher in un-shaded treatment compared to shading treatment. However, there was no significant difference among the treatments. Nitrogen fertilizer indicated a significant difference between the different concentrations, and increasing its concentration resulted in higher sugar content in leaves, as nitrogen is essential for ADP-glucose pyrophosphorylase involved in starch biosynthesis. Widodo et al. (2019) reported that applying nitrogen fertilizer to *Pennisetum purpureum* could increase its total digestible nutrients, such as protein and carbohydrates. Zhang et al. (2021) also reported a similar result which showed an increase in sucrose and sorbitol levels in crabapple shoots due to a high concentration of nitrogen Chang and Zhu (2017) revealed that low nitrogen fertilizer concentration caused the cells to be unable to increase the size of leaves cell. Leaves cell weight is mostly covered by vacuole weight, and the vacuole also acts as a storage place for sugar in the sink tissue. However, Previous research reported an opposite result or decreased sugar content of the leaves due to high nitrogen application (Kano et al., 2007; Braun et al., 2016).

Conclusion

Based on this research, shading treatment decreased the growth of gandarusa by reducing photosynthetic capacity, as indicated by plant height, number of leaves, and number of branches.

However, nitrogen fertilizer significantly affected the number of branches, Pn, Sc, and Tr. It was found that optimal plant growth was achieved through the interaction between these treatments. Chlorophyll content was significantly affected by shading treatment but not by nitrogen fertilizer, and total sugar content was not significantly affected by either treatment. These findings suggested that the effects of shading and nitrogen fertilizer on plant growth and physiology vary depending on the plant species, and their interaction should be considered for optimal growth.

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Research Article

The Pull and Push Factors of Farm Income Diversification among Fluted Pumpkin *(Telfairia occidentalis* Hook*)* Farmers in Akwa Ibom State, Southern Nigeria

Sunday B. AKPAN*[1], Edet J. UDOH[2], Veronica S. NKANTA[3]

[1]Department of Agricultural Economics and Extension, Akwa Ibom State University, Mkpat-Enin, Akwa Ibom State, Nigeria
[2,3]Department of Agricultural Economics and Extension, University of Uyo, Uyo, Akwa Ibom State, Nigeria

[1]https://orcid.org/0000-0002-0458-028X, [2]https://orcid.org/0000-0002-4550-4320, [3]https://orcid.org/0000-0001-7035-7978

*Corresponding author e-mail: sundayakpan@aksu.edu.ng

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Telfairia

Abstract: The study examines the magnitude and factors influencing agricultural income diversification among small-scale fluted pumpkin (*Telfairia occidentalis*) farmers in Akwa Ibom State in the south-south region of Nigeria. The required information was collected from *Telfairia* farmers using a structured questionnaire. A regression analysis tool was used to analyse the specific objective. The finding revealed an average farm income diversification index of 2.29 suggesting that agricultural income diversification among small-scale *Telfairia* farmers is high and disturbing. The empirical results revealed that farmers' household size, hired labour, and educational qualification are the major "push factors" of agricultural income diversification. In contrast, "the pull factors" are farmers' age, extension visit, membership in a social group, land size, the quantity of fertilizer and manure, and household labour. To intensify farm income earnings among small-scale vegetable farmers, it is recommended that the government should encourage child spacing and family planning among fluted pumpkin farmers as these would reduce the household size and family burden always carried along with agricultural expenditures. Providing input subsidies to small-scale vegetable farmers is important to cushion the adverse effect of increased production costs. Agricultural extension services should be strengthened to render more effective services to vegetable farmers. The formation of social groups should be encouraged, primarily through cooperative farming. The government of Akwa Ibom State should set up tractor hiring centres in all the local government areas; these would help reduce the hard time vegetable farmers encounter hiring labour.

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1. Introduction

Several purposes and factors in recent times have prompted many rural farm families to diversify their livelihood or income (Sallawu et al*.,* 2016, Alobo and Bignebat 2017; Akpan et al., 2017a; Yusuf et al*.,* 2019). From the literature, the two significant reasons rural farm households diversified farm income can be grouped into two broad categories, namely "push" or factors that encourage farm income diversification and the "pull" or factors that discourage farm income diversification (Akpan, 2010; Nagler and Naudé, 2017; Akpan et al, 2017a). Farm income diversification provides opportunities for

alternative options to most rural farm households to avert farming risks and escape the venomous monster called poverty that is currently rampaging most of the rural communities in Sub-Saharan Africa, including Nigeria (Fan et al., 2013, Akpan et al., 2017b; Akpan et al., 2019b). The failure of governments of the region, including Nigeria, to provide sufficient economic incentives and security as well as the non-inclusion of farmers in the enunciation and implementation of agricultural policies/programs have aggravated farmers' desire to diversify their farm income sources (Ofuoku et al., 2019; Iraoya and Isinika, 2022, Ayana et al., 2022). Compared to a well-managed farm economy, farmers in Nigeria generally suffer low farm earnings, increased risk in production, poor commodity pricing system, low output and an undeveloped agro-economy (Oyewole et al., 2015; Akpan et al., 2016; Odemero and Gbigbi, 2019; Akpan et al., 2019a; Akpan and Monday, 2021). Being rational and for the need for survival, most farmers in the country have resorted to adjusting their livelihood strategies to cushion the effects of these adverse shocks, reduce poverty and increase their survival capacities (Akpan, et al., 2017b; Akpan et al., 2019b). Additionally, the issues of increasing population pressure emanating from rapid urbanization and the inability of market forces to allocate farm resources efficiently also explain the need for agricultural income diversification as the most preferred survival option among vulnerable rural farm households in the country (Hazell et al., 2007; Abdoulaye and Bekele, 2016; Akpan and Ebong, 2021).

Most recently, in Nigeria, farmers' decision to diversify income sources also stems from the mounting insecurity in the farm environment, volatile macroeconomic variables and wavering political environments as well as the climatic variability in the country (Agri et al., 2019, Fadare et al., 2019, Olagunju et al., 2020). The continuous occurrence of these factors has intensified the poverty incidence of most farm households and further deteriorates the well-being of the rural dwellers in the country. As noted by Dixon et al*.,* (2004), creating a dynamic environment built on the framework of livelihood diversification options is one of the potent responses needed to eradicate the current suffering of the rural people in the developing World. In another submission, Regasa, (2016) noted that insufficient access to critical farm assets, cost-effective technologies, credit and lack of arable land induces rural farm families or households to engage in low-yielding/return opportunities. Hence, farm income diversification among small-scale farmers evolves to mitigate the harsh economic weather and the changing nature of our surroundings. For instance, the incessant attacks on farmers by the herdsmen and the activities of kidnappers, as well as poverty and political-driven terrorism, have further stimulated the echoes of farm income diversification among farmers in vulnerable farm communities in the country.

The continuous pervasiveness of farm income diversification among small-scale farmers is anticipated to worsen the already saturated labour market following the inability of the secular sectors to provide sufficient jobs for the unemployed. This could compound unemployment problem and further weaken the country's agro-economy. The leafy-fluted pumpkin-based farmers are critical in providing a cheap and easily accessible source of vegetables to millions of Nigerians, especially in the south-south region (Akpan et al*.,* 2018; Akpan and Okon, 2019; Akanni-John et al., 2020, Adepoju et al., 2020; Utobo et al., 2022). This agro-enterprise is practised mainly on a small-scale basis and has constantly been affected by the economic environment, and pressure from alternative land uses (Ada, 2017, Akpan and Ebong, 2021, Nkanta et al*.,* 2022a). Farm income diversification among *Telfairia* farmers is magnified because farm resources are rarely allocated efficiently in small-scale farming. This is because the production system is undeveloped and is characterized by using less efficient techniques than modern and improved methods. Anchored on these facts, many authors have attributed the resilience of *Telfairia* and other crop farmers' to multifaceted factors related to their socio-economic status and farm-specific characteristics, among others (Ada, 2017; Adeyonu et al., 2019; Ofuoku and Ekorhi-Robinson, 2020; Oyibo, 2020; Nkanta et al*.,* 2022b). Hence, identifying these factors are vital to achieving a sustainable policy framework for production now and in the future for Telfairia and other vegetable farmers in the region and Nigeria.

Many researchers in developing countries have delved into this critical issue of farm income diversification to generate appropriate policy variables to boost agricultural production/intensification. Among them, Ahmed (2012) in Borno State, Nigeria reported that rural farmers' educational attainment and ownership of assets significantly impact farmers' income diversification drive. Later, Agyeman et al., (2014) in Ghana identified farmers' age, years of formal education, per capita household income, female-headed households, agricultural extension agent contact, assets owned, and access road as significant factors that influenced income diversification of farm households. Furthermore, Ogbanje et

al., (2014) and Oyewole et al., (2015) stated that farm size, farmers' age, educational qualification, non-farm income, credit utilized, number of livestock owned and household size are positive determinants of agricultural income diversification; while membership in a social organization, number of agricultural extension agent visits, farm size, farm income, leisure hours and farm asset owned were identified as negative determinants. Also, Ababbo (2015), in Leemo district, Hadiya zone, Ethiopia, found the educational qualification of household heads, farm size and income, farmers' social capital, and distance to the selling point as significant variables that influenced farm income diversification. In a similar vein, Sallawu et al*.,* (2016) in Niger State, in the central region of Nigeria revealed that farmers' age, farm area, educational attainment, farm income and non-farm income, access to farm credit, household size, livestock owned, farmers' poverty position, and non-farm job were critical policy variables influencing farm income diversification or rural farmers. Similarly, Akpan et al., (2017a) identified factors influencing agricultural diversification among small-scale arable crop farmers in one of the southern States of Nigeria. The outcome showed that the educational attainment, cost of labour and the poverty level of farmers were positive determinants of agricultural income diversification. Contrarily, an increase in farming experience, fertilizer usage, farm size, household size and farm output was negative drivers. In addition, Etuk et al*.,* (2018) found the amount of farm credit, family size, farm size and farmers' marital status as the significant variables affecting the farmers' livelihood diversification in Cross River State, Nigeria. In a similar study, Adeoye et al*.,* (2019) showed that land ownership, educational qualification, access to electricity and farm location are significant dynamics influencing income diversification in rural farm households in the Western region of Nigeria. Besides, Yusuf et al*.,* (2019) in northern Nigeria identified farmers' age, educational qualification, household size and farming experience as determinants of income diversification. Moreover, Tyenjana and Taruvinga (2019) identified the sex of the household head, educational attainment, family size, and livestock ownership as factors influencing livelihood diversification in South Africa.

In furtherance of the research on farm income diversification, Teji (2020) in Southern Ethiopia stated that human capital, household assets and infrastructure-related variables were significant determinants of farm income diversification. Also, Kwizera (2021) in Burundi opined that household income, access to the market, and age of household head are positive factors of income diversification of rural households. Recently, Ayana et al., (2022) in western Ethiopia revealed that educational qualification, household dependency ratio, access to irrigation, and household-urban linkage are significant predictors of farm income diversification.

The evidence from the literature reviewed revealed scanty empirical studies on farm income diversification in small-scale farm production in the south-south region of Nigeria. The region is known for its distinct characteristics and challenges (such as oil spillage, gas flaring, increasing soil infertility and land fragmentation, tides and ocean waves, among others) that often push farmers to extreme conditions. Due to the differences in the environmental and climatic as well as the edaphic conditions among regions in the country, research inferences from other regions might not yield the appropriate policy direction needed to uplift the well-being of the rural farmers in the southern region of the country. As Wang (2018) noted, livelihood activities are fundamentally interwoven with the environment. Therefore, there is an overwhelming need to expand the frontier knowledge about farm income diversification among farmers in the State and the south-south region. Also, given the current downturn in the country's economy and the continuous deterioration of agricultural productivity, there is a need to revalidate previous results to sustain vegetable production in the State and the country. The choice of small-scale fluted pumpkin-based farmers is vital in that the leafy pumpkin constitutes the major dietary component of the vast population of the State and the region. Moreover, small-scale farmers are mostly rural dwellers and are more vulnerable to the scourge of poverty and hence have a high probability of diversifying their livelihood sources. Hence, the study is specifically designed to estimate the farm income diversification indices of fluted pumpkin-based farmers and identify the factors that influence the indices in Akwa Ibom State in the southern region of Nigeria.

2. Material and Methods

2.1. Study area

The study was conducted in the Oruk Anam local government area of Akwa Ibom State. The area lies on latitudes $4^{o}\,40^{1}$N and 5^{o} N, and longitude $70^{o}\,30^{1}$E and $70^{o}\,50^{1}$E. The total land area is about 511.73km sq., equivalent to 7.23% of the total land area of the State. The mean annual rainfall lies from 2000mm to 4000mm. The area has an average annual temperature range of 26^{0}C – 28^{0}C. Most of the population is engaged in farming, while others are involved in trading, fishing, and craft making etc. The estimated population composition of the Oruk Anam in 2021 consists of 86,239 males and 86,415 females and a total population of 172, 654 (NPC, 2022).

2.2. Selection of sample size

Based on the specification of Cochran (1963); the study derived a representative sample size from a relatively large population of fluted pumpkin farmers in the study area by applying the equation (1) specified thus:

$$S_x = \frac{\phi^2 P(1 - P)}{D^2} \quad (1)$$

Where S_x is the required sample size needed from a large population of fluted pumpkin farmers; "ϕ^2" is the area under the acceptance region in a standard distribution curve (1 – α), (at 95% confidence interval, type 1 error; 1.96). "P" is the estimated proportion of *Telfairia occidentalis* farmers in the total population of farmers in the study area. İt is estimated that about 85% of arable crop farmers in the study area cultivate fluted pumpkins (AKS Ministry of Agriculture, 2022). The farmers cultivate fluted pumpkins either in the home garden, as sole cropping or mixed cropping, and in fragmented lands. İn the dry season, the crop is planted near a water source. "D" is the desired level of precision at 5% (type 1 error). The sample size (S_n) is estimated following the substitution shown in equation 2.

$$S_n = \frac{(1.96)^2 0.85(1 - 0.85)}{(0.05)^2} = 196 \quad (2)$$

However, for convenience, the estimated representative sample was scaled up from 196 to 200. This was done to ease sampling.

2.3. Sampling technique and sources of data

The study used multi-stage random sampling methods to select respondents. The first phase involved a random selection of 5 clans from the nine (9) clans available in the Oruk Anam Local Government area. In the second phase, two villages renounced for fluted pumpkin production were randomly picked from each clan. Therefore, ten (10) villages were randomly selected 210iterat data collection. The third phase used a random sampling technique to select twenty (20) fluted pumpkin-based farmers from each of the previously selected ten (10) villages. Hence, a total of two hundred (200) leafy-fluted pumpkin-based farmers were randomly selected and used for information collection. Note that the required respondents or fluted pumpkin-based farmers were vegetable/arable crop farmers majorly involved in cultivating leafy fluted pumpkin as sole or mixed cropping system. The required information was collected from the respondents using a structured questionnaire instrument. Primary information was obtained from the respondents, covering a wide range of cross-sectional data such as social features, farm characteristics and economic status of farmers, among others. The data were collected during the first planting season of the 2022 planting year.

2.4. Measuring farm income diversification

Farm income diversification is how rural farm families in rural areas generate additional income by engaging in non-farm economic activities. In the literatüre, authors have employed different methods to measure income diversification based on the data available and the target population. Some

techniques used and the respective authors include the Simpsons Index of Diversity (Ibrahim et al., 2009; Agyeman et al., 2014; Olugbire et al., 2020; Ayana et al., 2022). The use of the number of non-farm income-generating activities by farm households (Halliru and Bara'u, 2018); the share of farm income from off-farm activities (Sallawu et al., 2016); binary method (Ababbo, 2015). The third method is the Herfindahl-Hirschman Index (Akpan et al*.,* 2015a; Akpan et al, 2017a; Teji, 2020; Iraoya and Isinika, 2022). Some scientists also employed the entropy diversification index and Ogive diversification index approaches (Akpan et al., 2015a and 2015b). Few authors have utilized the share of non-farm income generated in the total household income (Awoyemi 2004; William 2016; Odoh et al., 2019).

However, this study adopted the weighted share of non-farm income method, similar to the last method to measure the income diversification index of fluted pumpkin farmers. The approach is simple to analyze and well-fitted with the farmers' data. The method also allows the farmer's non-farm income to be compared with the total farm income. The index is described implicitly as follows:

$$Summation\ of\ non-farm\ income\ of\ a\ farmer\ = \sum_{i=1}^{n} X_{1i} + X_{2i} + X_{3i} + X_{kn} \quad (3)$$

Where X_i represents the stream of income (Naira) from non-farm sources available to a fluted pumpkin-based farmer. In the study, X's is either or a combination in no particular order and number of the following:

X_{1i} = income from salary paid as a civil servant
X_{2i} = income obtainable from pension
X_{3i} = income from okada/bus driving business
X_{4i}= income obtained from artisan activity
X_{5i} = income from petty trading
X_{6i} = income from remittances
X_{ki}= income obtained from any other non-farm source of livelihood

Equation 3 was weighted by the respondent's total farm income and expressed as follows:

$$Diversification\ Index\ = \left(\sum_{i=1}^{n} X_{1i} + X_{2i} + X_{kn}\right) \Big/ \left(\sum_{i=1}^{n} Y_{1i} + Y_{2i} + Y_{ni}\right) \quad (4)$$

Where Ys' is farm income (Naira) defined as;
Y_1 = income from the sales of pumpkin leaves (Naira)
Y_2 = income from the sale of pumpkin fruits (Naira)
Y_3= income from other farm sources other than fluted pumpkin production (for mixed crop farmers) (Naira). Note, the major source of farm income for the respondents is derived from the cultivation of fluted pumpkins.

The diversification index explains in equation 4 is farmer's specific, and if greater than one unity, it implies a farmer has a higher tendency to diversify his/her farm income sources. On the other hand, an index less than unity implies increasing agricultural income intensification and less diversification. A unity index suggests that a farmer is indifferent to income diversification and intensification.

2.5. The pull and push factors of farm income diversification in fluted pumpkin-based households in Akwa Ibom State

The multiple regression model was used to capture the pull and push factors associated with the farm income diversification of fluted pumpkin-based farmers. The push factors are conceptualized as the "positive determinants" (significant coefficients), while the pull factors are the "negative determinants" (significant coefficients) of farm income diversification. The estimation technique was based on the Ordinary Least Squares (OLS) method. The model is implicitly shown below:

$$DIV = \emptyset_0 + \emptyset_1 MAR + \emptyset_2 AGE + \emptyset_3 HHS + \emptyset_4 EXT + \emptyset_5 EXP + \emptyset_6 SOC + \emptyset_7 EDU + \emptyset_8 LAN + \emptyset_9 MAN + \emptyset_{10} FER + \emptyset_{11} HIL + \emptyset_{12} HHL + \emptyset_{13} GEN + \mu_i \quad (5)$$

Where,
DIV = Agricultural Income Diversification index as described in equation 4.
MAR = Marital status of a farmer (dummy; I for married farmers and 0 for the rest of the farmers)
AGE = Age of a fluted pumpkin farmer (years)
HHS = Family size of a fluted pumpkin farmer (number)
EXT = access to extension agent by a fluted pumpkin farmer in a year (Number of times)
EXP = Farming experience of a fluted pumpkin farmer (Years)
SOC = Member in social organization (number of years), Non-members are coded zero.
EDU = Educational qualification of a fluted pumpkin farmer (years)
LAN = Farm size of a fluted pumpkin farmer (ha)
MAN = Manure used by a fluted pumpkin farmer (Kg)
FER = Fertilizer used by a fluted pumpkin farmer (Kg)
HIL = Hired labour (number)
HHL = Household labour (number)
GEN = Gender of a fluted pumpkin farmer (1 for male and 0 for female)
μ = Error term

Note that the Ordinary Least Squares (OLS) estimation technique was preferred over other techniques because the distribution of the indices of diversification was non-zeros, continuous and normally distributed. The error term generated was also normally distributed.

3. Results and Discussion

3.1. Farm income diversification indices of fluted pumpkin farmers

The distribution of the estimated farm income diversification indices of fluted pumpkin farmers in the study area is presented in Table 1. The average farm income diversification index of 2.290 was discovered from the fluted pumpkin-based farmers/households. The finding suggests that farm income diversification among fluted pumpkin farmers is assuming an alarming dimension. Alternatively, the result reveals that most fluted pumpkin farmers rely on alternative non-farm income sources than income generated from the cultivation of fluted pumpkin crops. The minimum index of 0.096 units and maximum index value of 48.00 units were obtained from the pooled analysis. The breakdown of the income diversification index revealed that only 2.00% of the fluted pumpkin farmers had an index in the range of 0.001 – 0.200. This implies that no fluted pumpkin farmer solely depended on income generated from his/her farm. However, very few farmers were close to being dependent on farm income.

Table 1. Farm income diversification indices of fluted pumpkin farmers

Category of income diversification	Frequency	Percentage
0.001 – 0.200	4	2.00
0.201 – 0.400	12	6.00
0.401 – 0.600	14	7.00
0.601 – 0.800	4	2.00
0.801 – 1.000	24	12.00
Greater than one	142	71.00
Mean	2.29	
Minimum	0.096	
Maximum	48.00	

Source: Calculated by authors, data from field survey 2022.

The result also showed that, 6.00%, 7.00%, 2.00% and 12.00% of the farmers belong to the income diversification index range of 0.201 – 0.400, 0.401 – 0.600, 0.601 – 0.800 and 0.801 – 1.00 respectively. The result also showed that 29.00% of the fluted pumpkin-based farmers or households

generated farm income greater than their respective non-farm income. The finding suggests that farm income intensification among fluted pumpkin farmers is fast losing its mastery in the southern region of Nigeria. The findings revealed that more fluted pumpkin farmers are diversifying their sources of farm income to non-farm enterprises. The result further revealed that 71.00% of the farmers operated at an index greater than unity. This indicates that far more fluted pumpkin farmers have non-farm income greater than their farm income. The finding is in accordance with the reports of Djido and Shiferaw (2018), Odoh et al. (2019) and Iraoya and Isinika, (2022). They asserted that most rural farm households have increasing sources of farm income diversification or alternative non-farm income-generating activities in Nigeria.

3.2. Pull and the push factors of income diversification in fluted pumpkin-based farmers

The estimates of the multiple regression analysis representing the income diversification equation of fluted pumpkin-based farmers are presented in Table 2. The estimates represent the pull and push factors of income diversification of fluted pumpkin-based farmers. The diagnostic statistics revealed an R^2 of 0.778, which implies that about 77.83% of total variations in the calculated indices of income diversification are explained by the specified explanatory variables. The estimated F-calculated is about 14.0253 and is statistically significant at a 1% probability level. The outcome connotes that the R^2 is statistically significant. This means that the estimated equation has the goodness of fit. Also, the RESET test statistic's magnitude revealed the specification's adequacy. This implies that the estimate equation has structural rigidity. The null hypothesis defining the normality of the error term is strongly upheld, and this justifies the use of the OLS estimation technique.

The estimates revealed the following significant pull factors that influence farm income diversification among fluted pumpkin-based farmers/households: farmer's age, extension agent visits, membership in a social organization, farm size, the quantity of manure used, the quantity of fertilizer used, and the number of family labour. These variables negatively correlate with the farm income diversification index of fluted pumpkin-based farmers in the study area. These pull factors are the fundamental policy instrument that can effectively, efficiently and significantly tackle and address the issues related to farm income diversification in fluted pumpkin-based farmers. This means that an increase in these variables would lead to an increase in farm income intensification or a corresponding reduction in the diversification index of farm income of fluted pumpkin-based farmers/households in the study area.

The finding precisely revealed that a year increase in a farmer's age would result in a 0.07 unit decrease in the farm income diversification index of fluted pumpkin farmers in the region. This means that, as a farmer's age increases, the tendency to diversify declines. The result suggests that farm income diversification is predominant among youth farmers as opposed to intensification for older farmers. This finding aligns with the reports of Ahmed (2012), Agyeman et al. (2014), Ogbanje et al. (2014), Oyewole et al. (2015), Sallawu et al. (2016), Yusuf et al. (2019), Teji (2020) and Kwizera (2021).

Similarly, a 10% increase in agricultural extension workers' visits to fluted pumpkin farmers would decrease farmers' index of income diversification by 49.37 units. An increase in agricultural extension workers' visits to fluted pumpkin farmers will likely increase the rate of innovation adoption and also expose farmers to modern production techniques. Also, market potentials could be open through persistent interactions with the extension agents. This interaction will enhance the efficiency of resource use, farm productivity and farm income. As a result of an increase in farm income, farmers' well-being will be enhanced, and they will likely choose income intensification instead of diversification. The finding corroborates Agyeman et al. (2014), Ogbanje et al. (2014), Oyewole et al. (2015) and Teji (2020).

Table 2. The Push and pull factors of income diversification in fluted pumpkin farmers

Variable	Coefficient	Standard error	t-value	P-value
Constant	−3.8088	3.1813	−1.197	0.2345
Marital status	−0.9463	1.0273	−0.9212	0.3595
Age	−0.0674	0.0257	−2.6278**	0.0201
Household size	0.6788	0.3290	2.063**	0.0411
Extension agent	−4.9367	2.2443	−2.200**	0.0305
Experience	−0.0474	0.0783	−0.6063	0.5459
Social group	−0.7830	0.2926	−2.676***	0.0089
Education	0.2403	0.1381	1.740*	0.0804
Farm size	−4.0499	2.3882	−1.696*	0.0935
Manure quantity	−0.0018	0.0009	−2.000**	0.0497
Fertilizer quantity	−0.0221	0.0127	−1.736*	0.0861
Hire labour	0.1185	0.0253	4.685***	<0.0001
Household labour	−0.0158	0.0089	−1.775*	0.0793
Gender	−0.0291	1.1669	−0.0249	0.9801
		Diagnostic tests		
R-squared	0.7783	Adjusted R-squared		0.6843
F(calculated)	14.0253***	Mean dependent Var.		2.2904
Normality test	12.944(0.1263)	Log-likelihood		−275.4004
RESET test	10.354(0.2109)	White's test		7.136(0.1234)

Source: Data from Field Survey, 2022. Note *, ** and *** represent Significant level at 10%, 5% and 1% respectively.

According to the results, a one-year increase in farmers' membership in farm/social organizations reduces the income diversification index by 0.78 units. The increase in the year(s) of membership in a social organization would increase social capital formation among fluted pumpkin-based farmers. Farmers' encouragement, social interactions, sharing of farm production experiences and market information are quickly and effectively obtained in a social gathering. These social interactions create opportunities for farmers to expand production, the source for buyers and extend the value addition chain. Hence, increased social capital formation among fluted pumpkin farmers would breed opportunities for an enhanced farm income while reducing diversification activities. Ogbanje et al. (2014), Oyewole et al. (2015), Ababbo (2015) and Kwizera (2021) have submitted similar reports.

The findings also showed that the coefficient of farm size is statistically significant and negatively correlated to the index of farm income diversification at a 10% significance level. This connotes that a hectare increase in farm size cultivated by the fluted pumpkin-based farmer would reduce the farmers' diversification index by 4.05 units. The increase in farm size will likely enhance the economy of scale in production. Consequently, an increase in the size of production is often accompanied by increasing demand for credit and commercialization of farm activities vis-à-vis farm income. The increase in farm income and market share are possible incentives encouraging farm income intensification rather than diversifying. The finding aligns with the reports of Ogbanje et al. (2014), Oyewole et al. (2015), Ababbo (2015), Sallawu et al. (2016), Akpan et al. (2017a), Etuk et al. (2018), Adeoye et al. (2019), Yusuf et al. (2019) and Teji (2020).

Besides, the coefficients of manure and fertilizer were negative and significantly related to the index of income diversification. This implies that a kilogram surge in the use of manure and fertilizer by a fluted pumpkin-based farmer will lead to a 0.0018 kg and 0.0221 kg reduction in its income diversification index, respectively. Alternatively, an increase in the use of manure and or fertilizer/inorganic manure in the cultivation of leafy fluted pumpkin farms would increase the tendency of the farmers to intensify farm income generated. The increase in manure and fertilizer usage increase farm output and, subsequently, farm income. An increase in farmers' output and income would great incentives for farmers to intensify their production rather than diversification. The finding is similar to Akpan et al, (2017a).

Moreover, the finding revealed that a unit boost in household labour utilized by a fluted pumpkin farmer would result in a 0.0158 unit reduction in the index of income diversification. This connotes that the increase in family labour reduces the marginal effect of farm income diversification. Since hired labour is expensive and farm credit is difficult to get, most farmers rely so much on family labour. This reduces the cost of production but instead increases farm income, especially for small-scale producers

like fluted pumpkin farmers. This finding substantiates the previous research reports of Akpan et al. (2017a).

On the other hand, an increase in farmers' educational qualification, household size, and quantity of hired labour used was identified as the push factors to farm income diversification of fluted pumpkin-based farmers. This means that an increase in these variables increase fluted pumpkin farmers' tendency to increase their income diversification desires. For instance, a year increase in the formal education of a fluted pumpkin farmer will lead to a corresponding 0.2403 unit rise in its farm income diversification index. The boost in years of formal education qualification of fluted pumpkin-based farmers would likely expose them to wider job opportunities with better returns or earnings. Rationally, workers would migrate from one job to another based on wage differential and would likely be settled on the one that commands a higher wage rate. Since agriculture yields less income than other sectors, an advance in years of formal education of fluted pumpkin-based farmers would increase the potential of them moving away from agricultural production to better-yielding livelihood options. The result agrees with Ahmed (2012), Agyeman et al. (2014), Ogbanje et al. (2014), Oyewole et al. (2015), Ababbo (2015), Sallawu et al. (2016), Akpan et al. (2017a), Adeoye et al. (2019), Yusuf et al. (2019), Tyenjana and Taruvinga (2019) and Teji (2020).

The hired labour slope coefficient is positive and statistically significant at a conventional 1% significance level. The finding indicates that a number increment in the quantity of hired labour used by the fluted pumpkin-based farmer will increase the farm income diversification indicator by 0.1185 units. This means that the increased use of hired labour increases farm income diversification by the fluted pumpkin farmers in the State. The increased number of hired labour would increase the total production cost resulting in a decrease in farm returns. A continuous decrease in farm earnings would lead to an increase in income diversification. The result validates the submission of Akpan et al. (2017a).

Similarly, the coefficient of the household size exhibited a positive correlation with the income diversification index of fluted pumpkin farmers in the State. This connotes that an increase in a farmer's family size increase the possibility of increasing its diversification index. A person's increase in family size will increase the diversification index of a farmer by approximately 0.2403 units. The finding implies that an increase in household size would likely increase household expenditure and lower farm investment. In an attempt to better the family's well-being and break out from the captivity of poverty, diversification remains the best option. The finding is in agreement with the assertion put forward by Sallawu et al. (2016), Akpan et al. (2017a), Etuk et al. (2018), Tyenjana and Taruvinga (2019) and Teji (2020).

4. Conclusion

This study has shown overwhelming evidence of increased farm income diversification drive among fluted pumpkin-based farmers in the southern region of Nigeria. About 71.00% of the leafy-fluted pumpkin-based farmers in the study area derived their household income from non–farm sources. None of the sampled farmers depends solely on fluted pumpkin cultivation. The empirical results have revealed the important push and pull factors of farm income diversification of fluted pumpkin farmers in the study area. A designed policy framework based on these identified factors would help to slow the rate of farm income diversification among fluted pumpkin farmers and other stakeholders in the agricultural sector of the State and the region in general.

Based on the findings and the need to discourage agricultural income diversification, the following policy recommendations are wished-for: the Akwa Ibom State should encourage child spacing and family planning programmes among fluted pumpkin or vegetable farmers as these would reduce the household size, and family burden always carried along with agricultural expenditures. Input subsidy to small-scale farmers is important to cushion the adverse effect of increased production costs. Agricultural extension services should be reorganized to provide more effective services to farmers. The formation of social groups should be encouraged through cooperative farming. Also, there is an overwhelming need to provide the farming population in the State with farm inputs such as fertilizer and manure to encourage their adoption. This strategy can help minimise crop loss, and risks and sustainably increase yield. The government of Akwa Ibom State should set up tractor hiring centres in all the local government areas as these would help reduce the hard time farmers encounter in hiring manual labour.

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Research Article

Determination of Optimum Gamma Ray Irradiation Doses for Hulless Barley (*Hordeum vulgare* var. *nudum* L. Hook. f.) Genotypes

Namuk ERGÜN*[1], Güray AKDOĞAN[2], Saime ÜNVER İKİNCİKARAKAYA[3], Sinan AYDOĞAN[4]

[1,4] Central Research Institute for Field Crops, Department of Genetics and Breeding, 06170, Ankara, Türkiye
[2,3]Ankara University, Faculty of Agriculture, Department of Field Crops, 06110, Ankara, Türkiye

[1]https://orcid.org/0000-0002-6491-4149, [2]https://orcid.org/0000-0002-1192-3615, [3]https://orcid.org/0000-0001-8561-9002
[4]https://orcid.org/0000-0002-0971-1998

*Corresponding author e-mail: namuk.ergun@tarimorman.gov.tr

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Mutation

Abstract: The limited germplasm resources of hulless barley restrict the breeding of hulless barley with improved traits. Mutation techniques are an effective tool for generating variation for plant breeding studies. This study aimed to evaluate the impact of gamma-ray at different doses on certain seedling properties of M_1 plants of two hulless barley genotypes, as well as determine the effective dose (ED_{50}). The seeds of two hulless two-row barley genotypes, cv. Yalin and hulless barley line YAA7050-14, were irradiated with 100, 150, 200, 250, and 300 gray Gamma-rays delivered by a Cobalt 60 source along with non-irradiated control samples. Gamma-ray irradiation affects the seedling properties of M_1 plants of both hulless barley genotypes significantly. The significant effect varied based on the doses, traits, and genotypes. While lower doses were found statistically identical to the control in the majority of qualities in the M_1 generation, 250-300 gray gamma ray doses caused statistically significant decreases in the majority of characteristics studied in both genotypes. The effective doses (ED_{50}) for hulless barley genotypes were determined by plotting growth reduction values of seedling lengths, then the polynomial regression equations were calculated for each genotype. It was determined that 50% growth reduction in shoot length was reached at 214.1 Gy and 253.4 Gy for cv. Yalin and line YAA7050-14, respectively.

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1. Introduction

Barley (*Hordeum vulgare* L.), one of the oldest cultivated crops, is the cereal with the highest cultivation area and production in the world, after wheat, corn, and rice. Today, around 157 million tons of barley grain are produced in 51 million hectares worldwide (FAOSTAT, 2022). Approximately 60% of barley is globally utilized for feed, 40% for malt, 5% for seed, and 3% for food (Ullrich, 2011). It can be assumed that the majority of the produced barley is covered (hulled) barley (Meints et al., 2021).

Hulless (naked) barley, which threshes freely from the hull, accounts for a small proportion of total barley production. Hulless barley is primarily grown for food end-uses because it is rich in nutritional constituents like beta-glucan, starch, and total dietary fiber compared to its hulled types (Meints et al., 2021). While hulless barley has been mainly cultivated in Japan, Korea, Nepal, Tibet of China, and Bhutan (Shaveta and Simarjit, 2019), regarding potential feed for non-ruminants and potential health benefits as a food, the utilization of hulless barley has been in increments in the developed countries (Dickin et al., 2012; Shaveta and Simarjit, 2019; Meints et al., 2021). On the other hand, the number of released hulless barley cultivars in the world is still few compared to its hulled type. One of the reasons for the limited number of developed hulless barley cultivars is limited germplasm resources. For instance, it is reported by Meints et al. (2021) that out of the 36.734 barley accessions entered into The Germplasm Resources Information Network (GRIN), 3.003 are classified as hulless.

A wide genetic variation of genotypes provides an effective and efficient breeding program to develop new varieties. The breeding of hulless barley with improved traits is restricted because the number of two-row hulless barley genotypes is limited to find out genotypes carrying desired traits. Crossing hulless barley with hulled type is an option to generate variation, but effectiveness could be less because hullessness is controlled by the recessive allele *nud* on chromosome 7HL (Franckowiack and Konishi, 1997; Duan et al., 2015). The other option to generate variation is mutation techniques. Mutation techniques provide tools for rapidly creating desired traits if genetic variability or a specific character is not available in a germplasm collection (Maluszynski et al., 2009). Mutations are sudden changes that occur in the genetic structure of plants. Mutant plants can be released directly as a new variety or serve as a parent in crossing programs (Ahloowalia et al., 2004). Mutation breeding is one of the most effective strategies for creating genetic diversity as well as identifying critical genetic variants for economically significant traits toward crop development (Chaudhary et al., 2019). Moreover, integrating mutagenesis techniques into newly developed molecular biology technologies such as molecular markers, high-throughput mutation screening techniques, and next-generation sequencing techniques has also become more powerful and effective in crop breeding (Suprasanna et al., 2015).

In mutation breeding studies, there is a crucial ratio between plant deaths and the variation generated. In order to obtain the targeted variation, the mutagen to be applied must be at a dose that will provide a sufficient number of plants alive. Germination-viability rate (Kodym et al., 2012; Ahumada-Flores et al., 2020), growth decreasing in seedlings and the first leaf length (Kodym et al. 2012; Olgun et al., 2012), and chlorophyll mutations (Çiftçi and Şenay, 2005) are among the most accepted criteria to determine optimum irradiation dose. It is proposed that a dose that causes a 30% - 50% growth reduction could be accepted as the optimum dose. Currently, optimum irradiation doses for each type of mutagen have been determined in almost all cultivated plants, and for barley 150 - 400 gray (Gy) was reported (Suprasanna et al., 2015; FAO/IAEA, 2018). However, it is recommended that the optimum dose should be determined via a radiosensitivity test before large-scale experiment because it varies with the plant species, the cultivars, the type and status of the material, and the stage at which lethality is measured.

This study aimed to determine the optimum irradiation Gamma-ray doses for hulless two-row barley genotypes before conducting a large-scale mutagenesis experiment on hulless two-row barley to generate a variation. Effects of different Gamma-ray doses applied to seeds were investigated at M_1 plants by measuring the germination rate, survival rate, and shoot and root growth parameters. Optimum irradiation doses were determined by a dose-response curve.

2. Material and Methods

The elite seeds of the two-row hulless barley cv. Yalin and the two-row hulless barley line YAA7050-14 developed by the Central Research Institute for Field Crops were used. Gamma rays were obtained from the 381 Gray (Gy) hour^{-1} Cobalt 60 (^{60}Co) source in the Turkish Atomic Energy Agency (TAEK), Ankara Nuclear Research and Training Center (ANAEM). Five hundred seeds, uniform in size and containing approximately 12% moisture, were prepared for each irradiation dose and control group for each genotype. The seeds were irradiated with gamma rays obtained from the Cobalt 60 (^{60}Co) source at 100, 150, 200, 250, and 300 Gy doses. The seeds in the control group and irradiated at different doses were sown in a randomized complete block design with three replications separately in the greenhouse to grow M_1 plants. One day after the irradiation, the seeds were sown by hand in plastic pods (7.5 cm in

diameter and 14 cm deep) containing 320 g of washed sand, with one seed per pod. Twenty-four seeds per replication of each treatment were planted. A nutrient solution containing 6% N, 5% K_2O, 4% P_2O_5,, 0.021% Fe (EDTA), 0.013% B, 0.011% Mn, 0.0058% Zn, 0.003% Cu and, 0.0011% Mo was given to the pods once a week to prevent seedlings from nutrient deficiency. The moisture of the pods was maintained with irrigation at two days intervals. During the four-week growing period, the temperature of the greenhouse was around 20 °C, and the seedlings were grown under daylight conditions.

Measurements on M_1 plants were conducted as described by Çiftçi and Şenay (2005), and FAO/IAEA (2018) as follows. The emergence rate (ER) was calculated as a percentage (%) by dividing the total number of plants that emerged four weeks after sowing by the number of seeds planted. The survival rate (SR) of the seedlings was determined as the ratio of the plants that survived at the end of the fourth week. The number of tillers (NOT), the number of leaves (NOL), the first leaf length (FLL), the seedling length (SL), the shoot fresh weight (SFW), and the shoot dry weight (SDW) were measured at four-week-old seedlings. The root length (RL), the root fresh weight (RFW), and the root dry weight (RDW) were determined after washing the roots of the same plant, of which seedling measurements were recorded. The root/shoot dry weight ratio (RSR) was obtained by dividing the root dry weight by the shoot dry weight. First leaf length, shoot and root length were recorded in centimeters (cm), root and shoot fresh weight, and root and shoot dry weight in miligrams (mg). The data obtained from M_1 plants were subjected to analysis of variance (ANOVA) according to the randomized complete blocks design, separately for the cv. Yalin and the line YAA7050-14. The significance level of the differences among the investigated parameters was determined according to the F test, and means were separated according to Duncan's multiple range test (Montgomery, 2013). In addition, regression analysis was performed to demonstrate the relationship between gamma-ray doses and the traits examined (Freund et al., 2006). The effective dose (ED_{50}) was determined according to the regression value, taking into account the value of seedling growth reduction (GR) and seedling survival rate (SR) by 50% compared to the value obtained with the control in both genotypes (Kodym et al., 2012). The shoot length values measured at the end of the fourth week were used to calculate the seedling growth reduction.

3. Results

To determine the effective irradiation doses on hulless barley seeds to generate a variation for hulless barley breeding studies, five gamma-ray doses were evaluated on two hulless barley genotypes. The data obtained from each genotype were individually subjected to analysis of variance (ANOVA). The differences among the irradiation doses were found to be statistically significant in terms of all measured traits (Table 1).

Table 1. ANOVA table of data obtained from M_1 plants

	Yalin				YAA7050-14			
	Replication	Doses	Error		Replication	Doses	Error	
DF	2	5	10	CV	2	5	10	
	Mean square			(%)	Mean square			CV (%)
ER	117.4	163.4*	31.3	5.9	191.5	279.7*	52.9	7.9
SR	66.1	3130.8**	20.2	5.9	52.6	2289.1**	18.6	5.5
NOT	0.01	0.5**	0.01	7.1	0.002	0.05**	0.004	5.5
NOL	0.03	2.9**	0.08	6.5	0.19	0.92**	0.11	8.1
FLL	0.004	8.3**	0.06	5.9	0.25	11.3**	0.12	5.9
SL	0.96	212.6**	0.4	4.3	5.60	258.0**	2.3	7.1
RL	6.97	182.3**	2.2	6.4	1.79	114.1**	2.2	5.8
SFW	0.003	0.3**	0.005	11.4	0.005	0.7**	0.012	12.0
RFW	0.002	0.16**	0.002	11.8	0.01	0.4**	0.009	13.8
SDW	131.08	10865.5**	153.6	12.6	76.33	17124.4**	175.0	10.3
RDW	69.19	1803.1**	32.9	11.6	92.60	2449.2**	42.7	9.6
RSR	0.01	0.04*	0.01	14.4	0.05	0.37**	0.02	23.0

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ER Emergence rate; SR Seedling survival rate; NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio: CV Coefficient of variation (%); DF Degrees of freedom.

The emergence rate of the genotypes showed a different response to irradiation doses. The emergence rate in the cv. Yalin was significantly reduced over 200 Gy, while in the line, the YAA7050-

14 emergence rate was significantly reduced over 150 Gy (Figure 1.a). On the other hand, survival rates of both genotypes were significantly reduced at 200 Gy and overdoses, and at 300 Gy, only 12.3% and 25.5% of seedlings could be survived for four weeks, for cv. Yalin and YAA7050-14, respectively (Figure 1.b).

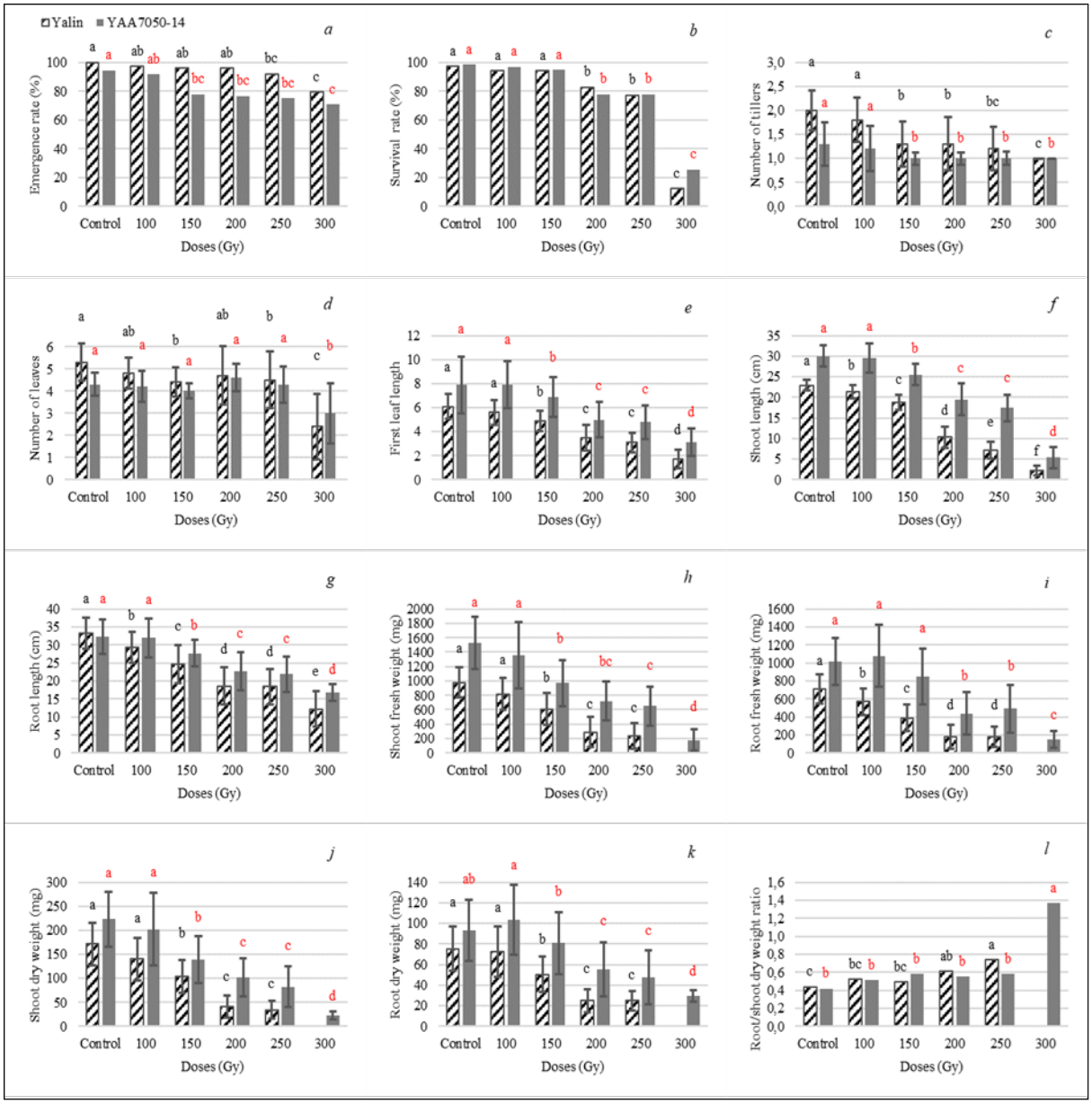

Figure 1. Effect of gamma irradiation on germination, shoot and root characters of hulless barley genotypes. *a*: Emergence rate (%); *b*: Seedling survival rate (%); *c*: Number of tillers; *d*: Number of leaves; *e*: First leaf length (cm); *f*: Shoot length (cm); *g*: Root length (cm); *h*: Shoot fresh weight (mg); *i*: Root fresh weight (mg); *j*: Shoot dry weight (mg); *k*: Root dry weight (mg); *l*: Root/shoot dry weight ratio, The same letters in bars are not significantly different at P 0.05 (black letters for cv Yalin, red letters for barley line YAA7050-14).

A significant decrease was found in the number of tillers of cv. Yalin and line YAA7050-14 at high doses compared to those in control, and even several plants were not tillering at these doses (Figure 1.c). Among the traits, the number of leaves was almost identical when the gamma-ray doses reached 250 Gy compared to those in control, and a significant reduction was only observed in both genotypes at 300 Gy doses, while the other traits investigated significantly affected the treatments at 150 Gy and overdoses (Figure 1.d). Growth reduction in seedling is a crucial indicator to determine optimal

irradiation doses (Maluszynski et al., 2009; Forster and Shu, 2012; FAO/IAEA, 2018). Therefore, several parameters related to seedling growth were measured, including first leaf length, shoot length, shoot fresh weight, and shoot dry weight. Growth parameters measured in 100 Gy treatments in both genotypes were almost identical to those in control. On the other hand, the increased gamma-ray doses gradually caused a significant reduction in growth parameters. The first leaf lengths measured in M_1 plants that completed their first development at four weeks after sowing the seeds under greenhouse conditions decreased significantly compared to those in control as the doses increased in both genotypes. The first leaf length decreased by 50% at 250 Gy dose in the cv. Yalin and 250 Gy and 300 Gy doses in the line YAA7050-14.

