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Research Article

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Investigation of Cold Storage Possibilities in Mass Production of Adult Stages of *Nesidiocoris Tenuis* Reuter (Hemiptera: Miridae)

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HIGHLIGHTS

- This study will help prevent the irregular use of pesticides in agriculture and increase the application of biological control techniques.
- This study aims to increase the availability of *Nesidiocoris tenuis*, the natural enemy of *Bemicia tabaci* and Tuta absoluta pests, which are economically important in agriculture.

Abstract

In this study, the effect of exposing the adult stages of the predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) to cold temperatures at different times was examined. In the experiments, individuals that reached the adult stage on the same day were kept in the dark for 5, 10, and 15 days at 7, 10, and 15°C. Adults kept at low temperatures were fed three times per week with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and honey water. Individuals of *N. tenuis* that survived after cold exposure were reared at 25°C under a 16:8 (L:D) day-night cycle. The lowest adult survival rate after cold exposure was 65.00% when held at 7°C for 15 days. The highest survival rates were 94.00% and 98.00% when stored at 7 and 10°C, respectively, for 5 days. The most extended longevity in individuals reared following cold exposure was 14.06 days at 7°C and 14.94 days at 10°C, respectively, while the shortest lifespan was 8.10 days in adults held at 15°C for 15 days. The average number of nymphs produced by adults kept at 7°C for 15 days was 322.4, while the number produced by adults kept at 15°C for 15 days was 47.6. Except for the nymphs acquired from adults held at 15°C for 15 days, the number of nymphs obtained from adults stored at other temperatures and durations did not differ statistically from the control group. The study concludes that those who intend to mass-produce *N. tenuis* adults should store them at temperatures between 7 and 10°C for up to 10 days.

Keywords: *Nesidiocoris tenuis*, Biological Control, Mass Production, Refrigerated Storage

1. Introduction

The primary objective in the management of numerous pests that cause product loss in terms of quality and quantity in agriculture is to produce a high yield per unit area and to cultivate crops that are suitable for sustainable agriculture techniques sensitive to the environment, human, and animal health (Uygun et al. 2010). The intensive use of pesticides increases the development of resistance in pests. Besides, it poses a threat to human and animal health, causes environmental pollution, and deteriorates the natural balance. However, the

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high cost of pesticides indicates that alternatives to chemical control should be developed. Therefore, biological control agents have been commercialized, mass-produced, and released for the control of pests.

Biological control is one of the most promising methods that have been widely used and demonstrated to be environmentally friendly (Abdel-Baky et al. 2015). In recent years, it has been utilized extensively, particularly in ecologically based integrated pest management programs.

It is widely acknowledged that mass production is a vital instrument of biological control programs (Kui et al. 2014). It focuses on the mass-rearing of insects, known to be effective in biological control, in climate-controlled rooms where optimal circumstances may be modified, in sufficient numbers to meet demand (Sürücü 2009). In terms of application, the cold storage of these laboratory-grown natural enemies is crucial for the advancement of biological control (Pitcher et al. 2002; Ayvaz et al. 2008).

Cold storage refers to the practice of storing beneficial insects or their hosts at low temperatures for a set period. This method enables natural enemies to be stored in the freezer to extend their shelf life. Simultaneously, it allows for the collection or stockpiling of natural enemies in acceptable climatic conditions, enabling the synchronized release of sufficient quantities of natural enemies to production regions during critical occasions such as epidemics (Sürücü 2009).

Several parameters of natural enemies, such as morphology, behavior, physiology, development, egg production, fecundity, lifespan, nutritional effects, adult-larvae emergence, and sex ratio, should not degrade after cold storage (Leopold 1998). Accordingly, an effective storage temperature and duration significantly contribute to the sustainability of natural enemy populations. As a result, numerous experiments have been conducted to preserve natural enemies employed in biological control. For example, JeongHwan et al. (2009) indicated that *Orius laevigatus* (Fieber), Bueno et al. (2014) demonstrated the same for *Orius insidiosus* (Say), Yanık and Ünlü (2015) for *Anthocoris minki* (Dohrn), Yaz and Özder (2016) for *Ephestia kuehniella* eggs parasitized by *Trichogramma pintoi* (Voegele), and Zhang et al. (2020) for *Tamarixia radiata* (Waterston), all of which can be stored in cold conditions.

Predator *N. tenuis* is a significant zoophytophagous species that is commonly and commercially used in both greenhouses and open vegetable areas as part of biological control programs. Whiteflies (*Bemisia tabaci* (Hem.: Aleyrodidae)) and tomato moths (*Tuta absoluta* (Meyrick) (Lep.: Gelechiidae)) can be controlled by releasing this predator (Sanchez 2009; Sohrabi and Hosseini 2015). In addition to these two essential pests, *Nesidiocoris tenuis* helps to control thrips, mites, leaf gallery moths, and other lepidopteran pests in greenhouses (Urbaneja-Bernat et al. 2013).

No research is found on the storage of the commercially released predator *N. tenuis* at low temperatures in the literature. The aim of this study is to investigate the effects of low temperature storage on post-storage survival rates, life spans and egg production in *N. tenuis* adults. The two most important factors for natural enemy cold storage are the level of cold storage and the duration of cold exposure, therefore these parameters were studied in this work. This study thus attempts to expand the usability of *N. tenuis* efficiently by achieving the best combination of low temperature and storage time.

2. Materials and Methods

The eggs of the predator bug *N. tenuis* and the laboratory host *E. kuehniella* were the primary study materials. Climate chambers with adjustable temperatures were employed in the experiment. In addition, green bean pods, binoculars, various glass and plastic containers were used. *Ephestia kuehniella* was grown using a mixture of flour and bran.

2.1.Rearing of Nesidiocoris tenuis

Adult *N. tenuis* was obtained from Biopheropoint Biological Control Systems Agriculture Chemical Industry Company. The stock culture was reared in climate rooms with 25±1°C temperature, 65±10% relative humidity, and 16:8 hours light-dark conditions in 12 cm diameter and 13 cm high plastic containers with ventilation holes in the laboratory. As food, *E. kuehniella* eggs and green bean pods were placed in the containers during experiments. Simultaneously, cottons soaked with 10% honey water were placed in plastic containers to suit the water needs of *N. tenuis* nymphs and adults (Urbaneja-Bernat et al. 2013). Bean pods containing *N. tenuis* eggs were removed every two day and transferred to another container in the controls, and new fresh pods were placed into the container for oviposition. Adult *N. tenuis* individuals ranging in age from 0 to 24 years old were collected from the laboratory colony and used in the experiments.