Due to increasing gamma-ray doses, significant decreases were detected in the seedling lengths measured in M_1 plants of both genotypes. Compared to the control, the seedling lengths obtained at 200 Gy doses in the cv. Yalin and 250 Gy in the barley line YAA7050-14 were approximately 50% less. Shoot fresh weight of M_1 seedling were gradually decreased by increasing irradiation doses. Shoot fresh weight of the cv. Yalin was 980 mg in control, while it was reduced to 240 mg at 250 Gy dose. The shoot dry weight of cv. Yalin gradually decreased by increasing irradiation doses from 171.7 mg at control to 104.5 mg at 150 Gy and drastically decreased to 41.3 mg when the dose was 200 Gy. On the other hand, the shoot dry weight of line YAA7050-14 showed gradual decreases with increasing gamma-ray doses from 222.9 mg to 22.5 mg. It should be noted that some destructive parameters such as shoot fresh weights, root fresh weights, shoot dry weights, and root dry weights were not able to be determined in cv. Yalin at 300 Gy treatment since very few seedlings of cv. Yalin treated with 300 Gy gamma-ray dose were able to survive.

The impact of gamma-ray doses on root parameters of hulless two-row barley genotypes was evaluated. The root length of cv. Yalin and line YAA7050-14 in control were 33.3 cm and 32.3 cm, while at the highest irradiation dose, it reduced to 12.2 cm and 16.8 cm, respectively. The root fresh weight of cv. Yalin gradually decreased from 710 mg in control to 180 mg in 200 Gy. However, the root fresh weight of line YAA7050-14 at 100 Gy and 150 Gy doses was identical to those in control, then dramatically reduction was observed with 440 mg and 150 mg at 200 Gy and 300 Gy irradiation doses, respectively. The root dry weight of both genotypes was significantly affected from the irradiation doses at 150 Gy, 200 Gy, and 300 Gy compared to those in control and respective irradiation doses. Contrary to all parameters, root/shoot dry weight ratios increased in parallel with irradiation doses. The lowest root/shoot dry weight ratios were observed in control with 0.44 and 0.42, while at 300 Gy, the highest ratios were 0.74 and 1.37 in cv. Yalin and YAA7050-14, respectively.

Table 2. Descriptive statistics for traits measured in M_1 plants of cv. Yalin

Doses		NOT	NOL	FLL	SL	RL	SFW	RFW	SDW	RDW
	min-max	1.0-3.0	2.0-8.0	3.7-8.4	10.5-28.9	21.8-42.5	300-1700	100-1000	47.2-317.9	22.1-135.0
Control	SD	0.41	0.83	1.01	2.59	4.40	212.3	160.5	44.53	21.54
	CV	19.8	15.7	16.6	11.3	13.2	21.6	22.7	25.9	28.5
	min-max	1.0-3.0	2.0-7.0	1.4-7.4	5.5-24.5	12.2-37.8	100-1300	100-900	13.9-261.1	18.2-138.7
100 Gy	SD	0.46	0.70	1.06	3.29	4.33	246.7	153.7	44.32	24.60
	CV	25.3	14.6	18.9	15.5	14.6	26.9	27.0	31.6	34.0
	min-max	1.0-2.0	2.0-6.0	2.7-6.3	8.0-24.5	4.5-34.8	100-1100	100-700	19.4-171.0	10.5-86.8
150 Gy	SD	0.47	0.65	0.86	3.71	5.32	219,1	146.2	33.92	17.25
	CV	35.6	14.8	17.6	19.7	21.4	35.6	36.4	32.5	33.7
	min-max	1.0-3.0	1.0-7.0	1.0-7.0	1.0-20.4	6.0-27.5	10-800	10-500	6.4-92.7	5.7-58.4
200 Gy	SD	0.55	1.31	1.06	5.28	5.12	212.9	130.7	23.47	11.00
	CV	42.3	27.7	29.9	51.2	27.5	72.5	72.2	56.6	44.1
	min-max	1.0-3.0	1.0-7.0	1.0-4.8	1.0-20.5	4.5-28.2	10-700	10-400	9.9-90.2	8.4-47.0
250 Gy	SD	0.45	1.28	0.83	4.20	4.93	182.3	106.7	18.78	9.08
	CV	38.5	28.2	27.2	58.4	26.4	74.1	58.7	53.7	36.2
	min-max	1.0-1.0	1.0-5.0	1.0-3.3	1.0-9.6	6.7-18.8	-	-	-	-
300 Gy	SD	0.00	1.47	0.78	2.24	4.97	-	-	-	-
	CV	0.0	67.0	47.6	99.0	40.5	-	-	-	-

NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio; SD Standard deviation; CV Coefficient of variation.

This study aimed to determine optimal gamma-ray doses for hulless two-row barley genotypes by comparing the mean values of the data collected from each treatment. On the other hand, we observed significant variation in terms of investigated parameters within each treatment. In order to reveal these variations, the standard deviation and coefficient of variation values of each parameter for each treatment were calculated and presented in Table 2. and Table 3. with the minimum and maximum values of the parameters measured. As it can be seen in Table 2. and 3. that, increased gamma-ray doses caused a higher coefficient of variation value, resulting in a wider variation within each treatment. In particular, the shoot lengths, one of the most representative parameters for determining optimal irradiation dose, ranged between 10.5 cm and 28.9 cm, 5.5 cm and 24.5 cm, 8.0 cm and 24.5 cm, 1.0 cm and 20.4 cm, 1.0 cm and 20.5 cm, and 1.0 cm and 9.6 cm in cv. Yalin at control, 100 Gy, 150 Gy, 200 Gy, 250 Gy, and 300 Gy gamma-ray doses, respectively. On the other hand, the calculated coefficient of variation values for shoot length of cv. Yalin were 11.3, 15.5, 19.7, 51.2, 58.4, and 99.0 at control, 100 Gy, 150 Gy, 200 Gy, 250 Gy, and 300 Gy gamma-ray doses, respectively. 200 Gy and over irradiation doses caused a wide variation within each treatment, resulting in higher CV values in parallel with our observations. Similar results were observed in the other genotype and the other parameters investigated in both genotypes.

Table 3. Descriptive statistics for traits measured in M_1 plants of the line YAA7050-14

Doses		NOT	NOL	FLL	SL	RL	SFW	RFW	SDW	RDW
Control	min-max	1.0-2.0	3.0-6.0	2.5-11.1	18.5-37.6	24.1-39.7	900-2500	500-1500	115.5-345.4	40.0-160.3
	SD	0.45	0.53	2.35	5.17	4.76	365.0	261.8	57.42	29.57
	CV	35.3	12.4	30.0	17.2	14.8	23.9	25.7	25.8	31.8
100 Gy	min-max	1.0-3.0	1.0-6.0	1.0-10.3	10.0-37.8	9.8-39.7	300-2900	50-1600	36.4-448.6	6.0-154.7
	SD	0.47	0.69	1.94	7.09	5.42	456	335.7	75.89	34.07
	CV	38.0	16.5	24.7	24.0	17.0	33.4	31.1	37.5	33.0
150 Gy	min-max	1.0-2.0	3.0-5.0	2.2-9.6	9.0-32.7	18.0-35.2	300-1800	200-1400	51.6-244.5	21.8-162.9
	SD	0.13	0.34	1.65	5.25	3.70	319.8	311.6	48.64	30.24
	CV	12.5	8.5	23.9	20.6	13.4	33.1	36.7	35.1	37.4
200 Gy	min-max	1.0-2.0	3.0-6.0	1.0-7.6	3.4-32.8	10.0-33.8	200-1300	10-1100	27.6-193.7	17.7-116.3
	SD	0.13	0.63	1.48	7.71	5.29	270.0	239.0	39.24	26.22
	CV	13.2	13.6	29.6	39.8	23.4	37.3	54.9	38.4	47.3
250 Gy	min-max	1.0-2.0	1.0-6.0	2.5-7.5	2.7-27.1	8.5-29.8	50-1300	10-1000	16.3-214.1	8.3-128.5
	SD	0.14	0.82	1.39	6.45	4.87	267.3	272.0	41.82	26.35
	CV	13.9	19.0	28.9	36.9	22.3	41.4	55.8	50.7	55.2
300 Gy	min-max	1.0-1.0	1.0-5.0	1.3-6.1	1.3-23.6	12.2-20.5	50-500	10-300	10.1-36.7	18.5-38.4
	SD	0.00	1.36	1.15	5.11	2.28	146.9	0.09	7.83	5.56
	CV	0.0	46.8	38.1	94.6	13.7	79.7	60.5	34.6	18.9

NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio; SD Standard deviation; CV Coefficient of variation.

The effective doses (ED_{50}) for hulless two-row barley genotypes were determined by plotting growth reduction values of seedling lengths on the chart given in Figure 2. and 3., then the polynomial regression equations were calculated for each genotype. Effective dose 50 (ED_{50}) was accurately calculated for cv. Yalin (R^2=0.9529) and the line YAA7050-14 (R^2=0.9653) using polynomial regression equations. It is revealed that a 50% growth reduction in shoot lengths was reached at 214.1 Gy and 253.4 Gy for cv. Yalin and the line YAA7050-14, respectively. The survival rates of genotypes were also shown in Figure 2 and 3 to demonstrate the seedling's survival rate in response to increased gamma irradiation doses. At ED_{50}, the survival rates of the seedlings were over 70% and around 60% for cv. Yalin and the line YAA7050-14, respectively. Moreover, we also determined the ED_{30}, recommended in reduced background mutation frequencies. The ED_{30} for cv. Yalin and line YAA7050-14 were calculated as 163.8 Gy and 206.3 Gy, respectively.

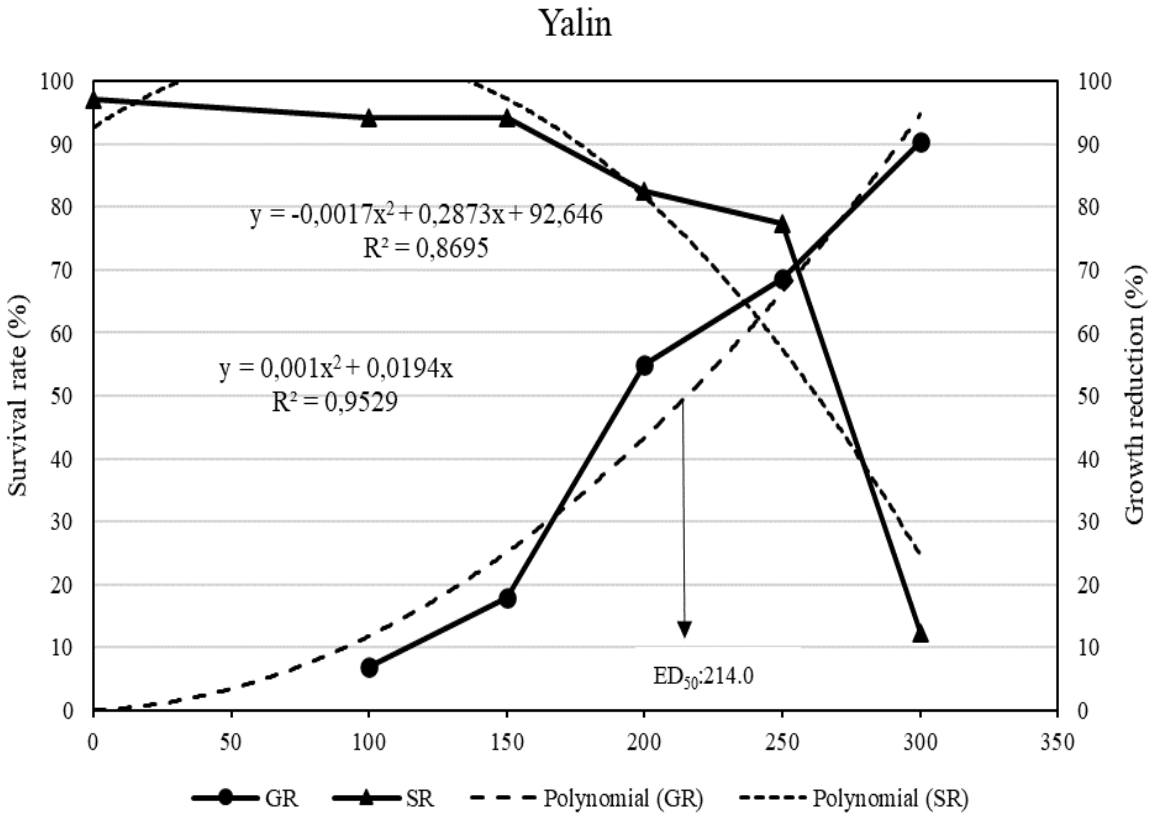

Figure 2. Polynomial regression graphic of survival rate and growth reduction of cv. Yalin.

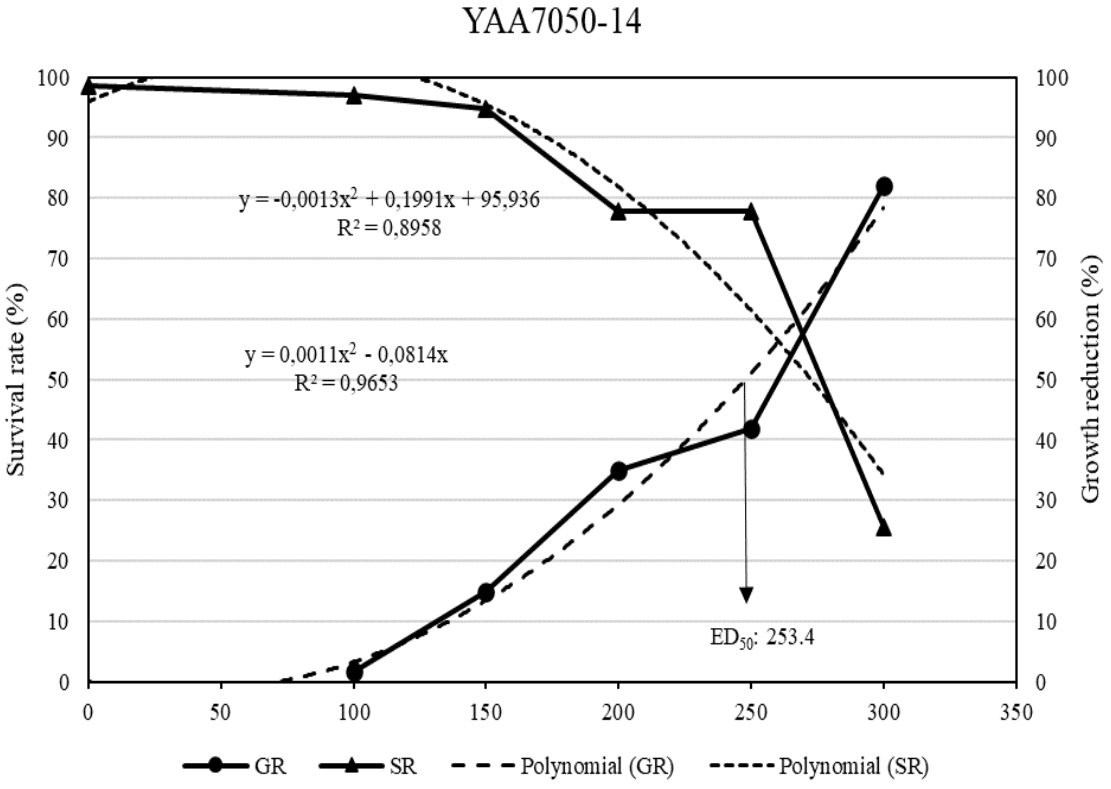

Figure 3. Polynomial regression graphic of survival rate and growth reduction of hulless barley line YAA7050-14.

4. Discussion

One of the most restricting factors for developing new varieties is the absence of variation in hulless barley breeding research; therefore, the mutation approach can be a potential strategy for developing new varieties in breeding research (Dyulgerova and Dyulgerov, 2020). The most critical step of mutation approach is the determination of the optimum dose of mutagen agent (Maluszynski et al., 2009). In this study, gamma rays at different doses (Control, 100, 150, 200, 250 and 300 Gy) applied to the seeds of hulless barley cv. Yalin and hulless barley line YAA7050-14. Germination, seedling, and root characteristics of both hulless barley genotypes were affected by increasing the dose of gamma rays. However, the effects of gamma rays varied based on the dose, genotype and the characteristics of plants.

According to our results, most of the investigated parameters in both hulless barley genotypes were found to be almost identical statistically at 100 Gy irradiation dose compared to those in controls. Moreover, the emergence rate of plants at the dose of 100 Gy was higher than that of control which could be the stimulating effect of gamma rays at 100 Gy. Researches demonstrated that low doses of gamma irradiation can break dormancy (Beyaz et al., 2016; Volkova et al., 2019), increase germination and emergence rate (Rozman, 2015), and have a growth-stimulating effect on seedling and root development in plants (Geras'kin et al., 2017; Volkova et al., 2019; Gorbatova et al., 2020). The positive effect of 100 Gy on plants can be explained by the hormesis phenomenon. Hormesis is known as an adverse effect on plant growth and development; nevertheless, it has a stimulating influence of low doses of harmful chemicals on plant growth and development (Małkowski et al., 2020; Jalal et al., 2021).

Low irradiation doses in all variables evaluated under greenhouse conditions were mainly close to the control, while 250 and 300 Gy doses negatively influenced germination, seedling, and root traits in both genotypes. The statistically significant differences in emergence and survival rate were found in M_1 plants at 250 and 300 Gy doses. Similar research on barley indicated that when mutagen doses increased, germination and emergence rates decreased (Sarduie-Nasab et al., 2010; Rozman, 2015; Navid et al., 2021). Possible reasons for the decrease in germination and emergence rates at higher gamma-ray doses can be structural damage to the embryo (Wang and Yu, 2011), disruption of cell differentiation resulting from mutations in DNA during germination (Daran, 2013), abnormalities at the chromosome and DNA level (Stoilov et al., 2013; Hong et al., 2022), changes on the plant hormones and DNA synthesis (Hong et al., 2022), disruptions in the synthesis and balance of plant growth regulators such as auxins and cytokinins that play a role in cell division and differentiation (Mok and Mok, 2001).

In addition to germination characters, above-ground plant parts were also negatively affected by high gamma-ray doses. The number of tillers, number of leaves, first leaf length, shoot length, fresh and dry weight of the shoot all reduced as the gamma-ray dose increased. Our results have been supported by other researchers showing that higher doses of mutagens diminish the number of tillers (Khah and Verma, 2015) and the number of leaves (Başer et al., 2005). DNA and chromosome damage (Stoilov et al., 2012) and damage to the lateral (axillary) meristems are two potential causes for the decline in tillers and leaves in M_1 plants grown in greenhouses (Hussien et al., 2014; Ye et al., 2019). Possible other explanations include disturbances in the synthesis and balance of hormones active in tillering in barley, such as auxin and cytokinin group hormones (Marzec and Alqudah, 2018) and enzymes (Bitarishvili et al., 2018). Gamma-ray doses over 150 Gy demonstrated a negative effect on leaf characteristics. Previous studies show that as the mutagen dose increased, the length of the first leaf and the shoot decreased (Borzouei et al., 2010; Ahumada-Flores et al., 2020; Navid et al., 2021). Leaves are formed from cells located in the periphery and are suitable for differentiation. Auxin plays an important role in the initiation of leaf formation by identifying these cells (Du et al., 2018). High doses of mutagen applications can cause disturbances in the synthesis and release of auxin group plant hormones (Bitarishvili et al., 2018; Hong et al., 2022.). In addition, mutagens at high doses have genotoxic and mutagenic effects, which may cause a decrease in mitotic index (İlbaş et al., 2006). The reductions in shoot length at high doses might be explained by the reduction of the rate and amount of cell division in apical meristems by mutagens (Oney-Birol and Balkan, 2019), reduction in DNA content (Yamaguchi et al., 2008), and disturbances in the synthesis and balance of auxin group plant hormones (Bitarishvili et al., 2018). The decrease in seedling wet and dry weights at increasing doses occurred as an indirect consequence of decreases in the number of tillers and leaves and seedling height. In other

words, abnormalities and damages at chromosome and DNA levels at high mutagen doses (Kiong et al., 2008; Stoilov et al., 2013), disruption of cell division and growth in apical meristems (Oney-Birol and Balkan, 2019), adverse effects on the synthesis and release of enzymes and plant hormones (Bitarishvili et al., 2018; Wang et al., 2018), carbon exchange in the leaves and negative effects on mineral uptake and utilization in the root (Singh et al., 2013). The exact reasons can be listed among the causes for the decrease in shoot wet and dry weights in the study.

Like the seedling traits in this study, root characters also showed a dramatic decrease at gamma-ray doses of 250-300 Gy. A significant negative effect of high irradiation doses on root length was found. Other researchers also found that root lengths decreased parallel with the increase in mutagen doses (Alghamdi et al., 2010; Olgun et al., 2012). Moreover, high mutagen doses reduced root fresh and dry weight of cv. Yalin and the hulless barley line YAA7050-14. The results of previous studies (Borzouei et al., 2010; Grover and Khan, 2014; Navid et al., 2021) also indicate that high mutagen doses have a negative effect on root fresh and dry weight. High gamma irradiation doses decreased root wet and dry weight, which was indirectly caused by a reduction in the number of tillers and root lengths. As gamma-ray doses increase in barley, the balance in plant hormones such as indoleacetic acid, indolebutyric acid, abscisic acid, and zeatin is disrupted, and the effect of growth-promoting hormones is lost (Bitarishvili et al., 2018). In addition, high gamma-ray doses reduce cell division in root tip meristems (Oney-Birol and Balkan, 2019) and mitotic index (Eroğlu et al., 2007). Since adventitious roots did not grow when tillering did not occur in M_1 plants at high doses, this might cause weaker root system development (Geçit, 2016).

Both seedling and root dry weight decreased significantly at high irradiation doses compared to the control and doses less than 200 Gy. However, the reduction in seedling dry weight was greater than the decrease in root dry weight. While both traits declined, the root/shoot dry weight ratio increased compared to the control and other doses. It can be assumed that the negative effect of high doses on root dry weight was less effective than on shoot dry weight. Furthermore, the coefficients of variation in all seedling and root characteristics were higher at the highest doses of 250 and 300 gray compared to control plants and plants irradiated with lower doses. In other words, high gamma-ray doses increased the deviations of the traits studied from their mean values.

Overall, the effect of different doses of gamma rays applied to the seeds on the seedling and root characters varied based on the hulless barley genotypes. Gorbatova et al. (2020) found that the genetic structure of the varieties was an important factor in their response to low doses of gamma rays. Some other research results indicate that the effects of mutagens may vary according to varieties (Nazarenko and Lykholat, 2020; Hong et al., 2022).

Conclusion

Hulless barley is in demand on the market because it has desirable nutritional properties such as high beta-glucan and total dietary fiber content. The number of released hulless barley varieties is limited compared to hulled barley. Wide genetic diversity helps breeders create new cultivars; nevertheless, germplasm shortages restrict the number of hulless barley varieties released. This research investigated different gamma ray doses (0, 100, 150, 200, 250, and 300 Gy) for hulless two-row barley genotypes (Yalin and YAA7050-14) to determine optimum irradiation doses before executing large-scale mutagenesis. The optimal gamma-ray irradiation dosages were determined via constructed dose-response curves. Based on the results, the effective doses (ED_{50}) for the hulless barley genotypes examined in the study ranged from 214.1 to 253.4 Gy gamma rays. At these gamma ray doses, where the mutagenic effect is optimum, the number of surviving plants around 60-70% may help to increase the chance of reaching the desired genotypes in following selection studies.

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Research Article

Profile of Secondary Metabolites in Different Parts of the Butterfly Pea (*Clitoria ternatea*) Plant with Antioxidant Activity

Waras NURCHOLIS*[1], Tenku Muhammad IQBAL[2], Sulistiyani SULISTIYANI[3], Novia LIWANDA[4]

[1,2,3,4]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java 16680, Indonesia
[1]Tropical Biopharmaca Research Center, IPB University, Bogor, West Java 16128, Indonesia

[1]https://orcid.org/0000-0001-7047-5093, [2]https://orcid.org/0000-0002-6593-1901, [3]https://orcid.org/0000-0001-9820-9723
[4]https://orcid.org/0000-0002-0238-949X

*Corresponding author e-mail: wnurcholis@apps.ipb.ac.id

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Secondary metabolites

Abstract: The butterfly pea flower (*Clitoria ternatea*) is widely used in traditional medicine and has the potential to be an antioxidant. The study aimed to compare the antioxidant capacity of the ethanol extract of the butterfly pea flower and the metabolite profile of the n-hexane fraction in different parts of the plant. The butterfly pea flower was planted for 12 weeks, and as many as 30 plants were in the Tropical Biopharmaca Research Center Cikabayan experimental garden, Bogor, Indonesia. Plant measurements included plant height, number of leaves, stem diameter, and plant production, which always increased during the experiment. The root had the highest phenolic content of each part of the plant (roots, flowers, leaves, and stems), with a phenolic content of 83.45 mg GAE/g. At the same time, the highest flavonoid content was in the leaves, with a total flavonoid value of 5.96 mg QE/g. Flowers and leaves only have anthocyanin content. The root had the highest antioxidant activity (low IC_{50} value) of each part of the plant, with an IC_{50} value of 106.973 µg/mL. The GC-MS results from the roots showed 13 compounds identified: 12 in the flower parts, 11 in the leaf parts, and 9 in the stem parts.

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Footnote: This study is largely based on T. Muhammad Iqbal's BSC thesis, which was completed in 2019. Sulistiyani and Waras Nurcholis supervised this thesis.

1. Introduction

Traditional medicine using plants has been carried out for a long time by the community, so much research on the efficacy of medicinal plants presents a great opportunity as an alternative treatment for various diseases by making them traditional medicines (Rabeta and Nabil, 2013). The butterfly pea flower is one of the plants used by the community as traditional medicine by boiling it. The butterfly pea flower has the potential to be a raw material for medicine because it has many bioactive compounds and is efficacious in its use (Esmail, 2016; Lijon et al., 2017). The manufacture of medicinal raw materials uses solvents that can attract bioactive compounds in plants, such as ethanol. This solvent is

often used in the pharmaceutical raw material industry because it has the same polarity level as bioactive compounds in plants (El-Maati et al., 2016).

The butterfly pea flower (*Clitoria ternatea*), also known as the blue flower or butterfly pea, is a vine belonging to the Fabaceae family (also known as Leguminosae), which has light blue, white, pink, and blue flowers. Water extracted from butterfly pea flowers in the traditional way also has the potential to be a good antioxidant. The ethanol extract of butterfly pea flowers also has properties as an antioxidant, so it can prevent oxidative stress in the body (Adwas, 2019). In addition, ethanol extract is also thought to have efficacy as an antidepressant from the roots, which regulates the serotonergic system and acetylcholine (Parvathi et al., 2013). Parts of the butterfly pea plant can also be used as an anti-microbial, anti-inflammatory, anti-cancer, and antidiabetic (Esmail, 2016). Applying butterfly pea flowers as animal feed is also very beneficial for livestock businesses because it is suspected of increasing livestock production (Suarna & Wijaya, 2021). The efficacy of the butterfly pea flower is the activity of the content of various compounds present in the butterfly pea plant, one of which is anthocyanins (Thuy et al., 2021). The blue color in the butterfly pea flower is an anthocyanin compound that is owned by the butterfly pea flower. Anthocyanins can also treat cancer, diabetes mellitus, and heart attacks (Alam et al., 2021). This content is an accumulation of secondary metabolite compounds produced by plants.

Secondary metabolites are compounds produced by a plant's metabolism in order to defend itself, and they have a wide range of biological functions for human health (Thirumurugan, 2018). It is this biological activity that gives a plant its properties. Secondary metabolite compounds can be taken with certain solvents and identified using compound separation techniques using certain tools. Hexane is an efficient solvent that dissolves in samples to extract non-polar secondary metabolites in plants, even though they are classified as pollutant compounds (Yara-Varon, 2016). Hexane takes up non-polar compounds found in plants well because of its polarity (Ghazali and Yasin, 2016). Identify secondary metabolites using compound separation tools such as Gas Chromatograph – Mass Spectrophotometer (GC-MS) (Alp et al. 2022). In research by Neda et al. (2013), water and methanol extracts of butterfly pea flowers were identified as having the main compound, inositol, which is effectively used as an anticancer against several types of cancer.

The butterfly pea flower (*Clitoria ternatea*) is an ornamental plant and has the potential to be a medicinal plant. Butterfly pea plants can be used as an alternative source of antioxidants, that contain secondary metabolites. There is no scientifically based information about the antioxidant capacity of roots, flowers, stems, and leaves in ethanol solvents or comparisons of plant secondary metabolite profiles with n-hexane solvents. Information on secondary metabolites in the butterfly pea plant is needed so its potential can be seen. Secondary metabolites are very important in determining the biological activity of plants, so the identification and isolation of secondary metabolites are mostly done to determine the content and structure of secondary metabolites (Sholikhah, 2016). This study aims to compare the antioxidant capacity of each part of the ethanol extract of the butterfly pea plant and the content of secondary metabolites in the n-hexane fraction in each part of the butterfly pea plant (*Clitoria ternatea*), namely the roots, flowers, stems, and leaves. This research is expected to provide information about the content of secondary metabolites in the butterfly pea flower (*Clitoria ternatea*) through the analysis of the separation of metabolites. In addition, this study also provides information about the extract of the part of the butterfly pea plant that has the most potential as an antioxidant.

2. Material and Methods

2.1. Plant materials and preparations

The *Clitoria ternatea*, or butterfly pea, plant used in this study was grown at the Tropical Biopharmaca Research Center Experimental Garden, IPB University, Bogor, Indonesia. Thirty samples of butterfly pea flowers were planted, and the morphology of the plants was observed until the age of the plants reached 12 weeks. Observations were made on the leaves, stems, flowers, and roots. Parameters calculated on the leaves include the number of leaves and leaf shape, while the parameters on the stem include stem length, stem color, diameter at the base of the stem, and stem shape. Interest observations are calculated from the sixth week when the flowers have appeared. The calculated flowers

are bud flowers, blooming flowers, and overblown flowers. Furthermore, the roots were observed when the butterfly pea plant was 12 weeks old to determine the plant's root system.

The different parts of the plants were harvested and separated. Each plant part is weighed using an analytical balance (Kenko) to determine the fresh weight. Next, each plant part was dried in an oven (EYELA NDO-700) at 50°C for 3 × 24 hours. The dry samples were then weighed again to determine the dry weight of each plant part. Each plant part was then crushed to become simplicia powder (60 mesh).

2.2. Water content analysis (Depkes RI 2008)

The water content is measured by weighing a porcelain cup that has been desiccated at 105°C for 30 min using an oven (EYELA NDO-700) and a desiccator. A total of 1 gram of sample was put into a cup and weighed. The sample and cup were heated at 105°C for 5 hours using an oven, and the weight was again weighed. The moisture content was calculated using the following formula.

$$\% \text{ Water content} = \frac{A - B}{A} \times 100 \quad (1)$$

Note: A = Sample weight before drying (g)
B = Weight of sample after heating (g)

2.3. Sample extraction

Sample extraction was carried out by the maceration method based on Lee et al. (2011). As much as 20 g of simplicia powder was macerated with 200 mL of 96% ethanol (Merck KGaA Germany) at a ratio of 1:10. The mixture was macerated in a dark room using a water bath shaker (Memmert WNB 22 With a Shaking Device) for 2 × 24 hours at 110 rpm. The filtered sample filtrate was taken and concentrated using a rotary vacuum evaporator (Hahn-Shin, HS-2005 V) to obtain an extract paste. The extraction yield calculation uses the following formula.

$$\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Simplicia weight (g)} \times (1 - \text{Water content})} \times 100 \quad (2)$$

2.4. Analysis of total anthocyanins (Tonutare et al., 2014)

A total of 10 mg of sample extract (in 10 mL of 96% ethanol) was added with 0.1 M HCl at a ratio of 85%:15% (v/v). The solution was centrifuged for 15 minutes at 5000 rpm. 3 mL of the centrifuged supernatant was diluted with 5 mL of buffer solution pH 1.0 (0.1864 g KCl in 95 mL distilled water and 5 mL concentrated HCl) and a buffer solution pH 4.5 (0.1864 g KCl in 95 mL distilled water and 5 mL concentrated HCl). Both solutions were measured at a wavelength of 520 nm and 700 nm using a UV-VIS spectrophotometer (Genesys 10 UV, Thermo Scientific). The absorbance calculation uses the following formula.

$$A = (A_{520}\text{-}A_{700})_{\text{pH 1.0}} - (A_{520}\text{-}A_{700})_{\text{pH 4.5}} \quad (3)$$

Meanwhile, monomeric anthocyanin levels (cyaniding-3-glucoside equivalent in mg/L) are calculated using the formula:

$$\text{Anthocyanin levels} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (4)$$

Keterangan: A = Absorbance (A)
MW = Molecular Weight *cyaniding-3-glucoside* (449.2 g/mol)
DF = Dilution Factor

ε = Molar Absorptivity *cyanidin-3-glucoside* (26.900 molar)
L = Cuvette Width (cm)

2.5. Total phenolic analysis

The total phenolic content was measured based on the procedures of the Indonesian Ministry of Health (2011). A total of 15 mg of the extract was dissolved in 25 mL of methanol (pro analysis). As much as 1 mL of the extract solution was added to 5 mL of Folin-ciocialteu reagent (7.5% in water) in a test tube. Solution incubation was carried out for 8 minutes in a dark room (room temperature). Then, 4 mL of 1% NaOH was added to the solution and incubated again for 1 hour in a dark room (room temperature). The total phenolic content was measured using a UV-VIS spectrophotometer (Genesys 10 UV, Thermo Scientific) with a wavelength of 730 nm.

2.6. Analysis of total flavonoids

Measurement of total flavonoids refers to BPOM RI (2008) procedures. A total of 200 mg of the extract was added with 2 mL of 25% HCl, 1 mL of 0.5% w/v HMT, and 20 mL of acetone in an Erlenmeyer glass (50 mL PYREX). The solution was shaken and refluxed at 90°C for 30 minutes. The solution was added with a little acetone (pro analysis) and filtered into a measuring flask (PYREX 100 mL). A total of 20 mL of the filtrate was added with 20 mL of distilled water and 15 mL of ethyl acetate in a separatory funnel. The separated ethyl acetate fraction was collected in a measuring flask (50 mL PYREX). A total of 1 mL of the fractionated solution was added to 1 mL of 2% $AlCL_3$ and calibrated with 5% (v/v) glacial acetic acid to a volume of 25 mL in a measuring flask (25 mL PYREX). Measurement of total flavonoids using a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific) with a wavelength of 426 nm.

2.7. DPPH antioxidant activity analysis (Vats, 2014)

50 mg of the sample extract was dissolved in 50 mL of 96% (v/v) ethanol to obtain a stock solution of 1000 ppm. Stock solutions were diluted in stages to concentrations of 800, 600, 400, 200, and 100 ppm. A total of 1 mL of sample solution from each concentration was added to 1 mL of DPPH reagent and then incubated in a dark room for 30 minutes. The absorbance of the solution was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific).

2.8. GC-MS analysis

GC-MS analysis was carried out using the services of the Jakarta Regional Health Laboratory (Labkesda). A total of 10 mg of crude extract (in 10 mL of hexane solvent) was extracted in a sonicator for 30 minutes. The solution was filtered using a millipore, and the hexane fraction was taken for GC-MS analysis. GC-MS analysis used Agilent Technologies GC-MS 7890 and MS 5975 series equipped with HP INNOWAX capillary columns (internal diameter: 30 m x 0.25 mm, film thickness: 0.25 µm). The carrier gas flow rate (He) was 0.6 mL min^{-1}. 1 µL sample was injected into the GC-MS at an injection temperature of 250°C. The temperature of the GC-MS was set for the operating conditions, namely an initial temperature of 60°C (held for 0 minutes), increased at a rate of 2°C min^{-1} until the final temperature was 150°C (held for 1 minute), then increased at a rate of 20°C min^{-1} (hold for 10 minutes). The mass spectrophotometer was operated at 70 eV. GCMS analysis was compared using the Chemistation Data System to see the results.

2.9. Statistical analysis

ANOVA was carried out using statistical analysis based on a completely randomized design using the Minitab 16 program. The results of each plant part and the comparison of each tests were carried out using ANOVA and Tukey's follow-up test. The data is presented in terms of replication ± standard deviation, which is visualized as a bar chart.

3. Results

3.1. Morphology, growth and production of butterfly pea flower

3.1.1. Morphology of butterfly pea flower

The morphology of the butterfly pea plant can be seen in Table 1. The leaves of the butterfly pea flower are compound and have 3, 5, and 7 leaflets (Figure 1a), but they only have 2 leaflets when they are young. The roots of the butterfly pea plant have main roots, primary roots, secondary roots, and tertiary roots (Figure 1b). The stem of the butterfly pea plant has primary, secondary, and tertiary branches and tendrils. The plant has a brown stem base when it is 8 weeks old (Figure 1c).

The flower of the butterfly pea plant generally has three phases. The first is the initial phase when the flower is still in the form of a bud, which lasts 4-5 days. The second is the blooming phase (which lasts only 1 day) when the flowers fully bloom and have the perfect color (Figure 1d). The third stage is the late blooming phase, which will result in pods. This phase lasts for nine days. However, in this study, only 2 out of 6 flowers produced pods.

Table 1. The morphology of the butterfly pea plant

Sample	Morphology	Results
Stem	Stem Color	Green
	Stem Shape	Straight creeper (Round)
	Amount / plant	2 – 4 clumps
Leaf	Leaf Color	Green
	Leaf Shape	Oval (lanceolate when young)
	Bone leaves	Pinnate
	Leaf tip	Tapered
Root	Root Color	White
	Root Shape	Fiber
Flower	Flower Shape	Symmetry
	Flower Color	Purple blue gradation

(a) (b) (c) (d)

Figure 1. The parts of the butterfly pea plant (a) Leaves (b) Roots (c) Stems and (d) Flowers.

3.1.2. Flower production of butterfly pea flower plant

The calculated interest is the bud and bloom flowers counted daily (Figure 2). Flower blooms and buds increased every week but decreased to 345 flower buds and 374 flower blooms in the eighth week. Week 9 saw an increase in flower buds of 96.24% from before, but blooms still decreased to 298 blooms.

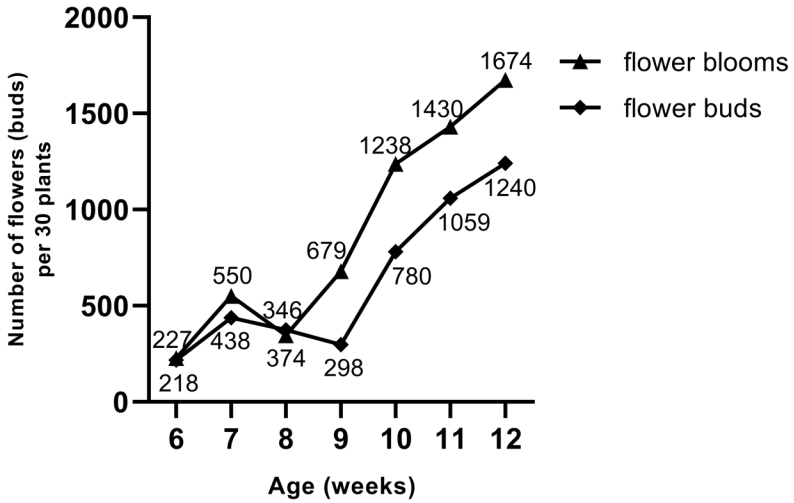

Figure 2. Graph of the number of flowers of the butterfly pea plant every week.

3.1.3. Growth of Butterfly Pea Flower

Agronomic measurements of the butterfly pea plant are carried out every week, including plant height, number of leaves, stem diameter, and number of flowers calculated every day, as well as the weight of each part of the plant, which will be calculated at the time of harvest. The results of the growth of butterfly pea plants that were measured included plant height, number of leaves, and stem diameter (Figure 3). Plant height was measured in the 2nd week using a measuring tape, and the results are shown in Figure 3a. Plant height reached its highest point in the 11th week with a height of 101.47 cm and decreased in the 12th week to 98.83 cm. Plant height in week 5 experienced a surge of 153.64% from the previous week.

On the leaves that blossomed, the number of leaves was counted to determine the photosynthesis capability of plants. The number of leaves every week has increased without a decrease in the number of leaves. Figure 3b shows the results of the number of leaves, which always increases every week. The highest number of leaves was found in week 12, with a yield of 679 leaves per plant. Plant stem diameter was measured at week 4 using a caliper. Stem diameter is measured to see the thickness of the stem supporting the plant. The stem diameter increased weekly and reached 7.21 mm in week 12 (Figure 3c).

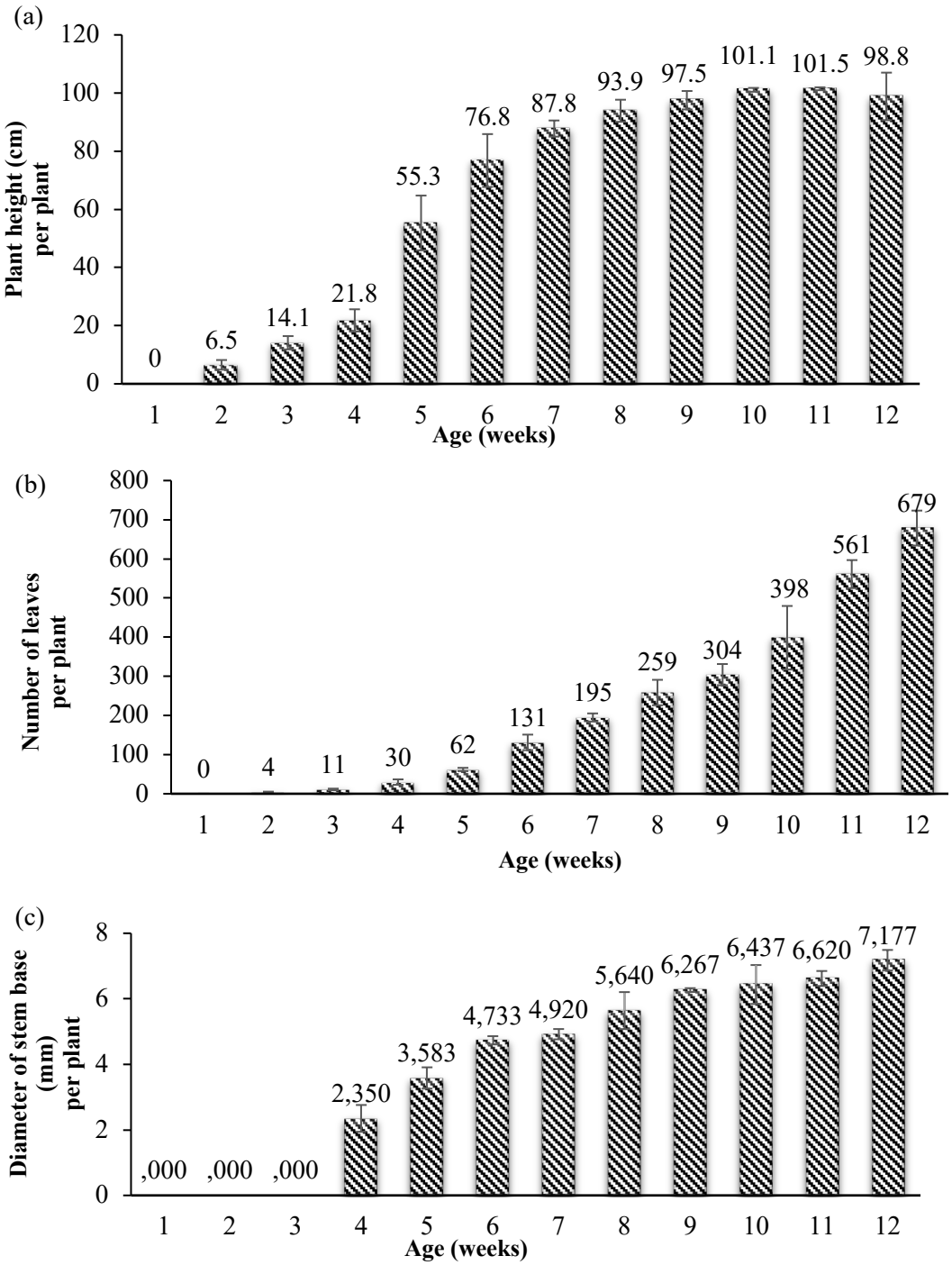

Figure 3. Growth of butterfly pea: (a) plant height, (b) number of leaves, and (c) stem diameter for 12 weeks.

3.1.4. Production of butterfly pea flowers

Yields consist of the fresh and dry weight of plant roots, stems, flowers, and leaves (Figure 4). The production yield is the total production of 30 butterfly pea plants. The highest dry weight obtained was in the stem sample, with a weight of 574.83 g, and the highest fresh weight was found in the leaves, 1425.64 g. The dry weight of the leaves is lower than that of the stems. The leaf and flower samples had significantly different wet and dry weight values.

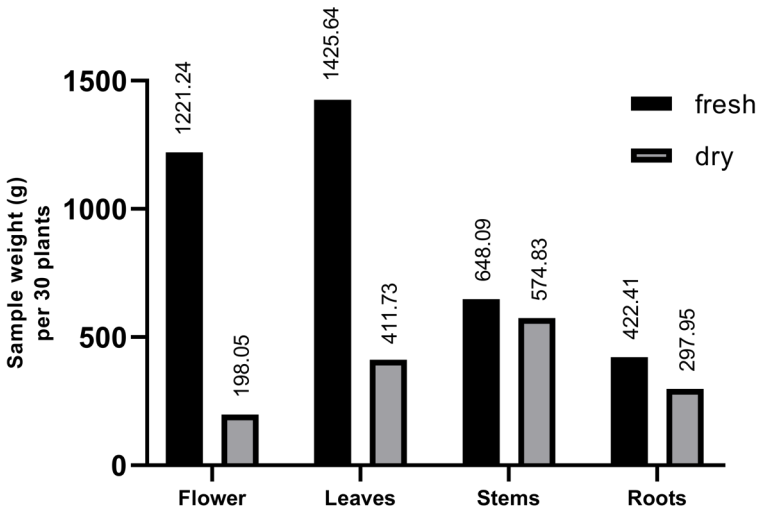

Figure 4. Fresh weight and dry weight of 12 weeks old butterfly pea.

3.2. Standardization of butterfly pea plant ethanol extract

Moisture content is one of the quality parameters of simplicia as a raw material for drugs used in treatment (Depkes RI, 2008). Water content was measured on all parts of the butterfly pea plant, namely the stems, roots, flowers, and leaves. The water content of the pea flower simplicia has a value between 3.52 – 8.98% (Figure 5). The results of the water content of all parts of the plant have a water content of <10%. These results are in accordance with the standard for simplicia as a medicinal ingredient, namely that the water content of simplicia is not more than 10% (BPOM RI, 2008).

The simplicia is then extracted using ethanol as a solvent to produce a yield. The yield comes from the process of concentrating simplicia using a solvent to obtain metabolites that match the polarity of the solvent (Erviani and Arif, 2017). The solvent used is 96% ethanol. The extract yield was calculated based on the ratio of the final weight of the extract produced to the weight of the simplicia used. The yield results showed that the butterfly pea flower sample had the highest yield, at 50.21% (Figure 6).

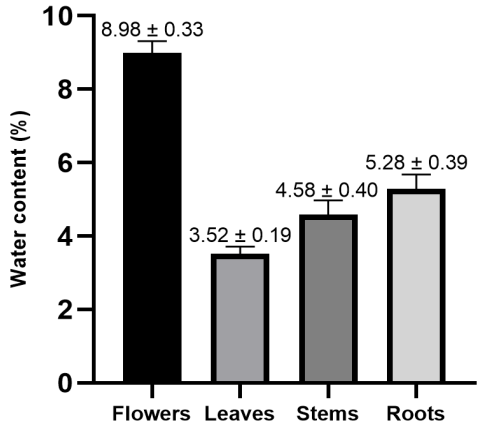

Figure 5. Water content of different parts of butterfly peat. Each value is presented as the mean of three replicates ± standard deviation (SD).

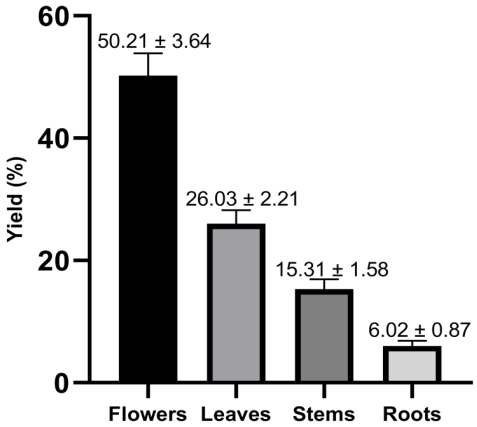

Figure 6. Yield of ethanol extract of different parts of butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD).

3.3. Secondary metabolite content of butterfly pea flower plant

An analysis of secondary metabolite content was carried out to quantitatively determine the secondary metabolite content. The samples analyzed were ethanol extract to determine polar compounds

and the n-hexane fraction to determine non-polar compounds. The secondary metabolites measured in the ethanol extract samples were total phenolics, flavonoids, and anthocyanins. In contrast, the profile of secondary metabolites was identified using GC-MS for the n-hexane fraction.

3.3.1. Total phenolic and total flavonoids content

The highest total phenolic content was found in the root sample with a value of 83.45 mg GAE/g, and the lowest total phenolic content was found in the stem sample with a value of 37.09 mg GAE/g (Figure 7). The value of 83.45 mg GAE g^{-1} means that there is 83.45 mg of phenolic equivalent to gallic acid in one gram of sample. The phenolic content in flowers and stems is not significantly different ($p>0.05$), with values of 37.58 mg GAE g^{-1} and 37.09 mg GAE g^{-1}, respectively.

The analysis of the total flavonoid content was to determine the total flavonoid content in the sample in milligrams of quercetin equivalent per gram of sample. The highest total flavonoid content was found in leaf samples, with a value of 5.96 mg QE g^{-1}, and the lowest was in root samples, with a value of 0.27 mg QE g^{-1} (Figure 8). The value of 5.96 mg QE g^{-1}means there are 5.96 mg of flavonoids equivalent to quercetin in one gram of sample.

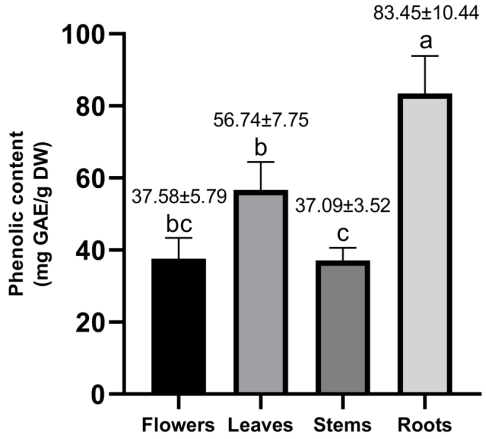

Figure 7. The total phenolic content of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significant differences at the 5% test level (Tukey test).

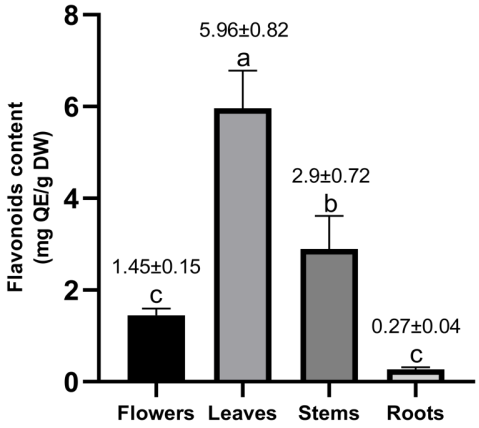

Figure 8. The total flavonoid content of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significant differences at the 5% test level (Tukey test).

3.3.2. Total Anthocyanin Content

The calculated anthocyanin levels are monomeric anthocyanin levels, namely cyaniding-3-glucoside equivalents in mg/L. The anthocyanin content of flower samples had the most anthocyanins compared to leaves. Stem and root samples obtained negative results in the calculation, so it can be said that the stem and root samples did not contain anthocyanins (Table 2). The flower anthocyanin level is 4.72 mg/L of cyaniding-3-glucoside equivalent.

Table 2. Anthocyanin content of butterfly pea

Sample	Total Absorbance	Total Anthocyanin (mg L^{-1})
Flowers	0.12 ± 0.01	4.72 ± 0.39
Leaves	0.07 ± 0.01	2.92 ± 0.33
Stems	-0.14 ± 0.03	N/A
Roots	-0.14 ± 0.02	N/A

Note: Each value is presented as the mean of three replicates ± standard deviation (SD); N/A: not available.

3.3.3. Identification of secondary metabolite compounds of n-hexane extract by GC-MS

A GC-MS analysis was carried out to determine the content of non-polar compounds in the sample. The analysis of the compound content of the flower samples showed that 12 compounds were identified (Table 3). In the chromatogram of the flower sample, there is one most dominant peak with a percent area of 31.95%, namely the citronellal compound, which belongs to the terpenoid compound group. The compounds identified in the flower samples contained many terpenoids such as sabinene, cymene, and alpha-terpinolene. Another compound identified is a vitamin K1 compound with a halogen element, fluoro.

Table 3. Non-polar compounds in the flowers of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**2-methyl-4-pentenal**	3.639	$C_6H_{10}O$	98.145	8.99
2	**2-Pentanone-4-methyl (Methyl isobutyl ketone)**	3.832	$C_6H_{12}O$	100.161	8.99
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	31.95
4	**Sabinene**	4.094	$C_{10}H_{16}$	136.238	1.78
5	**Cymene**	4.866	$C_{10}H_{14}$	134.22	1.12
6	**Isodurene**	4.907	$C_{13}H_{20}$	134.22	2.19
7	**240α-terpinolen**	6.424	$C_{10}H_{16}$	136.238	1.35
8	**Tetradecamethylhexasiloxane**	30.889	$C_{14}H_{42}O_5Si_6$	458.995	3.61
9	**Heptacosane**	31.296	$C_{27}H_{56}$	380.745	1.03
10	**15-methoxymaysine**	39.853	$C_{29}H_{37}ClN_2O_8$	577.071	1.98
11	**Zinc, allyl-crotyl-**	42.183	$C_7H_{12}Zn$	161.553	3.35
12	**Vitamin K1 (20) Heptafluorobutyric**	48.934	$C_{35}H_{47}F_7O_3$	648.747	2.07

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The analysis of the compound content of the stem samples showed that nine compounds were identified (Table 4). In the chromatogram of the stem sample, the dominant compound is the unsaturated fatty acid 9,12-Octadecadiynoic acid, with an area of 19.99%. Terpenoid group compounds were also identified in the stems: beta-humulene, squalene, and D-Friedoolean-14-en-3-ol(3.beta).

Table 4. Non-polar compounds in the stems of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	12.79
2	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	19.99
3	**Squalene**	33.874	$C_{30}H_{50}$	410.73	1.08
4	**β-stigmasterol**	39.928	$C_{29}H_{48}O$	412.702	1.19
5	**γ-sitosterol**	41.073	$C_{29}H_{50}O$	414.718	4.37
6	**D-Friedoolean-14-en-3-ol,(3.beta.)-**	41.583	$C_{30}H_{50}O$	426.729	9.09
7	**α-(P-chlorobenzoyl)-P-chloroacetophenone**	41.949	C_8H_7ClO	154.593	1.00
8	**Isomultiflorenone**	42.480	$C_{30}H_{48}O$	424.713	12.10
9	**β -Humulene**	43.480	$C_{15}H_{24}$	204.357	10.45

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The results of the chromatogram on the root sample (Figure 12) showed 13 compounds identified (Table 5) and 2 dominant compounds, namely 9.12-Octadecadiynoic acid and D-Friedoolean-14-en-3-ol(3.beta)-. D-Friedoolean-14-en-3-ol(3.beta)- belongs to the terpenoid group. Apart from D-Friedoolean-14-en-3-ol(3.beta), there are other terpenoid group compounds, namely citronellal, and taraxerone. Steroid group compounds were also identified in the root samples, namely Stigmasta-5.23-dien-3-beta-ol, and gamma-sitosterol.

Table 5. Non-polar compounds in the roots of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Heptane,2,4-dimetyhl**	3.832	C_9H_{20}	128.259	1.68
2	**Pyrrolidine,3-methyl-**	3.942	$C_{12}H_{17}N$	175.275	2.03
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	1.67
4	**Neophyadiene (Neophytadiene)**	27.896	$C_{20}H_{58}$	278.524	1.67
5	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	9.17
6	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	27.16
7	**Stigmasta-5.23-dien-3-beta-ol**	39.866	$C_{29}H_{48}O$	412.702	1.23
8	**Delta14-taraxen3-3-one (Taraxerone)**	40.770	$C_{30}H_{48}O$	424.713	1.5
9	**γ -sitosterol**	41.073	$C_{29}H_{50}O$	414.718	4.96
10	**D-Friedoolean-14-en-3-ol,(3.beta.)-**	41.583	$C_{30}H_{50}O$	426.729	37.1
11	**Thunbergol**	42.204	$C_{20}H_{30}O$	290.491	1.51
12	**2,2,3,7-tetramethyltricyclo [5.2.2.01.6]**	42.852	$C_{12}H_{26}O$	246.38	6.03
13	**9,17-octadicadienal**	29.854	$C_{18}H_{32}O$	264.453	1.84

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The leaf samples contained 11 compounds identified by GC-MS (Table 6). The most dominant compound was an unsaturated fatty acid compound, 9.12-Octadecadinoic, with an area of 31.22%. In addition, the leaf samples contained isoprenoid group compounds, namely vitamin E, with an area percent of 13.23%. The terpenoid compound group was also identified in the squalene leaf samples, with an area of 11.78%. In addition, there are also otochilone compounds that are included in the steroid class.

Table 6 Non-polar compounds in the leaves of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Heptane,2,4-dimetyhl**	3.832	C_9H_{20}	128.259	4.89
2	**Pyrrolidine,3-methyl-**	3.942	$C_{12}H_{17}N$	175.275	11.01
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	9.93
4	**Neophyadiene (Neophytadiene)**	27.896	$C_{20}H_{58}$	278.524	3.19
5	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	4.35
6	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	31.22
7	**2.2 metilenebis**	32.061	$C_{25}H_{36}O_2$	368.561	1.78
8	**Squalene**	33.874	$C_{30}H_{50}$	410.73	11.78
9	**Vitamin E**	37.529	$C_{29}H_{50}O_2$	430.717	13.23
10	**Otochilone**	42.183	$C_{30}H_{48}O$	424.702	6.40
11	**Lanost-7-en-3-one**	42.797	$C_{30}H_{50}O$	426.729	5.16

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

3.3. Antioxidant activity (DPPH method)

With the DPPH method and vitamin C as a positive control, antioxidant activity was measured. Evaluation of antioxidant activity using ethanol extract samples from each butterfly pea plant part. Antioxidant activity can be measured by Inhibitory Concentration 50 (IC_{50}), the solution concentration required to inhibit 50% of free radicals. The lower the required IC_{50}, the stronger the antioxidant activity in the sample. Antioxidant activity was classified into very strong (IC_{50} < 50 µg mL^{-1}), strong (IC_{50} 50-100 µg/mL), moderate (IC_{50} 101-150 µg mL^{-1}), weak (IC_{50} > 150 µg mL^{-1}) (Fidrianny et al., 2015).

Vitamin C has an IC_{50} value of 3,888 µg/mL; the lowest IC50 value was obtained from a root sample of 106,973 µg mL^{-1} (Figure 9). The antioxidant activity of vitamin C is very strong, while the root samples are moderate antioxidants. Other plant parts, such as leaves, stems, and flowers, are classified as weak antioxidants. The results showed that the root has a stronger antioxidant content than other plant parts.

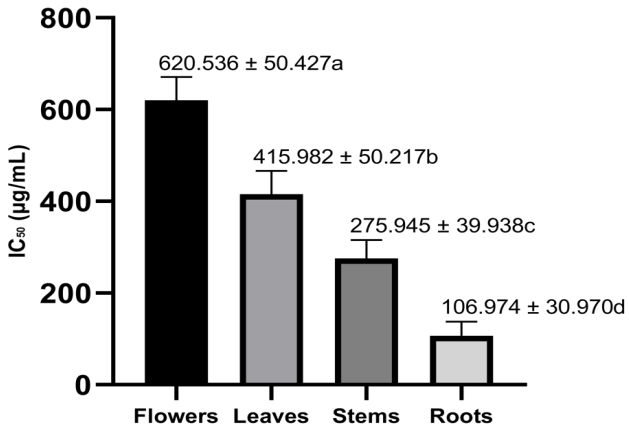

Figure 9. Antioxidant activity (IC_{50}) of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significantly different results at the 5% test level (Tukey test).

4. Discussion

4.1. Morphology, growth, and production of butterfly pea flower

The results of morphological observations are consistent with the literature of Suarna and Wijaya (2021), but the butterfly pea plant that was planted only produces blue flowers. The butterfly pea flower is a monocot plant, an annual shrub plant. Its main characteristic is that it has predominantly blue flowers, even though it also has white flowers (Artiyani et al., 2023). The butterfly pea plant has fibrous roots and deep, and woody roots. The stems of the butterfly pea plant are erect and slightly uphill, with a height of 20 cm to 90 cm, and have fine hairs. This plant can propagate with stems up to 0.5 to 3 meters long. The butterfly pea plant has oval leaflets with a hairless upper surface. The lower surface has scattered hairs and has a length of 1.5 cm to 7 cm and a width of 0.3 cm to 4 cm. Butterfly pea flowers have purple to almost white petals and an oval, funnel-shaped fruit 6 cm to 12 cm long and 0.7 to 1.2 cm wide. The egg-like structure of flower seeds is round.

4.2. Standardization of the butterfly pea flower plant simplicia

The standardization carried out in this study was the water content. The calculated water content is the total percentage of water in a sample. The standard for water content in plant samples dried is below 10% (BPOM RI, 2008). The water content in all samples is below 10%, so the Simplicia is good quality. The high-water content can affect the durability of the Simplicia. High water content can be damaged by microorganisms such as fungi or mold, so the water content of Simplicia cannot be too high (Taslim et al., 2021). Simplicia that complies with the standard is extracted with ethanol.

Extraction is the process of separating active compounds in samples, such as secondary metabolites. Extraction using ethanol as a solvent aims to obtain a more diverse compound due to its polarity. Ethanol is also a safe solvent for extracting samples as pharmaceuticals, especially phenolic compounds (El-Maati et al., 2016). The results of the extraction of the largest butterfly pea flower samples were flowers, leaves, stems, and roots. The yield of flower part has the highest yield compared to the other samples at 50.21%. This result is because flowers have compounds that have a polarity that matches the solvent. The yield of roots was lower compared to the study by Kadam and Ahire (2011), while the yield of stems and leaves had higher yield than in previous studies. According to Kadam and Ahira (2011), yields are influenced by several factors, such as the plant's environmental conditions, the yield processing, and the solvent used.

4.3. Secondary metabolite content of the butterfly pea flower plant

The total phenolic test used the folin-ciocalteu method with standard gallic acid, with the equation of the standard curve line $y = 0.008x + 0.0078$ and a value of $R^2 = 0.9968$. The plant parts with

the highest total phenolic yields were roots, leaves, flowers, and stems. These results indicate that roots have a higher phenolic content and are significantly different at 83.45 mg GAE g^{-1} compared to leaves, flowers, and stems. However, the total phenolic yield was not significantly different in flowers and stems. According to Setford et al. (2017), environmental factors, processing, and plant age resulted in differences in the content of phenolic compounds in different plants. The different phenolic content due to phenolic compounds in plants is a defense response carried out in plants, which can affect every part of the plant. The principle of the total phenolic test using folin-ciocalteu is that the reduction process of the folin-ciocalteu reagent, namely tungstate phosphomolybdate, reacts with phenolic compounds, which reduces the reagent to produce a blue complex. The absorbance of the color complex can be measured at a maximum wavelength of 730 nm, so the higher the absorbance value, the higher the total phenolic content in the sample. Gallic acid is used as a standard because it is stable and can be obtained easily and cheaply (Treml and Smejkal, 2016).

Testing for total flavonoids using the colorimetric method with $AlCl_3$ reagent and standard quercetin with the equation of the standard curve line $y = 0.0685x - 0.0071$ and the value of $R^2 = 0.9984$. The total flavonoid content results from the highest sources: leaves, stems, flowers, and roots. Leaves contain high levels of flavonoids and are significantly different at 5.96 mg QE g^{-1} compared to stems, flowers, and roots. The root contains very little flavonoids, at 0.27 mg QE g^{-1}, and is significantly different from other plant parts. This difference is due to the different functions of each part of the plant, environmental factors, and sample processing (Borges et al., 2013). Flavonoids are derivatives of phenolic compounds that have a defense function in plants and have antioxidant properties because they have hydroxyl groups (Gengaihi et al., 2014). The principle of measuring flavonoid levels using $AlCl_3$ reagent will form orange to red compounds with flavonoids in an alkaline state and then measured at a wavelength of 426 nm. Quercetin standard is a flavonol compound with very strong radical scavenging activity, so it can be a strong antioxidant compound because it has many hydroxyl groups. Quercetin is a flavonol group widely distributed in every part of the plant to be used as a standard (Kumar et al., 2017).

The total anthocyanin test used the pH comparison method with a buffer of pH 1 and pH 4.5. The principle of this test is to compare the results of the color change at pH 1 and pH 4.5 measured at 520 nm and 700 nm. Anthocyanins will change color from red to orange at an acidic pH, so the measured anthocyanin levels are at monomeric anthocyanin levels (Tonutare et al., 2014). The results of anthocyanin content showed that only flower and leaf parts had anthocyanin, while roots and stems did not contain anthocyanin. Flowers have a higher anthocyanin content than leaves. The butterfly pea flower plant has anthocyanin-type delphinidin in its flower parts, making the flowers' color turn bluish. Delphinidin has more OH groups than other types of anthocyanidins (Lijon et al., 2017).

Anthocyanins are included in the class of flavonoids, the largest group of phenolic compounds found in nature. Anthocyanins are known as natural dyes because these compounds are in the form of glycosides which cause colors ranging from red, blue, violet, and yellow found in plants (Kamiloglu et al., 2015). Anthocyanins are derivatives of flavylium or benzyl flavylium salts, which are easily soluble in water. There are eighteen types of anthocyanidins, but only six play an important role in food coloring, namely pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Šulc et al., 2017).

Identification of metabolite compounds using GC-MS was carried out to determine the content of metabolite compounds which are included in volatile compounds, non-polar compounds, and have a relatively low molecular weight (Wijit et al., 2017). Identification of compounds using GC-MS (Table 7) shows that the flower parts have a high content of citronellal compounds. The leaves and stems contain the dominant compound, linoleic acid. The root contains the dominant compound of taraxerol.

The compounds in the ethanol extract of butterfly pea flower identified by GC-MS have useful biological activity. Linoleic acid (Figure 10a) is an unsaturated fatty acid compound that functions as a precursor metabolite for forming EPA (eicosapentaenoic acid) in plants and is found in many parts of the leaves (Rajram, 2014). Citronellal (Figure 10c) is an aldehyde monoterpenoid with an aldehyde group abundant in eucalyptus trees. Citronellal compounds are thought to have biological activity as antifungal and antibacterial (Ho et al., 2020). According to Mus et al. (2022), taraxerol (Figure 10b) is a steroid-derived terpenoid with anti-cancer, antioxidant, and antimicrobial activity. Taraxerol is produced in plants via the mevalonate pathway in the cell cytoplasm with its precursor, squalene. Taraxerol is thought to have high toxicity and effectiveness in inducing apoptosis (Surapaneni and Prakash, 2018).

Table 7. Metabolites identified by GC-MS in flower of butterfly pea

No.	Compounds	Class	Biological Activity
1.	**Citronellal**	Terpenoids	Anti- inflammatory, Anti- microbial (Ho et al., 2020)
2.	**9.12-Octadecadiynoic acid (asam linoleat)**	Unsaturated fatty acid	Antidiabetic, Anti-inflammatory (Ferraz et al. 2018)
3.	**D-Friedoolean-14-en-3-ol,(3beta) (Taraxerol)**	Terpenoids	Anti-tumor, anti-microbial, anti-inflammatory agent (Mus et al., 2022)

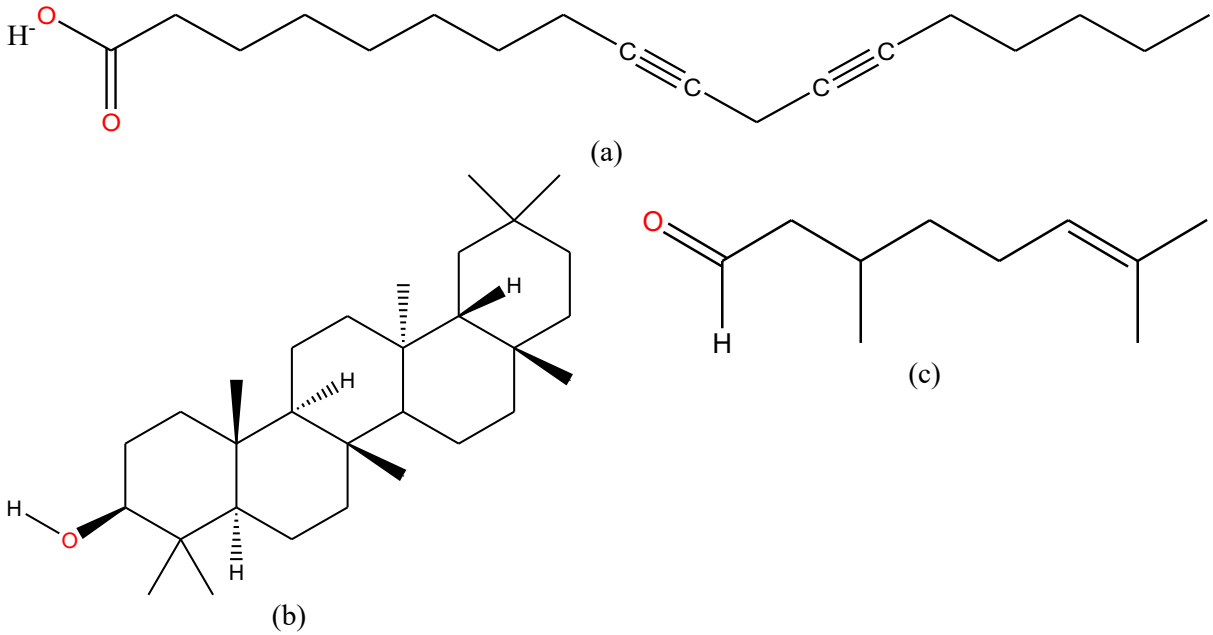

Figure 10. Structures of compounds identified by GC-MS (a) linoleic acid, (b) taraxerol and (c) citronellal.

4.4. Antioxidant activity of butterfly pea plant

According to Gengaihi et al. (2014), the DPPH method is easy and fast for determining non-enzymatic antioxidant activity. DPPH is a purple free radical with maximum absorption at a wavelength of 517-520 nm. DPPH will change color to yellow when antioxidants are added by donating electrons and transferring H atoms from antioxidants to DPPH. The results of the highest antioxidant activity were roots, stems, leaves, and flowers. The part that belongs to the root is a moderate antioxidant because it has an IC_{50} value between 100-150 µg mL^{-1}, while the leaves, stems, and flowers are included in a weak antioxidant because it has an IC_{50} value of more than 150 µg mL^{-1}. The IC_{50} value is inversely proportional to the antioxidant activity. The lower the IC_{50} value, the higher the antioxidant activity (Kaur et al., 2019).

The results of the antioxidant activity show the same thing as the research by Desmukh et al. (2013). The highest antioxidant activity was found in the root sample with an inhibition percentage of 82.87% at a concentration of 500 µg mL^{-1}, then the stem sample, and finally, the leaf sample. The antioxidant activity of a sample can be determined by knowing the compounds that can scavenge free radicals, such as phenolics, flavonoids, and anthocyanins. The highest analysis of phenolic compounds was found in the roots, but the roots had the lowest flavonoid content compared to other parts, and the highest antioxidants were found in the roots. The results of the content of secondary metabolites in the roots, which have properties as antioxidants, are thought to be non-flavonoid phenolic compounds. The phenolic mechanism inhibits antioxidants by directly transferring hydrogen atoms and producing more stable compounds due to the resonance of aromatic rings (Mohandas and Kumaraswamy, 2018). The GC-MS results showed that the compound that could act as an antioxidant was taraxerol (Table 7). Terpenoid compounds can inhibit antioxidants by capturing different antioxidant activities, which can

be caused by synergistic or antagonistic effects between the active components in a sample (Gengaihi et al., 2014).

Conclusion

The profiles of volatile compounds differed between different parts of the butterfly pea. Significant constituents of the flower and root were citronellal and taraxerol, respectively, while linoleic acid was found in the leaves and stems. The root had the greatest total phenolic content and antioxidant activity, whereas the leaves contained the highest total flavonoid content. In flowers and leaves, anthocyanins have been identified.

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Research Article

Imaging Techniques of Tomatoes (*Solanum lycopersicum* L.) Grown with Different Organic and Conventional Fertilizer Applications

Selma KIPCAK BITIK*[1], Suat SENSOY[2]

[1]Van Yuzuncu Yil University, Baskale Vocational School, 65090, Van, Türkiye
[2]Van Yuzuncu Yil University, Faculty of Agriculture, Horticulture Department, 65090, Van, Türkiye

[1]https://orcid.org/0000-0002-0563-1130, [2]https://orcid.org/0000-0001-7129-6185

*Corresponding author e-mail: selmakipcak@yyu.edu.tr

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Abstract: In this study, which was carried out in field conditions in Van in 2019 and 2020, different image generation methods (biocrystallization method, and circular and rising picture chromatography methods) were used to determine the difference between various organic and conventional fertilizer applications. 3% frozen tomatoes and 16% $CuCl_2.2H_2O$ were applied in the biocrystallization method, 80% frozen tomatoes and 1% silver nitrate solution were used in the circular chromatography method, and 100% frozen tomatoes and 0.5% silver nitrate and 0.5% iron sulfate solution were employed in the rising picture method. In light of the visual findings obtained at the end of the study, it has been determined that there are some differences between organic and conventional fertilizer applications. As the alteration between organic and conventional products; the center number difference in the copper crystallization method; the smoothness of the rings formed in the circular chromatogram and the vividness of the colors; in the rising painting method, it clearly reveals the transitions between colors and the difference in light and dark tones that occur in colors.

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Footnote: This study was produced from the doctoral thesis of the first author.

1. Introduction

Carbs in tomatoes, organic acids, amino acids, vitamins, various mineral substances, and phenolic compounds have an important place in human nutrition (Georgé et al., 2011; Sönmez and Ellialtıoğlu, 2014; Erdinc et al., 2018). Tomato is one of the most used vegetables in human nutrition. It is known that tomato cultivation is carried out almost everywhere in the world except Antarctica (Özcan, 2016). Tomato is from the Solanaceae family spread to other continents from the New World; it is known that tomatoes enter Türkiye from Adana, and there were numerous studies on it (Şalk et al., 2008; Uçar and Şensoy, 2022). World tomato production is 186 million tons (FAO, 2020). China produces approximately 64 million tons and ranks 1st with a share of 35% of the total world production, while India ranks second with 20 -million tons, and Turkey ranks 3rd with 13 million tons of production (FAO, 2020).

Chemical fertilizers, hormones, and pesticides (İlter and Altındişli, 1998), used unconsciously and excessively in order to meet the food needs of the increasing population, cause pollution of water and air, decrease in soil fertility, destruction of agricultural areas, and serious damage to both human and animal health (Saber, 2001; Hossain et al., 2003; Çakmakcı, 2005; Öztemiz, 2008; Akbay, 2012; Kapakci, 2013).

The most successful way to improve the soil structure and ensure its sustainability is to enrich the soil with organic matter (Tüzel et al., 2011; Zhang et al., 2012). With organic fertilizers, the physical and chemical structure of the soil is improved, and the vital activities of living things and microorganisms living in the soil are increased. Thus, soil quality, fertility, and continuity are ensured (Beşirli et al., 2009; Nesmeyanova et al., 2013; Patil et al., 2014; Ragozo et al., 2014; Xie Feng et al., 2014). With the start of microorganism activities in the soil, various events such as the mineralization of nutrients important for plant growth, nitrogen fixation, phosphorus solubility, and prevention of harmful microorganisms occur (Altın and Bora., 2005; Alagöz et al., 2020).

In recent years, to reveal the difference between organic and conventionally grown products, a new method of creating images, which was used by some researchers and has become increasingly important, has been used (Andersen et al., 1999; Balzer-Graf, 1999; Koepke et al., 2001; Huber, 2006; Abdollahi, 2008; Kuşçu, 2008; Unluturk et al., 2014). As a result of quantitative analysis, it was not observed between organic and conventional products in some methods; on the other hand, the pictures obtained from the products in question with these methods may differ. Image creation methods can be grouped under three main headings as biocrystallization method, circular chromatogram method, and rising picture method (Abdollahi, 2008; Kuşçu, 2008).

With the increasing consumer awareness day by day, the taste and aroma properties, biochemical contents, nutritional contents, pesticide residues, nitrate nitrite accumulation, the effects they have left on the soil, human health, etc., of the products obtained by organic and conventional agriculture it is seen that comparisons have been made in many subjects such as (Abdollahi, 2008; Kuşçu, 2008; Ninfali et al., 2008; Hallmann, 2012; Mditshwa et al., 2017; Suja et al., 2017; Erdinc et al., 2018). While many features are revealed separately with quantitative analysis, these features are stated as reflecting the whole with picture creation methods.

The aim of the study was to determine whether the difference between the two cultivation methods used by some organic and conventional fertilizers can be distinguished by using image formation methods in the Rio Grande tomato variety grown under field conditions in 2019 and 2020.

2. Material and Methods

2.1. Materials

The seeds of the Rio Grande standard tomato variety, the main material of the study, were obtained from Yalova Atatürk Horticultural Research Institute. Chemical fertilizer (18:18:18 NPK compound fertilizer), plant activator containing seaweed, organic mineral fertilizer, solid worm, and liquid worm were obtained from private companies. Cattle manure was obtained from farmers residing in the Van-Tusba district, sheep manure from the Van-Edremıt district, and chicken manure from the Van-Gurpınar district.

Tomato seeds were sown on 11.04.2019 in viols containing sterile peat: perlite mixture at a ratio of 2:1 in the greenhouses of Van Yuzuncu Yil University, Faculty of Agriculture, Department of Horticulture. The healthy seedlings were planted on 08.06.2019 in the first year and on 14.06.2020 in the second year. The field experiment was established in the village of Göllü, which has a latitude of 38.7202^{o} and a longitude of 43.3124^{o}, within the borders of the Tusba district of Van, in a randomized block design with 4 repetitions, a control group, and 8 different fertilizer applications, with a total of 9 applications.

The study area consisted of 36 plots in total. The plots were prepared with a length of 7 m x 6 m, and a 1.5 m distance was left between the blocks and the plots. Tomato seedlings were planted at intervals of 100 cm x 50 cm on the rows. There were 84 plants were used in each plot and a total of 336 plants were used for one application. The drip irrigation system was used for irrigation.

2.1.1. Fertilizer applications

1. Control application (No fertilizer)
2. Chemical fertilizer (CF) (based on 12 kg da^{-1}N: 12 kg da^{-1} P_2O_5: 18 kg da^{-1} K_2O) 1st Year (as a base fertilizer compound fertilizer of 18N:18P:18K, potassium sulfate, and triple super phosphate were applied); the rest of the nitrogen was divided into two and applied in the flowering and fruit setting periods as ammonium sulphate.
3. Chemical fertilizer (CF) (12 kg da^{-1}N: 12 kg da^{-1} P_2O_5: 18kg da^{-1} K_2O)+ Plant activator (100 ml of plant activator (seaweed) in 100 liters were sprayed four times in 20 days intervals after planting).
4. *Organomineral fertilizer (based on 11 kg da^{-1}N: 11 kg da^{-1} P_2O_5:, 11kg da^{-1} K_2O)
5. *Solid vermicompost 1. Year (589 kg da^{-1}); 2.Year (590 kg da^{-1})
6. *Liquid vermicompost 1. Year (84.82 l da^{-1}); 2.Year (85.89 l da^{-1}).
7. *Sheep manure 1. Year (784.67 kg da^{-1}); 2.Year (786 kg da^{-1}).
8. *Cattle manure 1. Year (1528.57 kg da^{-1}); 2.Year (1531.16 kg da^{-1}).
9. *Chicken manure 1. Year (435.93 kg da^{-1}); 2.Year (436.67 kg da^{-1}).

*: In all applications, the fertilizer rates were adjusted approximately to be equivalent to the amount of chemical fertilizer nitrogen.

2.2. Methods

2.2.1. Samples preparation for imaging techniques

For each application, 10 fruits were taken randomly from the fruits harvested from the 6 plants in the middle, and they were pureed and stored at -20 °C until the analysis day. Frozen tomato samples of fertilizer applications were homogenized with the help of a blender. Then the extract is centrifuged at 4000 rpm for 20 minutes. After centrifugation, the extract was filtered using coarse filter papers, and obtained filters were employed in imaging techniques (biocrystallization method, circular chromatography, and rising picture method).

2.2.1.1. Biocrystallization method

The biocrystallization method, which is described as the method of sensitive copper chloride crystallization, has been modified according to Balzer-Graf and Balzer (1991). The copper chloride solution was prepared at a concentration of 16% and the sample extract at a concentration of 3%. Each sample was prepared in triplicate. Petri dishes were left to dry in the climate room of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Horticulture, in a flat place at 25-30 °C and 60-65% relative humidity conditions. After approximately 14-16 hours, the crystal patterns formed by drying on the petri surface were evaluated.

2.2.1.2. Circular chromatography method

The circular chromatogram method was modified according to Balzer-Graf (1999). In the circular chromatogram method, 1% $AgNO_3$ solution and 80% concentration sample extract were used. The colors and patterns that emerged due to the drying of the paper emerged in a period of a few hours to a few days.

2.2.1.3. Rising picture method

This method, called the capillary rising picture method, capillary dynamolysis, or steigbild, has been modified according to Balzer-Graf (1999). In the method, 100% tomato extract, silver nitrate 0.5 %, and iron sulfate were prepared at 0.5% concentrations. In this method, a certain amount of sample extract is given to the chromatography paper. After the drying stage, the silver nitrate solution was added to a height of 1 cm from the sample extract, and after the second drying period, the iron sulfate solution was given to a total height of 12 cm.

2.2.2. Examining patterns

The crystal patterns formed in the glass petri dish, the circular chromatogram, and the patterns obtained from the ascending picture methods were taken with the Cano 7d DSLR digital camera. The

evaluation of the photographs was made according to the morphological features of the crystals, and the colors and patterns that emerged in the circular chromatogram and ascending picture method.

3. Results and Discussion

3.1. Biocrystallization images

The zones formed in biocrystallization are demonstrated in Figure 1. Pictures obtained by the biocrystallization imaging technique in frozen tomato pulps are given in Figure 2. The biocrystallization method was prepared by choosing the most appropriate sample concentration and copper solution, taking into account the previous studies on tomatoes.

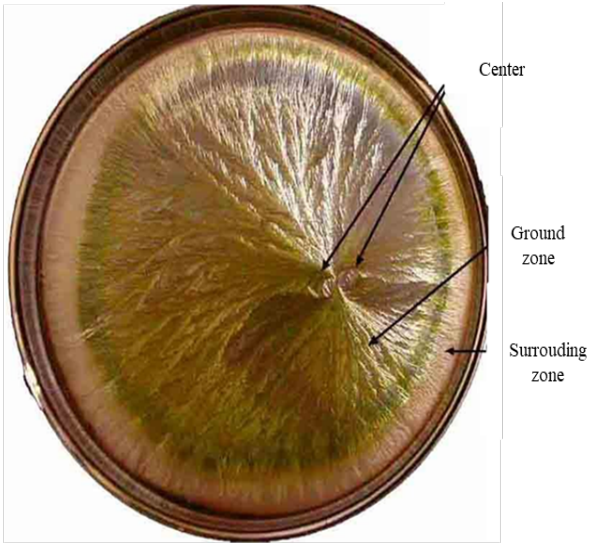

Figure 1. Regions formed in biocrystallization imaging (Abdollahi, 2008).

To determine the different products between organic and conventional fertilizer applications, when the cryptograms were examined in the study conducted in 2019 and 2020, deformation and quality loss in the crystal structure were observed in both organic and conventional samples. It is seen that the number of crystal centers obtained in CF+plant activator application and organomineral fertilizer, excluding chemical fertilizer, is higher than in the organic samples. Due to the quality loss of frozen tomatoes during storage, breakage, and deformation are observed in the crystal branches. When organic and conventional frozen samples are compared, there are still differences between organic and conventional samples although the cryptograms of frozen samples do not reveal as much difference as the cryptograms of fresh samples. However, it is more difficult to reveal crystalline differences due to deformation in organic and conventional samples than in fresh samples. As a matter of fact, in a study by Abdollahi (2008), the cryptograms of fresh, frozen, and pulp-treated samples were examined, and it was determined that the crystal structures of fresh samples more clearly reflected the difference between organic and conventional. He determined that the frozen samples did not form strong crystals as they were exposed to losses during storage. It is quite difficult to reveal the difference between organic and conventional crystals in frozen tomatoes and tomato pulp compared to the fresh sample. In the present study, the samples were frozen and kept at -20 ºC until the day of imaging techniques. It has been determined that the crystal structures of organic fertilizers in solid vermicompost, sheep manure, and chicken manure applications form smoother, tighter, and more lively tissues compared to conventional fertilizers. Although it was frozen, it was determined that there were differences, though not very obvious, in tomato samples grown with organic and conventional fertilizer applications.

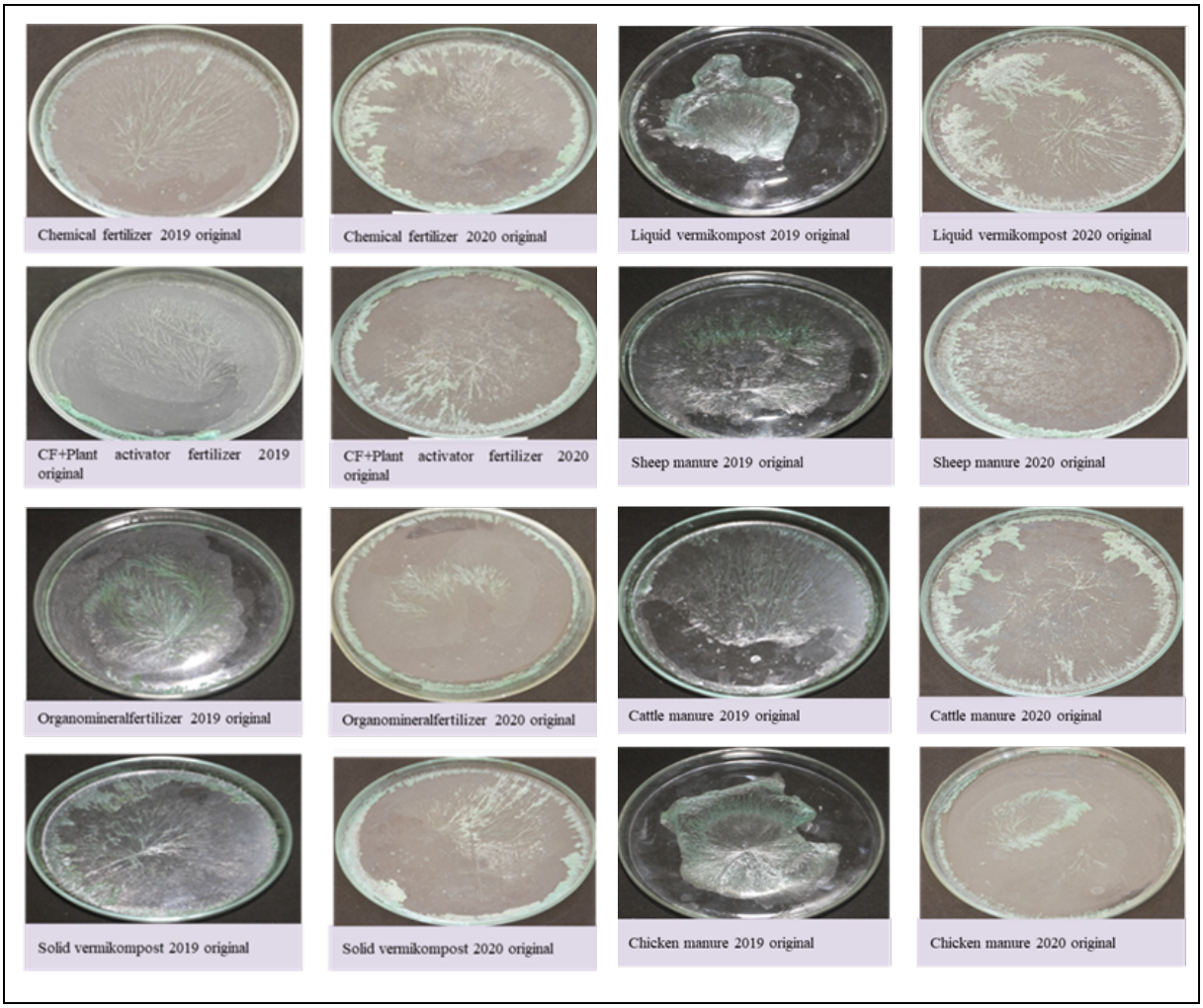

Figure 2. Crystallization images of some frozen tomato samples in 2019 and 2020 (3% frozen tomatoes, 16% $CuCl_2.2H_2O$).

3.2. Circular chromatography images

As seen in Figure 3, circular chromatogram images are seen to consist of four regions: the central zone, the middle (ground) zone, the edge zone, and the end zone (Medina Saavedra et al., 2018). Pictures obtained in the circular chromatogram imaging technique applied to frozen tomato pulps are given in Figure 4.

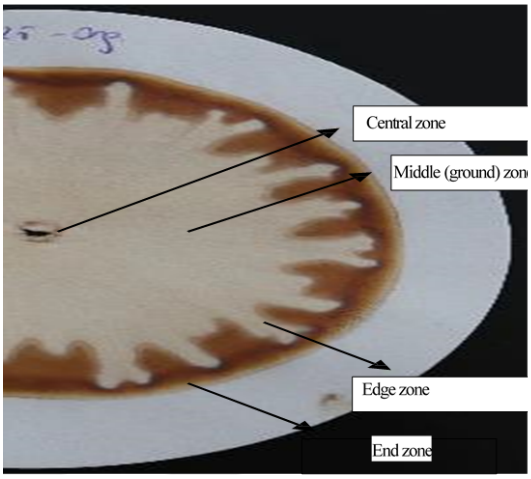

Figure 3. Regions formed in circular chromatography imaging (Medina Saavedra et al., 2018).

The patterns formed in the chromatograms differed in 2019 and 2020, the end zone formed in 2019 was dark brown, and the edge zone was not very clear; In 2020, it is seen that the end zone is light brown and the edge zone is more prominent and clear.

It has been determined that the patterns obtained from the five different organic fertilizers studied show almost the same characteristics, and the patterns obtained in 2020, in particular, are smoother, clearer, and clearer than those obtained from conventional fertilizers. The central and middle zone of the organic and conventional frozen tomato sample chromatograms were separated from each other in both years and in 2019, they acquired a color close to lilac; In 2020, it is seen that it has an orange color. When the edge zone is examined; N: P: K (12:12:18), which is one of the conventional examples, shows almost non-existent patterns in the application, while in CF+ plant activator and organomineral applications, it is seen that there are close to each other and more prominent structures in the second year. In organic fertilizer applications; when the patterns obtained in 2020 are smoother and more distinct, and the corrugated (indentation) structure is examined, it is seen that they are longer than the conventional samples. Among the organic fertilizer applications, especially the patterns obtained from the sheep manure application are clearer than both conventional fertilizer and other organic fertilizer applications, pronounced, and the indentations were determined to be longer. In the tip region, it was observed that the samples obtained from conventional fertilizer applications took a lighter brown color in the second year compared to organic fertilizers, conventional fertilizer applications other than organomineral were longer and irregular, and the color formed in the tip area was darker and shorter in organic fertilizer applications. In light of the data we have obtained, the images obtained from the circular imaging technique in the second year, excluding cattle manure, are smoother than conventional applications, and the rings formed between the center and the periphery are more prominent. The fact that the tip is darker in the circular imaging technique is due to the protein amount of the products grown with organic fertilizer.

Actually, when the protein content of 2020 is examined (data is available in the thesis), it has been determined that the protein content is higher in organic fertilizer applications than in conventional applications, except for chicken manure. In the circular chromatogram technique, circular small and light-colored rings indicate biomaterials, and the shapes extending from the periphery to the center show the protein quality and amount in the sample (Pfeiffer, 1960). In a study in which a circular chromatogram was used for protein detection with different fertilization conditions, it was stated that chromatograms obtained from organic fertilization were more frequent and deep (Knorr, 1982). Reported that in the circular chromatogram technique, circular small and light-colored rings indicate biomaterials, and shapes extending from the periphery to the center indicate the quality and amount of protein in the sample. Kucukyasar (2019), reported that the vivid dark color formed at the ends of circular chromatograms indicates high protein and enzyme activity. Velimirov (2003) stated that in the chromatograms obtained from organic apple samples, the widths of the corrugations are narrower and a more vivid orange color is dominant in the end region. In the images obtained from the studies performed on fresh and frozen red pepper and tomato samples, it was determined that the corrugations formed in the organic chromatograms differed from the conventional ones and were in a deeper and narrower structure (Abdollahi, 2008; Kuşçu, 2008). As a result of some previous studies, they stated that dark gray spots occur between dark brown corrugations in organic chromatograms, while light brown corrugations appear in conventional chromatograms (Knorr, 1982; Abdoollahi, 2008; Kuşçu, 2008).

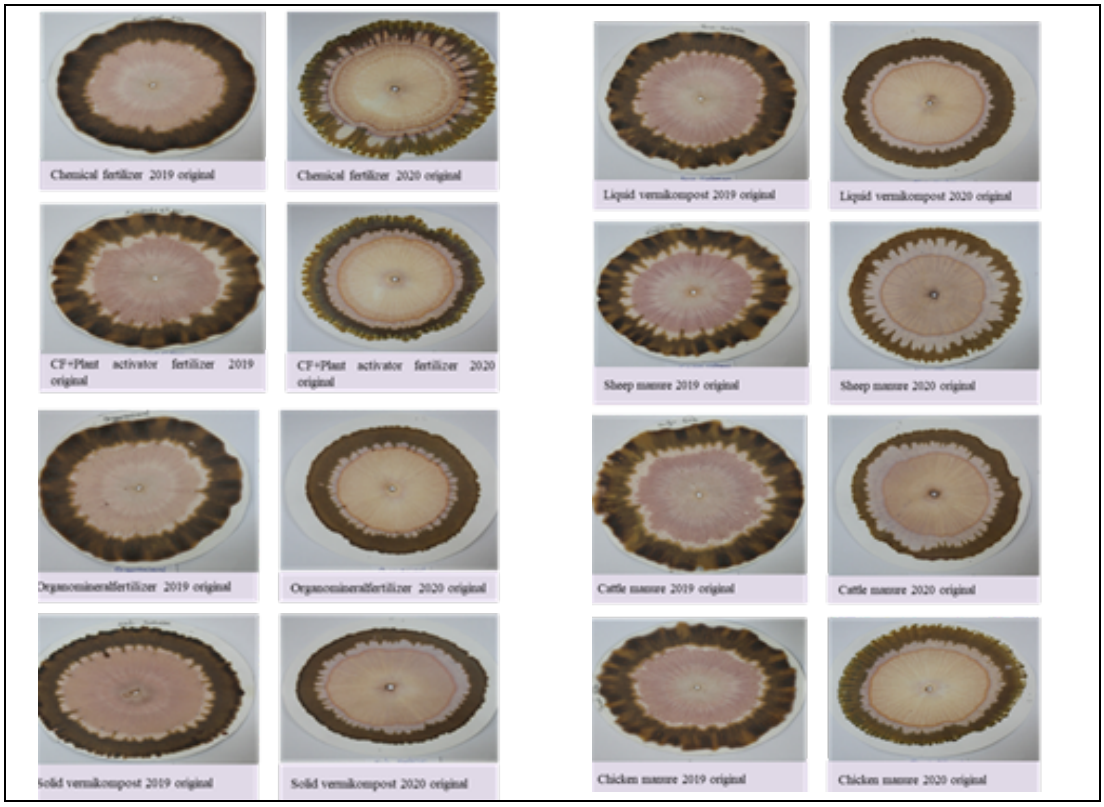

Figure 4. Circular chromatogram of some frozen tomato samples in 2019 and 2020 (80% frozen tomatoes, 1% silver nitrate solution).