2.2.The effect of cold storage on the biological properties of Nesidiocoris tenuis

The post-storage survival rates, life spans and nymph numbers of *N. tenuis* were determined after storage at 65±5% relative humidity at 7, 10 and 15±1ºC for 5, 10 and 15 days, respectively. The studies were conducted in plastic containers with a diameter of 12 cm and a height of 13 cm with ventilation holes on them, 10 males and 10 females from the stock culture, with 10 replications for each distinct holding time of each temperature. The control group consisted of 10 males and 10 females produced immediately at 25±1ºC without being kept cold. During the controls, *E. kuehniella* eggs were added to the containers every two days, and bean pods containing *N. tenuis* eggs were removed and replaced with new pods. Dried cottons were re-moistened with honey water.

After storing *N. tenuis* at various temperatures and times, the viability rates were evaluated by recording the dead individuals in each container subsequent to the controls.

The adult samples that died in the containers were counted in the controls every two days, and their life span was computed at 25±1ºC following storage. At the same time, the bean pods with eggs were placed in separate containers and stored until the nymphal emergence was complete, then the number of nymphs emerging was recorded. Because *N. tenuis* lays its eggs in plant tissue, which is difficult to observe even with binoculars, the number of nymphs was determined rather than egg count in both application and control dishes.

2.3.Statistical analysis

Analysis of variance to determine whether there is a difference between the averages of survival rates, life spans and nymphal numbers of *N. tenuis*. Since the insects used at each storage temperature and time are different, the measurements obtained here are not dependent on each other. Due to nymph numbers do not show normal distribution and are obtained by counting, square root (√X) transformation was applied to these data (Düzgüneş et al. 1987). The Randomized Plot 3x3 Factorial Experimental Desing (ANOVA) was used in the study. Tukey's multiple comparison test was applied to determine the different overall and interaction averages.

3. Results and Discussion

3.1.The effect of cold storage on the life span of Nesidiocoris tenuis

The effect of storing adult individuals of *N. tenuis* for 5, 10 and 15 days at 7, 10 and 15°C on the life span is given in Table 1.

Table 1. The effect of cold storage on the life span of *Nesidiocoris tenuis* (day)

Storage period (day)	Temperature			
	7°C	10°C	15°C	Control
5	13.83±0.73^{ABC}	12.53±0.87^{ABC}	11.42±0.83^{BCD}	15.83±1.30^{A}
10	14.06±0.42^{AB}	14.94±0.49^{AB}	9.83±0.70^{CD}	15.83±1.30^{A}
15	12.52±0.24^{ABC}	13.09±0.47^{ABC}	8.10±0.61^{D}	15.83±1.30^{A}

[1] The difference between the averages shown with the same uppercase letters in the same row and column is not statistically significant (A-D: p<0.01).

In table 1, the values in the inner part of the table show the averages of the interactions. In the study, the interaction effect between cold storage times and degrees was found to be significant. The most extended longevity of *N. tenuis* following storage was 14.06 and 14.94 days at 7 and 10°C in adults kept for 10 days, respectively. The difference between the life spans of the adult samples kept at these two temperatures was not statistically significant (P<0.01). Adults kept for 15 days had the shortest life duration of 8.10 days at 15°C, and this difference was statistically significant (P<0.01) (Table 1). The difference in the samples' storage lifespans at 7 and 10°C for 5 and 15 days was determined to be statistically negligible (P<0.01) (Table 1).

Different cold storage studies were carried out with *Orius* spp. and *Anthocoris* spp., which are in the same subfamily with *N. tenuis*. According to Bueno et al. (2014), the most extended longevity for *O. insidiosus* was 14.1 days when kept at 8°C for up to 10 days. The study showed a similar result found in the literature, as the adult life span of *N. tenuis* kept for 10 days at 10°C was 14.9 days. Kim et al. (2009), reported that after 20 and 40 days of storage at 10°C, the female lifetime of *O. laevigatus* was 19.8 and 23.7 days at 25°C, respectively. Female *O. majusculus* was stored for 30 and 50 days at 9°C, followed by 25.9 and 19.8 days at 22°C, according to Rudolf et al. (1993). According to Yanık and Ünlü (2015), the longest life span of *A. minki* for females was 33.30 days when held at 11°C for 20 days, and 29.75 days for males when stored at 15°C for 20 days. Furthermore, they indicated that female samples kept in the cold had a shorter life duration (54.66 days) than the control group reared at 25°C. Similar to earlier experiments, this study discovered that an increase in the waiting period lowered the life span of *N. tenuis* adults.

3.2.The effect of cold storage on the survival rate of Nesidiocoris tenuis

The survival rates of *N. tenuis* adults after storage at 7, 10 and 15°C for certain periods are given in Table 2.

Table 2. The effect of cold storage on the survival rate of *Nesidiocoris tenuis* (%)

Storage period (day)	Temperature		
	7°C	**10°C**	**15°C**
5	94.00 ± 4.00^{A}	98.00 ± 3.54^{A}	89.00 ± 4.47^{AB}
10	90.00 ± 1.22^{AB}	89.00 ± 3.67^{AB}	86.00 ± 3.40^{AB}
15	65.00 ± 2.45^{C}	77.00 ± 2.45^{BC}	88.00 ± 2.00^{AB}

[1] The difference between the averages shown with the same uppercase letters in the same row and column is not statistically significant (A-C: p<0.01).

In Table 2, the values in the inner part of the table show the averages of the interactions. According to the experimental results, the interaction effect was found to be significant. The highest survival rate of *N. tenuis* was observed to be 94.00% and 98.00%, respectively, at 7 and 10°C in adults held for 5 days, according to the experimental data. Adults kept at 7°C for 15 days had the lowest viability rate of 65.00%, which was statistically significant (P<0.01) (Table 2).

According to Colinet and Boivin (2011), storage time is an essential factor in cold storage research. Several studies on cold storage at various holding times have found that natural enemy survival rates decrease inversely as storage time increases (Abd El-Gawad et al. 2010; Tunca et al. 2014; Yanık and Ünlü 2015). Similarly, in this study, the average survival rates of *N. tenuis* were 93.67% after 5 days of storage, 88.33% after 10 days of storage, and 76.67% after 15 days of storage, indicating that survival rates decrease while cold storage period increases.

When adults of *O. laevigatus* were kept at 6, 8, 10, and 12°C, Kim et al. (2009) reported a survival rate of 70% after 36 days at 10°C. According to Rudolf et al. (1993), adults of *O. majusculus* had a 50% survival rate after 42 days of low-temperature storage, while adults of *O. laevigatus* had a 75-80% survival rate after 40 days of storage at 9°C. In this study, survival rates of 77% were achieved in individuals of *N. tenuis* held at 10°C for 15 days, which is consistent with the literature. Bueno et al. (2014) found that adult *O. insidiosus* survival rates following storage at 8, 10, and 12°C were generally greater than 70% for both females and males. According to the study's findings for *N. tenuis*, a viability rate of more than 70% was recorded at all low temperatures and waiting times. According to Yanik and Ünlü (2015), the lowest survival rate (7.33%) was recorded in the 1-3 nymphal stages when held at 7°C for 40 days, while the maximum survival rate (90.0-92.0%) was recorded in the 1-3 nymphs and adults during the period when they were kept at 11°C for 10-30 days.

3.3. The effect of cold storage on the number of nymphs of Nesidiocoris tenuis

The effect of storage at 7, 10 and 15°C for 5, 10 and 15 days on the number of nymphs of *N. tenuis* was investigated and the results are presented in Table 3.

Table 3. The effect of cold storage on the number of nymphs of *Nesidiocoris tenuis*

Storage period (day)	Temperature			
	7°C	10°C	15°C	Control
5	188.0±14.98^{B}	181.6±15.12^{B}	185.6±44.27^{B}	250.2±13.60^{AB}
10	194.6±6.40^{B}	216.4±23.10^{B}	172.0±15.25^{B}	250.2±13.60^{AB}
15	322.4±14.82^{A}	166.6±9.37^{B}	47.6±8.05^{C}	250.2±13.60^{AB}

[1] The difference between the means shown with the same capital letters in the same row and column is not statistically significant (A-C: p<0.01).

In Table 3, the values in the inner part of the table represent the averages of the interactions. The interaction effect was found to be significant. The average number of nymphs produced by *N. tenuis* adults kept at 7°C for 15 days was 322.4, while it was 47.6 for adults kept at 15°C for 15 days. According to the statistical analysis, this difference is significant (P<0.01). The study observed no difference in the nymph numbers of the other groups compared to the control group, except for the adults kept at 15°C for 15 days (P<0.01) (Table 3).

Kim et al. (2009) found that after 20 and 40 days of storage at 10°C, females of *O. laevigatus* deposited 109.2 and 69.2 eggs, respectively, at 25°C. In contrast, the control group laid 224.5 eggs. According to Rudolf et al. (1993), females of *O. laevigatus* laid 145 and 72 eggs, respectively, at 22°C after 20 and 50 days of storage at 9°C, while the control group laid 190 eggs. When kept at 11°C, adults of *A. minki* laid 155.85 eggs, whereas the control group laid 207.51 eggs, according to Yanik and Ünlü (2015). In this study, the number of *N. tenuis* nymphs decreased with increasing waiting time, as determined by the researchers' previous studies (Table 3).Cold storage is critical in the large production of beneficial insects. The ideal storage conditions for predators should be found to maximize nymph productivity, life span, and viability. When we examined the results of survival rates after cold storage for *N. tenuis* (Table 2), we see that the normal life expectancy in the control group is 15 days. Therefore, 15 days of storage at different low temperatures will not be suitable for adults, but 5 and 10 days of storage will be more beneficial. The effect of cold storage on life span indicates that it is established that adults stored at 15°C have the shortest life span, making storage at this temperature unsuitable for mass production (Table 1). Besides, when we evaluated the effect of cold storage on the number of nymphs, we detected no difference between the nymph numbers of the other groups and the control group, except for the nymph numbers of the adults stored at 15°C for 15 days (Table 3). The viability rate after storage, life span, and nymph yield showed that *N. tenuis* could be preserved at 7 and 10°C for up to 10 days.

4. Conclusions

Cold storage has proven to be a valuable method for preserving predator *N. tenuis*, extending its shelf life, and enabling synchronized releases of sufficient numbers of natural enemies to production areas under favorable weather conditions, especially during critical moments such as epidemics and during off-season periods when demand is low. Furthermore, cold storage allows for the aggregation or stockpiling of natural enemies, which benefits mass manufacturing in a cost-effective manner. This study aimed to increase the efficiency of *N. tenuis* in biological control by determining the best combination of low temperature and storage period.

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Research Article

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Description of The Phenotypic Characteristics of Some Tomato Genotypes

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HIGHLIGHTS

- Tomato (*Solanum lycopersicum* L.), is one of the most economically valuable vegetable crops
- Phenotypic characterization studies are important for the appropriate assessment of many factors, such as plant genetic diversity and yield potential.

Abstract

This study was carried out in order to determine some morphological characteristics of 240 tomato genotypes at S2 level and to demonstrate the usability of these materials in breeding programs. Accordingly, the average fruit weight was between 553.11 g and 74.83 g. The mean pericarp thickness, carpel number, and soluble dry matter content of the genotypes were 7.38 mm, 3.6 pieces and 4.04% brix, respectively. While the fruit color of 165 genotypes was determined as red, the longitudinal section shape of the fruit was determined as slightly flattened in 106 genotypes. The flowering period of the genotypes was determined as medium flowering. The leaf posture of the genotypes was determined as attitude, and the leaf width was determined as medium and broad leaves. Based on these measurements and observations, tomato genotypes were also investigated using the Principal Component Analysis (PCA) methods. According to the PCA results, the first component explained 24.2% of the total variance, and the number of fruit carpels, average fruit weight, and fruit width were the most highly explained parameters. The second and third main components constituted 13.1% and 11.2% of the total. Based on the results of fruit characterization studies, genotypes 233, 447, 126, 22, 391, 118, 71, 100, 340, 102 were determined as superior. Overall, a high degree of phenotypic variation was detected among the characterized tomato genotypes.