3.3. Rising picture chromatogram

The patterns formed when the image is viewed from bottom to top in the rising picture chromatogram consist of the lower region (ground), thin white line, bowl region, tailing region, fall region, branches, and drops (Figure 5).

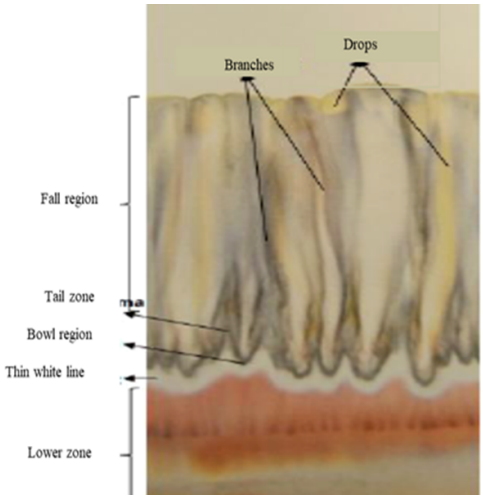

Figure 5. Regions formed in rising picture chromatogram (Kucukyasar, 2019).

It is seen that the regions formed in the second year of the rising picture images obtained in the study and the thin yellow and white lines formed between these regions are more clearly separated than in the first year (Figure 6). In the 2019 images of the ground area, it is seen that a matte coloration occurs in organic fertilizer applications, darker and more vivid colors are formed in conventional fertilizer applications, but the thin yellow line is not very clear in both organic and conventional manure

applications. However, it was photographed that the thin yellow line occurred prominently in the second year. In the pictures obtained from conventional fertilizer applications, it is seen that a thin yellow line is formed in the ground region in the second year, followed by a white thin line. The bowl area is formed and has a vivid dark brown color. It has been determined that tailing, branching, and drops are more prominent in the first year. It was seen that gray tones are more dominant in the decline region in the second year.

It was determined that the yellow line formed in the soil area in organic fertilizer applications was not very pronounced compared to conventional fertilizer applications, and the coloration in all regions was dominated by dull and gray tones. Since the thin white line is wider than in conventional applications and the tailing region is clear, the bowl region has a recessed structure. In almost all organic fertilizer applications, it has been photographed that the branches forming the fall zone become clear towards the tailing zone, but there is no clarity where the drops have entered each other.

Kucukyasar (2019), reported that in the chromatograms created by the rising picture method of potatoes obtained by organic and conventional applications, organic applications took a darker, more pronounced color in the ground region, a very intense gray color in the tailing region, and indistinct branching in the fall region. Schilperoord (2004) examined the ascending picture chromatograms of wheat samples grown as a result of organic and conventional applications and found that for organic wheat samples, a burgundy-colored wavy region, more dense and narrow, red bowl structures were formed in the lower region, and more vivid colored drops were formed in the fall region for conventional samples. Fresh red pepper samples grown with organic and conventional fertilizer applications were examined by imaging techniques, It has been reported that in fresh peppers obtained from conventional fertilizer applications, the width of the bowl area is wider, the tail area is clear, the thin white line separating the ground and the bowl area is wider, and the drop formations in the fall zone are darker and wider (Kuşçu, 2008). In a study that adhered to organic and conventionally grown green olives, the images obtained from the rising image chromatograms also determined that the ground region had different colors in the samples obtained from both applications, the conventional applications samples had wider bowl structures and the thin white line was more prominent and wider (Kucukyasar, 2019). It has been reported that the thin white line separating the ground and bowl area is wider and the drop formations in the fall zone are darker and wider (Kuşçu, 2008).

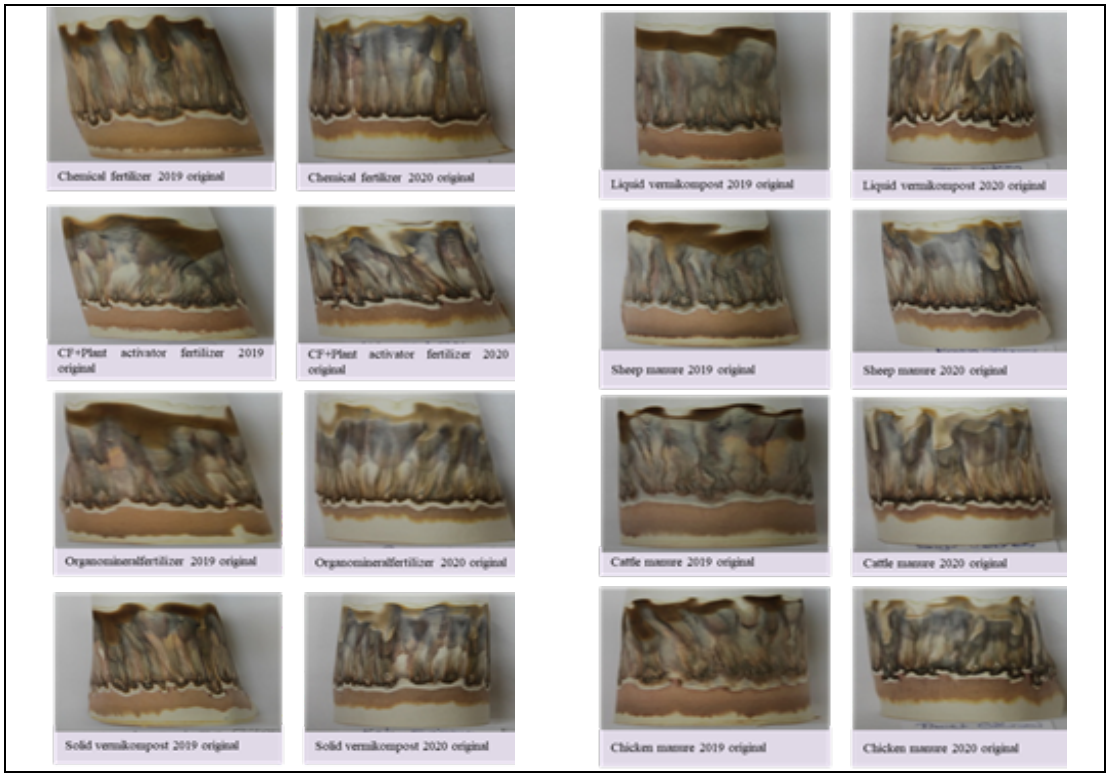

Figure 6. Rising picture chromatogram of some frozen tomato samples in 2019 and 2020 (100% frozen tomatoes, 0.5% silver nitrate, 0.5% ferrous sulfate solution).

5. Conclusion

The components (vitamin C, antioxidant capacity, etc.) that can be determined by analyzing them one by one with analytical methods have brought a different perspective in that they reflect the entire sample with holistic methods.

Painting methods could be applied very easily because they are both easy and repeatable to reveal the difference between organic and conventional products. However, since both organic and conventional crystals are deformed in frozen products, especially in tomatoes, the crystal branches formed began to resemble each other. However, the change in the number of centers in the Biocrystallization method, the smoothness of the rings formed in the circular chromatogram and the vividness of the colors, the transitions between the colors and the light and dark tones in the colors in the ascending dyeing method reveal the difference between organic and conventionally grown tomato fruits.

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Research Article

Phenotypic Diversity of Super Local Durian (*Durio zibethinus* Murr.) Varieties from South Kalimantan, Indonesia: A Case Study

Dindin Hidayatul MURSYIDIN*[1]

[1]University of Lambung Mangkurat, Faculty of Mathematics and Natural Sciences, Laboratory of Genetics and Molecular Biology, South Kalimantan, 70714, Indonesia

[1]https://orcid.org/0000-0002-1200-0927

*Corresponding author e-mail: dindinhm@gmail.com

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Abstract: *Durio zibethinus*, known as durian in several Southeast Asian countries, is a prospective horticultural commodity to cultivate and develop. This study aimed to determine the phenotypic diversity and relationship of superior durian varieties from South Kalimantan in Indonesia based on morphological characteristics. Here, 20 varieties of durian (*D. zibethinus*), including an outgroup, were used. Meanwhile, 57 morphological characteristics, comprising 35 qualitative and 22 quantitative, were observed. The Shannon index (H') method was applied for phenotypic diversity, and the relationships were by the UPGMA. The results show that durians of the region have low phenotypic diversity. However, some morphological characteristics show high ones, e.g., crown shape, fruit skin color and thickness, fruit flesh thickness, and fruit spine length, including tree age. In this case, the highest fruit skin and flesh thickness are present in Malutu and Bamban Birin, respectively. In addition, the fruit spine length and tree age are also in 'Malutu'. The UPGMA revealed that the durians were separated into seven clusters and near-corresponding to geographic origin. In this case, 'Gentarbumi Uya' is the closest to 'Taradak Uya', whereas the farthest is 'Malutu' with 'Tapai Idaman'. Thus, this information is essential in promoting the future durian-breeding program in local and global coverages.

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1. Introduction

Durio zibethinus, known as 'durian' in several Southeast Asia countries, is a very prospective horticultural commodity to cultivate and develop. It is related to the demand for the durian market that continues to increase every year, both for the domestic and foreign markets (Mursyidin et al., 2022). Indonesia, for example, is one of the second largest durian-producing countries in the world after Thailand, which can generate more than 1 million metric tons of durian every year. Even today, this country can export this product to several Asian, European, American, and Middle Eastern countries with a fantastic value of more than 230 thousand USD. However, the Indonesian quality of durian is generally low competitive to such commodities from neighboring countries, i.e., Thailand and Malaysia (Durian Harvests Indonesia, 2021). Thus, the improvement included in the strategic plan of the durian-breeding program is necessary to employ.

Taxonomically, *D. zibethinus* is one of nine durian species whose fruit flesh is edible. Meanwhile, this plant is native to Southeast Asia, especially Malaysia, Thailand, and Indonesia, with tremendous phenotypic diversity. According to Husin et al. (2018), there are 15 varieties of durians registered in Malaysia, such as 'D24', 'D99', 'D123', 'D145', 'D158', and 'D159'. In Thailand, the Thai Agricultural Standard has reported seven commercial varieties of durian, including 'Chanee', 'Karnyao', 'Kradoomthong', 'Longlublae', 'Monthong', 'Nualthongchan', and 'Puangmanee'. In addition, more than 102 durian varieties have been released and registered in Indonesia with different characteristics of fruit flesh, color, aroma, and taste (Bayu and Ashari, 2019).

South Kalimantan is part of the Indonesia region with a high genetic diversity of durian. Unsurprisingly, this region is known as the center of diversity of the durian germplasm of the world. In this region, not only *D. zibethinus* is present, but also several other wild durian species, e.g., *D. dulcis*, *D. excelsus*, *D. kutejensis*, and *D. lowianus* (Mursyidin and Daryono, 2016). This germplasm has beneficial traits to the breeding program, such as a high tolerance to specific environmental conditions and diseases, like acid soils and patch cankers (Mursyidin et al., 2022). This study aimed to determine the phenotypic diversity and relationship of superior local durian varieties from this region based on morphological markers. While these traits have certain disadvantages, such as multigenic inheritance, time-consuming, and strongly influence by environmental parameters (Wu et al., 2021), they are customary to assess the genetic diversity of durian (Sundari et al., 2019; Sihaloho et al., 2021).

In brief, determining genetic diversity and the relationship of germplasm is critical for plant genetic preservation and breeding program, particularly in developing new superior varieties (Acquaah, 2007). In addition, such studies provide valuable information for understanding the relationship among varieties, evaluating the likelihood of mixing or repetition in germplasm collections (Delfini et al., 2021), and the parental selection of crossbreeding in breeding and preservation programs (Wu et al., 2021). From a global perspective, determining genetic diversity would provide an essential foundation for promoting the future durian-breeding program (Roy et al., 2016). However, on the local scale, such studies will reveal the complicated interaction between the germplasm and the local wisdom of the community (Mursyidin et al., 2019).

2. Material and Methods

2.1. Plant materials

A total of 20 superior varieties of durian (*D. zibethinus*), including an outgroup (*D. dulcis*), were collected by a purposive sampling method from seven regions of South Kalimantan, Indonesia (Figure 1) and used in this study (Table 1). Generally, naming and selecting varieties based on the shape and taste of the durian fruit in the community, then registering it with the government through the authorized agency. In this case, all sampling locations were characterized by daily temperature and humidity, ranging from 22-31ºC and 65-95%, respectively (BMKG, 2023).

2.2. Phenotypic analysis

In total, 57 morphological traits, comprising 35 qualitatives (Table 2) and 22 quantitative (Table 3), were observed and evaluated in determining durian phenotypic diversity. This characterization was followed by the standard durian (*D. zibethinus*) protocol from Bioversity International (2007).

2.3. Data analysis

The qualitative and quantitative data were tabulated and converted into multivariate values. Then, all were standardized and analyzed with the assistance of the MVSP ver. 3.1 (Kovach, 2007). The phenotypic diversity was determined by the Shannon diversity index (*H'*) following the equation (Mursyidin and Khairullah, 2020).

$$H' = -\sum_{i=1}^{n} pi \ln pi \quad (1)$$

Where *H'* is the diversity index; p_i is the proportional frequency, and ln p_i is the ratio of the natural logarithm. The diversity was categorized into high ($H' > 0.60$), moderate ($0.40 \leq H' \leq 0.60$), or low ($H' < 0.40$) levels (Mursyidin and Khairullah, 2020).

Before further analysis, the distance matrix was proceeded using the Euclidean approach, whereas the phenotypic relationship was by the unweighted pair group with the arithmetic average (UPGMA) and PCA methods. All analyses were employed by a similar software, i.e., MVSP ver. 3.1 (Kovach, 2007).

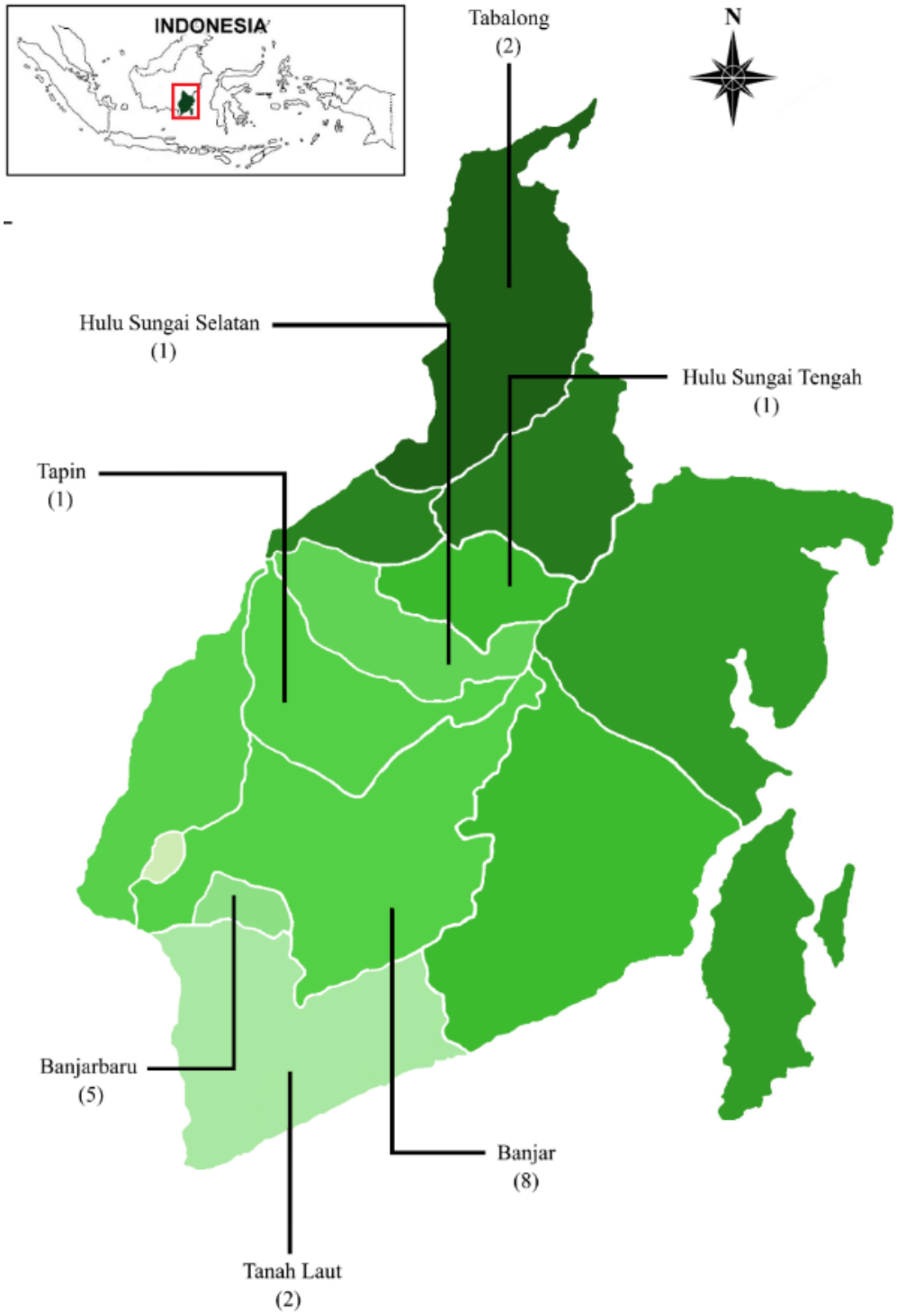

Figure 1. Map of South Kalimantan in Indonesia, showing seven sampling locations where 20 varieties of superior local durian (*D. zibethinus*) were sampled and used in this study.

Table 1. List of superior local durian (*D. zibethinus*) varieties, including an outgroup (*D. dulcis*), used in this study and their origin

Name of cultivar	Origin (village, district, regency)	Geographic ordinate
'Coklat'	Alat, Hantakan, Hulu Sungai Tengah	2°37'44.55"S; 115°28'44.69"E
'Lunas'	Alam Subur, Bati-Bati, Tanah Laut	3°34'26.83"S; 114°50'11.13"E
'Nonong'	Suato Baru, Salam Berbaris, Tapin	3°03'55.05"S; 115°11'30.77"E
'Penganten'	Beruntung Jaya, Cempaka, Banjarbaru	3°33'10.92"S; 114°50'57.69"E
'Siput'	Bentok Darat, Bati-Bati, Tanah Laut	3°35'42.88"S; 114°52'12.07"E
'Tapai Idaman'	Beruntung Jaya, Cempaka, Banjarbaru	3°33'10.92"S; 114°50'57.69"E
'Bamban Birin'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Taradak Uya'	Lumbang, Muara Uya, Tabalong	1°55'18.87"S; 115°39'25.51"E
'Dodol Mascinta'	Balau, Karang Intan, Banjar	3°24'02.77"S; 115°00'07.76"E
'Gading Abirau'	Abirau, Karang Intan, Banjar	3°24'24.99"S; 115°01'00.36"E
'Panyangat Kuning'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Gantang 88'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Garuda Idaman'	Sei Abit, Cempaka, Banjarbaru	3°31'26.20"S; 114°54'04.12"E
'Gentarbumi Uya'	Lumbang, Muara Uya, Tabalong	1°55'18.87"S; 115°39'25.51"E
'Hadangan Idaman'	Sei Abit, Cempaka, Banjarbaru	3°31'26.20"S; 114°54'04.12"E
'Hintalu Biih'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Idangan Biih'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Kuning Janar'	Biih, Karang Intan, Banjar	3°24'05.50"S; 114°58'52.86"E
'Dino Banjarbaru'	Gunung Kupang, Cempaka, Banjarbaru	3°28'45.62"S; 114°51'59.14"E
'Malutu'*	Malutu, Padang Batung, Hulu Sungai Selatan	2°52'43.50"S; 115°16'40.19"E

3. Results and Discussion

In this study, the superior durians of South Kalimantan, Indonesia, have low phenotypic diversity following morphological traits, both quantitative and qualitative traits. It is pointed out by diversity index values of 0.389 and 0.324, respectively (Tables 2 and 3). Such level of diversity was also reported by Sihaloho et al. (2021) from durian varieties of Central Tapanuli Regency, North Sumatra, Indonesia, and from Sawang and Langkahan of North Aceh, Indonesia (Handayani and Ismadi, 2018). According to Gao et al. (2017), this case may be due to natural selection, the founder effect, genetic isolation, including inbreeding.

However, although the durians have a low level of diversity, several others show a high, such as branching density (0.79), crown shape (0.982), petal color (0.921), fruit skin color (0.83), and fruit flesh texture (0.692) (Table 2). Quantitatively, four traits also presented a high diversity, i.e., fruit skin thickness (0.754), fruit flesh thickness (0.785), fruit spine length (0.636), and tree age (0.624) (Table 3). In general, the fruit characteristics are commonly used, as a marker, to differentiate durian varieties (Susila et al., 2021). However, these traits could only be applied precisely when the plant reaches a minimum of 8 years old, particularly by seed propagation (Retnoningsih et al., 2016). Figure 2 shows morphological differences among durian varieties of South Kalimantan, Indonesia.

According to Sawitri et al. (2019), the superior durian has several criteria, such as: (1) the fruit shape is an ellipse and regular; (2) the flesh fruit is smooth, fluffy, dry, thick, and sweet; (3) the fruit spine is large and pyramid-shaped. For domestic consumers, durians with a sweet taste, medium to strong scent, attractive yellow color, thick flesh, and high fruit productivity are favorable. Meanwhile, foreign consumers prefer durian with an unscented, sweet taste, slightly bitter, thick flesh, and yellowish flesh color (Susila et al., 2021). Following our results, the 'Malutu' is durian with the highest value of fruit skin (2 cm), fruit spine length (1.9 cm), and tree age (100 years). Meanwhile, flesh thickness by 'Bamban Birin', is 1.95 cm (Table 3).

Conceptually, the high diversity level of durian is due to the self-incompatibility or cross-pollination events (Handayani and Ismadi, 2018). In other words, plants with a cross-pollination mechanism (like durians) are derived from natural crosses and are very difficult to obtain with potentially superior character (Kurniadinata et al., 2019). In this context, plant breeders utilize this aspect to assemble new-improved varieties with desired traits, particularly for abiotic and biotic stress adaptation, including consumer-preferred (Swarup et al., 2021; Mursyidin, 2022). In addition, only populations with high genetic diversity can adapt rapidly to environmental changes (Lloyd et al., 2016).

Thus, tasks in expanding the genetic diversity of durians are essential and can be achieved by several approaches, e.g., hybridization, introgression, mutagenesis, or transgenic (Allier et al., 2020).

Table 2. Phenotypic diversity of superior local durian (*D. zibethinus*) varieties for qualitative characters

Character	Code	*H′* Index	Category of Diversity
Tree growth habit	A	0.432	Moderate
Bark color	B	0.445	Moderate
Branching density	C	0.790	High
Branching position	D	0.537	Moderate
Crown shape (canopy)	E	0.982	High
Trunk surface	F	0.707	High
Bark shape	G	0.000	None
Leaf upper surface color	H	0.000	None
Leaf lower surface color	I	0.354	Low
Leaf-blade shape	J	0.432	Moderate
Arrangement of leaf	K	0.356	Low
Leaf apex shape	L	0.512	Moderate
Leaf-blade margin	M	0.432	Moderate
Leaf texture	N	0.432	Moderate
Flower clustering habit	O	0.432	Moderate
Flower bud shape	P	0.138	Low
Flower bud apex shape	Q	0.432	Moderate
Petal color	R	0.921	High
Sepal color	S	0.256	Low
Stigma color	T	0.140	Low
Anther color	U	0.102	Low
Time of flowering	V	0.432	Moderate
Time of harvesting	W	0.432	Moderate
Fruit shape	X	0.221	Low
Fruit skin color	Y	0.830	High
Fruit spine shape	Z	0.127	Low
Fruit flesh color	AA	0.123	Low
Seed shape	AB	0.145	Low
Seed color	AC	0.507	Moderate
Fruit flesh taste (level of sweetness)	AD	0.421	Moderate
Fruit flesh taste (fatty)	AE	0.109	Low
Fruit flesh taste (sugarless)	AF	0.238	Low
Fruit flesh texture (fiberless)	AG	0.692	High
Fruit flesh texture (moisture)	AH	0.135	Low
Fruit aroma	AI	0.357	Low
Average		**0.389**	**Low**

In South Kalimantan, Indonesia, durians are not planted in monoculture but in polyculture. In concept, polyculture is the cultivation practice or model where more than one crop species is grown in a similar location and time. It attempts to mimic the diversity of natural ecosystems (Altieri, 1999). Due to climate change, polyculture is popular in more developed countries and has a beneficial impact on certain aspects, for example, controlling the improvement of some pests, weeds, and diseases while reducing the need for pesticides (Iverson et al., 2014). However, polyculture has a limitation, particularly in reducing crop yields due to competition among species for light, water, or nutrients (Capinera, 2008). It also makes management complex because different species have different growth rates, maturation times, and yield requirements (Loomis, 2022).

Table 3. Phenotypic diversity of superior local durian (*D. zibethinus*) varieties for quantitative characters

Character	Code	Lowest value	Durian cultivar	Highest value	Durian cultivar	*H'* Index	Category of diversity
Leaf blade length (cm)	AJ	11.45	'Panyangat Kuning'	17.05	'Hadangan Idaman'	0.331	Low
Leaf blade width (cm)	AK	3.60	'Panyangat Kuning'	11.35	'Lunas'	0.200	Low
Tree height (m)	AL	15.00	'Kuning Janar'	59.00	'Coklat'	0.125	Low
Tree trunk (first branching of the stem) (m)	AM	1.50	'Siput'	17.00	'Bamban Birin', 'Malutu'	0.109	Low
Crown/canopy diameter (m)	AN	9.00	'Gantang 88'	22.00	'Malutu'	0.240	Low
Trunk circumference (cm)	AO	90.00	'Gantang 88'	242.00	'Coklat'	0.262	Low
Bark diameter (cm)	AP	28.70	'Gantang 88'	77.00	'Coklat'	0.259	Low
Number of flowers/cluster	AQ	8.50	'Kuning Janar'	25.00	'Malutu'	0.107	Low
Number of fruit/cluster	AR	1.00	'Tapai Idaman', 'Dodol Mascinta'	3.00	'Gentarbumi Uya'	0.506	Moderate
Fruit length (cm)	AS	12.00	'Gantang 88'	26.00	'Idangan Biih'	0.236	Low
Fruit diameter (cm)	AT	9.50	'Gading Abirau'	58.20	'Idangan Biih'	0.336	Low
Fruit spine length (cm)	AU	0.40	'Dino Banjarbaru'	1.90	'Malutu'	0.636	High
Number of seeds per fruit (unit)	AV	2.50	'Kuning Janar'	25.00	'Panyangat Kuning'	0.354	Low
Fruit weight (kg)	AW	0.60	'Gading Abirau'	2.40	'Idangan Biih'	0.108	Low
Fruit skin thickness (cm)	AX	0.35	'Coklat'	2.00	'Malutu'	0.754	High
Number of fruit segment	AY	4.00	'Gading Abirau'	5.00	Most of durian	0.135	Low
Number of fruit flesh	AZ	4.00	'Gading Abirau, 'Dodol Mascinta'	25.00	'Panyangat Kuning'	0.172	Low
Seed length (mm)	BA	15.15	'Hadangan Idaman'	52.18	'Coklat'	0.208	Low
Fruit flesh thickness (cm)	BB	0.65	'Hintalu Biih', 'Siput', 'Penganten', 'Kuning Janar', 'Idangan Biih'	1.95	'Bamban Birin'	0.785	High
Fruit production per plant (unit)	BC	110.00	'Hintalu Biih'	600.00	'Panyangat Kuning'	0.454	Moderate
Storability at room temperature (day)	BD	3.00	'Penganten', 'Tapai Idaman'	5.00	'Coklat'	0.187	Low
Tree age (year)	BE	20.00	'Gantang 88', 'Hadangan Idaman', 'Dodol Mascinta', 'Dino Banjarbaru'	100.00	'Malutu'	0.624	High
Average						**0.324**	**Low**

Figure 2. Morphological fruit differences among (11 of 20) superior local durian (*D. zibethinus*) varieties of South Kalimantan in Indonesia.

The UPGMA revealed that the durians were separated into seven clusters (Figure 3). In this case, the clustering or grouping is near-correspond to geographic origin. Such a study have reported by Wallace et al. (2015) in the *Echinochloa colona* population. Dwivedi et al. (2020) stated that geographic origin does not commonly correspond to the emergence of genetic diversity. It implies that samples coming from the same area are not always grouped or clustered together. In concept, however, a region can be viewed as a cluster, where all members should have a close similarity (Rey et al., 2020). In this case, durian 'Gentarbumi Uya' has very closely related to 'Taradak Uya' at a coefficient of 0.92, whereas the farthest by 'Malutu' with 'Tapai Idaman' (0.56) (see Figure 4). Following a qualitative trait, all durian have sweet flesh tastes, except for 'Gentarbumi Uya' with a high amount of fruit per cluster (Table 3). Yet, this knowledge is crucial for advancing the durian-breeding program in the future, especially in assessing the phenotypic diversity of the progeny (Acquaah, 2007).

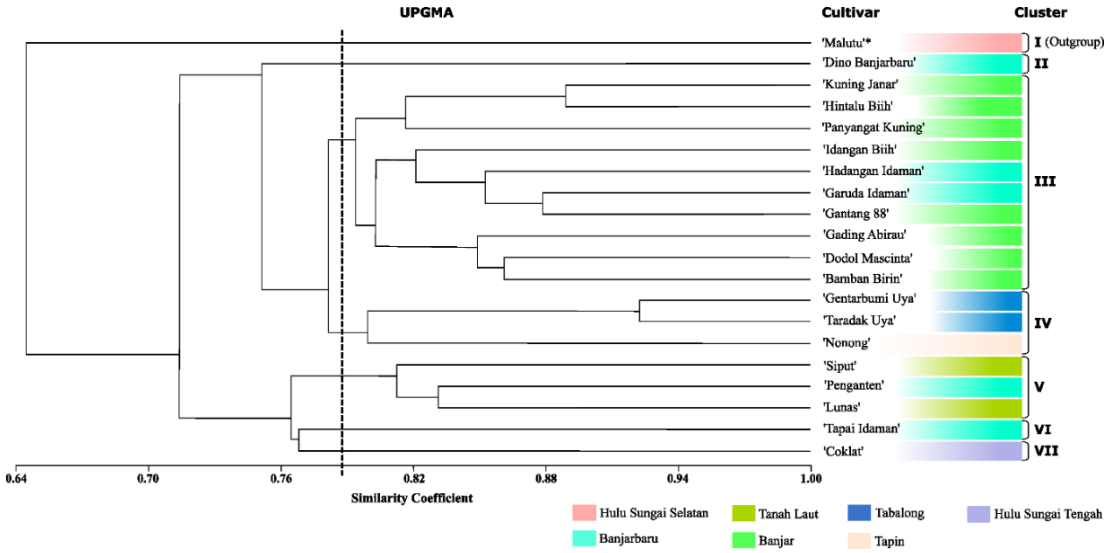

Figure 3. The phenotypic relationship of superior local durian (*D. zibethinus*) varieties revealed by morphological characters. The box color next to the cultivar's name indicates the origin of the samples (see Table 1 for details).

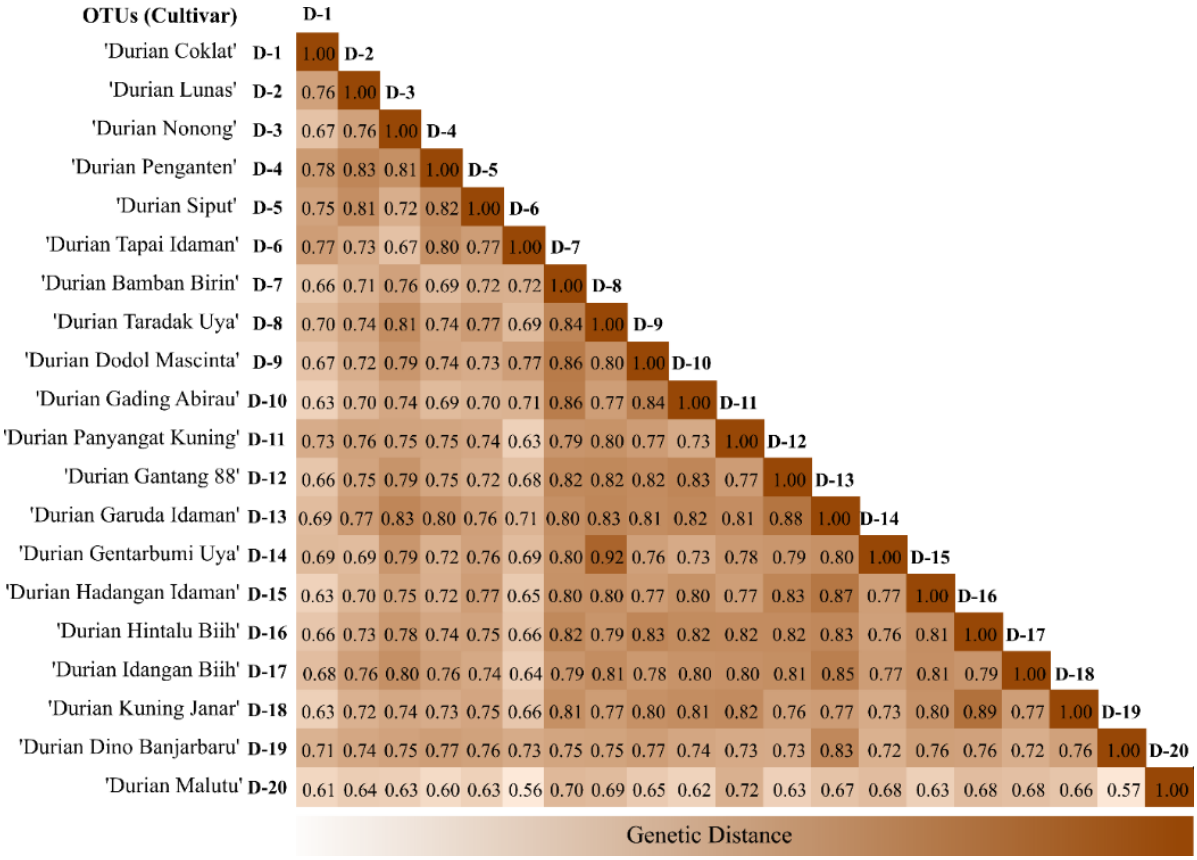

Figure 4. Phenotypic distance among superior local durian (*D. zibethinus*) varieties based on morphological characters. Colors indicate a relationship: dark color = strong relationship; light color = low relationship.

4. Conclusion

In conclusion, although the superior local durian (*D. zibethinus*) germplasm of South Kalimantan in Indonesia has low phenotypic diversity, several morphological characteristics show high ones, e.g., crown shape, petal color, and fruit skin color. Further, the UPGMA revealed that the durians were separated into seven clusters and near-corresponding to geographic origin. In this case, 'Gentarbumi Uya' has very closely related to 'Taradak Uya,' whereas the farthest by 'Malutu' is with 'Tapai Idaman.' Thus, this information is essential in promoting the future durian-breeding program, particularly in developing new superior varieties.

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Research Article

Characterization of Nepalese Bread Wheat Landraces Based on Morpho-Phenological and Agronomic Traits

Ajaya KARKEE[1], Ram Prasad MAINALI[2], Krishna Hari GHIMIRE[3], Pradip THAPA[4] Bal Krishna JOSHI[5], Sudeep SUBEDI[6], Jiban SHRESTHA*[7]

[1,2,3,4,5]Nepal Agricultural Research Council, National Agriculture Genetic Resources Centre, Khumaltar, Lalitpur, Nepal
[6,7]Nepal Agricultural Research Council, National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal

[1]https://orcid.org/0000-0002-5074-7079,[2]https://orcid.org/0000-0002-4637-3676,[3]https://orcid.org/0000-0002-3393-290X
[4]https://orcid.org/0000-0002-2080-6517, [5]https://orcid.org/0000-0002-7848-5824, [6]https://orcid.org/0000-0002-4478-704X
[7]https://orcid.org/0000-0002-3755-8812

*Corresponding author e-mail:jibshrestha@gmail.com

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Quantitative traits,
Wheat landraces

Abstract: Due to the presence of valuable genes that contribute to a variety of functional traits, landraces kept in Genebank can be extremely important in wheat breeding. A study was conducted based on agro-morphological traits of Nepalese bread wheat landraces to explore genetic diversity among them. Using a replicated rod row design, 200 landraces were evaluated during the winter season of 2018 and 2019 at Khumaltar, Lalitpur, Nepal. The degree of variations among landraces was determined using univariate and multivariate statistical tools. The Shannon-Weaver diversity index (H') showed a wide range of variations among the studied landraces, ranging from 0.55 to 0.91 in quantitative traits and 0.63 to 0.85 in qualitative traits.Principal component (PC) analysis with an eigenvalue greater than 1 reveals that 68% of the variability for quantitative traits is contributed by the first five principal components whereas 67% of the variability of qualitative traits is governed by the first four principal components. UPGMA (Unweighted pair-groups methods through arithmetic average) clustered 202 landraces into five groups according to quantitative charterers.Identified advantageous adaptive traits through the analysis of variability within the accessions, will be used by breeders for crosses in the breeding or used directly by farmers.

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1. Introduction

The annual plant *Triticum aestivum* L., commonly known as bread wheat, has 21 chromosome pairs arranged into three sub-genomes, A, B, and D. It is a hexaploidy species (2n=6x=42) (Genome BBAADD). It belongs to the grass family Poaceae's Triticeae subtribe (Sears, 1952). Around 8 500 years ago, it was created by Aegilopstauschii, the domesticated tetraploid progenitor (genome BBAA) and the diploid donor of the D sub-genome (Levy and Feldman, 2022). The most significant cereal crop in the world is wheat, with production and productivity estimates of 760.93 million tons and 3.47 mt ha^{-1}, respectively. It is cultivated on 219 million ha of surface area (FAOSTAT, 2022). In terms of

production and area, wheat is Nepal's third-largest cereal crop after rice and maize (Karkee et al., 2019). It is grown on 711 000 ha, with a productivity of 2.99 mt ha^{-1} and a total production of 2.127 million tons (MoALD, 2021).

Primitive cultivars, landraces, and wild relatives of crop plants make up a source of valuable genetic diversity needed for successful breeding programs (Routray et al., 2007). Landraces are essential for overcoming the effects of climate change on agriculture because they are locally adapted to the environmental conditions and provide valuable genetic resources for breeding programs (Mainali et al., 2020). Due to their genetic diversity, unique adaptation to local environmental conditions, and presence of genes that confer resistance to biotic and abiotic stresses, wheat landraces are important geneticresources (Lopes et al., 2015; Robbana et al., 2019). These are a mixture of different genotypes that were selected and evolved over time using both natural and artificial selection techniques (Gharib et al., 2020).

Despite the fact that there are many genetic resources available on a national and international level, breeders tend to focus only on adapted and improved materials while avoiding landraces and wild and weedy relatives in their breeding program (Upadhyaya et al., 2014). Traditional varieties, also known as landraces, have highyield stabilityunder low-input agricultural systems and have the capacity to tolerate biotic and abiotic stresses despite having a lower yield than improved and hybrid varieties (Manohara et al., 2018). One of the primary causes of the low utilization of landraces is a lack of knowledge about particular characteristics of them (Thapa et al., 2021). In order to improve the use of specific traits in breeding programs, it is crucial to evaluate the diversity of wheat that is currently available. Economically significant traits require evaluation, characterization, and tagging (Joshi et al., 2020). Agro-morphological characterization (Ghimire and Magar, 2017) and genotyping-by-sequencing (GBS) results in both showed that there is a high genetic diversity among Nepalese wheat landraces (Khadka et al., 2020). A crucial step in managing and utilizing the genetic diversity of any crop and for the development of better varieties is agro-morphological characterization (Manzano et al., 2001; Ali et al., 2022; Yüce et al., 2022). Smith and colleagues (1991) stated that significant traits of local landraces need to be assessed, characterized, and labeled. In the current study, agro-morphological traits were used to describe the diversity among landraces and explored the wheat intra-varietal diversity collected from different parts of Nepal.

2. Materials and Methods

2.1. Plant materials and site description

At the National Agriculture Genetic Resources Centre (NAGRC), Khumaltar, Lalitpur, Nepal. The genetic material consists of 200 landraces of bread wheat that have been preserved. As checks, two varieties, WK1204 and Morocco, were used. The landraces came from 42 different Nepalese districts, ranging in altitude from 148 to 3 353 meters above sea level (masl) (Figure 1). The study area is situated in the sub-tropical mid-hill region at latitude 27°40'N, longitude 085°20'E, and elevation 1 368 m (Genebank, 2018; Karkee et al., 2021). The research site's soil is of the black loamy variety (Ghimire and Magar, 2017). Figures 2 and 3 display the meteorological data for the wheat growing seasons of 2019 and 2020.

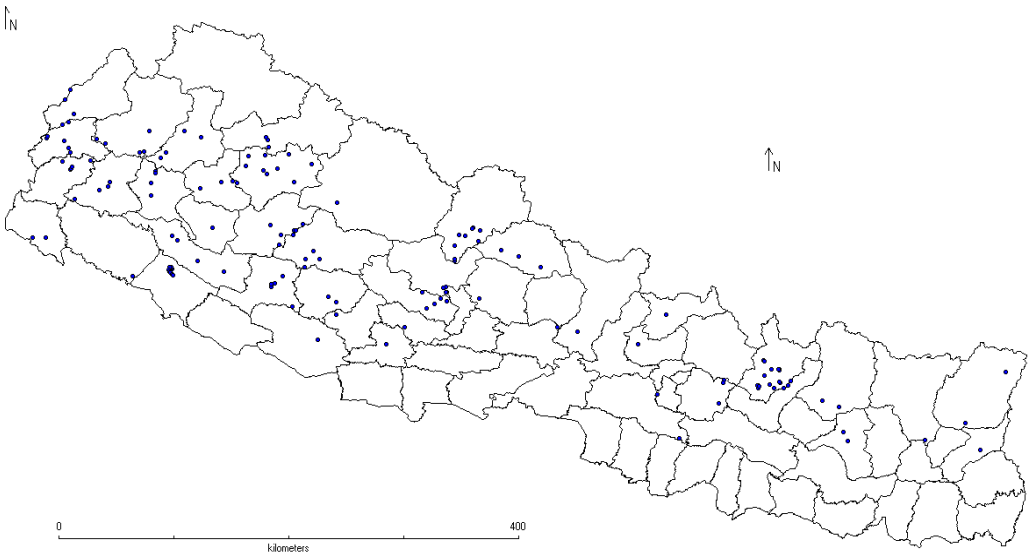

Figure 1. Collection sites of wheat landraces.

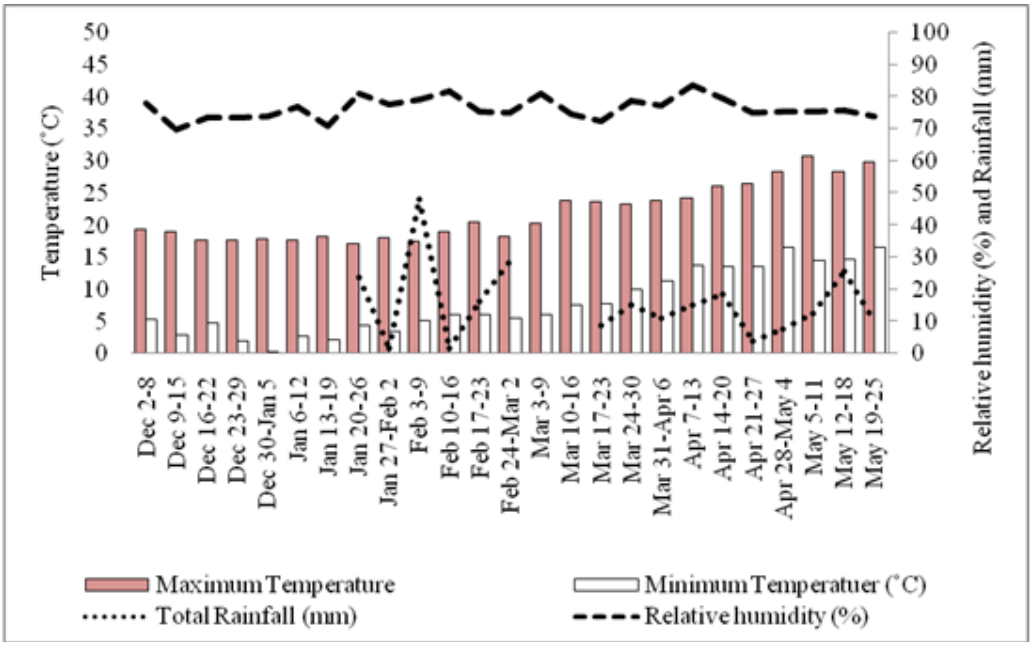

Figure 2.Average maximum and minimum temperatures (°C), total precipitation (mm), and average relative humidity (%) measured in Khumaltar, Lalitpur, Nepal, between December 2018 and May 2019 (Source: National Agronomy Research Centre, Khumaltar, 2019).

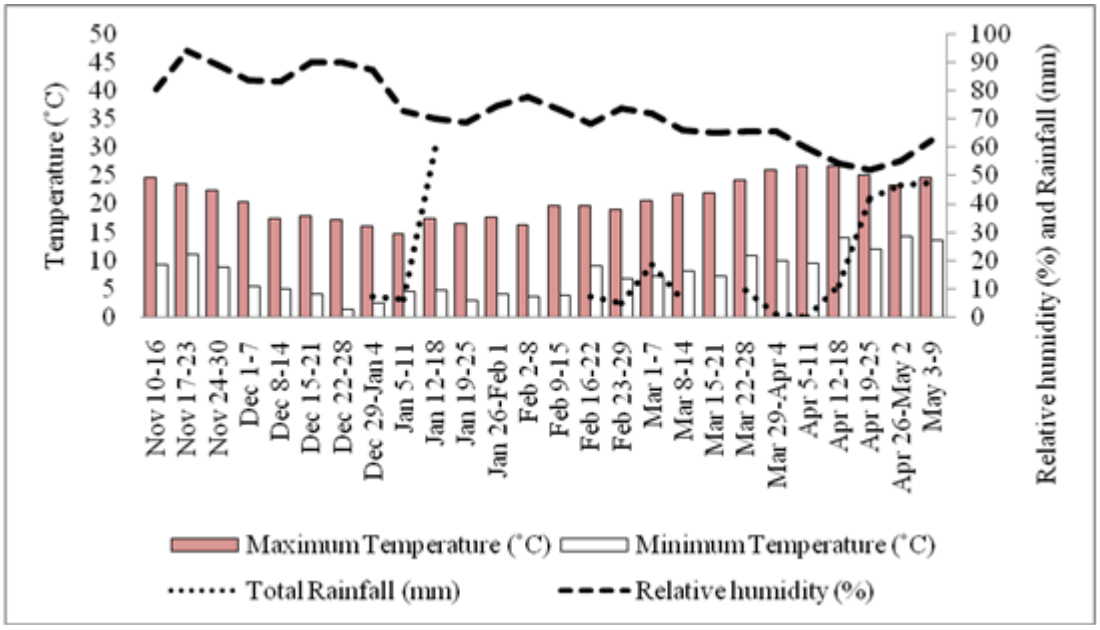

Figure 3. Average maximum and minimum temperatures (°C), total precipitation (mm) and average relative humidity (%) measured at Khumaltar in Lalitpur, Nepal, between November 2019 and May 2020 (Source: National Agronomy Research Centre, Khumaltar, 2020).

2.2. Field experiment and cultural practices

The tests were carried out at NAGRC Khumaltar in Lalitpur, Nepal, during the 2018–19 and 2019–20 crop seasons. They were arranged in a rod row design with two replications. The sowing was finished on 6th December 2018 and 15th November 2019. Two rows of each landrace, each measuring two meters in length, were continuously sown with 25 cm row spacing. The fertilizer was applied during the land preparation with 6 mt ha^{-1} FYM and with N:P_2O_5:K_2O (100:50:0 kg ha^{-1}) provided by di-ammonium phosphate (DAP), urea, and murate of potash. A full dose of P_2O_5 and K_2O and half a dose of N were applied during the land preparation process. The remaining N was distributed in two split doses during the tillering and flowering phases. Intercultural operations, such as irrigation, weeding, and other agronomic procedures, were carried out in accordance with the National Wheat Research Program, Bhairahawa, and the National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal (NWRP, 2017; Yadav et al., 2020).

2.3. Data collection and data analysis

Eight qualitative and sixteen quantitative morphological data were recorded according to IPGRI (1985) wheat descriptor. The diversity of the landraces was calculated and examined using the Shannon-Weaver diversity index (H'), frequency distribution, and some descriptive statistics.

Multivariate principal component analysis (PCA) was carried out in MINITAB version 17 for landrace classifications. 2-D plots were created based on the first and second principal components (PCs). Hierarchical clustering was carried out using the Average Euclidean distance method. The dendrogram was produced utilizing the unweighted pair group average method (UPGMA). R version 4.2.1 was applied to create the dendrogram.

3. Results and Discussion

3.1. Diversity based on quantitative characters

A higher coefficient of variations with more than 20% CV for four parameters among 16 quantitative characters was found, which suggests that the variability exists in a wider range in the studied landraces. The parameters with lower CV (20%) were found on days to emergence, heading, flowering, and maturity; plant height; flag leaf length and width; spike length; the number of seeds per spikelet and spike, and seed length and seed width (Table 1). This indicates that the estimate of this

identified parameter disperses minimally and estimates accurately. Together, genetic and environmental factors may have an impact on the variability of a crop's quantitative traits (Karkee et al., 2020). The diversity of quantitative characters in wheat is an important factor in practical wheat breeding, as it provides breeders with a wide range of traits to select from and can help to improve the overall performance and adaptability of wheat varieties. Typically, the yield of wheat grains is analyzed in terms of several yield components, including the number of spikes per square meter, the number of grains per spike, the number of grains per square meter, and the thousand kernel weight. These components are interrelated and compensate for each other to contribute to overall yield (Beral et al., 2020) and the diversity of these components will help to increase the choices available for breeders to select from.

Table 1. Descriptive statistics and Shannon-Weaver diversity indices (H') for 16 quantitative traits

Traits	Minimum	Maximum	Mean	CV (%)	SD	H'
Days to emergence	4	13	12	6.9	0.79	0.77
Days to heading	104	123.00	112	3.7	4.17	0.90
Days to flowering	109	129	119	3.3	3.96	0.91
Days to maturity	139	169	154	4.5	6.93	0.86
Plant height (cm)	98	173	122	7.5	9.15	0.85
Flag leaf length (cm)	10.8	45.5	17.2	18.9	3.24	0.78
Flag leaf width (cm)	0.81	2.53	1.26	19.2	0.24	0.83
Spike length (cm)	6.9	14.8	9.9	11.1	2.16	0.89
Spike per square meter	223	450	367	21.9	40.88	0.87
Number of seed per spikelets	7	12	9	8.2	0.69	0.84
Number of seed per spike	25	49	33	13.7	4.51	0.91
Awn length (cm)	0.48	5.98	3.82	39.6	1.51	0.55
Thousand grain weight (g)	15	56	36	22.8	8.18	0.86
Seed length (mm)	4.41	7.23	5.90	8.0	0.47	0.86
Seed width (mm)	1.95	3.29	2.71	8.7	0.23	0.88
Yield (mt ha^{-1})	1.43	5.81	3.51	25.0	0.88	0.90

SD = Standard Deviation, CV = Coefficient of Variation, H' = Notation for Shannon–Weaver diversity index.

3.2. Diversity based on qualitative characters

Shannon-Weaver diversity indices, frequencies, and proportions are shown in Table 2 for each qualitative trait. The studied genotypes of wheat have higher diversity for the qualitative traits, as evidenced by the diversity index (H') from 0.63 to 0.85 with a 0.76 average value. In terms of qualitative characteristics, 75% of landrace plants had higher tillering capacity, 54% had red seeds, 59% had intermediate seeds, 48% had lax-type spike density, and 59% had awns. They also had white glumes, 67% had hairy glumes, 54% had white glumes, and 54% had red seeds. Qualitative characters are traits that are controlled by one or a few genes and exhibit clear-cut phenotypic differences. These traits are often important for determining important agronomic traits such as end-use quality and consumer preferences. Therefore, the diversity of qualitative traits in wheat can be useful in a practical wheat breeding program, as these traits can be manipulated through controlled crosses and selections.

The awning characteristic in wheat, which contributes to spike photosynthesis, is crucial for increasing yield potential as the amount of assimilate produced and primarily derived from the spike, flag leaf, and leaf sheath, also determines the grain yield of crops (Borner et al., 2005). According to Rebetzke et al. (2016) awns participate in photosynthesis when the canopy assimilation is limited due to water scarcity and Bruening (2019) suggests that awned-type genotypes offer protection benefits, recommending them to farmers who face wildlife problems such as deer damage when choosing a wheat variety. Some wheat characteristics can serve as field markers to identify desirable traits, making it more practical to select Glu-B3 alleles that contain elite lines by observing their 'glume color' in the breeding field instead of using DNA-MAS, which require laboratory procedures (Kiyoshi et al., 2011). The Glu-B3b and Glu-B3g alleles are becoming increasingly prevalent due to their association with bread-making quality (Si et al., 2013). Moreover, the breeding program can utilize the findings of the current study on the glume color of the relevant genotypes.

The function of glume hairiness in wheat is a topic of debate, but some researchers suggest that it plays a role in enhancing crop resilience and disease resistance. Warham (1988) suggests that hairy glumes may confer resistance to Karnal bunt disease. Studies by Reynolds et al. (1999), Skovmand et

al. (2003), and Trethowan et al. (1998) have demonstrated that leaf pubescence can improve drought tolerance and cold tolerance in wheat.

Grain traits such as shape and size play a crucial role in determining wheat quality and yield, as they are linked to the crop's initial vigor (Kehel et al., 2020). Aparicio et al. (2002) reported that seed size has a direct impact on the growth of the first two leaves, which in turn affects how emergence and development of seedlings. The color of wheat seeds may also varies depending on consumer habits and preferences for the final product. Pre-harvest sprouting is less likely to occur in colored grains; this may be due to the pleiotropic effect within these traits or the genetic linkage between them (Groos et al., 2002). The majority of our landraces have colored grains due to the beneficial effects of color genes.

In our study, young plants with an upright growth habit were more prevalent (149 out of 202) than those with a prostrate habit. The same situation is described by Laino et al. (2015), who discovered that landraces with prostrate growth habits were less common than those with erect habits in their study. Overall, the diversity of qualitative characteristics in wheat is an important resource for wheat breeding, as it can help to improve the performance, quality, and adaptation of wheat varieties, making it a valuable tool for breeders to achieve their breeding objectives.

Table 2. S-W diversity index (H′), phenotypic class and their frequency and proportion for eight qualitative traits

Characters	H'	Observed phenotypic class	Frequency	Proportion %
Awnedness	0.85	Awnless	120	59
		Awnletted (short awns)	26	13
		Awned (Conspicuous awns)	56	28
Glumes colour	0.80	White	109	54
		Red to brown	80	40
		Purple to black	13	6
Glumes hairiness	0.78	Absent	136	67
		Low	31	15
		High	35	17
Tillering capacity	0.82	Low	52	26
		High	150	74
Seed colour	0.63	White	93	46
		Red	109	54
Seed size	0.76	Small	32	16
		Intermediate	119	59
		Large	44	22
		Very large	7	3
Spike Density	0.65	Very lax	12	6
		Lax	96	48
		Intermediate	83	41
		Dense	11	5
Growth habit of young plant	0.83	Upright	149	74
		Prostrate	53	26

3.3. Principal component analysis (PCA)

The genetic diversity of the studied genotypes was demonstrated by using PCA. The PCA can be used to quantify the independent influence of specific characteristics on overall variance, and each proper vector's coefficient illustrates the involvement of individual variables that every principal component is connected to each other (Nachimuthu et al., 2014). The better they are at separating the landraces, irrespective of sign, the higher the value. The distribution of landraces along the axes was quantified using quantitative characters' principal components 1 and 2, and the level of phenotypic variation in the collection was shown (Figure 4).

The total variance (68%) of the quantitative traits comes under the initial five Principal components with eigenvalues >1 (Table 3), demonstrating that the identified traits within the axes had a significant impact on the quantitative parameters of the genotype under study. Thousand-grain weight, seed length, spike length, yield, and seed width are the traits responsible for this variation in the first PC which covered 29% total variance. The second PC in which days to heading and flowering, the number of seed per spike, and flag leaf width were the primary determinants that cover 15 variances. Likewise,

9% total variance accounted for the third PC, which is affected by both the number of spikes/m^2 and the number of seed/spikes.

Among the qualitative traits, the first four PCs explained 67% of the total variance with less than 1 eigenvalue, indicating a significant impact of the identified traits on the genotypes under study (Table 4). The first principal component, which comprises 25% of the overall variation, was made up specifically of awareness and seed size. Important traits like spike density, glume color, and glume hairiness made up the second component, which had a 15% variance. The third variable, which covered 14% overall variation, was composed of seed color as well as tillering capacity. Growth habit, the last variable, accounted for 13% of the variance.

Table 3. Principal component analysis for sixteen quantitative characters of wheat landraces

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	4.60	2.43	1.42	1.37	1.17
Proportion	0.29	0.15	0.09	0.09	0.07
Cumulative variance (%)	0.29	0.44	0.53	0.61	0.68
Days to emergence	0.07	0.07	-0.29	-0.31	0.18
Days to heading	-0.13	0.51	-0.32	0.08	0.12
Days to flowering	-0.17	0.48	-0.36	0.12	0.12
Days to maturity	-0.16	0.02	-0.22	-0.34	-0.03
Plant height	-0.27	-0.12	-0.09	0.27	0.16
Flag leaf length	0.13	0.05	-0.04	0.09	0.60
Flag leaf width	0.13	0.25	0.03	0.20	0.14
Number of spike m^{-2}	0.01	-0.05	0.40	-0.20	0.66
Spike length	0.33	0.19	-0.09	-0.39	-0.02
Number of seed/ spikelets	-0.22	0.38	0.28	0.02	-0.13
Number of seed/ spikes	-0.08	0.41	0.50	-0.03	-0.23
Awn length	-0.30	0.14	0.00	-0.49	-0.11
Thousand-grain weight	0.41	0.12	0.10	0.18	0.03
Seed length	0.39	0.09	0.03	0.11	-0.02
Seed width	0.33	0.13	0.13	0.29	0.00
Yield	-0.37	0.11	0.31	-0.28	0.15

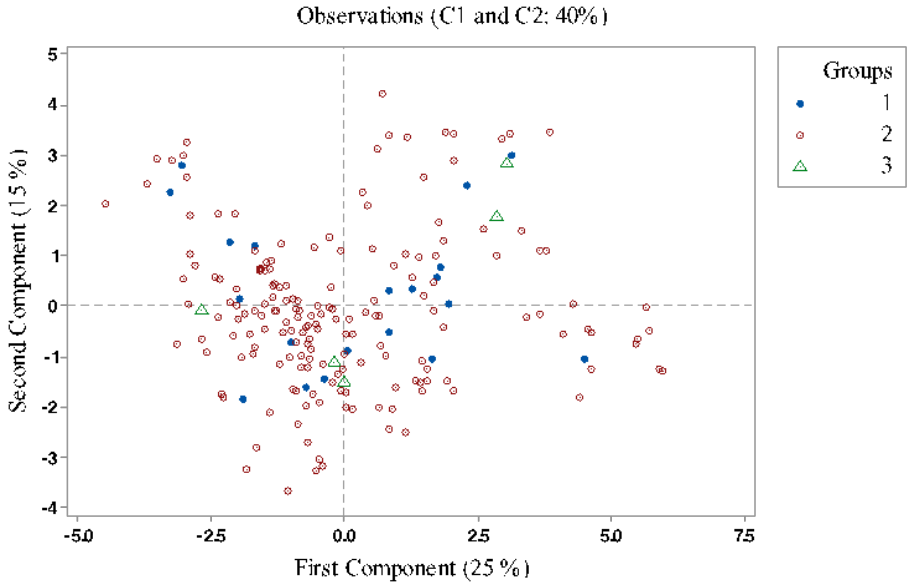

Figure 4. First two Principal Component Analysis (PCA) plots of the wheat landraces based on the 16 quantitative characters.

Table 4. Principal component for eight qualitative characters of wheat landraces

Qualitative traits	PC1	PC2	PC3	PC4
Eigenvalue	2.03	1.17	1.15	1.04
Proportion	0.25	0.15	0.14	0.13
Cumulative	0.25	0.40	0.54	0.67
Growth habit	0.03	0.22	0.23	0.88
Tillering capacity	0.25	0.14	0.61	0.08
Spike density	0.39	0.44	0.29	0.01
Awnedness	0.47	0.01	0.00	0.07
Glume colour	0.07	0.61	0.48	0.10
Glume hairiness	0.37	0.45	0.04	0.42
Seed colour	0.43	0.22	0.44	0.10
Seed size	0.49	0.35	0.27	0.17

3.4. Cluster analysis

Based on the estimated relatedness or kinship, a dendrogram was produced using UPGMA. The dendrogram is shown in Figure 5. The wheat landraces could be divided into five clusters. According to cluster analysis, there are differences in how different landraces are grouped based on agro-morphological traits. The reresulting dendrogram revealed five distinct groups: Groups I, IV, and V are comprised of 19 accessions (9%) in each, group II of 55 accessions (27%) and largest group III consists of 90 (45%) accessions. The descriptive statistics of the distinct cluster are presented in Figure 5. Based on theresults of the cluster analysis, cluster-I was determined to be superior in quantitative characteristics when compared to the other cluster. On the basis of cluster analysis, Cluster-I was found superior in terms of quantitative character as compared to the other clusters. Cluster-I includes landraces having higher mean values for the number of seeds per spikelets, number of seeds per spike, and yield but lower mean values for flag leaf length and width, number of spikes per square meter, spike length, and seed length. On the other hand, Cluster II landraces have higher mean values for flag leaf length and width, but lower mean values for the number of seeds pers pikelet and total yield but moderate mean values for other traits. Likewise, Cluster-III has a higher mean value for the number of spikes per square meter and a moderate mean value for all remaining traits. Cluster-IV has higher values for days to maturity and plant height, but lower values for the number of seeds per spike, Awn length, thousand-grain weight and seed width while Cluster-V includes landraces with higher values for spike length, awn length, thousand-grain weight and seed width, but lower values for days to maturity and plant height (Table 5). Using cluster analysis, Singh and Dwivedi (2002) also reported the genetic divergence among the tested wheat genotypes. These materials that have been identified can serve as candidates for selective breeding programs aimed at developing traits to interest in the future. It is likely that the crosses between members of clusters separated by inter-cluster distances would be advantageous for further improvement (Yatung et al., 2014).

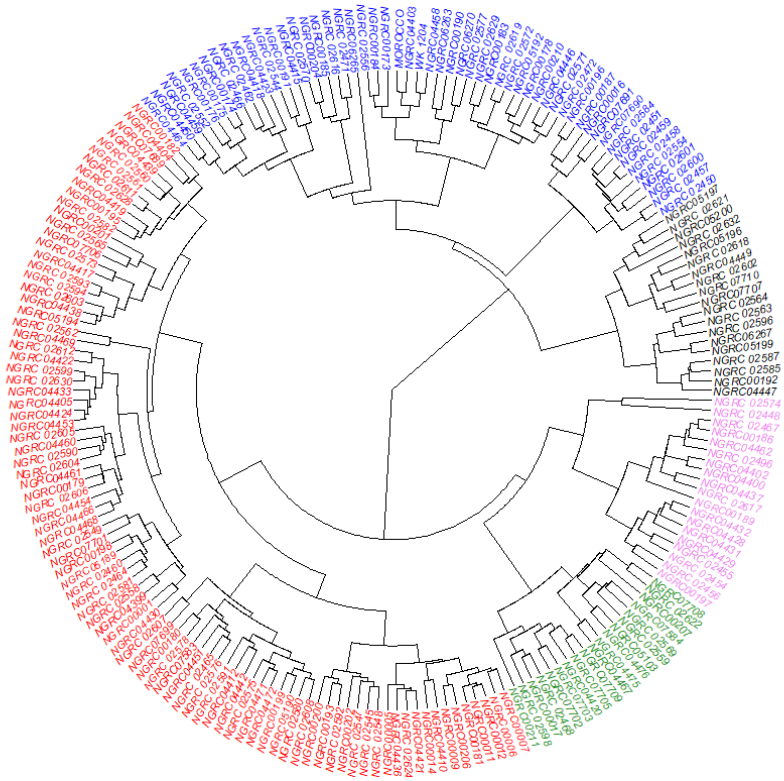

Figure 5. Dendrogram of 202 bread wheat landraces by UPGMA cluster analysis.

Table 5. Descriptive statistics of quantitative traits within clusters of 202 wheat genotypes

Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
No of landraces	19	55	90	19	19
Days to Emergence	11±1.86 (4-13)	12±0.5 (11-13)	11±0.5 (10-13)	12±0.4 (11-13)	12±0.5 (11-13)
Days to Heading	115±4.04 (110-121)	110±3.8 (104-119)	111±3.3 (104-118)	116±3.7 (111-123)	116±2.1 (112-121)
Days to Flowering	122±3.15 (117-128)	117±4.0 (110-124)	118±3.1 (109-125)	123±3.0 (120-129)	122±1.9 (117-125)
Days to Maturity	157±5.02 (148-166)	153±7.4 (139-169)	153±6.3 (139-169)	161±5.4 (151-168)	152±6.9 (141-165)
Plant height (cm)	126.0±8.1 (110.5-143.9)	119.6±7.4 (100.8-136.5)	124.4±8.1 (109.9-173.2)	127.3±6.3 (115.1-135.4)	107.7±5.3 (97.6-117.0)
Flag leaf length (cm)	16.1±2.01 (13.2-20.7)	18.4±4.5 (13.4-45.5)	16.4±2.4 (12.1-25.4)	17.8±2.5 (14.2-23.1)	17.3±3.1 (10.8-22.7)
Flaf leaf width (cm)	1.2±0.13 (1.0-1.4)	1.4±0.3 (0.8-2.5)	1.2±0.2 (0.8-1.6)	1.2±0.1 (1.0-1.4)	1.4±0.3 (1.0-1.9)
No of Spikesm^{-2}	349±33.65 (280-415)	367±41.5 (223-440)	374±38.0 (223-441)	360±40.8 (250-424)	369±53.9 (223-450)
Spike Length (cm)	8.4±1.27 (6.9-12.5)	11.2±2.0 (7.1-14.7)	8.5±0.8 (6.9-11.3)	11±1.9 (8-14)	13.3±0.8 (11.8-14.8)
Number of seed per spikelets	9.6±0.87 (8.5-12.3)	8±0.5 (7-9)	8.4±0.4 (6.7-9.3)	8±0.7 (7-10)	9±0.5 (8-10)
Number of seed per spike	40±4.21 (33-49)	31±2.5 (27-40)	32.4±2.8 (25.8-38.4)	28±2.9 (25-33)	38±4.7 (31-45)
Awn Length (cm)	3.1±0.98 (2.1-4.1)	4.7±0.8 (2.8-6.0)	1.9±1.3 (0.5-5.3)	2.4±1.2 (0.5-4.8)	4.7±0.7(3.0-5.6)
Thousand Grain Weight	30.2±3.80 (22.0-36.0)	43.6±7.7 (29.7-56.1)	32.8±3.5 (24.8-39.9)	25.7±5.4 (15.4-34.7)	44.3±4.5 (35.5-51.7)
Seed length (mm)	5.6±0.21 (5.2-6.0)	6.3±0.5 (5.6-7.2)	5.7±0.3 (5.0-6.5)	5.6±0.4 (4.4-6.3)	6.3±0.4 (5.5-7.2)
Seed width (mm)	2.6±0.14 (2.2-2.8)	2.9±0.2 (2.4-3.3)	2.7±0.2 (2.2-3.1)	2.4±0.2 (1.9-2.7)	2.9±0.2 (2.5-3.2)
Yield (mtha^{-1})	4.7±0.62 (3.7-5.8)	2.7±2.7 (1.4-3.8)	3.7±0.6 (2.3-5.4)	4.0±0.7 (2.6-5.3)	3.2±0.9 (1.9-4.8)

Conclusion

The cluster analysis as well as principal component analysis(PCA) showed the presence of genetic diversity in the evaluated landraces. Overall, this study offers a novel insight into the genetic diversity of wheat landraces in Nepal, which may support efforts to improve wheat breeding programs.

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' contribution statement

AjayaKarkee: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Ram Prasad Mainali, Krishna Hari Ghimire, Pradip Thapa, Bal Krishna Joshi, SudeepSubedi, and Jiban Shrestha: Contributed data analysis, Reviewed the initial draft of the manuscript.

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Research Article

Evaluation of Some Sorghum (*Sorghum bicolor* L.) Genotypes by Principles Component Analysis

Mehmet ÖTEN*[1], İlker İNAL[2], Semiha KİREMİTCİ[3]

[1]Department of Field Crops, Faculty of Agriculture, Sakarya University of Applied Sciences, Sakarya, Türkiye
[2]Department of Field Crops, Eastern Mediterranean Agricultural Research Institute, Adana, Türkiye
[3]Department of Field Crops, West Mediterranean Agricultural Research Institute, Antalya, Türkiye

[1]https://orcid.org/0000-0002-3737-2356, [2]https://orcid.org/0000-0002-5891-8004, [3]https://orcid.org/0000-0001-5533-0849

*Corresponding author e-mail: mehmetoten@subu.edu.tr

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Abstract: To improve sorghum productivity, farmers need high-yielding sorghum cultivars. A field experiment was conducted in the 2020 cropping season to determine the interaction effect of genotypes in Adana and Antalya locations in Turkey. Two lines plus three checks cultivars were evaluated at both locations in Turkey. The analysis of variance showed highly significant (P≤0.01) differences among the genotypes for all traits. The analyzed result indicates that the genotypes gave a higher yield in the Antalya location in terms of forage yield. The lowest forage yield was obtained from Line 2 and Line 1 with 4735.7 and 6212.9 kg da^{-1} in Adana, while the highest forage yield was recorded from all genotypes in Antalya. Moreover, forage yield and plant stalk ratio were positively and significantly associated with hay yield. The first two principal components (PC) accounted for 86.42% of the total genotypic variation.

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1. Introduction

The population of Turkey is increasing rapidly, as in the whole world. Increasing urbanization in parallel with population growth is another factor that causes the decrease in pasture areas. Global climate change and the change in precipitation regimes have caused approximately 70-75% of Turkey's rangeland to be located in semi-arid and arid regions. In these regions, yield potential has decreased considerably due to insufficient or no summer precipitation and limited irrigation facilities. In order to meet the need for roughage, it is necessary to open new rangelands or to increase the forage crop ratio and production in field crops. It has been demonstrated by many scientific studies that it is a necessity to choose the plants that will be included in the product pattern from the plants that can provide the highest yield per unit area and that have the least water consumption due to global climate change. Sorghum is the most produced plant after corn among the hot climate cereals, which is used both for human and animal nutrition, for alcohol production, and as a renewable energy source. Sorghum cultivation is carried out in an area of approximately 40 074 667 ha in the world (FAO, 2021). Sorghum can be easily grown in areas that are not ideal for corn cultivation. The most common types of sorghum, which have many types suitable for their intended use, are grain and silage. The stem and leaf surfaces

of sorghum, which is a typical C4 plant, are covered with a waxy layer, which helps the plant to benefit more from daylight in hot periods and to increase the rate of photosynthesis. Besides, sorghum has a high tolerance to drought and soil salinity (Avcioglu et al., 2009). For this reason, its cultivation as a second product is increasing in our regions where irrigation opportunities are relatively low in Turkey. In this regard, some studies have been carried out with different genotypes in different locations. In the experiment conducted by Acar et al. In 2002 in the fields of Konya Livestock Research Institute, with five sorghum-sudangrass hybrids (Elrey, Gmss, Grazer, Jumbo, and Sweet), the average plant heights in two harvests were 215.53 cm (Sweet) and 231.02 cm (Jumbo), dry matter yield was found between 4 486.8 kg da^{-1} (Grass) and 5 745.2 kg da^{-1} (Jumbo) and total green herbage yield was between 14641.3 kg da^{-1} (Grass) and 19038.7 kg da^{-1} (Jumbo). In the study by Cakmakci et al. (1999), which was carried out in the West Mediterranean Agricultural Research Institute (Antalya) trial area for 2 years to investigate the effects of different cutting cycles on yield and quality; Rox variety was harvested at five different developmental stages and it was concluded that it is appropriate to harvest silage sorghum in the yellow maturity stage in Antalya region. In another study conducted by Guneş and Acar in 2005, four sorghum cultivars (Grazer, El Rey, Grass II, Jumbo) were used as material in order to determine the cultivation possibilities of silage sorghum varieties as a second product under irrigated conditions in the Nursery Field of Karaman Province Directorate of Agriculture and their green yields were 6483.73 kg da^{-1} (Grazer) and 7 671.23 kg da^{-1} (Jumbo), dry matter yields were between 2 093.50 kg da^{-1} (Grazer) and 2 321.40 kg da^{-1} (Jumbo), crude protein ratios were between 4.41% (Grazer) and 5.15% (El Rey). All sorghum cultivars included in the experiment were determined as cultivars that could be grown as a second crop.

The correlation, determine the correlation between variables and measures the power of the linear relationship between two variables (Kumar et al. 2022).. Also Correlation is of great importance in evaluating the most effective characters in the selection of superior yielding genotypes. When the main characters to be used in selection are positive, selection will be very effective in terms of breeding, if it is negative, it will be difficult to make simultaneous selection (Nemati, et al. 2009).

Statistical analysis of multivariate methods has wide use in summarizing the genotypes. The principal component analysis is an important multivariate analysis method (Oyelola, 2004). PCA is a technique for increasing interpretability with this minimizing information loss. The principal component analysis reduces to solving an eigenvalue and new variables are defined by the dataset, consequently, PCA is an adaptive data analysis technique (Jolliffe and Cadima, 2016).

The current study aimed to determine by correlation and principal component analysis, some sorghum genotypes that are suitable for both regions, with high yield and quality in Antalya and Adana locations.

2. Material and Methods

Two experiments were carried out at the West Mediterranean Agricultural Research Institute's (Altitude 30 m above sea level, average annual rainfall 963.4 mm and the average temperature is 19.03°C) field area in Antalya and Eastern Mediterranean Agricultural Research Institute (Altitude 12 m above sea level, average annual rainfall 757.4 mm and the average temperature is 19.1°C) in Adana in 2020. The soil of the research area was clay-loam-silt in texture, non-saline and rich in calcium carbonate, and slightly alkaline in pH, in Adana. The land of the research area of Antalya had silty-clay loam soil and contained adequate organic matter.

Two candidate varieties of sorghum genotypes (Line 1 and Line 2) along with the tree registered varieties (Erdurmus, Uzun, and Leoti) were arranged in randomized block design with four replications. Experimental materials were sown on May 06, 2020, in Antalya and on May 20, 2020, in Adana. The plot size was 14 m^2 (4 rows, 5 meters length, and 0.7m inter-row). Data were collected for the parameters of days to 50% flowering day, plant stalk ratio, plant leaf ratio, forage yield, and hay yield. For determining green forage yield, harvested plants were weighed quickly, then for determining green forage yield, 0.5 kg fresh samples were oven-dried at 70 ºC for 48 hours until reached constant weight for estimating the dry matter content. Data on days to 50% flowering were recorded from the whole plot, but plant stalk ratio and plant leaf ratio components were recorded from randomly selected 10 plants in each plot. Statistical analysis of obtained data was done using the SPSS software version 16.0 and means were compared using Duncan's test at the $p \leq 0.05$ probability level.

3. Results and Discussion

3.1. Days to 50% flowering

Analysis of variance results showed that days to 50% flowering was significantly affected due to the main effect of variety, location ($P \leq 0.01$), and variety*location interaction ($P \leq 0.05$). The highest days to 50% flowering was recorded in Line 2 with both in Antalya (98.3 days) and Adana (95.8 days) locations (Table 1). In both locations, the lowest days to 50% flowering was obtained from the Erdurmus variety. The candidate variety Line 1 also showed a value (87.8 days) close to the Erdurmus variety (87 days). With this result, it showed an earlier performance than Uzun and Leoti varieties. Days to flowering was affected due to variety and location, moreover, it depends both on the genotypes/varieties and the environment. This could change for the same genotypes/varieties at the different locations if the planting date were changed, as we did. This is in agreement with the finding of different results of researchers (55.0-82.0 days in Saglamtimur et al., (1988) and 55.0-99.1 daysinYucel et al., (2020)).

Table 1. Performance of the varieties and lines in the two locations

Location	Variety	Days to 50% flowering (Day)	Plant leaf ratio (%)	Plant stalk ratio (%)	Forage yield (kg da^{-1})	Hay yield (kg da^{-1})
Adana	**Line 1**	87.8 de*	14.3 ab	84.7 c	6 212.9 cd	2 631.3 c
	Line 2	95.8 ab	15.3 a	84.7 c	4 735.7 d	1 361.2 d
	Erdurmus	87.5 de	11.2 c	88.9 a	11 144.5 a	4 029.7 a
	Uzun	90.5 c	15.3 a	84.7 bc	6 800.0 bc	2 740.2 c
	Leoti	90.0 cd	13.4 b	86.6 b	8 095.4 b	2 851.4 bc
Antalya	**Line 1**	87.0 e	14.4 ab	85.6 bc	10 862.6 a	3 193.4 bc
	Line 2	98.3 a	15.7 a	84.4 c	10 670.6 a	2 851.9 bc
	Erdurmus	87.0 e	14.8 ab	85.2 bc	11 162.6 a	3 501.3 b
	Uzun	93.8 b	14.5 ab	85.6 bc	10 532.3 a	3 200.6 bc
	Leoti	94.0 b	15.4 a	84.6 c	11 152.0 a	2 888.2 bc
F Probability	**Variety**	**	**	**	**	**
	Location	**	**	*	**	ns
	Variety*Location	*	**	**	**	**
CV (%)		2.0	6.8	1.5	12.0	15.0
LSD		1.28	0.58	0.77	719.89	324.47

LSD= Least Significant Difference; CV= Coefficient Variance; ns= Non Significant;*= $P \leq 0.05$; **= $P \leq 0.01$ *The means in the same column with same letters are in the same group.

3.2. Plant leaf ratio

The data obtained from the plant leaf ratio in the research are shown in Table 1. According to the statistical analysis results; variety, location, and variety*location interaction were found significant. Plant leaf ratio values ranged from 11.2% to 15.7%. Line 2 had the highest plant leaf ratio in both Adana and Antalya locations, the Erdurmus variety in the Adana location, and Line 1 at the Antalya location had the lowest value. Our results for plant leaf ratio value have broadly similar to the findings reported by İptas, (1993) with 17.7%, Hosaflioglu (1998) with 17.8%, Yılmaz, (2000) with %18.6, Acar et al., (2002) with 15.5%, and Geren and Kavut, (2009) with 16.1%. According to Kır (2014), the high plant leaf ratio is an indicator of the quality of the plant. Furthermore, the leaf/stem ratio is an important quality criterion for forage crops.

3.3. Plant stalk ratio

Among the traits studied plant stalk ratio had the highest coefficient of variation. The mean value for plant stalk ratio ranged from 84.4%-88.9%. The Line 1, Line 2 and Leoti variety had the least plant stalk ratio and the Erdurmus variety showed the highest rate. Our results are in agreement with the results by Acar et al. (2002) (74.7%), Gul and Basbag (2005) (70.7%), and Salman and Budak (2015) (77.4%).

3.4. Forage yield

There were strong genotypic differences (P<0.001) for forage yield (Table 1) in the study. Forage yield ranged from 4 735.7 kg da^{-1} to 11 162.6 kg da^{-1} averaged across locations and genotypes. Erdurmus variety had the highest forage yield with 11162.6 kg da^{-1}, whereas Line 2 had the lowest with 4 735.7 kg da^{-1}. While all the materials used in the experiment in the Antalya location were obtained from more than 10 tons of yield, it was yielded more than 10 tons only from the Erdurmuş cultivar in the Adana location. We can explain the high performance of the Erdurmuş cultivar in both locations as "the high performance of a cultivar in different ecologies shows that it is stable" (Keser et al., 1999). Especially among the cultivar candidates, Line 2 had the lowest yield with 4 735.7 kg da^{-1}. It can be said that the two weeks difference between planting dates and the annual precipitation difference between locations are effects on forage yield between locations.

Some researchers have obtained different yield values in different ecological conditions, such as; Acar et al. (2002) 14 641.3-19 038.7 kg da^{-1}, Karadas, (2008), 6 296.3-7 613.2 kg da^{-1} and Ozkose et al. (2014), 5 356.5-13 446.4 kg da^{-1} in Konya ecological condition, Yolcu (2015), 7 499.5-15 213.8 kg da^{-1} in Çanakkale ecological condition. Balabanlı and Turk (2005), 4 371.2-6 831.5 kg da^{-1} in Isparta ecological condition.

Forage yield was affected due to variety and location. This might be due to the genetic difference of the genotype in response to genotype and location also shows a significant difference in forage yields.

3.5. Hay yield

In the current research, the highly significant effect of genotypes on hay yield was determined. The varieties also differentiated on the basis of locations in terms of the hay yield, similar to the forage yield. However, the difference between varieties in terms of hay yield was found to be insignificant between locations (Table 1). While all the varieties were in the same group in the Antalya location, the lowest hay yield was obtained from Line 2 with 1 361.2 kg da^{-1} in the Adana location. Erdurmus variety had the highest hay yield with 4 029.7 kg da^{-1} in the Adana location, whereas Line 2 had the lowest hay yield with 1 361.2 kg da^{-1} in the Adana location. The reason for the difference between the two locations in terms of hay yield of the Erdurmus cultivar may be that it was planted late in the Adana location and therefore the harvest date was delayed. This result is consistent with Avcıoğlu et al., (2000), who reported that dry matter yield increased with the advancement of harvest time in forage crops.

Similar results were obtained by Acar et al. (2002) 4 486-5 745 kg da^{-1}, Gunes and Acar (2005) 2 093.5-2 321.4 kg da^{-1}, Karadas (2008) 1 908.9-2 343.4 kg da^{-1} and Salman and Budak (2015), 5 210.3 kg da^{-1}.

3.6. Correlation analysis

The correlations among parameters are presented in Table 2. In the present investigation, correlation coefficients were worked out among five characters. Results of correlation analysis indicated that the hay yield was found to be significantly correlated with all characters. However, days to flowering was negatively and significantly correlated with stalk ratio forage yield and hay yield. Furthermore, plant leaf ratio was also negatively and significantly correlated with stalk ratio forage yield and hay yield. Hay yield showed a strong correlation with these traits, as well as with flowering days and plant leaf ratio negatively, and positively with plant stem ratio and forage yield.In many previous studies conducted on sorghum, positive and negative correlations were found among the investigated traits (Moyer et al., 2003; Khandelwal et al., 2015; Abate, 2016; Mesfin, 2016; Temesgen, 2018).

Table 2. The correlation coefficient of the characters

Parameter	Days to 50% flowering (Day)	Plant leaf ratio (%)	Plant stalk ratio (%)	Forage yield (kg da^{-1})	Hay yield (kg da^{-1})
Days to 50% flowering	1.0000	0.4590**	-0.3909*	-0.1393ns	-0.4582**
Plant leaf ratio		1.0000	-0.9400**	-0.1290ns	-0.5535**
Plant stalk ratio			1.0000	0.1717ns	0.5193**
Forage yield				1.0000	0.6962**
Hay yield					1.0000

3.7. Principal component analysis

In the present study, the first two eigenvectors have eigenvalues greater than one and cumulatively explained about 86.42% percent of the total variation among genotypes of sorghum (Table 3 and Figure 1). Therefore, PC 1 had an eigenvalue of 3.26 and accounted for 65.21 % of the variations. This represented an equivalent of five variables (Days to 50% flowering and plant leaf ratio negative correlation, plant stalk ratio, forage, and hay yield positive correlation) and indicated that were important contributing variables for the variation among the genotypes. This study shows that the PCA analysis is able to identify a few key traits that accounted for the largest variability in genotypes of sorghum. The aim of the PCA is to get more important information from the available data, simplify the characterization of the data, and analyze the structure of the observations on the variables. Ozdamar (2010) reported that to determine the appropriate number of principal components in PCA, components with an eigenvalue greater than one, or principal components that could explain at least 67% of the total variance should be considered. Additionally, Gozen, (2008) emphasized that eigenvalues greater than one are considered significant and component loading greater than 0.3 are considered to be meaningful. PCA is used as an effective size reduction method in multivariate data sets.

Table 3. Principal component analysis

Principal Components	**PC1**	**PC2**
Eigenvalue	3.26	1.06
Total Variance (%)	65.21	21.21
Cumulative Variance (%)	65.21	86.42
Factor loading by parameters		
Days to 50% flowering	-0.663	0.389
Plant leaf ratio	-0.906	0.329
Plant stalk ratio	0.912	-0.199
Forage yield	0.554	0.817
Hay yield	0.928	0.306

The maximum number of principal components that can be obtained in PCA is equal to the original number of variables. However, analysis of results is usually interpreted taking into account the first two or three basic components, not all components. Thus, most of the variation in the original variables can be explained by the first two principal components. In this way, the original variables can be provided that are largely summarized and easier to interpret (Demir et al., 2016). The present study is supported by earlier results by Ali et al. (2011) and Abraha et al. (2015).

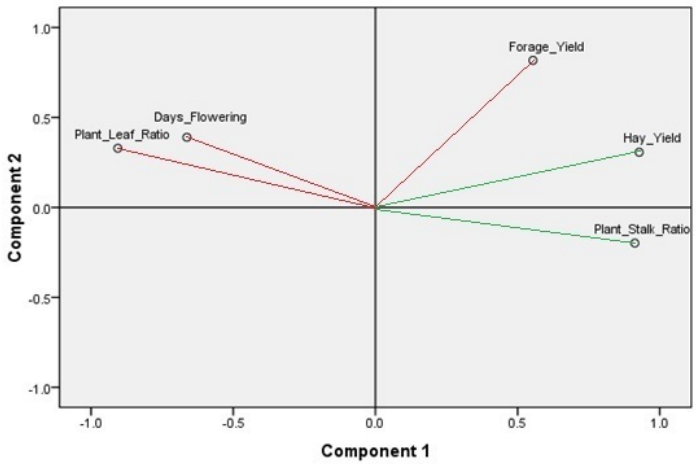

Figure 1. Grouping of studied sorghum traits based on factor analysis under variety*locations.

Conclusion

Despite its superior characteristics, sorghum does not receive the attention it deserves in Turkey. Variety candidates that have come to the forefront as a result of the breeding program carried out for

years, were tested in different locations and the differences in yield between locations have been identified. Variety candidates Line 1 showed similar characteristics with cultivars in terms of earliness and forage yield. Besides results of correlation analysis indicated that the hay yield was found to be significantly correlated with all characters. As a result of the principal component analysis used to define the variation, it was determined that 2/3 of the variance was defined in the first two principal components and the most important descriptive element was the yielding character.

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Research Article

Effects of Quince Rootstocks and Pear Cultivars on Fruit and Yield Characteristics

Tahsin KURT[1], Ahmet ÖZTÜRK*[2], Zaki Ahmad FAIZI[3], Yakup POLAT[4]

[1,2,3]Horticulture Department, Faculty of Agriculture, University of Ondokuz Mayıs, 55200 Samsun, Türkiye
[4]Horticulture Department, Faculty of Agriculture, University of Van Yuzuncu Yil University, Van, Türkiye

[1]https://orcid.org/ 0000-0002-1574-4083, [2]https://orcid.org/0000-0002-8800-1248, [3]https://orcid.org/0000-0002-1429-6493
[4]https://orcid.org/0000-0002-5831-8199

*Corresponding author e-mail: ozturka@omu.edu.tr

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Fruit breeding,
Marketable rate,
Pyrus communis,
Yield efficiency

Abstract: The research carried out to evaluate the effects of quince rootstocks [Quince Province BA29 (BA29), Quince A (QA), Quince C (MC)], pear cultivars, and research years on fruit quality and yield efficiency in the years 2020-2021. The highest fruit weight was obtained from BA29 (196.02 g), and the lowest was from MC (158.09 g). In the cultivars, the highest fruit weight was obtained from 'Abate Fetel' (210.85 g), the lowest from Santa Maria (156.73 g). The highest number of fruits (17.06 pieces tree^{-1}), yield per tree (3.13 kg tree^{-1}), yield per hectare (5982.8 kg ha^{-1}), and yield per trunk cross sectional area (0.30 kg cm^{-2}) obtained from BA29 followed by QA. In the cultivars, the highest number of fruits (19.60 pieces tree^{-1}), yield per tree (2.98 kg tree^{-1}), and yield per hectare (5685.00 kg ha^{-1}) were obtained from 'Santa Maria'. In the research years, the pre-harvest fruit drop rate (PHFDR 11.04%) and black spotted fruit rate (BSFR 13.79%) were observed to be higher in 2021, while the marketable fruit rate (MFR 77.03%) was observed to be higher in 2020. In the rootstocks, the highest PHFDR (11.24%) was observed on BA29 rootstocks, while the highest MFR (73.72%) was recorded on QA. In terms of cultivars, the highest PHFDR (10.73%) was observed in 'Williams', while the highest BSFR (16.41%) was in 'Deveci', and the highest MFR (76.31%) in 'Santa Maria'. As a conclusion, the highest yield and marketable fruit rate were obtained from the 'Santa Maria' cultivar and yield from BA29 rootstock. It could be suggested that semi-dwarf cultivars and rootstocks for suitably perform under high density pear orchards.

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1. Introduction

Pear is an important temperate fruit due to a great degree of adaptation to various climatic situations (Bhat et al., 2017). In 2021 world pear production recorded 25.7 million tons, 18 million tons of the production carried out by China followed by America, Argentina, and Türkiye in the 4th position (FAOSTAT, 2023). Türkiye's pear production in 2022 reported 551 086 tons (TSI, 2023). In modern orchards, pear cultivars are grafted on quince rootstocks to obtain more dwarf trees than those grafted on pear rootstocks (Ozturk and Faizi, 2022). Also, quince is recommended as rootstock for pear to come up with precocity, increasing fruit quality, and facilitating cultural processes (Francescatto et al., 2010). Pear orchards with around 2000 - 5000 trees h^{-1} can be established with the use of quince rootstocks

(Pasa et al., 2012; Jovanovic et al., 2022), and cause high yield per area as they own higher photosynthetic efficiency (Ladaniya et al., 2020), due to the enhanced utilization of solar energy, nutrients, and water (Ladaniya et al., 2021). Arbitrary conditions like temperature, humidity, and less sunshine in dense pear orchards could rally undesirable pest incidence in the orchards (Poornima et al., 2018). The long viability of trees is an unsolved problem of pear orchards grafted on quince rootstocks (Musacchi et al., 2021). It is possible to increase quality with quince rootstocks usage in pear cultivation, as cultivars on quince rootstocks produce less fruit per canopy volume and yield per area could be increased as possible to use about 8000 trees ha^{-1}. But economic life of the orchards with quince rootstocks was reported low (Zhang et al., 2016; Musacchi et al., 2021). The research aimed to consider the quince rootstocks, pear cultivars, and research years' main factors impact on fruit and yield characteristics during two consecutive research years 2020-2021.

2. Materials and Methods

2.1. Materials

In the research, 'Abate Fetel', 'Deveci', 'Santa Maria', and 'Williams' were used as cultivars, and BA29, MC, and QA as rootstocks. The research was done in 2020-2021. The experimental pear orchard was planted in 2018 in the fruit research station of Ondokuz Mayis University (altitude 20 m, 35° 52' 21'' E; 41° 33' 50'' N). The research area has a cool climate in winters, a hot & humid climate in summers, and precipitation mostly occurs in late autumn as well as early winter. The experimental area has an average temperature ranging between 3.3 to 26.2 °C (TSMS, 2022). The properties of experimental area soil were recorded as 2.73-10% clay (low), 13.21-20% silt (moderate), 6.5-20% sand (moderate), pH 7.5 (slightly alkaline), 0.2-0.3 dS m^{-1} salt (no salt), 0.3-0.5 organic matter (low), 3-6% lime ($CaCO_3$) (less), 0.03-0.06% N (less), 5-10 ppm P (medium) level and the soil depth was more than 1 meter. The plants were irrigated with drip irrigation between 15 May to 15 September. Fertilization was done with 15-30-15+ME fertilizer at the beginning of summer and 20-20-20 NPK-containing fertilizer in autumn with drip irrigation.

2.2. Methods

At 3.5 m x 1.5 m distances (1910 tree ha^{-1}) trees were planted and pruned according to the modified leader system. Young planted trees were supported by a supporting system of metal poles against the wind as well as tie up and bending of branches to prevent breaking at the yielding age. For this purpose, 3 rows of wire were tied to the poles at 0.5, 1.0, and 1.5 m from the ground. The trees' irrigation was done regularly with pressure compensating drippers at 1.20 m intervals, with 2 pipes per row on both sides of the trees. Also, weed control and pruning were done regularly in the experimental orchard.

2.3. Observations

Observations were done according to (Ozturk and Ozturk, 2014; Ozturk et al., 2022). The weight of fruit (g) was measured, taking 30 fruits into consideration in each replication with the help of 0.01 g sensitive digital balance (CAMRY L-500). Fruit width and length (mm) along with the fruit stalk length and thickness (mm) determined with a 0.01 mm digital caliper (Mitutoyo CD-20CPX). Fruit skin color properties (L*, a*, b*, chroma, and hue angle) were determined by colorimeter (Minolta, CR-300; Japan) as explained by Erdem and Ozturk (2012). Fruit firmness (kg cm^{-2}) was evaluated with a hand penetrometer (EXTECH FHT 200- with 5/16 head). The total soluble solids content (%) was determined with a digital refractometer (ATAGO, PAL-1), and acidity (%) observed by using the colorimetric method (Ozturk and Faizi, 2022). The number of fruits (pieces $tree^{-1}$), yield per tree (kg), yield per hectare (kg), yield per trunk cross sectional area (kg cm^{-2}), and yield per canopy volume (kg m^{-3}) were recorded. Pre-harvest fruit drop rate (%) was calculated by counting the total number of fruits that existed on the tree around one month before the harvest, then once a week the number of dropped fruits was counted and divided by the number of fruits that were recorded at the beginning. The rate of black spotted fruits (%) was determined by counting the number of fruits with infection in each replication and was expressed as percentage. Marketable fruit rate (%) was calculated by separating the unsound

fruits (over black spotted, fruits with *monilia*, fruit with worms and malformed) from healthy fruits in each replication and expressed as a percentage.

2.4. Data analysis

The research employed the Randomized Block Design (RBD) methodology with three replications and ten trees in each replication. The number of fruits used in each replication was 30 for pomological and biochemical investigation. IBM SPSS 21.0 was used to evaluate the data when they were collected (SPSS Inc. Chicago, ABD). In the case of ANOVA, the significance mean comparison with Duncan Multiple Comparison Test (DMRT) was calculated at a 5% level of significance ($p \leq 0.05$).

3. Results and Discussion

3.1. Pomological characteristics

Variance analysis of rootstocks, cultivars, and research years' effects on pomological characteristics of some standard pear cultivars on different quince clonal rootstocks were given in Table 1. Fruit width, fruit stalk length, fruit stalk thickness in the rootstocks, and fruit stalk thickness in the research years were found to be not significant. In the rootstocks, the highest values were observed on BA29 rootstocks, while the lowest were on MC. But in some properties, QA and MC showed the same values. In the cultivars, the highest fruit weight (F wt.), fruit length (FL) and fruit stalk thickness (FST) were observed on 'Abate Fetel' cultivar. While the highest fruit width (F wdt.) was observed on 'Deveci' cultivar.