Keywords: Principal Component Analysis; Morphological; Tomato

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically valuable vegetable crops. While 186 million tons of tomatoes are produced annually on 5 million hectares of land worldwide (FAO 2020), Türkiye ranks 4th with an annual production of 13 million tons on 181 thousand hectares (TURKSTAT 2022).

Tomato is a versatile vegetable that can be consumed both directly fresh and by processing in different ways, such as frozen, canned, paste, ketchup, pickled, sauce, dried, juice, pureed and chopped product (Günay 2005). As an important member of the *Solanaceae* family and a model plant, tomato is a widely studied species. The most essential element for improving tomato production and quality is for sure, high genetic performance. For this reason, tomato breeding studies are important for the sector and continuous breeding programs are carried out in order to develop new varieties. In addition to high yield and quality, stress resistance is often aimed to be establised in hybrid varieties (Kaloo 1988).

Detection of variation among parental genotypes is important in hybrid cultivar development for anticipating hybrid performance (Gözen 2008; Keskin 2014). In breeding studies, phenological characterization provide useful information in determining the desired traits. (Hernández-Villareal 2013). In this context, it is very important to determine the relationship between genotypes and to reveal the extent of genetic diversity (Oduor 2016). Phenotypic characterization is used to assess the diversity manifested by morphological traits (Bajracharya et al. 2006).

Fruit and plant characteristics come to the fore among tomato morphological characters (Altıntaş et al. 2016). When the relevant literature is examined, it is observed that research efforts continuously aim at improving the UPOV criteria in line with their aims (Kurt 2019). On the other hand, it is well known that the parameters examined in characterization studies are under the influence of many factors and features related to each other may require multiple factors to be considered.

Determination of phenotypic traits is time consuming and difficult due to the quantitative nature of the traits (Fiorani and Schurr 2013). Nevertheless, phenotypic characterization studies are required for appropriately assessing many factors such as plant genetic diversity and yield potential (Lopez et al. 1994; Dharmatti et al. 2001). In this context, multivariate analysis methods have been developed by examining more than one feature at the same time (Tahtalı 2005).

Principal component analysis is commonly performed in characterization studies (Karaağaç and Balkaya 2010). The aim of the present study was to reveal the diversity within a set of 240 S2 level tomato genotypes based on some morphological characters defined in UPOV criteria by clustering and principal component analysis.

2. Materials and Methods

In the study, 240 S2 level genotypes derived from Selko Arge company breeding gene pool were characterized. The seed sowing viols were filled homogeneously with 2 parts peat and 1 part perlite, and the seeds were sown in these viols. After the seedlings were grown according to the method, after the true leaves were formed on the seedlings, dilution was made in the viols. Seeds of the genotypes on July 22, 2019, under greenhouse conditions. Four weeks later, these seedlings were planted as double rows in 40x50x100 cm intervals, 18 of each genotype, in the greenhouse in Aksu district of Antalya province. During the fertilization period, 100 kg ha-1 N, 150 kg ha-1 P2O5 and 100 kg ha-1 K2O were applied in the experimental area in pure form. Weed control was carried out by hoeing, and diseases and pest management has been carried out by spraying regularly toprotect plant health and growth. 10 plants from each genotype were selected from the set of S2 level genotypes and the observations were taken according to some UPOV parameters (Tablo 1).

Growth type, number of flowers, inflorescence at first node, leaf attitude, leaf length, leaf width, presence of leaf blade, fruit width-length, cross-section, fruit shape in longitudinal section, fruit pericarp thickness, fruit locus amount, fruit presence of green neck, fruit maturity time, water soluble dry matter, fruit color were the observed characters. Measurements and observations were determined according to the upov criteria. In the study, leafing, leaf length and leaf width observations were determined by observations of plants in the whole plot relative to the seventh leaf. The number of flowers in the cluster was determined according to the 3rd brush. Average fruit weight, fruit length, fruit width, pericarp thickness, carpel number, fruit shape, fruit color, fruit longitudinal section and fruit cross-section shape were determined in the first 3 fruits in each brush.

Measurements and observations taken from 240 tomato genotypes were analyzed principal component analysis (PCA) using the JMP-14 software. Differences among genotypes were determined by examining the Score Plot graph created in line with the components obtained from the analysis.

Table 1. Measurements and observations made in tomato geno-types (UPOV 2011)

Features	Measurement and Observations
Plantgrowthtype	İndeterminate (2), Detarminate (1)
Leaf attitude	Semi-Erect (3), Horizontal (5), Semi-Dropping (7)
Leaf length	Short (3), Medium (5), Long (7)
Leaf width	Narrow (3), Medium (5), Broad (7)
İnflorescence at first node	Present (9), Absent (1)
Plant flowering time	Early (3), Medium (5), Late (7)
Fruit color	Light Pink (1), Pink (3), Light Red (5), Red (7), Dark Red (9)
Extent of green shoulder	Present (9), Absent (1)
Fruit shape	Flattened (1), SlightFlattened (2), Round (3), Ovate (6), Cordate (7),
Fruit diameter	Round (9), Nonround (1)
Fruit maturity time	VeryEarly (1), Early (3), Medium (5), Late (7), VeryLate (9)
Fruit weight (g)	
Fruit width (mm)	
Fruit length (mm)	
Number of locules	
Thickness of pericarp	
SÇKM	

3. Result and Discussion

3.1. *Morphological features*

Out of the 240 genotypes included in our study, 3 genotypes (1.2%) were determined as determinate and 237 (98.7%) were indeterminate. While flowering was observed in the first node in 7 genotypes (2.8%), it was not observed in 233 genotypes (86.2%). Small leaflets were detected in 207 genotypes (86.2%) and were absent in 33 genotypes (13.7%). Early flowering was detected in 39 of the genotypes (16.2%), flowering time was intermediate in 124 genotypes (51.6%), and late in 77 genotypes (32.2%). The number of flowers in a cluster was defined as 3-5 (44.1%) in 106 genotypes, 6-10 (44.1%) in 106 genotypes, and more than 10 (11.6%) in 28 genotypes. As a result of the observations made in the study, the leaf attitude of the genotypes was determined as semi-vertical (17%) in 41 genotypes, horizontal (53.3%) in 128 genotypes, and semi-drooping (29.5%) in 71 genotypes. According to leaf length observation values, 4 genotypes had short (1.6%), 62 genotypes had medium (25.8%) and 174 genotypes had long (72.5%) leaf structure. In terms of leaf width, 54 genotypes were narrow (22.5%), 100 genotypes were medium (41.6%) and 86 genotypes were determined as broad-leaved (35.8%) (Tablo 3).