Table 1. Effects of quince rootstocks, pear cultivars and research years on pomological characteristics

Main Effects		Weight of Fruit (g)	Width of Fruit (mm)	Length of Fruit (mm)	Length of Fruit Stalk (cm)	Thickness of Fruit Stalk (mm)
Rootstocks	**QA**	187.83 a*	63.99 a	89.14 b	14.64 a	4.15 a
	BA29	196.02 a	65.00 a	89.54 a	16.90 a	4.44 a
	MC	158.09 b	62.52 a	83.03 c	15.85 a	4.20 a
Cultivars	**Deveci**	188.16 b	68.68 a	67.74 d	15.13 b	3.77 b
	Williams	166.86 c	67.52 a	75.48 c	15.89 b	4.49 a
	Santa Maria	156.73 c	59.31 b	86.53 b	20.31 a	4.32 ab
	Abate Fetel	210.85 a	59.84 b	119.20 a	11.87 c	4.49 a
Years	**2020**	201.64 a	65.84 a	93.57 a	13.68 b	4.29 a
	2021	159.65 b	61.84 b	80.90 b	17.92 a	4.24 a
Significance						
Rootstocks		0.001	0.185	0.005	0.162	0.450
Cultivars		0.001	0.001	0.001	0.001	0.038
Years		0.001	0.001	0.001	0.001	0.854

* Means shown with different letters in the same column are statistically significant.

Fruit size in pears is a considerable factor in marketing (Ozturk and Faizi, 2022). Ideal rootstock selection for pear is a necessity in increasing the average fruit size for each cultivar (Pasa et al., 2017; Askari et al., 2019). Kucuker et al. (2015) recorded that the weight of fruit varied according to cultivars and research years. And they reported that fruit weight in 'Santa Maria' grafted on BA29 ranged from 147.5 to 169.4 g. Erdem and Ozturk (2012) reported 140.00-156.20 g. Lepaja et al. (2014) reported a fruit width of 61.18 to 81.86 mm and a fruit weight of 183.00 to 290.00 g. 'Santa Maria' fruit weight on different rootstocks in semi-arid conditions was reported 265.49 to 290.37 g (Ikinci et al., 2014). In calcareous soil and semi-arid conditions, Ikinci et al. (2016) reported a fruit weight of 304.1 g. Jovanovic et al. (2022) reported weight of the fruit was 188.4 g, the fruit length 8.8 cm, and the fruit width 6.5 cm in 'Santa Maria' pear cultivar. Fruit stalk length and thickness observed respectively, 31.54 to 32.56 mm; 3.94 to 4.75 mm in 'Deveci' grafted on BA29 by Uysal et al. (2016) and 11.1 to 14.2 mm; 4.6 to 5 mm in 'Abate Fetel'grafted on QA by Ozturk et al. (2016). Pomological results of our study are generally in agreement with previous studies.

3.2. Fruit skin color characteristics

Variance analysis of rootstocks, cultivars, and research years on fruit skin color were given in Table 2. All the color characteristics including L*, a*, b*, Chroma, and hue angle (h°) in the case of rootstocks and h^o in the research year found not significant, out of those characteristics mentioned above all the observed data found significant statistically.

Table 2. Effects of quince rootstocks, pear cultivars and research years on fruit skin color characteristics

Main Effects		L*	a*	b*	Chroma	h^o
Rootstocks	QA	65.71 a*	-13.34 a	31.66 a	33.01 a	107.73 a
	BA29	64.72 a	-16.75 a	30.67 a	34.20 a	112.05 a
	MC	65.24 a	-12.68 a	32.16 a	34.48 a	109.31 a
Cultivars	Deveci	76.41 a	-18.62 b	38.98 a	39.97 a	104.13 b
	Williams	56.05 b	-8.38 a	27.75 c	29.28 b	105.21 b
	Santa Maria	73.95 a	-19.89 b	34.88 b	38.39 a	120.83 a
	Abate Fetel	54.50 b	-10.15 a	24.36 d	27.95 b	108.62 b
Years	2020	53.57 b	-11.27 b	24.89 b	28.61 b	110.81 a
	2021	76.88 a	-17.24 a	38.10 a	39.18 a	108.58 a
Significance						
Rootstocks		0.679	0.269	0.344	0.748	0.443
Cultivars		0.001	0.001	0.001	0.001	0.001
Years		0.001	0.005	0.001	0.001	0.425

* Means shown with different letters in the same column are statistically significant.

Color is an important quality characteristic and variations in the color are mentioned to be related to the crown structure and leaf area of the trees as a result of vegetative growth and development. So, the trees with lower canopy achieved more sunlight which caused the formation of the red blush of pear bark color. L* and b* are among the best identifiers showing the degree of maturity in fruits of pear trees. As, increasing in the b* value which expresses the yellow color, indicated higher sugar content (Ozturk and Faizi, 2022). Many studies were shown that rootstocks have important effects on fruit color and other quality aspects of pear fruits (Erdem and Ozturk, 2012; Kucuker et al., 2015; Askari et al., 2019).

3.3. Fruit firmness and chemical properties

Analyzed data of rootstocks, cultivars, and research years' effects on fruit firmness and chemical characteristics were illustrated in Table 3. All results were obtained to be statistically significant, except for TSS in the case of research years which obtained not significant. Fruit firmness was the highest in the MC rootstocks (9.15 kg cm^{-2}) and lowest in the BA29 (8.64 kg cm^{-2}) in terms of rootstocks, and lower in 'Abate Fetel' than the other cultivars in terms of cultivars. In terms of rootstocks, the highest TSS content was determined in the BA29 (12.21%), the lowest in the MC (10.92%), and the lowest TSS content was determined in the 'Santa Maria' (9.94%) in terms of cultivars. The highest titratable acidity was determined in the BA29 (0.46%) in terms of rootstocks, the lowest in QA (0.40%); the highest in the 'Santa Maria' (0.54%), and the lowest in the 'Abate Fetel' (0.35%) in terms of cultivars (Table 3).

To determine pear fruit maturity, firmness is an important consideration (Ozturk and Faizi, 2022), and reported to differ based on rootstocks, growing years, and cultural practices in the pear orchards (Ikinci, 2017). Lepaja et al. (2014) reported the fruit firmness at 4.96 kg cm^{-2} in the 'Santa Maria'. Ikinci et al. (2014) notified that the rootstocks significantly affected the fruit firmness of the 'Santa Maria' pear cultivar, as the flesh firmness was highest on BA29 and MA rootstocks and the lowest on pear seedling rootstocks. Ikinci et al. (2016) stated that fruit firmness was 22.3 lb. Pasa et al. (2017) mentioned that fruit firmness was 62.11 - 66.46 N in 'Santa Maria'. Total soluble solids are a crucial consideration in pear fruits ripening and have a positive correlation with maturity, while acidity decreased with the increase in maturity (Ozturk and Faizi, 2022). 'Santa Maria' TSS was reported highest in pear seedlings, and lowest in BA29 rootstock, while titratable acid was reported highest in BA29 and lowest in the pear seedling rootstocks (Ikinci et al., 2014). Rootstocks and research years reported to have an important effect on the TSS and acidity of the 'Shamiveh' pear cultivar on different

quince and pear rootstocks (Askari et al., 2019). Titratable acidity was reported at 0.46% in QA, 0.62% in BA29, and 0.56% in the MC rootstock while 'Santa Maria' grafted on them (Ozturk and Faizi, 2022). The average pH of 'Santa Maria' was reported 3.98 to 4 on BA29 rootstocks by Erdem ve Ozturk (2012); 3.75 to 3.85 on pear seedling rootstocks by Kellecioglu (2014); 3.98 to 4.06 on BA29 rootstocks by Kucuker et al. (2015); 3.40 on BA29 rootstocks by Ekinci and Akcay (2016).

Table 3. Effects of quince rootstocks, pear cultivars and research years on fruit firmness and chemical traits

Main Effects		Fruit Firmness (kg cm^{-2})	TSS (%)	Titratable Acidity (%)	pH
Rootstocks	**QA**	8.96 ab*	11.56 b	0.40 b	3.62 ab
	BA29	8.64 b	12.21 a	0.46 a	3.68 a
	MC	9.15 a	10.92 c	0.43 ab	3.56 b
Cultivars	**Deveci**	9.26 a	12.21 a	0.44 b	3.82 a
	Williams	9.08 a	11.91 a	0.39 bc	3.49 b
	Santa Maria	9.04 a	9.94 b	0.54 a	3.59 b
	Abate Fetel	8.28 b	12.18 a	0.35 c	3.59 b
Years	**2020**	8.51 b	11.63 a	0.49 a	3.68 a
	2021	9.32 a	11.49 a	0.37 b	3.56 b
Significance					
Rootstocks		0.025	0.001	0.041	0.044
Cultivars		0.001	0.001	0.001	0.001
Years		0.001	0.517	0.001	0.006

* Means shown with different letters in the same column are statistically significant.

3.4. Yield and yield efficiency

Analyzed results of rootstocks, cultivars, and research years' effects on the number of fruits (NF), yield per tree (YT), yield per hectare (YH), yield per trunk cross sectional area (YTCSA) and yield per canopy volume (YCV) gave in Table 4.

Table 4. Effects of quince rootstocks, pear cultivars and research years on yield and yield efficiency

Main Effects		Number of Fruits (pieces tree^{-1})	Yield per Tree (kg tree^{-1})	Yield per Hectare (kg ha^{-1})	Yield Efficiency (kg cm^{-2})	Yield per Canopy Volume (kg m^{-3})
Rootstocks	**QA**	11.75 b*	2.13 b	4070.9 b	0.26 ab	15.64 a
	BA29	17.06 a	3.13 a	5982.8 a	0.30 a	15.24 a
	MC	11.80 b	1.62 c	3098.7 c	0.24 b	15.09 a
Cultivars	**Deveci**	16.63 a	2.93 a	5591.6 a	0.23 b	12.29 b
	Williams	9.89 b	1.64 b	3133.0 b	0.33 a	21.08 a
	Santa Maria	19.60 a	2.98 a	5685.0 a	0.31 a	12.53 b
	Abate Fetel	8.01 b	1.64 b	3127.0 b	0.19 b	15.40 ab
Years	**2020**	7.76 b	1.51 b	2877.6 b	0.25 a	17.94 a
	2021	19.30 a	3.08 a	5890.7 a	0.28 a	12.71 b
Significance						
Rootstocks		0.001	0.001	0.001	0.037	0.980
Cultivars		0.001	0.001	0.001	0.001	0.031
Years		0.001	0.001	0.001	0.103	0.026

* Means shown with different letters in the same column are statistically significant.

YTCSA in the research years and YCV in the rootstocks were found to be not significant. Except for those mentioned above, all others were found to be significant. The highest NF was determined in the BA29 rootstock (17.06 pieces tree^{-1}) in terms of rootstocks; 'Deveci' and 'Santa Maria' cultivars (19.60 and 16.63 pieces tree^{-1}, respectively) in terms of cultivars. In terms of rootstocks, the highest YT, YH, and YTCSA were observed from the BA29 rootstocks (3.13 kg tree^{-1}, 5982.8 kg ha^{-1}, and 0.30 kg

cm^{-2}, respectively) and the lowest in the MC rootstock (1.62 kg $tree^{-1}$, 3098.7 kg ha^{-1} and 0.24 kg cm^{-2}, respectively). In terms of cultivars, the highest YT and YH were determined in the 'Santa Maria' and 'Deveci' cultivars. YCV was higher in the 'Williams' cultivar than the other cultivars (Table 4).

Rootstocks along with the cultivars' effects on the yield per tree, number of fruits per tree, and trunk cross-sectional area reported to be significant in the pear orchards in which the cultivars grafted on quince rootstocks (Ozturk and Faizi, 2022). The highest cumulative yield efficiency was reported in 'Santa Maria'/MC, and the highest cumulative yield was reported in 'Santa Maria'/BA29 and 'Santa Maria'/MC combinations (Ikinci et al., 2014; Ikinci et al., 2016). Cabrera et al. (2015) observed the rootstocks' significant effect on the 'Williams'/Farold40 yield (190 ton ha^{-1}). Pasa et al. (2015) reported that the number of trees per area had a significant effect on the number of fruits per plant, yield, and yield efficiency in the 'Santa Maria'. The highest yield efficiency in the 'Carrick' cultivar on different quince rootstocks was found in Portugal and MC rootstocks (Pasa et al., 2017). Pasa et al. (2020) observed the highest yield per tree (kg $tree^{-1}$), the number of fruits per tree, and yield efficiency (kg cm^{-2}) in 'Williams' grafted on Champion rootstock. Kucuker and Aglar (2021) reported a yield per plant of 3.80 to 7.60 kg $tree^{-1}$, a yield efficiency of 2.22 to 2.97 kg cm^{-2} in 'Santa Maria' grafted on QA rootstock. Number and quality of flowers, pollination efficacy, fruit set efficacy, the severity of natural or artificial fruitlet abscission, degree and rate of cellular proliferation and expansion in the persisting fruits, also genetical (scion cultivar and rootstock), environmental (climate and soil) and cultural practices (training, pruning, plant growth regulators, manuring) effect yield of pear trees (Pasa et al., 2012; Ikinci et al., 2016; Bhat et al., 2017; Pasa et al., 2020; Kucuker and Aglar, 2021; Jovanovic et al., 2022). Yield efficiency observed 0.07 to 0.49 kg m^{-3} in 'Santa Maria' combined with two research years, also reported 0.25 kg m^{-3} in QA, 0.22 kg m^{-3} in BA29, and 0.40 kg m^{-3} in the MC rootstock (Ozturk and Faizi, 2022). It can be said that the results obtained from this study are in accordance with previous researchers' findings.

3.5. Pre-harvest fruit drop rate, black spotted fruit rate and marketable fruit rate

Rootstocks, cultivars, and research years' effects on pre-harvest fruit drop rate (PHFDR), black spotted fruit rate (BSFR), and marketable fruit rate (MFR) are illustrated in Figure 1 and Figure 2. Except for BSFR in the rootstocks which were found not significant, all others were found to be statistically significant. The highest MFR was obtained from the QA rootstock and the lowest in the BA29 rootstock in the case of rootstocks. In terms of cultivars, MFR was lower in the 'Deveci' than the others (Figure 1). In terms of rootstocks, BA29 had higher PHFDR than the others. The highest PHFDR was determined in the 'Deveci' and 'Williams', and the lowest in the 'Abate Fetel' cultivars in the case of cultivars. In terms of cultivars, the highest BSFR was found in the 'Deveci' cultivar, and the lowest in the 'Williams' cultivar (Figure 2).

Many factors can cause the pre-harvest fruit drop rate, for example, short periods of high temperatures and water stress before harvest, different species and cultivars of fruit, cultural practices like irrigation, nutrition status of the trees, control of weeds, training system, and pruning affect the level of fruit drop. Ozturk et al. (2015) reported that NAA and AVG applications significantly reduced pre-harvest fruit drop (2.67-35.21%) in 'Breaburn' apple on M26 rootstock. Pre-harvest fruit drop of 'Deveci' on BA29 with the application of 1-Methylcyclopropene reported 1.75 to 26.50 pieces (Sakaldaş and Gündoğdu, 2016). Also, cumulative fruit drop was observed at 1.03 to 59.64% in the 'Scarlet Spur' on M26 rootstock (Unsal et al., 2017). 7.33 to 33.33% in the 'Starkrimson Delicious' on seedlings reported by (Sincan et al., 2020). Black spot is a fungal disease that occurs in moist conditions. Physical control, such as removing the infected fruits and leaves from the garden as well as chemical control can be done to decrease or prevent the disease. Our research garden is located in a flat area and humidity is high due to being close to the Kızılırmak River, which may have also caused to increase the rate of the disease. The severity of disease changes according to the climatic conditions and resistance level of cultivars and rootstocks, but can reach up to 100% in the absence of chemical spraying (Urbanovich and Kazlovskaya, 2008). 'Granny Smith Challenger' on M9 rootstock showed 2.7 to 12.3% of black spotted fruit in Samsun climate conditions (Ozturk et al., 2021). In the study, to determine the number of marketable products in pear fruit in Türkiye, was the first research done. Rootstock, cultivar, and research years were found effective on the amount of marketable yield of pear fruit. Total marketable fruit in three grade quality (extra, class I and II) obtained between 46.07 to 88.03%, while different apple

cultivars grafted on M9, MM106, and MM111 rootstocks (Ozdemir et al., 2009). 'Granny Smith Challenger' on M9 rootstock showed 46.73 to 82.23% of extra quality fruit, 6.20 to 35.30% of first quality fruit, and 3.00 to 13.00% of second quality fruit (Ozturk et al., 2021).

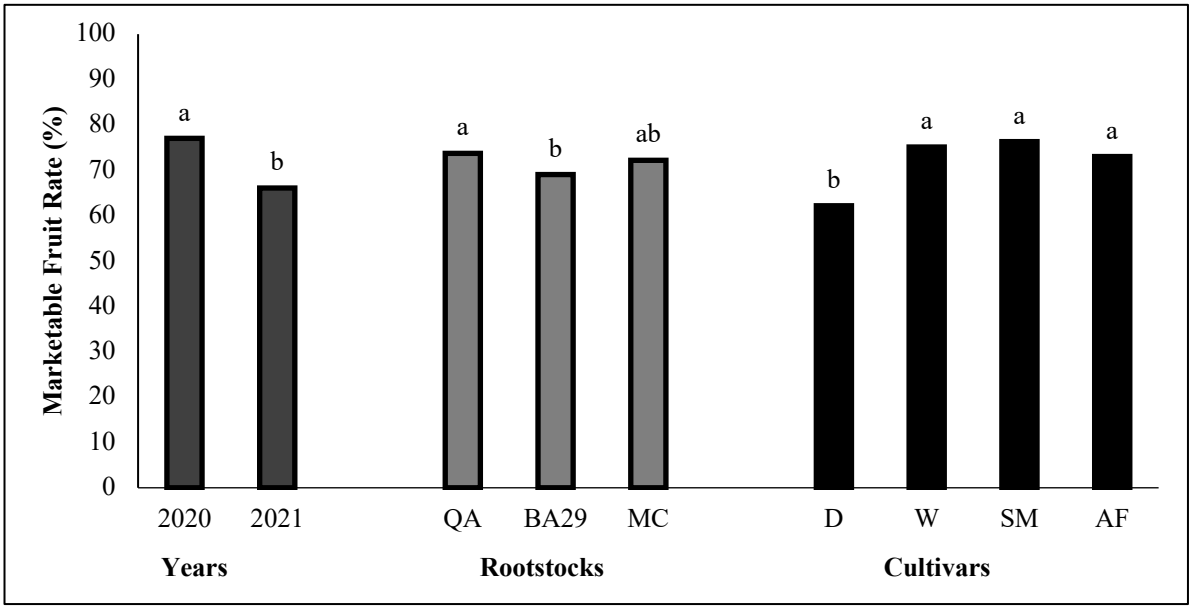

Figure 1. Marketable fruit rate (%) in the research years, rootstocks and cultivars. D= 'Deveci'. W= 'Williams'. SM= 'Santa Maria'. AF= 'Abate Fetel'.

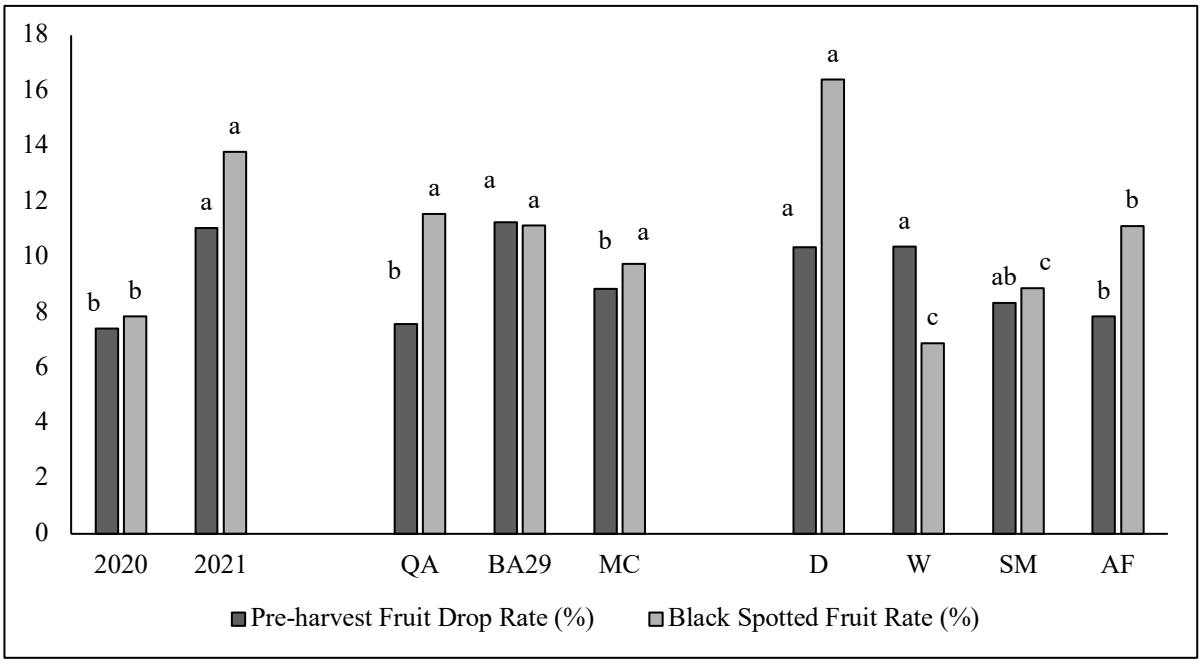

Figure 2. Pre-harvest fruit drop and black spotted fruit rate (%) in two research years, three rootstocks and four cultivars. D= 'Deveci'. W= 'Williams'. SM= 'Santa Maria'. AF= 'Abate Fetel'.

Conclusion

The highest yield and marketable fruit rate were obtained from the 'Santa Maria' cultivar and yield from BA29 rootstock. It could be said that early ripening cultivars are more suitable in regions with high relative humidity for better quality performances especially free of diseased fruit. And such cultivars are less prone to the adverse abiotic stress factors like water shortage in the summer season. Briefly, It could be suggested that semi-dwarf cultivars and rootstocks for suitably perform under high

density pear orchards. For precise results, it is recommended that the research could be continued for a longer period of time as the used trees were young.

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Interest Conflict

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Contributions of the Authors

All authors contributed to the research application, preparation of the research article, reading, and approval of the final manuscript.

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Research Article

Phosphorus-Enriched Organomineral Fertilizers Affect the Cation Exchange Capacity of the Soil: A Comparative Evaluation

Serdar TOPRAK*[1], Saime SEFEROĞLU[2]

[1]Agricultural Production and Training Center, Department of Plant Production, 09200, Söke, Aydın, Türkiye
[2]Aydın Adnan Menderes University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 09100, Koçarlı, Aydın, Türkiye

[1]https://orcid.org/0000-0003-3939-8530, [2]https://orcid.org/0000-0003-3550-5562

*Corresponding author e-mail: serdar.toprak@gmail.com

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Keywords

Cation exchange capacity, Dairy cattle manure, Exchangeable cations, Organomineral fertilization, Phosphorus

Abstract: The aim of this study is to determine the effects of phosphorus-enriched cattle manure applications on the exchangeable cations content, cation exchange capacity (CEC), and base saturation rate (BSR) of the lime soil. The research was carried out with four different levels (except control) of dairy cattle manure (M1: 10; M2: 20; M3: 30; M4: 40 t ha^{-1}) and with four different levels (except control) of phosphorus dose (P1: 10; P2: 20; P3: 30; P4: 40 kg P ha^{-1}) in the ecological conditions of Southwest Türkiye during the wheat vegetation period of 2019-2021. The study was carried out in medium calcareous soil (14.8%) with three replications randomized blocks experimental by composing organomineral fertilizer combinations. According to the results of the study, the highest change in exchangeable Ca and K content in soils was obtained from organomineral fertilizer applications by 11.2% and 29.7% respectively, and the highest change in exchangeable Mg and Na content was obtained from dairy cattle manure applications by 25.1% and 18.2%, respectively for M4P2 (40 t ha^{-1} dairy cattle manure + 20 kg P ha^{-1}). Among the fertilization systems, the highest increase in total exchangeable cations was 13.1% and the increase in CEC was 21.3% in organomineral fertilizer applications. The fastest decrease in the BSR was also obtained from the organomineral fertilization system. As a result, it has been determined that M4P2 application is the most economical and the most effective combination in the cation exchange capacity among organomineral fertilizer combinations.

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1. Introduction

Chemical fertilizers are used intensively at every stage from the beginning of plant production to fruit maturity. However, the problems it creates in the soil are worrisome for the future of sustainable soil fertility (Daneshgar et al., 2018). As a result of the excessive use of fertilizers, the cation exchange capacity (CEC) and the contents of exchangeable cations decrease, especially in soils that are very poor in organic matter (OM) and constantly become barren (Smith et al., 2020). Due to this decrease, there

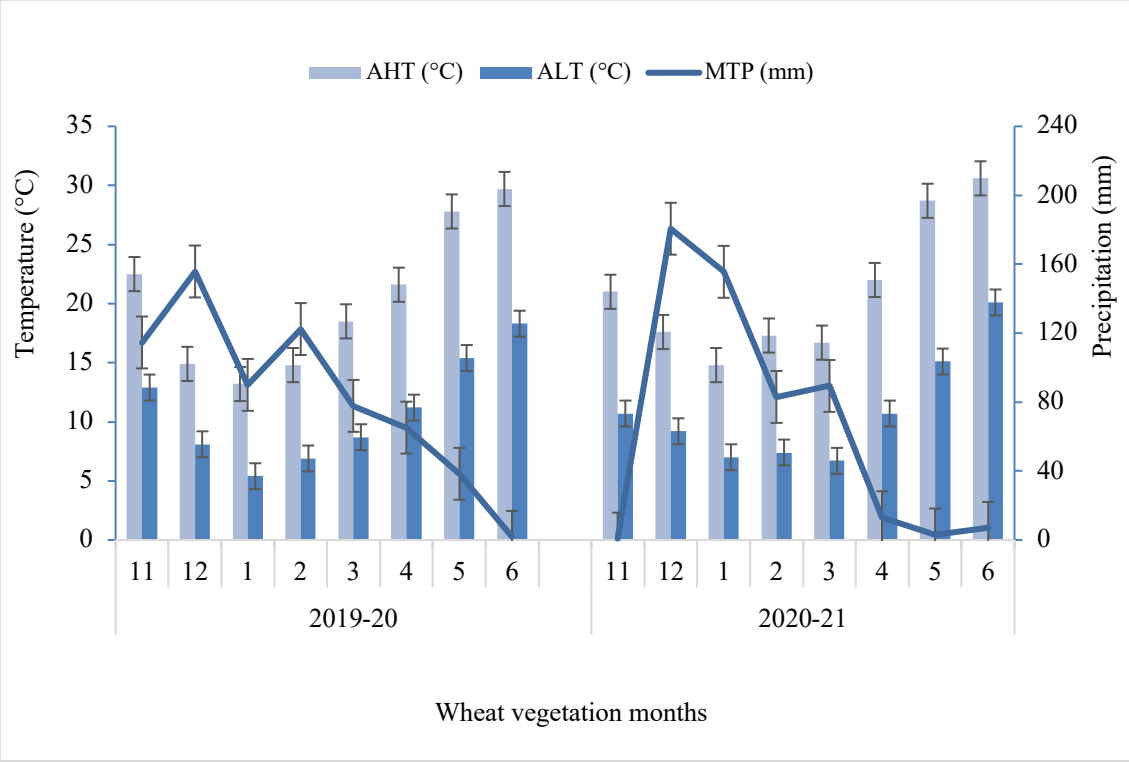
are big problems in the rhizosphere region, and the plants grown cannot complete their development because they cannot be fed adequately resulting in losses in yield and quality (Shen et al., 2011; Alamgir et al., 2012). However, organic fertilizers are the only indispensable key to increasing the CEC of the soil. Regardless of the origin of the organic fertilizer, organic fertilizers added to the soil increase the level of exchangeable cations, and high cation exchange capacities are detected in the rhizosphere region (Fernandez et al., 2016). In the transition to organic agriculture, organomineral fertilizers have created an alternative in plant production. In the last decades, many studies have been reported that detect yield and quality increases in plant production in many countries in the transition to organomineral fertilizers (Namlı et al., 2019; Toprak, 2019; Mounirou et al., 2020; Yossif and Gezgin, 2020). Organomineral fertilizers are composed of both mineral fertilizers and organic fertilizers in certain proportions. With the addition of organomineral fertilizers to the soil, both the mineral-rich chemical fertilizers easily supply the nutrients needed by the plant, while the organic matter in the organomineral fertilizer causes the rhizosphere to be improved and the nutrients offered by the chemical fertilizers to be benefited by the plant much more easily (Süzer and Çulhacı, 2017; Mounirou et al., 2020; Yossif and Gezgin, 2020). Phosphorus fertilizers, on the other hand, are plant nutrients that are highly related to the yield elements in the soil and ultimately to the plant's nutrition (Bai et al., 2017). Even if it is used in high amounts, the presence of high amounts of calcium in the soil transforms phosphorus into forms that plants cannot use (Shen et al., 2011; Malhotra et al., 2018).

Besides, the study primarily determines the effect of the surface electrostatic bond, which is formed by phosphorus mostly with carbon-bound cations in the soil, on the exchange capacity under different fertilization systems. As it is known, in recent years, soil and water pollution have reached dangerous levels due to the neglect of sustainable agriculture and soil management with the excessive use of mineral fertilizers in conventional agriculture. Since the use of organomineral fertilizers in the transition to organic agriculture reflects a positive perspective on both economic and environmental sensitivity, their use should be encouraged. In the future, the effect of studies carried out under different fertilization systems on soil properties should be considered comparatively. From this point of view, it is hoped that the conscious and effective use of phosphorus in addition to organic fertilization within the framework of different phosphorus fertilization systems will shed light on such fertilization programs in the future in terms of sustainable agriculture. In addition, P fertilization, which can be created with organic fertilizer combinations, can be a good alternative to mineral fertilization in the future, especially against P eutrophication caused by excessive P fertilizer consumption. This study targets to evaluate the effect of organomineral fertilization systems consisting of conventional phosphorus fertilization, organic fertilization, phosphorus, and organic fertilizer combinations on the exchangeable cations content and cation exchange capacity of the soil.

2. Materials and Methods

2.1. Test location and climatic properties

The experiment was established in the plant production areas with alluvial soil type and silty clay soil texture in the Agricultural Production and Training Center (TAYEM) located at an altitude of 38 m in Söke district of Aydın province (37.705309 North, 27.379727 East) in southwestern Türkiye. The experiment started in November 2019 and was completed in June 2021. Söke County has a warm and temperate climate with much more precipitation in winter than in summer. According to Köppen-Geiger climate classification, it is defined with the definition of Csa (temperate winter, hot and dry summer/Mediterranean climate) (MGM, 2016). According to the meteorological data, in the 2019-2020 wheat growing season, the annual average highest temperature was 20.4 °C, the annual average lowest temperature was 10.9 °C, and the annual average precipitation was 666 mm. On the other hand, the annual average highest temperature was 21.1 °C, the annual average lowest temperature was 10.9 °C, and the annual average precipitation was 532.8 mm (Fig. 1).



AHT: Average highest temperature, ALT: Average lowest temperature, MTP: Monthly total precipitation; The lines above the bars show the standard deviation.

Figure 1. Meteorological data of the research area during the wheat growth periods.

2.2. Fertilizer resources

In the experiment, dairy manures taken from the dairy farm of TAYEM were used as an organic fertilizer source. This organic fertilizer was taken from the dairy and separated for one year in a different area and free of weed seeds. DAP (Diamonniumphosphate: 18% N and 46% P_2O_5) fertilizer was used as a phosphorus source. In the study, organomineral fertilizers were created from different combinations of these two fertilizers. Also, the wheat plant was grown for two years in the experimental area, and Urea (46% N) 160 kg N ha^{-1} and Potassium Nitrate (13% N and 45% K_2O) 75 kg K ha^{-1} were applied as nitrogen sources for optimum growth and support (Kara, 2014).

2.3. Study design and application of fertilizers

The research was set up in a randomized block design with three replications. Block sizes are 2 x 6 m. In the experiment, chemical phosphorus (conventional fertilization-CF) doses (P_1: 10, P_2: 20, P_3:30, and P_4: 40 kg P ha^{-1}), dairy manure (organic fertilization-OF) doses (DM_1: 10, DM_2: 20, DM_3: 30, and DM_4: 40 t ha^{-1}), four different organomineral fertilization systems (P-OMFS) consisting of these two different fertilizer combinations were prepared and studied. These new organomineral fertilizers, composed of combinations of phosphorus and dairy manures, were incubated for one month before being applied to the soil. After all the fertilizers were laid in a layer on the soil 24 hours before wheat planting, they were mixed with a soil rotavator to a depth of 0-30 cm.

2.4. Soil and organic manure sampling and analysis methods

Before and after the study, 75 soil samples were taken from each block from a depth of 0-30 cm. Soil samples were air-dried ground with a soil crushing machine, passed through a 2 mm sieve, and prepared for physical and chemical analysis. Organic manure samples were randomly collected from 10 different places to represent the whole heap and samples were taken as 500 g by mixing. Then passed

through a 0.5 mm sieve and prepared for analysis (Kacar and Katkat, 2009). The analysis results of the soil and manure samples taken before the experiment are given in Table 1.

Cation Exchange Capacities (me 100 g^{-1}): The cation exchange capacities of the soils were determined by the ICP OES spectrophotometer (Perkin Elmer 8000) in solutions extracted with ammonium acetate (1 N, pH: 7.0) after sodium adsorption with sodium acetate (1 N, pH: 8.2) in the samples (Rhoades, 1982a). Exchangeable Cations (me 100 g^{-1}): The exchangeable cations (Na and K, Ca, Mg) of the soils were determined in the sieve after extraction with Ammonium Acetate (1 N, pH: 7.0) by ICP OES spectrophotometer (Perkin Elmer 8000) (Rhoades, 1982b).

The base saturation ratio was calculated as the ratio of the total exchangeable cations (Ca, Mg, K, and Na) to the cation exchange capacity and is given in equation 1 below (Doetterl et al., 2018).

$$\text{Base saturation rate: [Total cations / Cation exchange capacity] x 100} \quad (1)$$

The soils of the study area have silty-loam texture and slightly alkaline character; It has been determined that soils without salinity problems contain insufficient organic matter and moderate lime. It was determined that total nitrogen and available potassium were insufficient, available phosphorus was sufficient, exchangeable calcium was moderate, and available magnesium was high in the soils. However, according to the micro plant nutrients analysis in the soil, it was determined that all of them, except for the available manganese, were in sufficient amounts in the soil (Kacar and Katkat, 2010).

Table 1. Some physical and chemical properties of experiment field soils and organic manure before research

Properties	Soil	Analysis method	Manure	Analysis method
Texture class	Silty-Loam	Bouyoucos Hydrometer	-	
pH (1:2.5)	7.95	pH meter	6.92	pH meter
EC (dS m^{-1})	0.32	EC meter	3.40	EC meter
OM (%)	1.81	Walkley-Black	67.9	AOAC
N (%)	0.06	Mikrokjheldahl	1.98	Mikrokjheldahl
P (%)	0.001	MBC	0.45	AOAC
K (%)	0.011	AA	2.16	AOAC
Ca (%)	0.216	AA	0.85	AOAC
Mg (%)	0.077	AA	0.12	AOAC
Fe (mg kg^{-1})	10.9	DTPA	386.7	AOAC
Zn (mg kg^{-1})	1.75	DTPA	92.4	AOAC
Mn (mg kg^{-1})	7.84	DTPA	142.0	AOAC
Cu (mg kg^{-1})	2.86	DTPA	83.3	AOAC
B (mg kg^{-1})	1.82	Azomethine-H	5.60	Azomethine-H

EC: Electrical conductivity, MBC: Molybdophosphoric blue color, AA: Ammonium acetate, DTPA: Diethylene triamine penta acetic acid, AOAC: Association of Official Analytical Chemists.

2.5. Statistical analysis

Evaluation of all the findings obtained in the study was determined according to the randomized blocks experiment design with the Jump statistical program (JMP) ver.7 (JMP, 2007). In the analysis of variance, the significance of the differences between the means was determined by the Duncan's multiple range test in the same package programs. The variance analysis was created by determining the least significant difference according to the importance levels of the factors $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ probability values.

3. Results and Discussions

3.1. Organic fertilization system

In the organic fertilization system, an increase was observed in exchangeable cations with the increase in the amount of organic manure in the soil. As it is known, the increase in the content of exchangeable cations is closely related to the increase in organic matter (OM) in the soil. Regardless of its origin, OM added to the soil increases the amount of exchangeable cations in the soil (Fernandez et

al., 2016). The exchangeable Ca contents of the soil increased also for two years. However, the effect of applied organic manures on the exchangeable Ca content of the soil was not statistically significant in the first year, but significant in the second year ($p \leq 0.05$). With the addition of organic manure to the soil, the exchangeable Ca content increased by 6.6% in the first year and by 9.6% in the second year (Table 2). According to the data of both years, the highest exchangeable Ca content was obtained from the M4 application (8.71 me 100 g^{-1}). The greater increase in the second year may be due to the accumulation of OM in the soil and the mineralization of Ca. Ca bound in the soil is desorption by the effect of OM and can pass into the soil solution (Shen et al., 2011).

Table 2. Exchangeable cations of the soil as affected by manure applications

Manure	Ca (me 100 g^{-1})		Mg (me 100 g^{-1})		K (me 100 g^{-1})		Na (me 100 g^{-1})	
doses	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
M0	8.14a	7.95c	1.30e (ef)	1.27c (f)	0.58c (d)	0.65b (c)	0.042a	0.038a
M1	8.27a	8.08bc	1.46d (c)	1.34bc (de)	0.66b (c)	0.66b (c)	0.044a	0.039a
M2	8.26a	8.07bc	1.35c (de)	1.36b (d)	0.71a (bc)	0.66b (c)	0.046a	0.039a
M3	8.68a	8.52ab	1.50b (bc)	1.46a (c)	0.68b (bc)	0.70a (bc)	0.047a	0.043a
M4	8.67a	8.71a	1.68a (a)	1.53a (b)	0.79a (a)	0.72a (b)	0.051a	0.043a
Means	8.41A	8.27A	1.46A	1.39B	0.69A	0.68A	0.046A	0.040B
F-values								
M	2.74 ns	4.98*	211.9**	18.28***	9.62**	9.91**	1.07ns	0.65ns
Y	2.11 ns		31.28***		0.11 ns		7.51*	
M x Y	0.11 ns		7.05**		4.13*		0.20 ns	

M0 to M4: No manure, and 10, 20, 30,40 t ha^{-1} dairy manure applications, respectively; Statistically significant at (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test (p ≤ 0.05). Letters in parentheses indicate manure x year interaction. Capital letters indicate the results of the Duncan's test between years.

In the experiment, the exchangeable magnesium contents increased in both years with the effect of organic manure. This increase was statistically significant in both years ($p \leq 0.01$; $p \leq 0.001$). While the increase in the exchangeable Mg content in the soil was 29.2% in the first year, it was recorded as 20.5% in the second year (Table 2). However, the manure x year interaction was found to be statistically significant ($p \leq 0.01$). In both years, the highest exchangeable Mg content in the soil was determined in the M4 application in the first year of the experiment (1.68 me 100 g^{-1}). The differences in exchangeable Mg contents between years were also found to be statistically significant ($p \leq 0.001$). In the preliminary manure analysis, the Mg content in the organic fertilizer was already high. However, with the addition of OM, it became inevitable to increase the exchangeable Mg content in the soil when combined with the Mg existing in the soil (Angelova et al., 2013).

The exchangeable K contents in the soil increased with the increase of organic manure applied. Statistically significant relationships were found between the organic manure applied and the exchangeable K content of the soil ($p \leq 0.01$). However, organic manure x year interaction was also found to be statistically significant ($p \leq 0.05$). According to the data obtained at the end of both years, the highest exchangeable K content in the soil was obtained from M4 application in the first year (0.79 me 100 g^{-1}). Besides, organic fertilizers increased the exchangeable K content of the soil by 36.2% in the first year and by 10.8% in the second year (Table 2). The decrease in the rate in the second year compared to the first year may be related to the saturation of the potential K content in the soil. The amount of K in organic manures can increase the exchangeable K amount in the soil (Siregar et al., 2005; Shen et al., 2011; Whittinghill and Hobbie, 2012).

However, it increased the exchangeable sodium content of the soil by being affected by the existing salt ratio in the amount of organic manure applied. In addition, this increase was not statistically significant (Table 2). The exchangeable Na content of the soil increased by 21.4% in the first year and by 13.2% in the second year. However, the exchangeable Na content of the soil decreased in the second year compared to the first year, and this was statistically significant ($p \leq 0.05$). Depending on the types of organic manures, the Na content may also vary due to the amount of salt in them. Organic manures added to the soil can also increase the salt content of the soil (Demirtaş et al., 2012; Erdal et al., 2018).

According to the results of the experiment, it was determined that the organic manure added to the soil increased the cation exchange capacity (CEC) of the soil. However, organic manure's effect on

the soil's CEC was found to be statistically significant in both years ($p \leq 0.001$). While the increase in the first CEC was 19.6% in the experiment, it was recorded as 22.9% in the second year. In both years, the highest CEC was obtained from the M4 application (12.8 and 12.9 me 100 g^{-1}, respectively). However, no statistically significant difference was found between the years (Table 3). The CEC of the soil increased stably in both years. It is directly related to the amount of organic matter in the soil. Many studies have also reported that the organic matter added to the soil increases the CEC (Francou et al., 2005; Kasongo et al., 2011; Whittinghill and Hobbie, 2012; Gayathri et al., 2019).

Table 3. Cation exchange capacity and base saturation rate of the soil as affected by manure applications

Manure doses	CEC (me 100 g^{-1})		BSR (%)	
	2019-20	2020-21	2019-20	2020-21
M0	10.7c	10.5d	94.4a	94.1a
M1	11.5b	11.2c	90.6bc	90.3b
M2	11.3bc	11.3c	91.7ab	89.9b
M3	12.4a	12.2b	88.2c	87.8c
M4	12.8a	12.9a	87.5c	85.6d
Means	11.7A	11.6A	90.5A	89.6A
F-values				
M	11.64***	28.24***	7.79**	80.15***
Y	0.76 ns		3.72 ns	
M x Y	0.20 ns		0.58 ns	

CEC: Cation Exchange capacity, BSR: Base saturation rate; M0 to M4: No manure, and 10, 20, 30,40 t ha^{-1} dairy manure applications, respectively; Statistically significant at (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test ($p \leq 0.05$). Capital letters indicate the results of the Duncan's test between years.

On the contrary, the base saturation ratio (BSR) decreased as the amount of organic manure applied increased. Meanwhile, by 7.3% decrease was recorded in the first year of the study, and by 9.0% decrease was detected in the second year (Table 3). The BSR is the ratio of the total exchangeable cations in the soil to the cation exchange capacity, and this ratio increases with the increase in the amount of the total exchangeable cations in the soil, on the contrary, it decreases with the increase of the cation exchange capacity. This situation is closely related to the desorption of saturated cations and the exchange capacity of the cations separated from the solid phase increases and is offered to the plant in the forms that the plant can take, so the BSR in the soil decreases (Dengiz et al, 2007; Wuddivira and Camps-Roach, 2007; Osman, 2013).

3.2. Conventional phosphorus fertilization system

In the conventional phosphorus fertilization system, increasing doses of mineral P were applied and the effect on the content of exchangeable cations in the soil was not found statistically significant (Table 4). Although an increase in the exchangeable Ca content was noted up to the P2 application, decreases were detected at subsequent doses. Meanwhile, the effect of P doses on exchangeable Mg content was statistically significant in the first year of the study, it was insignificant in the second year ($p \leq 0.01$).

Exchangeable Mg content increased by 6.2% in the first year and increased by 3.1% in the second year. In addition, the difference in exchangeable Mg content between years was found to be statistically significant ($p \leq 0.01$). Compared to the first year, the exchangeable Mg content in the soil decreased in the second year. The effect of mineral P doses on the exchangeable K contents of the soil was not statistically significant for two years. However, the exchangeable K content of the soil increased in the second year of the study and this was statistically significant ($p \leq 0.01$). Moreover, the highest exchangeable K content was recorded at the P2 dose (0.69 me 100 g^{-1}).

Table 4. Exchangeable cations of the soil as affected by phosphorus applications

Phosphorus	Ca (me 100 g^{-1})		Mg (me 100 g^{-1})		K (me 100 g^{-1})		Na (me 100 g^{-1})	
doses	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
P0	8.14a	7.95a	1.30a	1.27a	0.58a	0.65a	0.042a	0.038a
P1	8.45a	8.26a	1.34a	1.31a	0.64a	0.68a	0.042a	0.040a
P2	8.53a	8.37a	1.30a	1.30a	0.69a	0.69a	0.042a	0.041a
P3	8.42a	8.32a	1.33a	1.31a	0.59a	0.68a	0.042a	0.041a
P4	8.08a	8.23a	1.38a	1.30a	0.64a	0.67a	0.040a	0.041a
Means	8.32A	8.23A	1.33A	1.30B	0.63B	0.67A	0.042A	0.040A
F-values								
P	1.89 ns	2.06 ns	2.84 ns	1.36 ns	2.03 ns	2.01 ns	0.11 ns	1.85 ns
Y	1.38 ns		13.39**		11.43**		1.81 ns	
P x Y	0.67 ns		2.41 ns		1.26 ns		0.34 ns	

P0 to P4: No phosphorus, and 10, 20, 30,40 kg ha^{-1} phosphorus fertilizer applications, respectively; Statistically significant at (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test (p ≤ 0.05). Capital letters indicate the results of the Duncan's test between years.

Table 5. Cation exchange capacity and base saturation rate of the soil as affected by phosphorus applications

Phosphorus	CEC (me 100 g^{-1})		BSR (%)	
doses	2019-20	2020-21	2019-20	2020-21
P0	10.7a	10.5a	94.4a	94.1a
P1	10.9a	10.9a	95.7a	94.4a
P2	11.2a	10.9a	94.7a	95.1a
P3	10.8a	10.9a	96.2a	94.9a
P4	10.6a	10.8a	96.2a	94.7a
Means	10.8A	10.8A	95.4A	94.6A
F-values				
P	1.81 ns	1.93 ns	0.60 ns	0.23 ns
Y	0.001 ns		1.50 ns	
P x Y	0.86 ns		0.34 ns	

CEC: Cation Exchange capacity, BSR: Base saturation rate; P0 to P4: No phosphorus, and 10, 20, 30,40 kg ha^{-1} phosphorus fertilizer applications, respectively; Statistically significant at (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test (p ≤ 0.05). Capital letters indicate the results of the Duncan's test between years.

Also, the effect of mineral P doses on the exchangeable Na content of the soil was not statistically significant. The CEC and BSR of soils were not affected by mineral P applications. According to the results obtained, the effect of mineral P doses on the CEC and BSR were not statistically significant during both study years (Table 5). The highest CEC was recorded at the P2 dose (11.2 me 100 g^{-1}) in the first year of the study. Cations dissolved from carbonate minerals (Ca, Mg, Na, and K) may tend to change by forming electrostatic bonds, especially with phosphorus at superficial levels, and/or cause precipitation of phosphates (Zhang et al., 2021). This depends on the alkaline potential of the soil. At high pHs, precipitation occurs by forming Ca and Mg-P phases. In some studies conducted in recent years, it has come to the fore that phosphorus applied in soils rich in Ca and Mg at high pH precipitates with electrostatic bonding rather than the change of these elements in the soil (Naveed et al., 2020; Geng et al., 2022). Therefore, it is reported that the effect of the phosphorus-to-cation exchange capacity is not alone but under some environmental factors (Zhu and Dittrich, 2016).

3.3. Organomineral phosphorus fertilization system

The effects of sixteen different organomineral fertilizer combinations comprised of dairy cattle manure and mineral P doses on the content of exchangeable cations, cation exchange capacity, and base saturation rate during the study period were examined. Its statistical evaluation is given in Tables 6 and 7. According to the results, although the effect of organomineral fertilizer combinations on the exchangeable Ca content of the soil was not statistically significant in both years, it was determined that the exchangeable Ca content of the soil increased. It was noted that organomineral fertilizers increased

the exchangeable Ca content of the soil by 13.3% in the first year and by 12.3% in the second year. Among the combinations, the highest exchangeable Ca content in the soil was determined in the first year in M4P3, and in the second year in M4P2 application. However, it was determined that the increase in the exchangeable Ca content increased with the increase in the organic fertilizer content of the organomineral fertilizers. Mainly, the exchange of Ca, a base cation, is due to the electrostatic interaction between the clay minerals and/or the negative charge on the organic matter, so the adsorption depends on variables such as pH and ionic strength (Rytwo et al., 2002). Decreased pH and increasing OM amount in the soil increased the exchange capacity of Ca and provided its adsorbent on the colloid surface. The amount of exchangeable Ca increasing in line with the increasing organic matter content supports the results obtained from the experiment, as in many studies (Gustafsson and Van Schaik, 2003; Meier et al., 2004; Whittinghill and Hobbie, 2012). The effect of organomineral fertilizer combinations on the exchangeable Mg content of the soil was found to be statistically significant ($p \leq 0.001$). It was determined that the exchangeable Mg content of the soil increased with the increase in the doses of organomineral fertilizers (especially the organic fertilizer content). The highest exchangeable Mg content was recorded in the combination of M4P3 in the first year and M4P1 in the second year (Table 6). However, it was determined that organomineral fertilizer combinations increased the exchangeable Mg content of the soil by 26.2% in the first year and 25.2% in the second year. The study also obtained statistically significant differences between the years ($p \leq 0.001$).

According to the findings, the amount of organic matter increased in the soil increased the exchangeable Mg amount of the soil in the same direction. This status can be explained due to the high organic matter content and the high electrostatic charge absorbency of Mg on colloid surfaces (Angelova et al., 2013). However, many studies have shown similarities with the findings in the experiment, in which the change of Ca and Mg elements increases with the increase of OM in the soil and the decrease in pH values in the soil (Dijkstra, 2003; Reich et al., 2005; Guckland et al., 2009; Whittinghill and Hobbie, 2012). In addition, it was determined that as the applied P doses increased, the exchangeable Mg capacity first increased and then decreased. P mineralization increased with the increase in OM in the soil, and the retained Mg element in the soil was activated for cation exchange. Therefore, it has been reported in some studies that phosphorus-bound Mg is stored in soil water in water-soluble forms for the benefit of the plant (Neirynck et al., 2000; Reich et al., 2005; Shen et al., 2011; Melenya et al., 2015).

Table 6. Exchangeable cations of the soil as affected by organomineral fertilizer applications

Organomineral	Ca (me 100 g^{-1})		Mg (me 100 g^{-1})		K (me 100 g^{-1})		Na (me 100 g^{-1})	
fertilizer doses	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
M0P0	8.14a	7.95a	1.30j	1.27j	0.58e	0.65f	0.042a	0.038a
M1P1	8.44a	8.25a	1.41gh	1.36hı	0.69d	0.68ef	0.040a	0.040a
M1P2	8.54a	8.35a	1.42gh	1.39gı	0.71d	0.71b-e	0.039a	0.041a
M1P3	8.40a	8.41a	1.38hı	1.35ı	0.73cd	0.69d-f	0.039a	0.040a
M1P4	8.40a	8.34a	1.33ıj	1.34ı	0.69d	0.68ef	0.039a	0.039a
M2P1	9.05a	8.39a	1.45e-g	1.41f-h	0.75cd	0.69d-f	0.043a	0.042a
M2P2	8.63a	8.59a	1.42gh	1.39gı	0.73cd	0.73a-c	0.039a	0.042a
M2P3	8.62a	8.71a	1.43f-h	1.37hı	0.75cd	0.71b-e	0.041a	0.043a
M2P4	8.15a	8.56a	1.38hı	1.35ı	0.75cd	0.70c-e	0.042a	0.042a
M3P1	9.08a	8.87a	1.51c-e	1.48de	0.82bc	0.73a-c	0.043a	0.043a
M3P2	9.08a	8.87a	1.53cd	1.50cd	0.77cd	0.73a-c	0.044a	0.043a
M3P3	8.97a	8.76a	1.48d-f	1.45ef	0.75cd	0.74ab	0.046a	0.042a
M3P4	8.51a	8.65a	1.46e-g	1.42e-g	0.73cd	0.74ab	0.041a	0.041a
M4P1	8.78a	8.78a	1.63a	1.59a	0.93a	0.74ab	0.048a	0.045a
M4P2	8.92a	8.93a	1.60ab	1.57ab	0.88ab	0.76a	0.045a	0.045a
M4P3	9.22a	8.80a	1.64a	1.53bc	0.77cd	0.76a	0.045a	0.044a
M4P4	8.67a	8.58a	1.56bc	1.52b-d	0.75cd	0.72a-d	0.044a	0.043a
Means	8.68A	8.58A	1.47A	1.43B	0.75A	0.72B	0.042A	0.042A
F-values								
OMF	1.20 ns	1.77 ns	23.34***	24.64***	5.19***	3.67***	1.01 ns	0.80 ns
Y	1.45 ns		31.59***		15.84***		0.20 ns	
OMF x Y	0.41 ns		0.72 ns		2.41 ns		0.38 ns	

Phosphorus x manure interactions are organomineral fertilizer combinations (OMF). Statistically significant at (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test ($p \leq 0.05$). Capital letters indicate the results of the Duncan's test between years.

The effect of organomineral fertilizers applied during the study period on the exchangeable K content of the soil was found to be statistically significant ($p \leq 0.001$). Depending on the doses of organomineral fertilizers, increases were recorded in the exchangeable K contents of the soils. In the study, organomineral fertilizer combinations increased the exchangeable K content of the soil by 60.3% in the first year and by 16.9% in the second year. This rapid increase in the first year compared to the second year may be due to the insufficient K content in the soil and the strong effect of both the K existing in the soil and the K present in the organic fertilizer together with the organomineral fertilizer combinations. In the second year, the situation became more stable, the potential K content in the soil became saturated and the rate of increase slowed down. Also, the highest exchangeable K content was determined in the M4P1 application in the first year, and in the M4P2 and M4P3 applications in the second year (Table 6). According to the findings obtained from the experiment, the increasing amount of OM in the soil increased the exchangeable K contents. It is reported that with the increase of OM and decrease in pH, the exchange of K, a base cation with high absorbance in soil, increases and it can be found in forms that can be taken by the plant (Siregar et al., 2005; Whittinghill and Hobbie, 2012). Many studies have stated that increasing OM in the soil increases the exchangeable K activation (Khoshgoftarmanesh and Kalbasi, 2002; Verma et al., 2005; Yalçın et al., 2018; Çimrin, 2020).

Table 7. Cation exchange capacity, and base saturation rate of the soil as affected by organomineral fertilizer applications

Organomineral	CEC (me 100 g^{-1})		BSR (%)	
fertilizer doses	2019-20	2020-21	2019-20	2020-21
M0P0	10.7e	10.5g	94.4a	94.1a
M1P1	11.5c-e	11.3f	91.8a-c	91.4b-d
M1P2	11.5c-e	11.4e-f	93.0ab	92.0b
M1P3	11.4de	11.4e-f	92.3a-c	91.8bc
M1P4	11.3de	11.3f	92.3a-c	91.7bc
M2P1	12.6a-c	11.7d-f	89.9c-e	90.3de
M2P2	12.0b-d	11.9c-f	90.4b-d	90.7c-e
M2P3	12.0b-d	12.0b-f	90.4b-d	90.3de
M2P4	11.4de	11.9c-f	90.4b-d	89.5ef
M3P1	13.0ab	12.6a-c	88.0d-f	88.0g
M3P2	12.8ab	12.6a-c	89.6c-e	88.6fg
M3P3	12.7ab	12.4a-d	88.5d-f	88.4fg
M3P4	12.1b-d	12.4a-d	88.5d-f	87.9g
M4P1	13.2ab	12.9a	86.1f	86.4h
M4P2	13.3a	13.1a	86.0f	86.4h
M4P3	13.4a	12.9a	87.2ef	86.0h
M4P4	12.7ab	12.7ab	86.6f	85.7h
Means	12.2A	12.1A	89.7A	89.4A
F-values				
OMF	4.76***	7.87***	5.78***	34.00***
Y	1.96 ns		1.73 ns	
OMF x Y	0.41 ns		0.23 ns	

CEC: Cation Exchange capacity, BSR: Base saturation rate; Phosphorus x manure interactions are organomineral fertilizer combinations (OMF). Statistically significant at (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns, not significant). Column means with the same letter are not significantly different by Duncan's multiple range test ($p \leq 0.05$). Capital letters indicate the results of the Duncan's test between years.

The effect of organomineral fertilizers applied during the study period on the exchangeable Na content of the soil was not statistically significant. However, it was determined that the increasing amount of organic fertilizer in organomineral fertilizer combinations increased the exchangeable Na content of the soil (Table 6). In the study, the highest exchangeable Na content was recorded in the M4P1 combination in the first year, and in the M4P1 and M4P2 combination in the second year. It was determined that the exchangeable Na content of the soils increased by 14.3% compared to the control in the first year, and by 18.4 in the second year. According to the findings, organomineral fertilizer combinations applied in both years increased the exchangeable Na content of the soils. Some studies reported that fertilizers of animal origin contain salt in different amounts depending on the type and

breed of animals (Demir et al., 2003; Demirtaş et al., 2012; Erdal et al., 2018). The presence of organic matter in the soil helps to disperse Na and can be mobilized in a soluble form, possibly in a colloidal form (Leogrande and Vitti, 2019). It was determined that the application of organomineral fertilizers increased the total exchangeable cation contents of the soil. Many researchers have reported that the increase in the content of exchangeable cations depends on the amount of OM in the soil (Whittinghill and Hobbie, 2011; Boethling, 2019; Timmer et al., 2020). The binding of cations to soil surface components results from the electrostatic interaction of the negative charge on OM, and thus cation adsorption is dependent on variables such as pH and ionic strength (Behera et al., 2010). The electrostatic charge and high surface area allow clay-sized particles to dominate mineral-mineral, mineral-organic, mineral-metal, and mineral-water interactions in soils (Kleber et al., 2021). However, with the increase in phosphorus doses, low-level increases were recorded in the exchangeable cations level. These increases are due to the increase in the saturation of colloid-bound Ca as the amount of water-soluble P in the soil increases, and the increase in the binding strength of Ca and Mg-bound P to the colloid as the soil pH decreases in lime soils (Manimel et al., 2013).

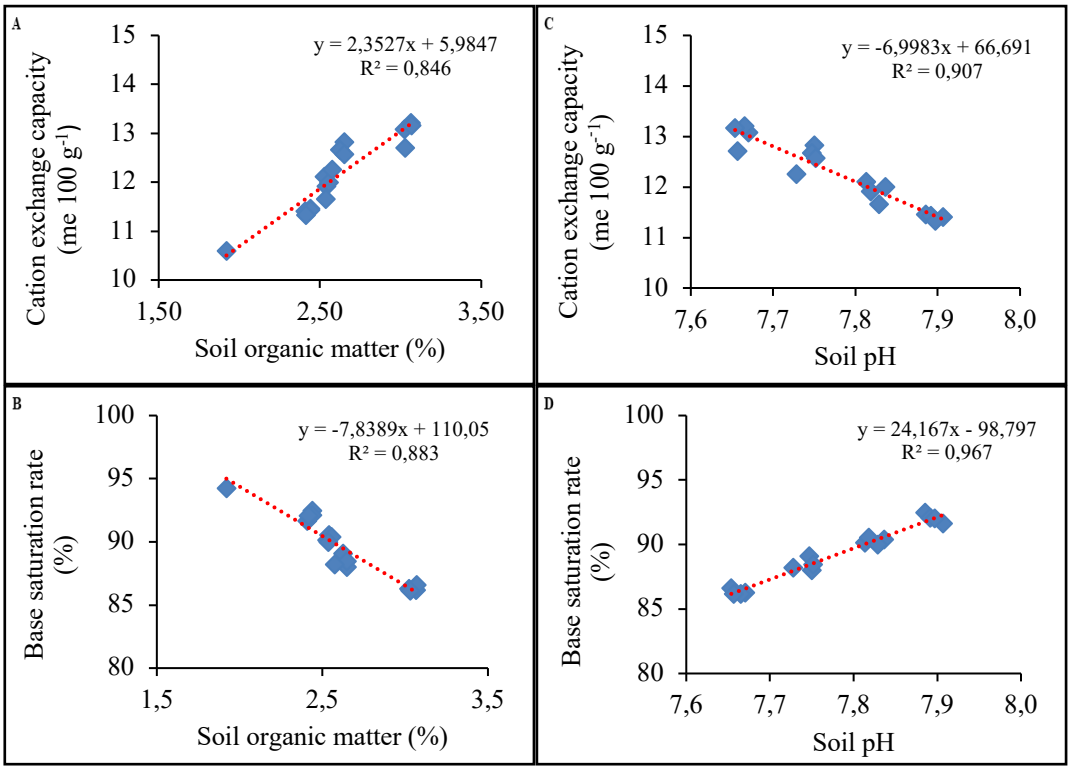


Figure 2. Relationship between cation exchange capacity and soil organic matter and pH (A-C) and the relationship between base saturation rate and soil organic matter and pH (B-D) affected by organomineral fertilizers.

Organomineral fertilizers composed of mineral phosphorus and dairy cattle manure combinations increased the cation exchange capacity of the soil for two years, and this was statistically significant ($p \leq 0.001$). Organomineral fertilizers increased the soil's cation exchange capacity by 25.2% in the first year of the study and by 24.8% in the second year. The highest cation exchange capacity in the soil was recorded in the M4P3 combination in the first year of the study, and in the M4P2 combination in the second year (Table 7). The difference between years was not statistically significant. According to the findings, it was determined that organomineral fertilizer applications in the soil increased the cation exchange capacity depending on the increasing amount of organic matter (Fig. 2A). Organic matter gives significant strength to the soil in terms of increasing cation exchange. As a result of the width of its surface area and its electrostatic state, it is the only substance that has the ability to bind large amounts of cations and increase their exchange activation (Oorts et al., 2003). Many researchers have reported that soils rich in organic matter have high cation exchange capacity (Francou et al., 2005; Kasongo et al., 2011; Gayathri et al., 2019; Demirel and Şenol, 2019; Liu et al., 2020). Base

saturation capacity is an important soil chemical property with implications for both soil taxonomic classification and soil fertility. Base saturation is defined as the sum of the four basic cations (Ca, Mg, K, and Na) relative to the total soil cation exchange capacity at pH 7.0 or 8.2 (Foth and Ellis, 2018). According to the results obtained from the experiment, it was determined that organomineral fertilizer applications reduced the base saturation capacity of the soil. The lowest base saturation rate was recorded in the M4P2 application in the first year and in the M4P4 application in the second year (Table 7). In the study, organomineral fertilizer combinations reduced the base saturation rate by 8.9% in both years. The effect of organomineral fertilizers on the base saturation ratio was found to be statistically significant ($p<0.001$). In many studies, it has been reported by researchers that the basic cations in the soil have a high exchange capacity in soils where organic matter is rich and decrease with the increase of organic matter (Fig. 2B) and decrease in pH values (Fig. 2C) in the soil and that basic cations pass into the soil solution in forms that the plant can take (Wuddivira and Camps-Roach, 2007; Osman, 2013; Blanco, 2017; Alkharabsheh et al., 2021).

Conclusion

According to the data obtained as a result of the study, the effects of organic fertilizer (dairy cattle manure), mineral P doses, and P-enriched organic fertilizer combinations (organomineral fertilizers) applied to the soil on the change of total cations, cation exchange capacity, and base saturation ratios in a calcareous soil at different levels as compare were evaluated. These comparative effects, by adding mineral fertilizers and organic fertilizers directly to the soil, or by the combination of these two fertilizers, the effects of the cation exchange capacity in the soil have provided some new ideas. In particular, mineral P doses had little or no effect on the cation exchange capacity in the soil. However, as the doses of organic fertilizer applied with the doses of mineral P were increased, the level of exchangeable cations and as a result, the CEC increased. Besides, cationic changes increased rapidly in organomineral fertilizers prepared by increasing the amount of organic matter.

The exchangeable Ca, Mg, K, and Na contents in organomineral fertilizer combinations were recorded as 8.67, 1.46, 0.74, and 0.042 me 100 g^{-1} on average, respectively. The cation exchange capacity and base saturation ratio were found to be 12.24 me 100 g^{-1} and 89.2% on average, respectively. With organic fertilizer applications, the exchangeable and exchangeable Ca, Mg, K, and Na contents of the soil were recorded as 8.41, 1.46, 0.70, and 0.044 me 100 g^{-1}, respectively. The cation exchange capacity and base saturation ratio were determined as 11.95 me 100 g^{-1} and 88.9% on average. With the addition of mineral P to the soil, the lowest cation changes, the content of total cations, and the highest base saturation rate were achieved compared to the other two fertilization systems. However, the fastest changes in the cation exchange capacity occurred with organomineral fertilizer applications. It was determined that the soil's exchangeable Ca, Mg, K, and Na contents increased by 11.2%, 22.8%, 29.7%, and 15.9%, respectively. In addition, organomineral fertilizer applications increased the cation exchange capacity by %21.3 and the base saturation rate decreased by 9.2%. As a result, it was concluded that M4P2 application (40 t ha^{-1} dairy cattle manure + 20 kg P ha^{-1}) could be the most economical and the most effective combination for CEC among organomineral fertilizer combinations.

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Research Article

Effect of Water Deficit at Different Growth Periods on Yield, Quality and Water Productivity of Sugarcane (*Saccharum officinarum* L.) under Central Sudan Agro-climatic Zone

Haitham Ageeb Mohammed ELBASHEIR[1], Samia Osman YAGOUB[2], Nahid KHALIL[3] Abdalbasit Adam MARIOD*[4]

[1]Guneid Sugar Factory, Sugarcane Research Center – Guneid, Sudan
[2,3]Weed Science Center, College of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Sudan
[4]University of Jeddah, College of Science, Jeddah, Saudi Arabia.
[4]Ghibaish College of Science and Technology, Indigenous Knowledge Center, Ghibaish, Sudan

[1]https://orcid.org/0000-0002-5730-3577, [2]https://orcid.org/0000-0002-9593-7941, [3]https://orcid.org/0000-0002-7415-3988
[4]https://orcid.org/ 0000-0003-3237-7948

*Corresponding author e-mail: basitmariod58@gmail.com

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Abstract: A field experiment was conducted during two consecutive seasons of 2020/21 and 2021/22 at the Sugarcane Research Center Farm – Gunied, (Central Sudan Agro-climatic zone), to evaluate the effect of water deficit irrigation at different growth periods on yield, quality and water productivity of sugarcane (*Saccharum officinarum L.*) Variety Co 6806. The study was designed in Randomized Complete Block Design (RCBD) and replicated three times. Irrigation deficit treatments were applied when available soil moisture content (ASMC) reached 25% in the root zone at eight different growth periods. The eight growth periods were begun from plant age 51th day to day100th at which the first deficit irrigation treatment was applied (DT1), age from day101th to day 150th the second deficit irrigation treatment was applied (DT2), age from day 151th to day 200th the third deficit irrigation treatment was applied (DT3), then at the same growth period length of 50 days fallow the other treatments till DT8 the eight irrigation deficit treatment was applied at crop age from day 401th to day 450th. These were compared with optimum irrigation (DT0) which was irrigated at 60% ASMC at the root zone. Results showed that all deficit irrigation treatments (DT1 to DT8) recorded significant cane and sugar yield reduction than the control (DT0) in the two growing seasons. In this sense, DT1, DT2, DT3, and DT8 treatments have recorded the highest cane and water productivity. Therefore, for sugarcane crop planting in November deficit irrigation must be avoided at the crop age of 6.7th month to age 13.3th month.

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1. Introduction

Sugarcane (*Saccharum spp*.) is a perennial crop, belongs to the family Poaceae, and is economically important for producing sugar and other products, such as electricity and bioethanol. For a long time, sugar is one of the essential components of the human diet (Ramiro et al., 2019). Its importance is realized due to its contribution towards meeting the individual energy requirement. The crop is cultivated in the tropical and subtropical region in an area of about 24.5 million hectares with a production of 1850 million cane tones' and 75.5 tc ha^{-1}. Recently the global harvest exceeded to175 million tons of sugar a year (FAO, 2016). Brazil was the largest producer of sugarcane in the world, followed by India, China, Thailand, Pakistan, and Mexico. While Brazil was still the largest sugar producing country in the 2020/2021 crop year, with 182 million metric tons of sugar as the total global production (FAO, 2021). Sugarcane is one of the most water-demanding crops after rice. For example, depending on the zone, it may take more than 1000 millimetres, i.e. 10 000 cubic metres of water, for a yield of 100 tons per hectare. Depending upon the agro climatic conditions and sugarcane yield water requirement varies (Choudhary et al., 2013). The average water requirement of sugarcane is to the tune of 1200 to 3000 mm. The number of irrigations in sugarcane depends upon the climatic conditions, type of soil, method of planting, and use of manures and fertilizers (Choudhary et al., 2013, Abu Alama et al. 2022). All the physiological and yield-related aspects of a crop were severely affected by drought from the very early stage of seedling to harvesting (Tawfik and El-Mouhamady, 2019). The proper irrigation interval can play a major role in increasing water use efficiency and productivity (Ethan et al., 2016). In India the highest yield was obtained when the crop was irrigated at eight-day intervals during early growth and at sixteen-day intervals during tillering and then every twelve days to maturity (Yahaya et al., 2010). On the other hand, Salgado et al. (2021) showed that suspending irrigation between 45 and 60 days before harvest increases the quality of the juices as well as the yields of the grinding stalks.

Sugarcane is grown as an irrigation and strategic crop in the central clay plain of Sudan; however, the sugar industry in Sudan faced several problems that lead to decreased sugarcane productivity from 93.57 tc ha^{-1} to 75.92 tc ha^{-1} to 49.74 tc ha^{-1} at crushing seasons 2010/2011, 2014/2015 and 2020/2021 respectively in Sudanese Sugar Company. That decrease in sugarcane productivity is due to high production and harvesting costs, lack of water poorly controlled, and the spread of weeds in both the ratoon and main crop (SSC, 2021). Water management plays an important role in enhancing sugarcane productivity stagnating in Sudan and demand for this crop is increasing. The scope for extending sugarcane areas in the country is limited. Under these circumstances, emphasis must be on water management issues that need to be addressed for increasing sugarcane productivity. Therefore, the present study was carried out to assess the effect of water deficit application at different growth periods on sugarcane yield, quality, and cane water productivity under the central Sudan Agro-climatic zone.

2. Material and Methods

2.1. Experimental site

A field experiment was conducted at the Sugarcane Research Center at Guneid farm, (14° 47″ N, 33° 19″ E, and an altitude of 386 m above mean sea level), during the 2020/021-2021/022 growing seasons. The objective was to evaluate the effect of water deficit irrigation at different growth periods on yield, quality, and water productivity of sugarcane (Saccharum spp.) crops, under the central Sudan Agro-climatic zone. The test crop was the sugarcane Co 6806 variety, which occupies around 90% of cultivated areas. Central Sudan's agro-climatic zone climate is classified as semi-arid the maximum air temperature ranges from 31.6 to 43.7°C, the minimum air temperature ranges from 12.8 to 25.7°C, relative humidity ranges between 22% to 83% (Table 1), also annual rainfalls were 191 mm and 236 mm at two growing seasons respectively (GMA, 2021). The field experiment soil has been described as Remaitab series (subclass S2v) which is Smectitic alluvium, clayey Vertisols with moderate chemical fertility, low infiltration rate, bulk density was 1.5, brown in colour, quite uniform, and alkaline in reaction (pH paste 8.1). It contained about

49% clay, 18% silt, and 33 % sand with a saturation of 59%, field capacity (FC) of 38.5%, welting point (WP) of 22 % and available water content was 16.5.

2.2. Experimental design

The experimental design was a Randomized Complete Block Design (RCBD). The field experimental unit was 112.5 m^2 (15m x 7.5m) consisting of five ridges. The sugarcane variety was planted in November and harvested in February at the age of 15 months. The recommended package of practices was followed to raise the crop. Furrow irrigation was used for the experiment and a Parshall flume was installed and a small pump to measure the quantity of water entering the field plot.

2.3. Deficit irrigation treatments

The treatments comprise two levels of water supply. The first was optimal irrigation (DT0) with full Irrigation water applied when the available soil moisture in the root zone reached 60% of the total available soil moisture (40% depletion). The second treatment was applied when available soil moisture content (ASMC) reached 25 % in the root zone (75% depletion).

Table 1. The second deficit irrigation treatments conducted at eight growth periods as following

Deficit irrigation treatment	Age of sugar cane
DT1	Plant age 51th days to day100th
DT2	Plant age from day101th to day150th
DT3	Plant age from day151th to day 200th
DT4	Plant age from day 201th to day 250th
DT5	Plant age from day 251th to day 300th
DT6	Plant age from day301th to day 350th
DT7	Plant age from day 351th to day 400th
DT8	Plant age from day 401th age to day 450th

2.4. Local climate, crop, and soil data

The reference evapotranspiration (ET0) for the Guneid area was computed using the FAO-Penman-Monteith approach (Smith, 1991) and CROPWAT software. Seasonal actual evapotranspiration (ETa) and the irrigation required throughout the growing season were calculated according to the method described by Doorenbos and Kassam (1979). The seasonal amount of water requirement (CWR) for sugarcane crop was determined as a function of the local climate, crop, and soil data according to Doorenbos and Kassam (1979) as:

$$CWR = ET0 \times kc \quad (1)$$

Where CWR is crop water requirement (mm day^{-1}), ETo is evapotranspiration of a reference plant under specified conditions, calculated by the class A pan evaporation method (mm day^{-1}), and Kc is the crop water requirement coefficient for sugarcane.

Soil samples were augured from each plot at a depth of 30 cm to determine the soil properties. Then soil moisture content was determined by gravimetric method (Farbrother, 1973) at 20 cm to 60 cm depth using an auger and Tensiometer. The sampling was made one day before irrigation and three days after irrigation throughout the growing seasons.

2.5. Agronomic parameters

Cane yield and quality parameters were recorded at the harvesting date.

2.5.1. Cane yield (TC/ha)

Cane yield and yield components viz; cane yield (tc ha^{-1}), stalk population (ha^{-1}), stalk height(cm), stalk diameter (cm), number of nodes per stalk, and internodal length(cm) were recorded.

2.5.2. Cane quality

A representative sample of 10 millable canes from each plot was taken randomly, stripped, cleaned, and squeezed by an electric mill and the extracted juice was screened to determine the following traits according to Gamechis., and Vighneswara, (2020) to *ICUMSA., (1997)*:

Pol % cane: Sucrose percentage (Pol %), which was determined by using a Saccharimeter device.

Purity % cane: It is the ratio between sucrose percent and the corrected brix (total solids) value expressed as percent purity of juice and calculated by using the formula of *(Spancer and Meade.,1963)*.

$$\text{Cane juice purity (\%)} = \frac{\text{Pol \% Juice}}{\text{Brix \% Juice}} \text{ x } 100 \quad (2)$$

Fiber content: Fiber was estimated at the time of harvest. Randomly selected, canes were cut into shreds with the help of shreds. 200 g pieces were weighed and taken in a bag and put under running water for 24 hours. After washing of all sugar, the remaining fiber was dried, weight was taken and the percent fiber was calculated by using the following formula.

$$\text{Cane fiber content \%} = \frac{\text{Dry weight of the washed shredded cane (g)}}{\text{Fresh weight of the shredded cane (200 g)}} \text{ x } 100 \quad (3)$$

2.6. Water productivity (WP)

It means the irrigation production efficiency which is defined as the ratio of crop yield to seasonal irrigation water applied including rainfall (Howell, 1994), it was calculated by using the following equation:

$$WP = Y/SI \quad (4)$$

Where WP is water productivity (kg $ha^{-1}m^{-3}$), Y is the yield (kg), and SI is the seasonal irrigation water applied including rain (m3).

2.7. Statistical analysis

Data collected were analyzed using the analysis of variance (ANOVA) technique to evaluate the differences among treatments. Means were separated using the least significant difference (LSD) at the 5% level of significance (USDA, NRCS, March 2007 USDA).

3. Results and Discussion

3.1. Crop water requirements (CWR)

Table 1 showed the climatic data of the experimental area (Guneid area) then the seasonal amount of water requirement for sugarcane crops was determined in Table 2. Moreover, it showed results that indicated that the highest period of sugarcane water requirements at the grand growth stage ranged from 6.3 to 10.3 mm day^{-1}, followed by the development stage with 4.3 to 6.7 mm day^{-1}, the initial stage with 2.90 mm day^{-1} to 3.2 mm day^{-1} and late season stage with a value of 3.9 to 4.0 mm day^{-1} water requirements,

respectively. Effective rainfall (Re) was recorded from June to September, the average ranged from 35.5 mm to 142 mm. Results also relieved that actual evapotranspiration (ET_a) reached a maximum value in March.

Table 1. Climatic data of the experimental area for the study years (2019-2021)

Years	Climatic data	Months											
		1	2	3	4	5	6	7	8	9	10	11	12
2019	Max. Temperature (°C)	36.1	36.1	37.5	41.7	43.1	38.46	37.4	32.7	34.9	35.0	37.2	33.9
	Min. Temperature (°C)	17.2	19.1	18.8	22.5	25.7	24.4	23.5	22.8	23.0	22.1	18.8	15.1
	R. humidity (%)	41.7	32.2	23.1	19.7	30.7	60.2	68.6	80.6	76.6	70.2	42.6	41.6
	Wind speed (m s^{-1})	1.9	2.1	1.9	1.7	2.4	4.0	4.0	2.5	2.8	1.0	1.1	1.5
	Evaporation (mm)	14.4	16.8	18.1	22.8	22.0	20.9	16.9	6.7	6.4	6.4	12.0	12.1
	Rainfall (mm)	-	-	-	-	-	15.6	43.4	129.7	69.7	8.4	-	-
2020	Max. Temperature (°C)	31.6	33.5	37.9	41.4	42.6	41.5	37.1	33.2	34.3	38.5	36.6	35.6
	Min. Temperature (°C)	12.8	14.4	24.8	22.0	25.6	24.9	22.2	20.1	22.7	24.7	18.3	16.4
	R. humidity (%)	37.2	32.7	24.1	22.0	31.3	47.4	67.4	83.1	76.9	62.3	41.3	44.1
	Wind speed (m s^{-1})	1.8	2.0	1.9	1.7	1.8	3.7	4.5	2.6	3.8	1.4	1.4	1.4
	Evaporation (mm)	13.2	14.7	23.9	18.9	17.9	18.2	18.2	6.3	7.2	11.2	14.4	12.8
	Rainfall (mm)	-	-	-	-	-	-	33.5	142.1	15.4	-	-	-
2021	Max. Temperature (°C)	33.3	34.1	40.2	39.2	40.0	40.5	35.9	34.9	35.5	39.0	38.2	32.5
	Min. Temperature (°C)	15.6	16.3	22.6	21.8	24.3	25.6	22.0	20.1	22.2	22.7	22.5	14.5
	R. humidity (%)	45.8	39.0	33.3	27.5	41.8	51.3	73.5	72.9	78.3	53.4	26.0	33.0
	Wind speed (m s^{-1})	6.82	1.90	2.27	1.97	2.21	2.77	4.3	2.4	2.3	0.8	0.9	1.5
	Evaporation (mm)	13.3	15.5	19.0	21.3	16.2	17.7	11.6	9.4	7.0	10.5	17.5	16.5
	Rainfall (mm)	-	-	-	-	10.3	40.0	58.7	79.6	47.4	-	-	-

Table 2. Sugarcane crop water requirements of the experimental area for two seasons

1st Season					2nd Season				
Month	**ET_0 (mm/day)**	**k_c**	**CWR (mm/day)**	**Rainfall (mm/month)**	**Month**	**ET_0 (mm/day)**	**k_c**	**CWR (mm/day)**	**Rainfall (mm/month)**
Nov 2019	5.13	0.6	3.08	-	Nov 2020	5.47	0.6	3.28	-
Dec	4.81	0.6	2.90		Dec	4.94	0.6	2.96	-
Jan 2020	5.32	0.8	4.26	-	Jan 2021	5.02	0.8	4.02	-
Feb	6.10	1.1	6.71	-	Feb	5.96	1.1	6.56	-
Mar	7.31	1.3	9.50	-	Mar	7.93	1.3	10.31	-
April	7.73	1.2	9.28	-	April	7.80	1.2	9.36	-
May	7.93	1.0	7.93	-	May	7.74	1.0	7.74	10.3
June	9.74	1.0	9.74	-	June	8.08	1.0	8.08	40.0
July	7.25	1.0	7.25	33.5	July	6.30	1.0	6.30	58.7
Aug	4.90	1.0	4.90	142.0	Aug	5.74	1.0	5.74	79.6
Sept	5.70	1.0	5.70	15.5	Sept	5.36	1.0	5.36	47.4
Oct	6.00	0.9	5.40	-	Oct	5.30	0.9	4.77	-
Nov	5.40	0.8	4.00	-	Nov	5.02	0.8	4.02	-
Dec	4.90	0.8	3.92	-	Dec	5.00	0.8	4.00	-
Jan 2021	Dry off	-	-	-	Jan2022	Dry off	-	-	-
Annual	-	-	-	191mm	Annual	-	-	-	236mm

CWR is crop water requirement (mm day^{-1}), *ET*o is evapotranspiration (mm day^{-1}), and *K*c is crop water requirement coefficient for sugarcane.

3.2. Effect of water deficit on different growth periods of cane yield

Effects of deficit irrigation at the different growth periods on cane yield parameters were represented in Tables 3, 4, and 5 for the first crop cycle (plant cane). It was clear that deficit irrigation displayed a negative effect on sugarcane cane yield parameters stalk height (cm), stalk diameter(cm), intermodal length, stalk population, and cane yield (tc ha^{-1}) during the two growing seasons.

3.2.1. Stalk height (cm)

Stalk height decreased whereas plant cane age increased in which deficit irrigation treatments were applied until peak, data illustrated in Table 3. The highest reduction in stalk high recorded at DT_5 treatments. Statistical analysis showed that water deficit treatments affected significantly stalk height. Stalk height was reduced when water deficit irrigation was applied at all eight growth periods compared to the optimum irrigation treatment which obtained maximum stalk height (222 cm). Deficit irrigation during grand growth periods of sugarcane reduced rates of stalk elongation and internode length (DT_3, DT_4, and DT_5). Similar results were found when Zhao et al. (2010) applied water stress, they observed reduction rates of plant elongation and node increment and there is a close relationship between plant height and stem diameter.

3.2.2. Stalk diameter (cm)

Analysis of variance showed that water deficit treatments significantly reduced stem diameter due to water stress restricted photosynthesis, elongation, and lateral enlargement. Data shown in Table 3 the finding agreed with Silva and Costa (2004).

Table 3. Effect of water deficit at different growth periods on cane stalk height and stalk diameter.

Treatments	Stalk height (cm)			Stalk diameter (cm)		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT_0	220.3^{a}	223.7^{a}	222.0	2.27^{a}	2.20^{a}	2.24
DT_1	211.0^{ab}	206.3^{abc}	208.7	2.02^{b}	2.20^{a}	2.11
DT_2	203.3^{ab}	206.3^{abc}	204.8	2.00^{b}	2.20^{a}	2.10
DT_3	198.7^{abc}	197.0^{abc}	197.9	1.90^{bc}	2.10^{a}	2.00
DT_4	179.3^{bc}	184.0^{bc}	181.7	1.70^{de}	2.10^{a}	1.90
DT_5	168.0^{c}	173.7^{c}	171.0	1.68^{e}	2.00^{a}	1.84
DT_6	184.0^{bc}	184.0^{bc}	184.0	1.73^{cde}	2.00^{a}	1.87
DT_7	181.7^{bc}	180.7 ^{bc}	181.2	1.73^{cde}	2.00^{a}	1.87
DT_8	201.0^{ab}	214.0^{ab}	207.5	1.87^{bcd}	2.20^{a}	2.04
Mean	194.2	196.0	195.1	1.87	2.10	1.99
CV%	9.49	9.9	-	5.60	6.20	-
LSD ($p \leq 0.05$)	31.91	33.6	-	0.18	0.23	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT0: Optimum irrigation, which was irrigated at 60% available soil moisture content (ASMC) at the root zone at all growing seasons. DT1 to DT8: Deficit irrigation treatments which were irrigated at 25% ASMC at the root zone at different growth periods.

3.2.3. Stalk population (1000ha^{-1})

Plant density is a major constituent of sugarcane yield. Tillering, which provides the plants with the optimum number of stalks needed for a good yield is known to be affected by the availability of the irrigation water. Water deficit treatments considerably decreased the sugarcane plant population compared with optimum irrigation treatment which produced an intensive plant population. The reduction of plant population when water deficit was applied to the sugarcane crop was probably due to a reduction in the number of tillers per plant. Zhao et al. (2010) reported that the water deficit reduced the number of tillers per plant. The reduction of the plant population in the second growing season was probably due to a reduction in total rainfall and the other climatic factors change (Table 1).

3.2.4. Internode length (cm)

The effect of Deficit irrigation application on intermodal length during 2020-21 and 2021-22 was significant (Table 4). The intermodal length significantly influences the yield of sugarcane. Optimal irrigation practice (DT_0 gave 10.8 and 9.5 cm intermodal length during the first and second seasons.

3.2.5. Stalk weight (kg)

Cane length displayed a positive correlation with one stalk weight and cane yield. No. of millable canes per hectare and yield are significantly correlated. Srivastava et al. (2005) found that the weight of one cane and cane yield were positively correlated. Table 5 showed that the effects of deficit irrigation on one cane stalk weight and total cane yield.

Table 4. Effect of water deficit at different growth periods on cane stalk population and intermodal length

Treatments	Stalk population (1000ha^{-1})			Internodal length (cm)		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT$_0$	148.0^{a}	172.0^{a}	160.0	10.8^{a}	9.5^{a}	10.2
DT$_1$	135.0^{ab}	169.0^{a}	152.0	10.3^{a}	8.3^{ab}	9.3
DT$_2$	126.0^{cb}	166.0^{a}	146.0	10.0^{a}	8.5^{ab}	9.3
DT$_3$	119.0^{bcd}	152.0^{a}	135.5	10.1^{a}	8.1^{ab}	9.1
DT$_4$	105.0^{d}	147.0^{a}	126.0	8.4^{b}	8.1^{ab}	8.3
DT$_5$	102.0^{d}	142.0^{a}	122.0	8.6^{b}	7.7^{b}	8.2
DT$_6$	117.0^{cd}	162.0^{a}	139.5	9.7^{ab}	8.6^{ab}	9.2
DT$_7$	110.0^{cd}	158.0^{a}	134.0	9.6^{ab}	8.6^{ab}	9.1
DT$_8$	118.0^{cd}	164.0^{a}	141.0	10.2^{a}	9.2^{a}	9.7
Mean	119.0	157.0	138.0	9.7	8.4	9.1
CV%	8.20	11.7	-	8.3	8.9	-
LSD (p≤0.05)	17.0	32.0	-	1.39	1.3	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT1 to DT8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400th to day 450th). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

3.2.6. Cane yield (tc ha^{-1})

There were significant differences in water deficit treatments on cane yield (Table 5 and Figure 1). DT$_0$ treatment recorded significant influence. The highest cane yield was (95.4 tc ha^{-1}), compared to DT$_4$, DT$_5$, and DT$_7$ treatments which recorded the lowest values of cane yield (81.4 tc ha^{-1}, 72.3 tc ha^{-1}, and 82.0 tc ha^{-1}) respectively, because high biomass crop requires large quantities of water for maximum production (Wiedenfed, 2008). Moreover, water stress reduced cane yield and dry weight of sugarcane (Basnayka et al., 2012). But DT$_6$ treatment recorded high values of cane yield (84.7 tc ha^{-1}) this is attributed to characterized of semi-arid regions during the rainy season by low temperatures, low evaporation rates, and high precipitation.

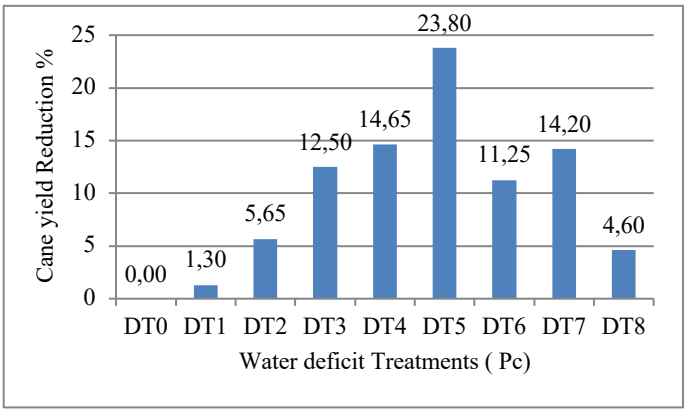

Figure 1. Effect of water deficit at different growth periods on cane yield reduction %, of two growing seasons (2020/021-2021/022).

3.3. Effect of water deficit at different growth periods on yield quality of cane plant

Tables 6, 7, and 8 showed the effects of deficit irrigation at different growth periods on yield quality parameters viz; Brix%, pol%, Purity%, Fiber%, ERS% cane, and Sugar yield (ts ha^{-1}), which affected by quality component. Moreover, water deficit negatively influenced sugarcane quality parameters. Control (DT_0) achieved the highest value of sugar yield (8.16) ts ha^{-1}. Furthermore, DT_8 recorded high in Brix% (16.1%), pol% cane (11.6%), ERS% cane (8.6%), and sugar yield values were (7.8 ts ha^{-1}) compared to DT_4, DT_5, and DT_7 treatments obtained the lowest values of sugar yield (6.55, 6.31, and 6.11 ts ha^{-1}, respectively). However, water deficits during the seven months of plant cane age (DT_1, DT_2, and DT_3 treatment) have significantly ($P \leq 0.05$) increased cane and sugar yield when compared to other treatments, this is attributed to the fact that deficit irrigation with the low level of water stress at tillering (DT_1, and DT_2, treatments) increase sugarcane plant numbers (Abdel-Wahab, 2005), while water deficit during the mid-season stage DT_4, and DT_5 were applied after fall significantly ($p \leq 0.05$) decreased cane and sugar yield compared to other treatments. This could mainly be because the mid-season stage is most sensitive to water stress (Eltayb, 2011). Deficit irrigation in the late season (DT_8) improves sugar cane quality and the crop is well ripened before harvest (Eltahir, 2002). But deficit irrigation before the drying off period after the rainy season has significantly ($p \leq 0.05$) decreased cane and sugar yield (DT_7), climatic data in Table 2 showed that in last October and November when deficit irrigation DT_7 was applied had high relative humidity % and high in evaporation(mm) that lead to a high reduction in sugar yield.

3.3.1. Total soluble solid (Brix% cane)

Total soluble solid is the main component determining the total sugar production. The deficit irrigation application method was failed to affect the brix % significantly. However, in the case of deficit irrigation treatments, brix % ranged from 16.42 to 16.10% during the first year and from 14.64 to 15.68% during the second year. These results agree with those of Jain et al. (2002) who reported that the quality of sugarcane did not vary. So that quality parameters such as brix and pol were not affected by cultural practices; Juice quality mainly depends on the genetic nature of the variety Wei and Eglinton (2022).

Table 5. Effect of water deficit at different growth periods on cane stalk weight and cane yield

Treatments	Stalk weight (kg)			Cane yield (ton ha^{-1})		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT_0	0.84^{a}	0.95^{a}	0.90	84.5^{a}	106.3^{a}	95.4
DT_1	0.83^{ab}	0.79^{ab}	0.81	82.8^{ab}	105.7^{a}	Mean
DT_2	0.83^{ab}	0.77^{b}	0.80	79.4^{b}	100.7^{b}	90.1
DT_3	0.80^{ab}	0.72^{b}	0.76	74.4^{c}	92.3^{c}	83.4
DT_4	0.74^{abc}	0.66^{b}	0.70	72.1^{c}	90.7^{c}	81.4
DT_5	0.65^{c}	0.62^{b}	0.64	67.5^{d}	77.0^{d}	72.3
DT_6	0.71^{bc}	0.75^{b}	0.73	75.3^{c}	94.0^{c}	84.7
DT_7	0.66^{c}	0.64^{b}	0.65	72.9^{c}	91.0^{c}	82.0
DT_8	0.81^{ab}	0.79^{ab}	0.80	81.7^{ab}	100.0^{b}	90.9
Mean	0.75	0.74	0.75	76.7	95.3	86.0
CV%	9.17	13.7	-	2.6	2.08	-
LSD ($p \leq 0.05$)	0.96	0.18	-	3.4	3.44	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT_0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT_1 to DT_8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400th to day 450th). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

3.3.1. Sucrose content in cane (pol% cane)

The data on sucrose content in cane, as influenced by different deficit irrigation treatments, are presented in Table 6. Gross carbohydrate i.e. pol% is another most important sugar yield-determining factor and is totally controlled by the genetic makeup of a variety and climatic conditions. Weather factors prevailing during the maturity stage play a major role in the quality parameters of sugarcane (DT_8). Thus, deficit irrigation application treatments did not exhibit any influence on the pol %. In this case, DT_5 DT_6, DT_8, and DT_0 deficit irrigation treatments, showed a high value of sucrose content in comparison to the other deficit irrigation treatments. Pol percent ranged from 12.28 to 10.81% and11.28 to 10.17% in the first and second year, respectively. These results are in line with those of Eltayeb (2011**)** who reported that juice quality parameters such as sucrose were not affected by deficit irrigation treatments.

Table 6. Effect of water deficit at different growth periods on cane Brix% and on cane Pol%

Treatments	Brix% cane			Pol% cane		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT_0	16.31^{a}	15.49^{ab}	15.90	11.90^{ab}	11.23^{a}	11.59
DT_1	16.20^{a}	15.49^{ab}	15.85	11.52^{bcd}	10.50^{ab}	11.01
DT_2	16.10^{ab}	15.41^{ab}	15.76	11.85^{ab}	10.60^{ab}	11.23
DT_3	16.07^{ab}	15.12^{ab}	15.60	11.89^{ab}	11.08^{a}	11.49
DT_4	16.34^{a}	15.18^{ab}	15.76	11.57^{bc}	10.62^{ab}	11.10
DT_5	16.25^{a}	15.61^{ab}	15.93	12.28^{a}	11.26^{a}	11.77
DT_6	16.24^{a}	14.79^{ab}	15.52	11.93^{ab}	11.18^{a}	11.56
DT_7	15.38^{b}	14.64^{b}	15.01	10.81^{d}	10.17^{b}	10.49
DT_8	16.42^{a}	15.68^{a}	16.05	11.97^{ab}	11.28^{a}	11.60
Mean	16.14	15.27	15.71	11.65	10.77	11.21
CV%	2.92	3.83	-	3.53	4.18	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT_0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT_1 to DT_8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400th to day 450th). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

3.3.3. Purity (%)

The data pertaining to cane juice purity, as influenced by different deficit irrigation treatments, are presented in Table 7. The results revealed that the purity of cane juice was affected significantly by deficit irrigation application. Under different deficit irrigation treatments, cane juice purity % ranged from 81.28 to 90.72 and 87.15 to 79.9 during 2020-21 and 2021-22. The results showed that DT_0 and DT_8 treatment obtained the highest purity% values 87.70 and 88.94 as the mean of the two growing seasons. While DT_4, DT_5, and DT_7 recorded the lowest purity% values of 84.88, 80.59, and 83.76 respectively. So, this means that there was a significant association between cane yield and traits for juice parameters like purity%.

3.3.4. Fiber (%)

Genetically Fiber (%) is a controlled feature of the sugarcane crop. The fact that fiber percent was mainly controlled by varietal genetic makeup was proved and thus fiber was not affected significantly during each year of study. Table 7 showed there was no significant difference between different water deficit treatments on fiber% cane in the second season clearly. DT_0 treatment recorded the lowest fiber% cane values in the mean of two growing seasons (16.62%) while DT_5, DT_7, DT_4, and DT_3 achieved the highest fiber% values of cane (18.97, 18.74, 18.33, and 17.92) respectively. However, for deficit irrigation treatments, the fiber% ranged from 16.62 to 18.97. Adoption of full irrigation resulted in an improvement in cane juice quality which was reflected in the reduced cane fiber percent in comparison to deficit irrigation treatments.