According to the relevant literature, different scales of diversity are reported for the above-mentioned traits, as expected. For example, Özbay (2021) reported that flowering time varied between 12-30 days; Anisa et al. (2022) reported that the number of flowers in a cluster varied between 6 and 8. Salim et al. (2020) reported 59.09% of their observed genotypes as horizontal, 27.27% as semi-upright and 13.64% as semi-drooping. Kayak et al. (2022) determined that 43.6% of the genotypes they examined had semi-drooping, 47.8% had horizontal and 8.5% had semi-upright leaves. Kal et al. (2020) reported 43% short-leaved, 31% medium, and 26% long leaved genotypes within their observed pool of materials. The observed variation among different studies obviously sources from both the genetic make up of the characterized gene pools and the environment. Numbered lists can be added as follows:

While green neck character was not observed for 97.09% of the genotypes, it was observed in 2.9% of the genotypes. The ripening time was medium for 25%, late for 36.6%, very late for 34.1% and early for 4.1% of the genotypes. When mature fruit color was observed, 10.4% of the genotypes were pink, 4.5% were light pink, 8.7% were light red, 68.7% were red and 7.7% were dark red (Tablo 3).

Since tomato is rich in terms of color diversity, it is natural to have these differences within and among studies. According to the results of a similar study by Kayak et al. (2022), 1.06% of the genotypes were light pink, 39.36% were pink, 22.3% were light red, 35.1% were red, 2.1% were dark red. Jin et al. (2019) determined that 57.72% of their genotypes were red and 36.42% were pink. Altintas et al. (2016) detected 1.6% of their genotypes as orange, 25% as pink, 73.4% as red. In the work by Öztürk (2022), the limited number of genotypes included in the study were grouped according to maturity color as 1 yellow, 3 pink, 1 light red, 16 red, 2 dark red, 1 brown.

When the fruit shape in longitudinal section was examined in the present work, 32 flat (13.3%), 106 slightly flattened (44.1%), 66 round (27.3%), 31 heart-shaped (12.9%), 4 oval (1.6%), 1 rectangular (0.4%) genotypes were observed. Fruit cross-section shapes were determined as 43.3% round and 56.6% not round (Tablo 3).

In the relevant literature, Bota et al. (2014) identified their genotypes as 50% flattened, 31% round, and 19% heart, long and rectangular. According to the results reported by Bhattarai et al.(2018b), the genotypes were 60% flat, 6% slightly flattened, 1% very round, 8% round, 4% heart-shaped and 21% cylindrical. Kurt (2019) reported 43.5% of flattened, 31% of oval, 20.5% of round, and 5% of long genotypes in his study with 39 tomato genotypes. Salim et al. (2020) determined 50% round, 9% heart, 31% flattened, 4% elliptical genotypes. In the work of Öztürk (2022), 12 genotypes were observed as round, 1 genotype was observed as heart, 5 as flat, 5 as slightly flat and 1 genotype as cylindrical.

In our study, while the average fruit weight was determined as 204.28 grams, genotype 118 (553.11 g) had the highest and 108 (74.83 g) had the lowest fruit weights. In the study by Öztürk (2022), the fruit weight was ranged between 307.99 g and 16.63 g. Figàs et al. (2018), reported a fruit weight that ranged between 62.6 and 446.6 g. Thus, variation was observed for the fruit weight trait in all the above-mentioned studies. In the present work, while the average fruit width was measured as 59.82 mm, the highest and lowest values were measured for genotype 22 (99.50 mm) and genotype 108 (46.30 mm), respectively. The average fruit length of the genotypes was 66.09 mm. For genotype 391 (103.45 mm) was fruit length was the highest, and for genotype 199 (40.42 mm), fruit length was the lowest (Tablo 4).

According to Salim et al. (2020), fruit length varied between 3.91 and 6.57 cm, and fruit width varied between 3.63 and 8.15 cm. Kouam et al. (2018) reported that fruit length and fruit width were 3.74 to 5.34 and 3.64 to 5.71 cm, respectively. Figàs et al. (2015) reported the variation in fruit length as 1.88-9.57 cm and fruit width as 2.15-11.40 cm in their study.

According to our results, the mean pericarp thickness and carpel number of the genotypes were determined as 7.38 mm and 3.6 pieces, respectively (Tablo 4). As a result of the tomato characterization study by Singh and Aakansha (2015), it was reported that the number of carpels in fruits varied between 2 and 11. Figàs et al. (2015) reported a carpel number range from 2.00 to 18.33. In our study, the amount of soluble dry matter was determined as 4.04% brix. Singh and Aakansha (2015), Khushboo et al. (2015) and Vishwanath et al. (2014) reported values of 4.00%, 5.60%, 3.25-6.32%, 4.00% and 5.60%, respectively. The relevant literature displays wide differences in all above mentioned parameters, which would naturally be both genetic and under environmental influence.

3.2. Principal Component Analysis

The principal component (PC) axes, eigen values, variation and cumulative variation ratios obtained as a result of principal component analysis, and factor coefficients indicating the weight values of principal components on the basis of features are presented in detail in Table 2. It has been stated in studies that PCA analysis can be used effectively when the first two components explain more than 25% of the variance.

In their study, Seymen et al. (2019) determined that the three components represented approximately 48.39% in terms of 11 morphological features as a result of PCA. The eigen values of the first three components were found between 1.00-2.65. An eigen value greater than 1 indicates that the principal component weight values are reliable (Mohammadi and Prasanna, 2003). In the work of Mohammadi and Prasanna (2003), the first component explained 24.1% of the total variance, and the number of fruit carpels, fruit average weight, and fruit width were the highest explained parameters. The second and third main components constituted 13.06% and 11.16% of the total. In a study, they reported that it explained 63.35% of the total variation (Jin et al., 2019). Zhou et al. (2015) reported that they explained 78.54% of the total variation.

Kal et al. (2020) studied 77 cherry tomatoes, and the total variance was found to be 16.8% in PC1, 12.6% in PC2 and 10.2% in PC3. Figàs et al. (2015) reported that the total variation in the first and second components of PCA in tomato genotypes Cherry, Borseta, Cor, Penjar, Plana, Pruna, Redona and Valenciana was 22.6% and 11.8%, respectively. Bhattarai et al. (2016) obtained 5 principal component axes in 71 tomato genotypes and were reported to explain more than 92% of the total variation.

Table 2. Revealed eigenvalue, variation and principal component (PCA) axes of the properties examined as a result of PCA

Eigen value	2.6595	1.4363	1.2274
Variance %	24.177	13.057	11.158
Cumulative variance %	24.177	37.234	48.392
Traits	**PC1**	**PC2**	**PC3**
FWC	-0.17366	0.10916	-0.20372
FMT	0.16377	-0.00461	0.22267*
FC	-0.14824	0.44027*	0.46521*
FW	0.34171*	0.41476	-0.39708*
FL	0.21458	-0.14225	0.60006*
SLS	-0.38755	0.05144*	-0.01321
FCS	-0.30346	0.03314	-0.08048
TP	0.05913	0.70866*	0.08749
CN	0.48645*	-0.25870*	-0.01390
SÇKM	-0.12980	-0.00752	0.39663*
AFW	0.50889*	0.17043	0.05417

FWC: Flower number of cluster, FMT: Fruit maturity time, FC: Fruit color, FW: Fruit widt, FL: Fruit lenght, SLS: Shape in longitudinal section, FCS: Fruit cross-section, TP: Thickness of pericarp, CN: Carpel number, SÇKM: Water soluble dry matter, AFW: Average fruit weight

When the results were examined, it was reported that it explained more than 48.3% of the first three components. The first component (PC1) explained 24.17% of the total variance and a high positive correlation was found between FW, AFW, and CN. The second component (PC2) explained 13.05% of the total variance and showed a high positive correlation between FC, TP nd while a strong negative relationship with CN. The third component (PC3) explains 11.15% of the total variance and shows a strong positive relationship between FMT, FL and while a strong negative relationship with FW. In another study with tomato breeding lines, it was reported that six principal component axes were independent from each other in terms of 17 traits, and these axes explained 63.35% of the total variation (Jin et al., 2019). To examine the interrelationships between features, a loading chart was created using principal component analysis. In the figure (Şekil 1), if <90º is a positive relationship, if >90º is a negative relationship, and if the angle is 90º, there is no significant relationship between vectors. When Figure 1 is examined, it is seen that there is a strong positive relationship between AFW, FW, FS and CN, while FLS and MT are explained with a strong negative relationship with them.

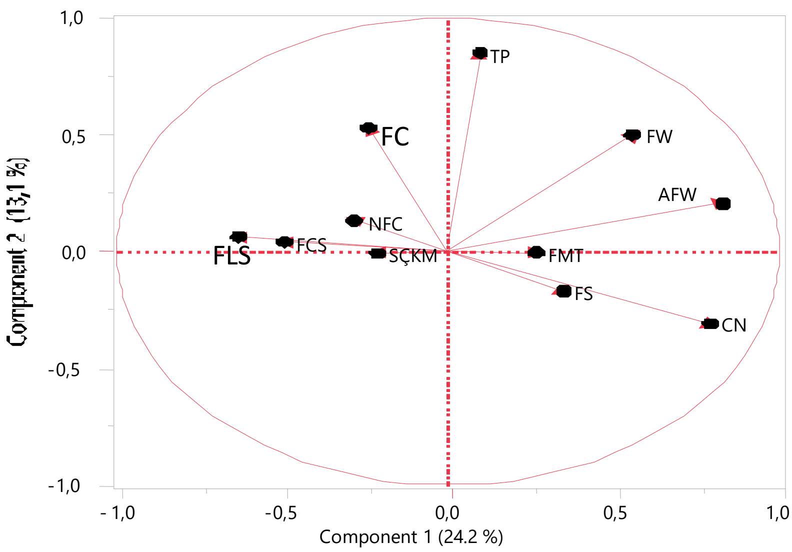

Figure 1. Loading plot graph obtained from PC1 and PC2 as a result of PCA made from fruit characteristics of tomato genotypes.

TP: Pericarp thickness, FW: Fruit width, AFW: Fruit average weight, FMT: Fruit maturity time, FS: Fruit size, CN: carpels number, FLS: Fruit longitudinal section, FCS: Fruit cross section, NFC: Number of flowers in cluster, FC: Fruit color, SÇKM: Water-Soluble Dry Matter (Brix)

A score graph was created for the evaluation of 240 tomato genotypes using PC1 and PC2 components (Figure 2). The fruit characteristics of the genotypes are located at the positive and negative intersection of both components. In terms of fruit characteristics, genotypes 233, 447, 126, 22, 391, 118, 71, 100, 340, 102 were determined as superior genotypes.