Table 7. Effect of water deficit at different growth periods on Purity% in Juice and Fiber% in cane

Treatments	Purity (%)			Fiber (%)		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT_0	90.01^{ab}	85.38^{ab}	87.70	16.27^{d}	17.57^{a}	16.62
DT_1	87.62^{abc}	86.21^{ab}	86.92	16.80^{d}	17.83^{a}	17.32
DT_2	88.74^{ab}	82.42^{ab}	85.58	17.17^{cd}	18.20^{a}	17.69
DT_3	88.74^{ab}	85.40^{ab}	87.07	17.53^{bc}	18.30^{a}	17.92
DT_4	86.23^{bc}	83.53^{ab}	84.88	18.33^{ab}	18.33^{a}	18.33
DT_5	81.28^{d}	79.90^{b}	80.59	19.43^{a}	18.50^{a}	18.97
DT_6	88.34^{abc}	86.04^{ab}	87.19	17.03^{cd}	17.83^{a}	17.43
DT_7	84.36^{cd}	83.16^{ab}	83.76	19.00^{a}	18.47^{a}	18.74
DT_8	90.72^{a}	87.15^{a}	88.94	16.97^{cd}	17.83^{a}	17.40
Mean	87.34	84.36	85.85	17.62	18.10	17.86
CV%	2.66	4.71	-	3.76	4.96	-
LSD ($p \leq 0.05$)	4.03	6.88	-	1.15	1.55	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT1 to DT8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400th to day 450th). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

3.3.5. Estimated recoverable sugar percentage (ERS%)

The results on estimated sugar recovery percentage clearly indicated that sugar recovery % was improved consistently during both the years of the study by treatments DT_0, DT_5, DT_6, and DT_8 compared to the other deficit irrigation, but the difference was low significant. The early development of millable canes with uniform maturity at harvest under deficit irrigation might have resulted in higher sugar recovery value. The differences between treatments didn't reach the significance level however, all deficit irrigation practices involved in the present investigation improved the percentage of cane juice recovery. Pure sugar is the goal of cane crop production and is mainly controlled by the genetic makeup of the variety. Thus, the water deficit factor has little effect on sugar recovery during each year of investigation.

3.3.6. Sugar yield (ton ha^{-1})

Perusal of data on sugar yield as influenced by deficit irrigation treatments revealed significant differences between the treatments (Table 8 and Fig 2). Sugar formation is dependent on climatic parameters and associated with an adequate water supply. The sugar yield is a function of cane yield and hence trend was similar as in cane yield. The sugar yields in various treatments followed the same trend as that of cane yield. Markedly the highest sugar yield was recorded in DT_0 which gave a significantly higher sugar yield (8.16 ton ha^{-1}) in the mean of both two years.

3.4. Effect of water deficit at different growth periods on water productivity of cane plant

Table 10 shows the effect of deficit irrigation on cane water productivity. High values of water productivity were recorded when deficit irrigation treatments DT_1, DT_2, DT_3, and DT_8 were applied followed by DT_6, DT_0, DT_4, DT_7, and DT_5 respectively. Moreover, cane yield reduction was not significant when compared to the benefits of saved water. This result agreed with Ayana (2011), who reported that deficit irrigation saved significant irrigation water without significant yield losses.

Table 8. Effect of water deficit at different growth periods on ERS% and Sugar yield

Treatments	ERS (%) cane			Sugar yield (ton ha^{-1})		
	1st Season	2nd Season	Mean	1st Season	2nd Season	Mean
DT$_0$	8.90^{ab}	8.28^{a}	8.57	7.52^{a}	8.80^{a}	8.16
DT$_1$	8.52^{bc}	7.50^{ab}	8.01	7.05^{ab}	7.93^{b}	7.49
DT$_2$	8.85^{ab}	7.60^{ab}	8.23	7.03^{ab}	7.65^{b}	7.34
DT$_3$	8.89^{ab}	8.08^{a}	8.50	6.61^{bc}	7.46^{bc}	7.04
DT$_4$	8.57^{bc}	7.62^{ab}	8.10	6.18^{c}	6.91^{c}	6.55
DT$_5$	9.28^{a}	8.26^{a}	8.77	6.26^{c}	6.36^{c}	6.31
DT$_6$	8.93^{ab}	8.18^{a}	8.56	6.72^{b}	7.69^{b}	7.21
DT$_7$	7.81^{d}	7.17^{b}	7.50	5.69^{d}	6.52^{c}	6.11
DT$_8$	8.97^{ab}	8.23^{a}	8.63	7.33^{a}	8.23^{a}	7.78
Mean	8.68	7.77	8.68	6.71	7.50	7.11
CV%	4.36	5.81	-	5.53	6.46	-
LSD (p≤0.05)	0.66	0.78	-	0.63	0.83	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT$_0$: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT$_1$ to DT$_8$: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400th to day 450th). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

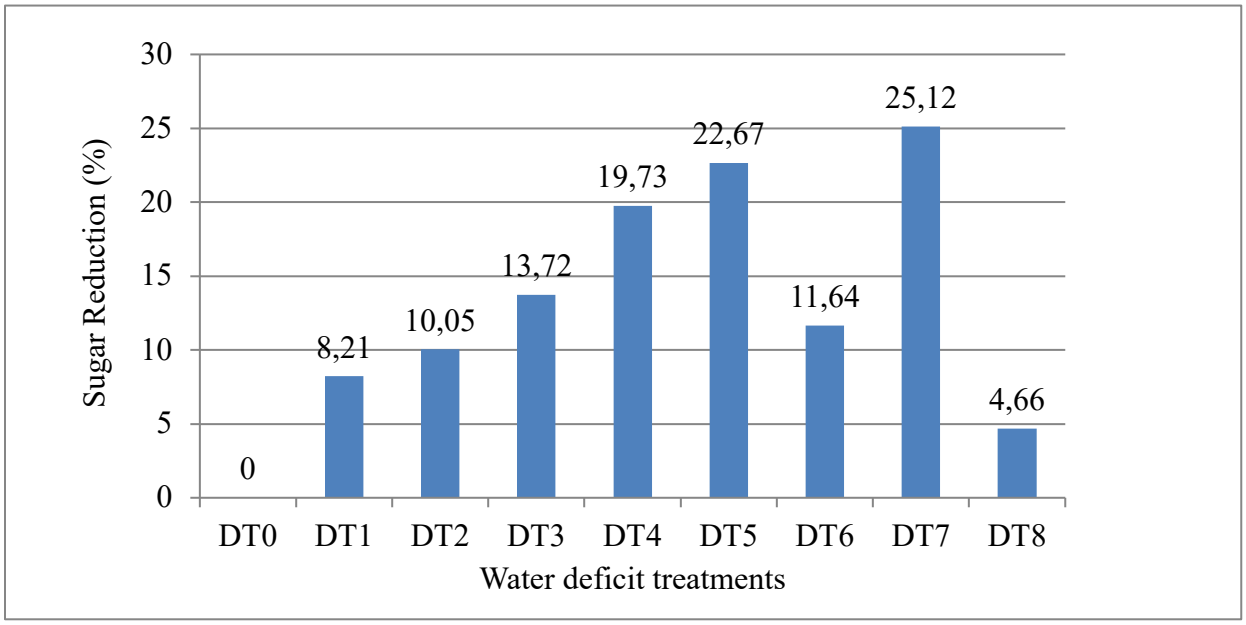

Figure 2. Effect of water deficit at different growth periods on plant cane sugar yield reduction %, mean of two growing seasons (2020/021-2021/022).

Table 9. Effect of water deficit on number of irrigations applied and water saved of plant cane at different growth Periods under Agro-Climatic zone (Sudan), seasons 2020/021- 2021-022.

Treatments	No. of irrigations applied	No. of Irrigations Saved	CWR $m^3(1000)\ ha^{-1}$/season	Water saved $m^3(1000)\ ha^{-1}$/season
$DT_{0\ (control)}$	34	0	23.3	0.0
DT_1	31	3	20.2	3.1
DT_2	31	3	18.2	5.1
DT_3	31	3	19.2	4.1
DT_4	31	3	20.2	3.1
DT_5	31	3	20.9	2.4
DT_6	31	3	20.5	2.8
DT_7	31	3	21.4	1.9
DT_8	31	3	21.6	1.7
Mean	31.3	2.67	20.6	2.7

Means sharing the same letters do not differ significantly at the 5% level of significance. DT_0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT_1 to DT_8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400^{th} to day 450^{th}). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC).

Table 10. Effect of water deficit at different growth periods on water productivity of cane plant

Treat.	CWR $m^3(1000)\ ha^{-1}$			Total sugarcane kg $(1000)\ ha^{-1}$			Water productivity (WP) kg $(1000)\ ha^{-1}\ m^{-3}$		
	1^{st} season	2^{nd} season	Mean	1^{st} season	2^{nd} season	Mean	1^{st} season	2^{nd} season	Mean
$DT_{0\ (control)}$	22.9	23.6	23.3	84.5 ^{a}	106.3^{a}	95.4	3.66	4.50	4.08
DT_1	20.1	20.2	20.2	82.8^{ab}	105.7^{a}	94.3	4.12	5.23	4.68
DT_2	17.9	18.4	18.2	79.4 ^{b}	100.7^{b}	90.1	4.42	5.48	4.95
DT_3	18.7	19.7	19.2	74.4 ^{c}	92.3^{c}	83.4	3.97	4.69	4.33
DT_4	19.6	20.8	20.2	72.1 ^{c}	90.7^{c}	81.4	3.69	4.36	4.03
DT_5	20.5	21.2	20.9	67.5 ^{d}	77.0^{d}	72.3	3.29	3.63	3.47
DT_6	20.0	21.0	20.5	75.3^{c}	94.0^{c}	84.7	3.75	4.48	4.12
DT_7	21.0	21.8	21.4	72.9 ^{c}	91.0^{c}	82.0	3.47	4.18	3.83
DT_8	21.2	22.0	21.6	81.7 ^{ab}	100.0^{b}	90.9	3.80	4.55	4.18
Mean	20.2	21.0	20.6	76.7	95.3	86.0	3.97	4.55	4.17
C.V %	-	-	-	2.6	2.08	2.34	-	-	-
LSD (P ≤0.05)	-	-	-	3.4	3.4	3.4	-	-	-

Means sharing the same letters do not differ significantly at the 5% level of significance. DT_0: Optimum irrigation, which was irrigated at 60% available soil moisture content at the root zone. DT_1 to DT_8: Deficit irrigation at first growth period to deficit irrigation at eighth growth period (from day one to day fifty after germination and from day 400^{th} to day 450^{th}). All these treatments were irrigated at 25% available soil moisture content at the root zone (ASMC). Means sharing the same letters do not differ significantly at the 5 % level of significance. CWR: Crop water requirement.

Conclusion

Deficit irrigation treatments (DT1 to DT8) recorded a significant effect on cane and sugar yield reduction than the control (DT0) in the two seasons (2020/2021 and 20 21/2022) under Gunied conditions, Central Sudan Agro-climatic zone.

DT3, DT4, DT5, and DT7 treatments recorded significantly the highest cane yield reduction was 12.50 %, 14.65 %, 23.8 %, and 14.20 %. Sugar yield reductions were 13.70 %, 19.70%, 22.67%, and 25.12% respectively compared to DT0 with full Irrigation.

High Sugarcane water productivity was recorded at deficit irrigation treatments DT1, DT2, DT3, and DT8 respectively compared to optimum irrigation (DT0) as plant cane.

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Research Article

Investigation of Foliar L-Glutamic Application on the Resistance to the Capacity of the SC2121 Tomato Variety (*Solanum lycopersicum* L.) to Long-Term Salinity Stress

Nezahat TURFAN*[1], Özlem DÜZEL[2]

[1,2] Kastamonu University, Science faculty, Biology department, Kuzeykent, 37150, Kastamonu, Türkiye

[1]https://orcid.org/0000-0002-5753-0390, [2]https://orcid.org/0000-0002-4824-5985

*Corresponding author: nturfan@kastamonu.edu.tr

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Abstract: Within the scope of this study, the effects of L-Glutamic acid (L-GLU: 250 mg L^{-1}, 500 mg L^{-1}) treatments on morphological and biochemical characteristics of SC2121 tomato variety under salt stress (50 mM, 100 mM, 200 mM NaCl) were compared. The morphological results obtained from leaves and fruits were found to peak at 500GLU, 50NaCl-500GLU, 250GLU, and 200NaCl-500GLU, whereas their lowest values were achieved with doses of 200NaCl, 200NaCl-250GLU, 100NaCl, and 100NaCl-500GLU Among the bioactive molecules, amino acid, and proline amounts increased in all the treatments, whereas total protein increased in 500GLU and 50NaCl- 250GLU, 50NaCl-500GLU. CAT activity increased in doses of 500GLU and 50 NaCl-250GLU, 50NaCl-500GLU, whereas POD and SOD activity decreased in high NaCl and 200NaCl+ 250GLU, 200NaCl-500GLU. Treatments caused an increase in MDA concentration, while NaCl (50-100 mM), GLU, and 100 NaCl-500GLU reduced the H_2O_2 concentration. In conclusion, 500GLU, 50NaCl-500GLU, 50NaCl-250GLU, 250GLU, and 200NaCl+500GLU stimulated the growth and development in the SC2121 tomato variety, as well as the leaf bioactive chemicals. However, 200NaCl-250GLU, 200NaCl, and 200NaCl-250GLU reduced the growth and development of the tomato and decreased the chemicals in the leaves. Given the results, it can be stated that yield and quality could be increased by making use of GLU treatments in tomato varieties under salt stress.

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1. Introduction

Salinity is one of the most progressive environmental factors suppressing the productivity of agricultural plants, excluding the salt-tolerant genotypes. Salinity problems can naturally develop in arid and semi-arid areas in regions with low precipitation or very uneven distribution and parameters such as excessive and unconscious irrigation in agricultural lands, the use of low-quality irrigation water, and high doses of fertilizers (Çelik et al., 2022; Kıpçak et al., 2019). It was researchers reported that approx. 20% of cultivated areas and 50% of irrigated areas worldwide are affected by salinity, 10 million hectares of land become unusable every year, and this amount will continue to increase. It was reported that data, approximately 6% of the world's land is threatened by salinity (Yılmaz et al., 2022). In Türkiye, 1.5 million hectares of land face salinity problems, and 60% of these areas are salty, 19.6% moderately

saline, 0.4% moderately alkaline, and 12% mildly saline (Gursoy, 2022). Salinity influences plant morphologically, physiologically, and biochemically, and lead to serious losses in yield and quality (Tuncer, 2017; Can et al., 2021). It was proven that physiological reactions promoting growth and development such as photosynthesis, respiration, nitrogen and carbohydrate metabolism, soil-water-plant relations, and cell cycle are suppressed due to salinity stress (Sadak et al., 2014; Gursoy, 2022). In the studies aiming to solve the salinity problem, methods such as 1) reclamation of saline soils, 2) traditional breeding methods, modern molecular biology methods, and 3) selection and development of salinity-resistant species and varieties are primarily used (Turhan and Şeniz, 2010; Can et al., 2021). The first option is expensive and difficult, the second option is time-consuming, and the methods obtained cannot guarantee genetically modified products. Therefore, it seems that the most suitable way in agricultural lands having high salinity levels is the third option, which is the exogenous application of biosimultans such as amino acids, hormones, jasmonates, polyamines, and minerals to plants (Bahjat et al., 2022; Alp and Şensoy 2023). Researchers reported that NH_4^+ in the amino acid structure participated in the structure of organic compounds directly and with less energy and that exogenous amino acid applications were more advantageous in increasing plant yield and stimulating stress resistance (Çakır, 2017; Sun et al., 2019). They have a carboxylic acid, hydroxyl, sülfidril, and histidine group, an amino group, and a side chain, which arrest some elements such as Cl, and Na, and chelate some of them (Hildebrandt, 2018; Kuşvuran et al., 2019). Moreover, since they inhibit the synthesis of reactive radicals or stimulate the antioxidative defense systems, they play an important role in protecting plant cell structures against salinity stress effects (Noroozlo et al., 2019). Glutamate, as the main NH_4^+ donor, is metabolized to other amino acids such as proline, glycine, and arginine, and also plays an important role in chlorophyll biosynthesis, activation of plant hormones, cell division, and phytochelatin activities (Septiyana et al., 2019).

In the present study, it was aimed to reveal the effects of salt stress and foliar L-Glutamic acid applications on seedling growth, pomological measurements in fruit, and the amounts of some bioactive components in leaves in SC2121 tomato (*Solanum lycopersicum* L.) variety. As a result, determining the most suitable GLU doses, which minimize the effect of salt stress on the growth capacity of shoot/root, leaf, flower and fruit, and leaf phytochemicals, is important to recommend tomato cultivation in salt-stressed agricultural lands. This study is also of significant importance as it is the first to examine the effects of L-Glutamic acid on salt stress resistance of the SC2121 tomato genotype.

2. Material and Methods

This study was carried out in a garden in Elmadağı district of Ankara province between 17 April-28 July 2022. SC2121 tomato variety was used as research material. It is one of the most preferred varieties because it is early growing. It is suitable for field cultivation, suitable for table consumption, round and red in color, and thin-shelled (Omar Bohalima, 2017).

2.1. Description of Study Area

Elmadağı is a district that is 31km away from Ankara province, has a surface area of 647 $km^{2,}$ and an altitude of 1088 m. The district has been established between 39° 55' 19.6716" N and 33° 13' 34.5540" E coordinates and it is the 492nd largest district in Turkey. This region has a continental climate, in which winters are cold and harsh. As presented in Table 1, the temperature ranged between 11.25 and 23.46 ºC during the study period; the highest temperature was found to be 38.46 ºC in July and the lowest one was found to be 2.55 ºC in April. The average level of precipitation ranged between 14.10 and 51% and peaked in May (Table 1). The characteristics of the soil and water samples used are presented in Table 2. For the soil and water samples used, pH ranged between 6.88 and 7.02 and conductivity ranged between 0.58 and 142.2, whereas N content (%) was found to be 1.68-5.14, K to be 33.46-8850 mg kg^{-1}, P to be 116.44-6076 mg kg^{-1}, Fe to be 4.40-345 mg kg^{-1}, Zn to be -4.64 28.4 mg kg^{-1}, and n to be 1.17-87.8 mg kg^{-1}.

Table 1. Monthly average meteorological values of the study area

Months	Mean (°C)	Maximum (°C)	Minimum (°C)	Precipitation (mm)	Humidity (%)
April	11.25	25.12	2.55	42.20	56
May	16.14	35.22	12	51.30	53
June	20.18	30.16	12.95	35.20	51
July	23.46	38.46	15.85	14.10	50

Table 2. Properties of soil and water used in the experiment

	pH	Conductivity ($\mu S\ cm^{-1}$)	N	K	P	Fe	Zn	Mn
Soil	7.02	0.58	5.14	8600	5064	255	28.4	43.2
Manure	6.88	1.33	1.68	8859	6076	345	24.6	87.8
Water	7.82	142.2	-	33.46	116.44	4.4	4.64	1.17

2.1.1. Arrangement of experimental design of the study

First of all, soil mortar was prepared by mixing garden soil and barnyard manure 2:1. First of all, soil mortar was prepared by mixing garden soil and barnyard manure 2:1. Some of the soils were filled in viols containing 32 cells (35x35 diameter, 48 mm depth, 38 cc) and some in polyethylene seedling growing tubes (25x50 cm). Viols and pots 1- Control (0), 2- 50 mM NaCl (50NaCl), 3-100 mM NaCl (100NaCl), 4- 200 mM NaCl (200NaCL); 5- 250 mg/L L-Glutamic acid (250GLU), 6-500 mg/L L-Glutamic acid (500GLU); 7- 50 mM NaCl + 250 L-GLU (50NaCl-250GLU), 8- 50 mM NaCl + 500 L-GLU (50NaCl-500GLU), 9- 100 mM NaCl + 250 GLU (100NaCl-250G), 10-100 mM NaCl + 500 GLU (100NaCl-500G), 11- 200 mM NaCl + 250 GLU (200NaCl-250GLU) and 12- 200 mM NaCl + 500 GLU (200NaCl-500GLU) were classified and labeled as 12 groups. Some of the seedlings grown from the viols were used for morphological measurements and chemical analyzes on shoots, roots, and foliage. Some of them were transferred to seedling growing bags when they were at the 2-3 leaf stage and they waited for 2 weeks for their adaptation to the new environment before starting the applications. On the other hand, the measurements of the flower, fruit, branch, and shoot of these plants were carried out with seedlings developed in plastic bags. In the pot trials, a total of 9 pots were used with 3 replications for each group. NaCl applications ($NaCl_2$: Sodium chloride-CAS 7647-14-5) were made by giving NaCl solutions dissolved in bottled water (viol: 10 ml and bag: 350 ml) from the soil, whereas L-GLU (L-Glutamic acid: Sigma-Aldrich-CAS 616281) applications were carried out by sprinkling GLU doses dissolved in pure water on the lower and upper surfaces of the leaves with a sprayer. Applications to seedlings in the viols and tubes were made twice a week for 8 weeks. After the termination of the applications, the seedlings in the viols were harvested and used in morphological and chemical analyses. The number of leaves, the number of branches, root diameter, and fruit characteristics were determined in plants grown in plastic bags. The L-GLU concentration applied in the study was determined by a preliminary study. For the GLU concentration, it was selected by considering the germination capacity of the seeds which caused an average 50% increase, while the NaCl concentrations were determined by considering the germination capacity that increased by more than 50% and caused more than 50% inhibition (Sadak et al., 2015; Yılmaz et al., 2022).

2.1.2. Morphological measurements

After the treatments, the seedlings in vials were removed by paying attention to not damaging the roots, the soil particles on the roots were eliminated by using tap water, and then they were used in morphological measurements. Shoot length and root length were measured using a ruler. The dry and fresh weights of shoots and roots, and also the fruit weights were measured using a precision scale, root collar, and fruit length and fruit width were measured by a digital caliper. And, the numbers of leaves, branches, and flowers were determined by counting per plant. While 10 plants were used for each character measured for seedling and leaf development, the measurements of fruit characteristics were carried out with 10 randomly selected fruits.

2.1.3. Biochemical analysis

The chlorophylls, carotenoids, and xanthophylls were homogenated with ethanol and estimated according to the methods described by Kukric et al. (2012) and Chang et al. (2013). Total anthocyanin level measures following the methods described by Burgos et al. (2013).

Total free proline concentration was determined by following Bates et al. (1973) method. A 500 mg fresh leaf was homogenized with 10 mL sulfosalicylic acid and filtered. Then 2 mL of filtrate was mixed with 2 mL acid ninhydrin solution, 2 mL glacial acetic acid, and 4.0 mL of toluene in a test tube, the absorbance was taken at 520 nm. Whilst the protein content was determined according to the Bradford method (1976), total free amino acid to the Spies (1957).

MDA content was estimated following the original method of Lutts et al. (1996). H_2O_2 level was measured spectrophotometrically according to the method of Velikova et al. (2000).

The superoxide dismutase (SOD) activity was assayed by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm (Çakmak 1994). The catalase (CAT) activity was assayed by monitoring the degradation of H_2O_2 at 240 nm over 2 min against a supernatant-free blank (Bergmeyer, 1970). The peroxidase (POX) activity was based on the method described by Lee and Lin (1995). All chemical analyses were done with three replications.

2.1.4. Statistical analysis

One-way ANOVA (Analysis of variance) was applied for analyzing the differences in the growth parameters, fruit characteristics, and chemical constituents in the leaf samples of tomato seedlings. The statistical analysis was performed using the SPSS program (Version 11 for Windows). Following the results of ANOVAs, Tukey's honestly significant difference (HSD) test (α= 0.05) was used for significance.

3. Results and Discussion

3.1. Variation of growth parameters of seedling

Foliar application of GLU is one of the most up-to-date agricultural approaches to improving the growth and development, yield, and quality of agricultural species under abiotic stresses (Fardus et al., 2021; Engin and Gökbayrak, 2022). In the present study, the effects of GLU treatments on the growth parameters of SC2121 tomatoes subjected to salt stress are presented in Table 3. The highest values for shoot length and root length, shoot fresh weight and root fresh weight, shoot dry weight and root dry weight, and numbers of leaves and branches per plant were obtained using GLU doses, 50NaCl+ 250GLU, 50NaCl-500GLU, and 200NaCl-500GLU (Table 3). Moreover, shoot length and root length values increased in all the treatment groups in comparison to the control, and shoot fresh weight and root dry weight showed significant increases with GLU and NaCl-GLU, whereas root fresh weight increased significantly in 500GLU and NaCl-GLU doses (Table 3). On the other hand, among the parameters examined, whereas fresh shoot weight reached the minimum level with 200NaCl, root fresh weight with 250GLU and 200NaCl, dry root weight with 100NaCl and 200 NaCl, and the number of leaves per plant with 200NaCl (Table 3). The results found for the effects of NaCl and GLU applications on the shoo-root development and leaf numbers are in parallel with the literature. The negative effects of salt stress were reported for tomato (Turhan and Şeniz, 2010), for common beans (Kıpçak et al. 2019), and wheat (Yılmaz et al., 2022). However, many researchers also reported that the exogenous applications of amino acids were effective in recovering the plant from salt stress or reducing the negative effects (Jannesari et al., 2016; Alp and Şensoy, 2023). Similar to the present study, Sadak et al. (2015) examining broad beans and Souri et al. (2017) investigating tomato, squash, and bean plants grown in calcareous soils determined that exogenous amino acid treatments stimulated the shoot and root length; shoot fresh weight, root fresh weight, shoot dry weight, root dry weight; the number of leaves, leaf development, and yield. In other studies carried out on okra (Greenwell and Ruter, 2018) and lettuce (Noroozlo et al., 2019), it was found that glutamine, arginine, and glycine/glutamine applications stimulated the shoot and root development in those plants.

Table 3. Variation of the growth parameters of SC 2121 tomato seedlings under salinity (L-GLU: Glutamic acid)

Group	Doses	Shoot length (cm)	Root length (cm)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Leaves /per plant
Control	**0**	20.33±1.20b*	5.26±0.68c	3.43±0.18c	1.04±0.03b	1.11±010c	0.21±0.03c	29.9±2.25c
NaCl mM	**50**	23.11±2.34b	5.72±0.44c	3.90±0.65c	1.02±0.07b	1.10±0.14c	0.20±0.03c	32.90±3.14c
	100	21.77±1.17b	7.45±1.12b	3.36±0.26c	1.02±0.05b	1.19±0.08c	0.20±0.04c	29.80±1.82c
	200	18.18±1.07c	7.36±0.80b	3.04±0.17c	0.94±0.04b	0.99±0.07c	0.23±0.05c	24.70±1.40
L-GLU mg L^{-1}	**250**	25.52±1.77a	8.91±0.89a	5.09±1.16b	1.34±0.19a	0.95±0.06c	0.31±0.03b	38.7±2.84
	500	28.61±3.50a	9.76±1.40a	7.83±2.09a	1.30±0.20a	1.53±0.24a	0.33±0.04b	44.90±7.12b
NaCl/L-GLU	**50/250**	26.01±2.17a	7.73±0.49b	6.44±1.05a	1.18±0.09b	1.66±0.11b	0.57±0.08a	40.40±3.83b
	50/500	25.54±1.56a	6.66±0.83b	6.76±0.65a	1.38±0.14a	1.61±0.14b	0.43±0.06b	51.10±5.90a
	100/250	15.96±1.88d	7.66±0.70b	3.77±0.73c	0.63±0.10c	1.15±0.14c	0.29±0.03b	34.80±4.99c
	100/500	14.76±1.01d	7.74±0.82b	3.43±0.41c	0.38±0.02d	1.38±0.12b	0.27±0.03b	27.80±3.47c
	100/250	17.92±2.76c	7.77±1.20b	4.49±1.20b	0.82±0.19bc	1.61±0.26b	0.35±0.07b	29.86±4.12c
	200/500	24.36±4.52b	9.10±1.34a	7.74±2.81a	1.47±0.4a	2.81±0.86a	0.41±0.10b	36.90±6.1b1
F		3.617	1.929	2.151	3.404	3.172	4.141	2.195
P		<0.001	<0.043	<0.022	<0.001	<0001	<0.001	<0.020

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$.

In the present study, the decrease in shoot height in 100NaCl-250GLU, and 100NaCl-500GLU, and 200NaCl-250GLU in comparison to the control group were thought to be a response to salt-related water deficiency in the soil, reduced shoot length. Results found on the number of leaves per plant confirm this finding (Table 3). When compared to the control group, the treatments decreased the seedling root collar, number of branches per plant, and number of flowers per plant but resulted in an increase in the number of leaves per plant (Table 4). The number of leaves peaked at 100NaCl, 500GLU, and 50NaCl-50GLU. The number of branches and the fruit characteristics found in the present study were similar to those reported by Kavasoğlu and Ceyhan, (2018) for beans, Civelek and Yıldırım (2019) for tomatoes, and Septiyana et al. (2019) for okra. In another study, Omer et al. (2013) examining the chamomile plants grown under salty conditions reported that the plant height, the number of branches, and the number of flowers decreased under salty conditions but branch number and flower yield increased in response to the amino acid treatments. The decrease in the number of flowers was also reported by Nahed et al. (2007). Geshnizjanı and Khosh-Khui (2016) and Septiyana et al. (2019), differing from the present study, stated that amino acid treatment increased the number of flowers. However, Jannesari et al. (2016) emphasized that it stimulated the fruit yield under salty conditions but amino acid treatments had no significant effect. In the present study, glutamic acid stimulated the number of leaves and fruit development but had no effect on blooming. In the present study, glutamic acid stimulated the number of leaves and fruit development but had no effect on blooming. It was thought to be because of differences in the reproductive proliferation differences of species, highness of treatment (NaCl, GLU) doses, and duration.

Table 4. Variation of root, leaf, branch, flower and fruit characteristics of SC 2121 tomato seedlings under salinity (L-GLU: Glutamic acid)

Group	Doses	Root collar	Leaf number	Branch number	Flower number	Fruit Width (cm)	Fruit Length (cm)	Fruit FW
Control	**0**	5.30±0.30a*	217.0±28.57h	45.8±7.06a	23.0±2.30a	4.06±0.30b	3.72±0.37c	36.8±7.78f
NaCl mM	**50**	4.32±0.11b	272.8±20.32d	24.6±1.66f	14.2±1.02c	4.76±0.25b	3.82±0.16c	41.8±6.79e
	100	4.94±0.22a	447.8±59.97a	27.8±2.78c	17.0±2.85b	4.86±0.65b	3.94±0.34b	53.4±14.99d
	200	4.46±0.08b	242.4±15.84g	22.8±1.16e	12.4±0.75d	5.74±0.36a	4.84±0.35a	75.4±10.91a
L-GLU mg L^{-1}	**250**	4.44±0.06b	221.8±25.55h	26.2±2.58c	16.8±2.91b	5.16±0.50a	3.90±0.19b	61.6±21.90b
	500	4.80±0.20b	374.6±12.94b	24.0±1.82e	14.8±1.32c	4.96±0.55a	3.96±0.17b	53.8±9.31d
NaCl/L-GLU	**50/250**	4.94±0.17a	318.2±25.64c	29.8±4.88c	18.6±3.78b	4.04±0.12b	3.46±0.30d	33.6±3.43d
	50/500	4.64±0.10b	263.2±25.14e	24.2±1.07f	18.0±1.38b	5.46±0.46a	4.70±0.36a	76.8±19.02a
	100/250	4.20±0.10b	253.8±26.22f	23.2±2.46e	13.8±0.92c	4.72±0.12b	4.26±0.09b	57.6±3.43c
	100/500	4.38±0.10b	304.0±5.57c	24.8±2.38f	14.8±0.59c	4.12±0.19b	3.74±0.05c	32.0±3.10f
	100/250	4.42±0.06b	231.2±22.98g	23.0±2.45e	14.0±1.14c	3.84±0.14b	3.52±0.15d	34.4±1.69f
	200/500	4.64±0.19b	299.2±7.69c	33.0±1.87b	15.6±1.12c	4.24±0.33b	3.80±0.22c	39.6±9.64e
F		4.225	6.113	4.370	2.163	2.732	2.915	2.003
P		<0.001	<0.001	<0.001	0.033	<0.008	<0.005	<0.049

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$.

3.2. Effects of NaCl and GLU treatments on the bioactive chemical constituents of samples

Chlorophyll molecules are the major light-harvesting pigment, involved in the conversion of carbon dioxide in the air into energy-rich compounds, which are necessary to survive the life cycle of plants (Rudiger, 1997; Greenwell and Ruter, 2018). In the present study, the salt concentrations other than the dose of 200NaCl did not cause any decrease in chlorophyll content. On the other hand, the doses of 100NaCl-250GLU, 100NaCl-500GLU, and 200NaCl-250GLU significantly reduced the chlorophyll a and b and total chlorophyll contents in comparison to the control group (Table 5). The ratio of chlorophyll a to chlorophyll b decreased in all the treatment groups when compared to the control group, whereas total carotenoid content decreased in 100NaCl-250GLU, 100NaCl-500GLU, and 200NaCl-250GLU. The highest level of decrease in the chlorophyll molecules was observed in 100NaCl + 250GLU, 100NaCl-500GLU, whereas the highest level of stimulation was found in 50NaCl + 250GLU, 50NaCl-500GLU (Table 5). The increase in chlorophyll pigments in low (50 and 100 mM) NaCl doses suggests that the SC2121 variety was tolerant to low salt concentrations (Turfan, 2017; Çelik and Karakurt, 2022). However, the fact that the highest level was found in 50NaCl+250GLU, and 50NaCl-500GLU showed that the low level of glutamic acid application reduced the damage caused by salt stress (Sadak et al., 2015; Rivera et al., 2022). The parameter affected by the treatments the most was the ratio of chlorophyll a to chlorophyll b (Table 5). Chlorophyll a has a high level of sensitivity to stress conditions such as light, drought, and salt (Rudiger, 1997). Transformation of chlorophyll a to chlorophyll b in the SC2121 tomato variety might be an adaptive mechanism. According to the results, it was revealed that exogenous GLU applications were found to be positively effective at low salt (50NaCl) concentrations, and the highest NaCl.

Table 5. Variations of the amount of chlorophyll pigment and total carotenoid under salinity

Group	Doses	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}	Total Chlorophyll mg g^{-1}	Chlorophyll a/ Chlorophyll b	Total carotenoid mg g^{-1}
Control	**0**	0.381±0.001d*	0.163±0.001g	0.544±0.002f	2.35±0.002a	7.36±0.01b
NaCl mM	**50**	0.399±0.001d	0.212±0.001e	0.611±0.001d	1.89±0.003c	7.20±0.01b
	100	0.446±0.002c	0.195±0.001f	0.640±0.001d	2.29±0.013a	7.68±0.01b
	200	0.274±0.001f	0.214±0.001e	0.488±0.001g	1.29±0.009e	5.39±0.01
L-GLU mg L^{-1}	**250**	0.532±0.001c	0.374±0.001c	0.905±0.001c	1.43±0.007d	9.38±0.01a
	500	0.499±0.002c	0.265±0.003d	0.764±0.0018d	1.88±0.030c	8.40±0.02b
NaCl/L-GLU	**50/250**	0.803±0.001a	0.481±0.002a	1.283±0.002a	1.67±0.005	10.89±0.01a
	50/500	0.773±0.002b	0.395±0.002b	1.167±0.004b	1.96±0.008b	10.58±0.01a
	100/250	0.184±0.001g	0.090±0.001h	0.273±0.001ı	2.05±0.014b	2.99±0.01d
	100/500	0.293±0.004e	0.143±0.001g	0.426±0.001h	1.89±0.003c	5.17±0.01c
	100/250	0.308±0.009e	0.149±0.001g	0.456±0.008g	2.07±0.076b	5.30±0.02c
	200/500	0.478±0.001c	0.223±0.001e	0.700±0.002d	2.14±0.006a	7.51±0.02b
F		4121.51	6664.93	12473.135	164.47	51519.996
P		<0.001	<0.001	<0.001	<0.001	<0.001

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$

Similarly, in studies aiming to decrease the damages of salt stress, it was observed that chlorophyll pigments decreased depending on genotype and salt concentration but exogenous bio-stimulant implementations caused an increase in the pigment content (Sadak et al., 2015; Fardus et al., 2021). The higher chlorophyll a, chlorophyll b, and total chlorophyll contents found in 250GLU, and 500GLU doses in the present study were related to the effectiveness of GLU in chlorophyll biosynthesis (An et al., 2019). Similar results were reported for lettuce (Noroozlo et al., 2019), and cucumber (Ikbal et al., 2021). The amount of xanthophyll was lowest in the control, conversely, it peaked in 200NaCl, 200NaCl-250GLU, and 200NaCl-500GLU doses (Figure 1A). This finding showed that this variety was tolerant to low salt concentrations but sensitive to high salt concentrations (Turhan and Şeniz, 2010; Fardus et al., 2021). Hence, Qiu and Lu (2003) carried out a study on *Atriplex centralasiatica*, a

halophilic plant, and reported that carotenoids stimulated salt tolerance and xanthophyll increased the herbal resistance by protecting the light-harvesting pigment systems.

GLU treatments might have exhibited their positive effects on xanthophyll via the synthesis of required compounds and the activation of enzymes (Johnson et al., 2010; Cirillo et al., 2021). Anthocyanin concentration was low in 100NaCl-500GLU, 200NaCl-250GLU, and 200NaCl treatment groups but GLU increased in 50NaCl + 25GLU, and 50NaCl-500GLU (Figure 1B). Glutamic acid solely stimulated the anthocyanin accumulation but its effect on salt damage was observed in low NaCl+ GLU doses (Figure 1B). This might be because GLU doses activated the enzymes that were responsible for anthocyanin (Gould et al., 2002; Turfan and Turan, 2023).

Anthocyanin accumulation was reported to be stimulated by mechanical injury in pepper plant in a study by Gould et al. (2002), by high temperature in Arabidopsis in a study carried out by Shao et al. (2007), and tobacco in a study carried out by Cirillo et al. (2021) and it was also reported to protect the plants from stress damages. Amino acids and total soluble protein are important osmolytes playing a significant role in cells in osmosis, regulation of turgor reactions, and also, being metabolized and forming the precursors of many compounds (Wang et al., 2014). In the present study, NaCl, GLU, and NaCl-GLU applications caused an increase in total amino acid and proline content, but the total protein content increased only in 500GLU, 50NaCl + 250GLU, 50NaCl-500GLU (Figure 1C, Figure 1D, Figure 2 A). It was expected that amino acid and proline content would be high in GLU doses (Figure 1C, 1D). However, the fact that these molecules were at a high level in NaCl treatments might be because they catalyze total protein into amino acids and proline (Wang et al., 2014; Bahjat et al., 2022). Moreover, GLU treatment might have increased salt resistance by stimulating the proline synthesis pathway (Sun et al., 2019).

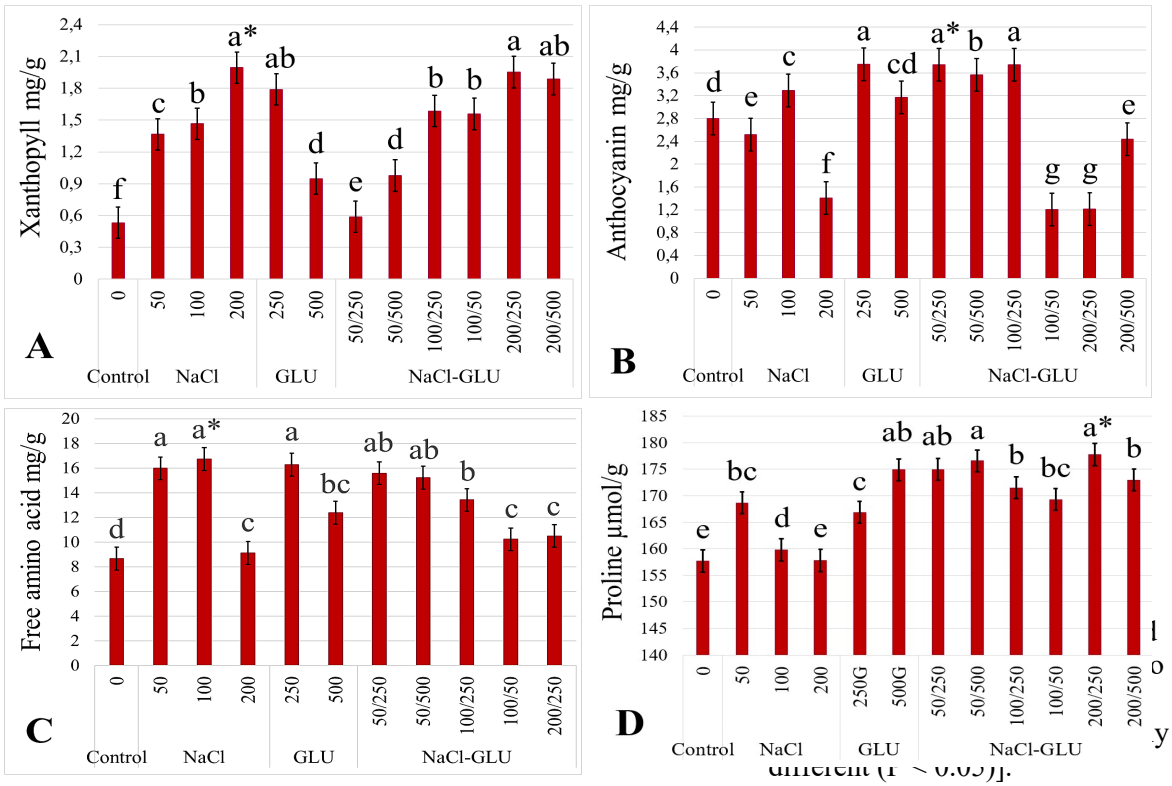

Figure 1. Variations of xanthophyll (1A), anthocyanine (1B), free amino acid (1C), and proline (1D) concentrations of SC2121 tomato seedling [(*: Means indicated with different letters within the same column are significantly different (P < 0.05)].

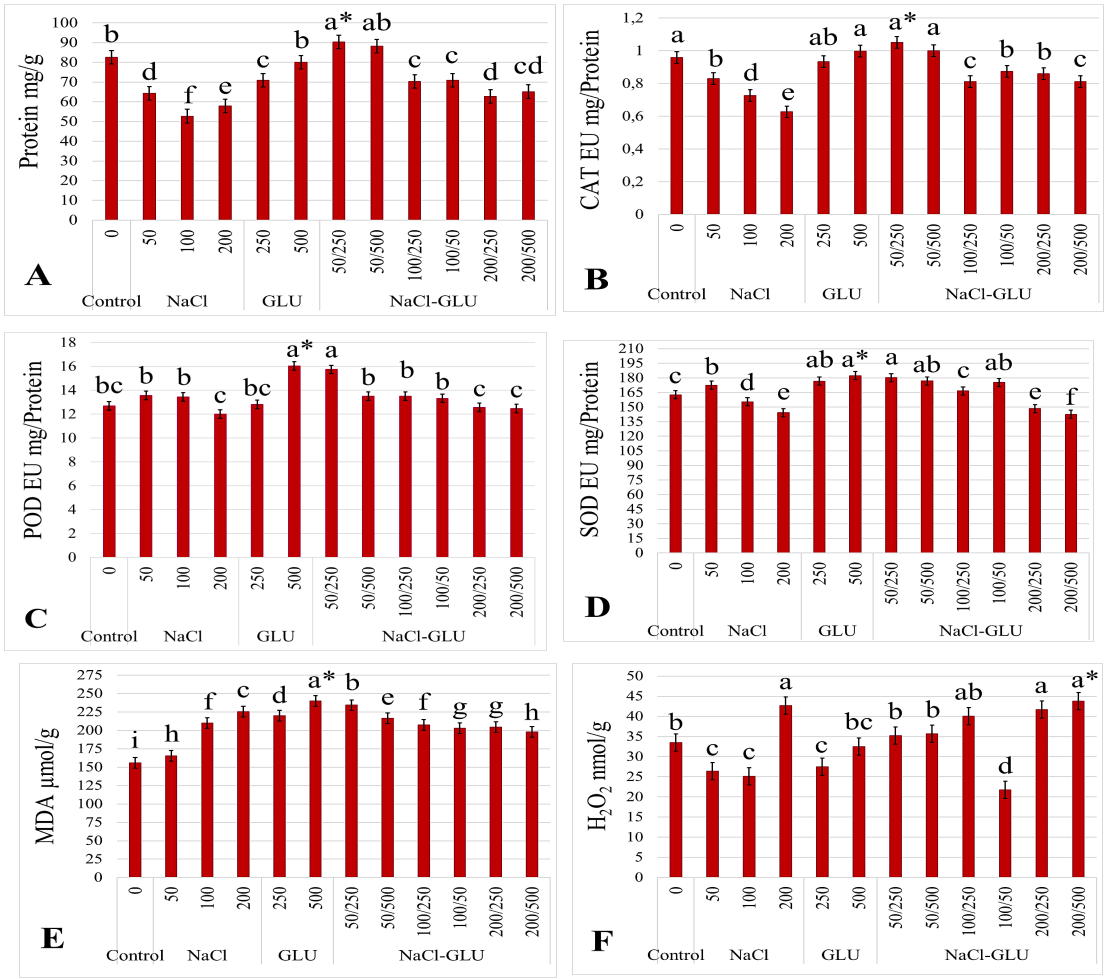

Figure 2. Variation of soluble protein (2A), CAT (2B), POD (2C) and SOD activities (2D), MDA (2E), and H_2O_2 concentrations of SC2121 tomato seedlings; [(*: Means indicated with different letters within same column are significantly different ($P < 0.05$)].

Many researchers reported that proline accumulation in cells increased in cases of water deficiency and salty conditions (Erdinc et al., 2018; Kıpçak et al., 2019). Besides them, proline might have induced the synthesis of nitrogenous compounds also by accelerating nitrate transportation (Bahjat et al., 2022). Similar to that study, Souri et al. (2017), Sun et al. (2019), and Turfan and Turan (2023) observed that GLU treatments caused increases in total amino acid and protein content in vegetative and reproductive organs. Similarly, Haghighia et al. (2020) also found that exogenous amino acid implementation given to squash seedlings significantly increased amino acid, protein, and proline content in comparison to the control group. Enzymatic molecules of defense mechanisms such as CAT, POD, and SOD suppress ROS production in plants under stress conditions or they scavenge these compounds from the cells (Ikbal et al., 2019; Yılmaz et al., 2022). In the present study, CAT activity was stimulated by 500GLU and 50NaCl +250GLU, 50NaCl-500GLU, and POD activity decreased in high NaCl and NaCl + GLU (Figure 2B, 2C). SOD activity was inhibited in 100NaCl, 200NaCl, 200NaCl + 25GLU, and 200NaCl-500GLU (Figure 2D). POD and CAT are the enzymes increasing the herbal resistance by reducing H_2O_2 into O_2 and H_2O (Figure 2E, 2F). Hence, in the present study, H_2O_2 content was found to be at a low level in high salt concentrations, in which POD activity was high. Moreover, the CAT, POD, and SOD activities were at the lowest level in the 200NaCl group and H_2O_2 content was high in this concentration (Figures 2B, 2C, 2D, 2F). Treatments stimulated CAT activity only in 500GLU and 50NaCl + 250GLU, and 50NaCl-500GLU (Figure 2B). In previous studies examining cucumber seedlings under Cd stress (Munawar et al., 2022) and lentil under salt stress (Fardus et al., 2021), it was reported that exogenous amino acid treatments stimulated the POD, SOD, and CAT activities. As in other organisms, plants also require oxygen (O_2) for an optimal life. However, during the metabolic reactions of cells and tissues, O_2 converts into toxic compounds and causes the

accumulation of molecules known as reactive oxygen species (ROS) and triggering destructions or degenerations in structures of cell membranes, proteins, chlorophyll, and other organic molecules (Kıpçak et al., 2019; Çelik and Karakurt, 2022). Deformation in cell membrane structure results in an increased intracellular MDA concentration and might reach very high levels, especially under stress conditions (Kuşvuran et al., 2019; Gursoy, 2022). Similar to the present study, Ikbal et al. (2019) observed that MDA content decreased in leaves of cucumbers under salt stress but MDA concentration increased again when seedlings were given salt + GLU treatment. Researchers claimed that exogenous GLU treatment stimulated fatty acid denaturation under salty conditions and induced an increase in MDA content (Hildebrandt, 2018; Fardus et al., 2021).

Conclusion

In the study, the effects of L-GLU treatments on morphological and biochemical characteristics of the SC2121 tomato variety under salt stress were investigated. It has been revealed that at the 500GLU, 50NaCl-500GLU, 250GLU, and 200NaCl-500GLU the morphological values were the highest, but they were the lowest with 200NaCl, 200NaCL-250GLU, 100NaCl, and 100NaCl-500GLU. In addition, amino acid and proline amounts were higher in all the treatment groups, whereas total protein and CAT activity generally increased with 500GLU and 50NaCl+250GLU, 50NaCl-500GLU. POD and SOD activity stimulated by high NaCl and 200NaCl+ 250GLU, and 200NaCl-500GLU. MDA level enhanced with all applications, while the H_2O_2 concentration reduced with lower NaCl and GLU doses. Finally, it can be stated that GLU doses and 50NaCl+250GLU, and 50NaCl-500GLU increased the growth and development in the SC2121 tomato, as well as bioactive chemicals, therefore, yield and quality could be increased by making use of GLU treatments in tomato varieties under lower salt stress.

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