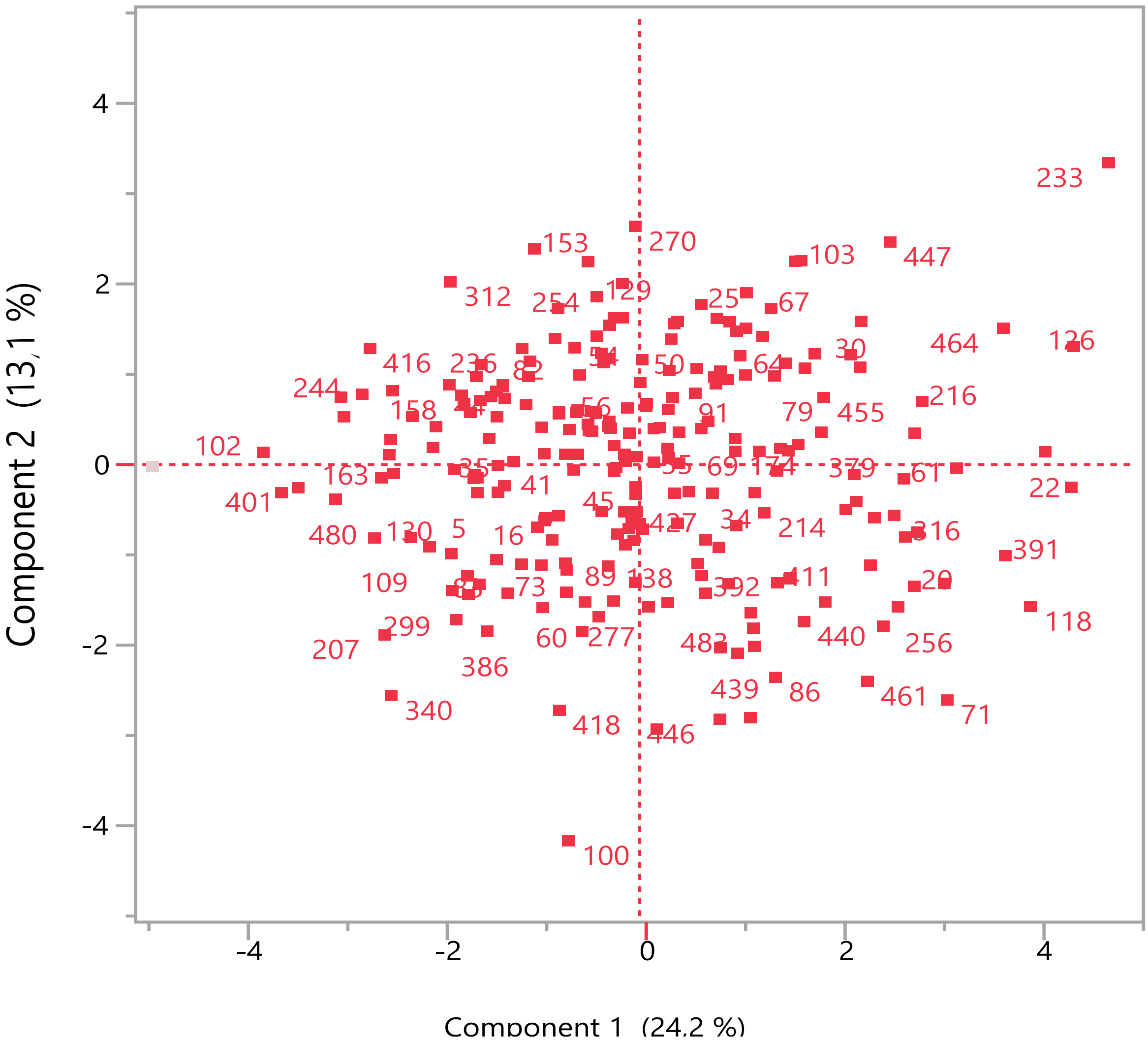

Figure 2. Score plot graph obtained from PCA result PC1 and PC2

4. Conclusions

In this study, some fruit and plant characteristics of 240 tomato genotypes in S2 stage were determined and the relationships between these characteristics were interpreted. As a result of the study, it was observed that there is a wide degree of variation between genotypes. As a result of the measurements, fruit weight differed between 553 and 74.83 grams. Fruit length varied between 103.45 and 40.42 mm and fruit width varied between 99.5 and 46.30 mm. It was determined that pericarp thickness ranged between 4.12 and 7.38 mm. The number of carpels in fruits varied between 2 and 7.

Based on morphological measurements and observations, 240 tomato genotypes were evaluated using Principal Component Analysis (PCA) method. As a result of the Principal Component Analysis (PCA), in terms of 11 morphological features, the three components represented approximately 48.39. The eigen values of the first three components were found between 1.00 and 2.65. As a result of PCA analysis, genotypes 233, 447, 126, 22, 391, 118, 71, 100, 340, 102 were determined as superior genotypes in terms of fruit characteristics. Overall, according to the data collected in the course of the present work it was determined that the variation between genotypes is high and, in this context, the evaluated gene pool qualifies as a potential core collection for future breeding programs.

5. Appendices

Table 3. Leaf and fruit characteristics of tomato genotypes

Genotype number	PGT	LA	LL	LW	IFN	FT	NFC	FC	PGN	GND	NS	FCS	FLS	MT
2	2	5	7	5	9	5	3	3	1	-	-	2	9	5
5	2	7	7	5	1	3	5	5	1	-	-	3	9	7
10	2	7	7	5	1	5	3	7	1	-	-	2	9	5
14	2	5	7	7	9	3	3	7	1	-	-	2	1	5
16	2	7	5	5	9	7	3	7	1	-	-	3	9	9
19	2	7	7	7	1	5	7	7	1	-	-	2	1	7
20	2	7	7	7	1	3	5	3	1	-	-	2	1	7
22	2	5	7	5	1	5	3	3	1	-	-	2	1	7
25	2	3	7	5	1	5	5	7	1	-	-	2	9	7
27	2	3	7	7	1	5	5	5	1	-	-	2	9	5
30	2	5	7	5	9	3	3	7	9	3	3	2	1	5
34	2	3	7	3	9	5	5	5	1	-	-	2	1	5
35	2	5	7	5	9	5	5	1	1	-	-	2	9	5
36	2	3	5	5	9	3	5	5	1	-	-	2	9	7
37	2	5	7	7	9	5	5	7	1	-	-	2	1	7
41	2	5	5	3	1	5	7	7	1	-	-	2	9	7
43	2	5	5	5	1	7	5	7	1	-	-	7	9	5
44	2	3	5	5	9	7	7	7	1	-	-	3	9	7
45	2	3	5	3	1	5	5	3	1	-	-	2	9	5
50	2	7	7	3	9	3	5	9	1	-	-	2	1	5
52	2	7	7	5	1	5	5	9	1	-	-	3	9	3
54	2	5	5	3	9	5	3	9	1	-	-	7	9	9
55	2	3	7	5	9	5	3	9	1	-	-	1	1	5
56	2	7	3	3	9	5	3	7	1	-	-	3	9	7
60	2	3	5	3	9	5	5	7	1	-	-	3	9	9
61	2	5	7	5	9	5	3	7	1	-	-	1	1	9
64	2	5	5	5	9	5	5	7	1	-	-	2	9	9
66	2	3	7	7	9	7	3	7	1	-	-	2	9	9
67	2	5	5	5	9	7	3	7	1	-	-	2	1	9
69	2	3	5	3	9	5	3	7	1	-	-	2	9	7
70	2	5	7	7	9	3	3	5	1	-	-	1	9	7
71	2	5	5	7	9	5	3	1	9	3	3	1	1	7
Genotype number	**PGT**	**LA**	**LL**	**LW**	**IFN**	**FT**	**NFC**	**FC**	**PGN**	**GND**	**NS**	**FCS**	**FLS**	**MT**
72	2	7	7	7	1	7	7	5	1	-	-	**3**	**9**	7
73	2	5	7	5	9	3	5	1	1	-	-	3	9	5
74	2	5	7	5	9	3	3	7	1	-	-	2	1	7
76	2	3	5	3	9	7	3	7	9	3	5	2	9	5
78	2	7	7	7	9	5	3	7	1	-	-	7	1	9
79	2	5	5	7	9	5	5	7	1	-	-	2	1	9
82	2	3	7	3	9	3	5	7	1	-	-	6	1	5
83	2	3	7	3	9	3	5	5	1	-	-	3	9	7
85	2	7	5	3	9	5	3	3	1	-	-	3	9	5
86	2	7	7	5	9	5	7	1	1	-	-	1	1	5
88	2	5	7	5	9	7	3	5	1	-	-	2	1	7
89	2	5	7	5	9	7	3	7	9	5	5	**2**	**9**	5
90	2	5	7	5	9	5	3	3	1	-	-	3	9	9
91	2	5	7	5	9	3	3	7	1	-	-	**2**	**9**	5
93	2	7	7	7	9	3	5	1	1	-	-	**3**	**9**	5
96	2	3	7	5	9	7	3	7	1	-	-	2	9	9
98	2	3	5	7	9	7	3	7	1	-	-	3	9	7

99	2	5	7	5	1	3	5	7	9	7	7	**3**	**9**	5
100	2	3	7	3	9	7	3	1	1	-	-	3	9	7
101	2	7	7	7	9	3	3	9	1	-	-	3	9	7
102	2	5	7	7	9	7	5	9	1	-	-	7	9	5
103	2	7	5	7	1	3	3	9	1	-	-	2	1	5
108	2	5	5	5	9	3	7	9	1	-	-	6	9	3
109	2	5	7	5	9	7	5	1	1	-	-	3	9	5
110	2	3	7	3	9	7	3	7	1	-	-	7	9	9
112	2	7	7	5	9	5	3	7	1	-	-	3	9	7
114	2	5	7	5	9	7	3	7	1	-	-	1	1	9
116	2	5	7	5	9	3	7	5	1	-	-	2	1	7
117	2	5	7	5	9	7	3	7	1	-	-	3	9	7
118	2	7	7	7	9	5	5	1	1	-	-	2	1	5
119	2	5	7	7	9	3	7	7	1	-	-	**2**	**9**	7
120	2	5	7	3	9	3	7	7	1	-	-	6	1	5
121	2	5	7	5	9	7	5	7	1	-	-	2	1	5
122	2	5	5	3	1	5	5	9	1	-	-	2	9	7
126	2	7	7	7	9	3	3	7	1	-	-	2	1	9
128	2	5	7	7	9	3	7	7	1	3	3	2	1	5
129	2	5	5	3	9	3	7	7	9	3	5	2	9	3
130	2	5	7	5	1	5	5	7	1	-	-	3	9	7
131	2	5	7	5	9	3	3	7	1	-	-	2	9	5
132	2	5	7	3	9	7	3	7	1	-	-	2	9	7
134	2	7	7	3	9	5	3	7	1	-	-	6	1	9
136	2	5	7	5	1	3	5	9	1	-	-	3	9	5
138	2	5	7	3	9	7	5		1	-	-	2	1	5
139	2	5	5	3	9	7	3	7	1	-	-	2	9	3
140	2	7	7	7	9	5	3	3	1	-	-	1	1	9
143	2	7	7	7	9	5	3	3	1	-	-	7	9	5
145	2	7	7	7	9	5	5	3	1	-	-	2	1	5
146	2	7	5	7	9	5	5	7	1	-	-	7	9	3
147	2	7	7	5	9	5	5	7	1	-	-	3	9	7
149	2	7	7	5	9	5	5	3	1	-	-	2	1	5
150	2	5	7	3	9	7	5	7	1	-	-	7	9	5
151	2	7	7	7	9	3	5	3	1	-	-	2	1	7
152	2	5	7	5	1	5	5	7	1	-	-	2	9	7
153	2	7	7	7	9	7	3	9	1	-	-	3	9	7
155	2	7	7	7	9	5	3	7	1	-	-	2	9	5
158	2	7	7	7	9	5	3	7	1	-	-	3	9	5
159	2	3	7	5	9	5	5	7	1	-	-	2	1	5
160	2	5	7	7	9	3	5	7	1	-	-	2	9	7
161	2	7	7	7	9	5	5	7	1	-	-	2	9	3
163	2	5	7	7	9	5	3	7	1	-	-	7	9	7
164	2	5	5	5	9	5	5	7	1	-	-	3	9	5
Genotype number	**PGT**	**LA**	**LL**	**LW**	**IFN**	**FT**	**NFC**	**FC**	**PGN**	**GND**	**NS**	**FCS**	**FLS**	**MT**
165	2	5	7	5	9	5	5	7	1	-	-	1	1	7
166	2	5	7	7	9	5	5	3	1			2	1	9
167	2	5	5	7	9	3	7	7	1	-	-	2	1	3
169	2	7	7	7	9	5	5	5	1	-	-	7	1	9
171	2	5	7	7	9	3	7	7	1	-	-	3	9	5
172	2	7	7	7	9	5	3	7	1	-	-	2	9	9
173	2	7	7	7	9	5	7	3	1	-	-	3	9	7
174	2	7	7	7	9	5	5	7	1	-	-	2	9	7
180	2	5	5	3	9	5	5	7	1	-	-	7	9	5
181	2	7	5	7	9	7	5	7	1	-	-	7	9	7
182	2	7	7	7	9	7	3	7	1	-	-	7	9	5
183	2	5	7	7	9	5	5	7	1	-	-	2	1	7
184	2	5	7	7	9	5	5	7	1	-	-	3	9	7
185	2	5	7	7	9	7	3	7	1	-	-	1	1	7

186	2	7	7	7	9	5	7	7	1	-	-	7	1	7
188	2	5	7	7	9	3	3	7	1	-	-	3	1	7
193	2	5	7	5	9	3	5	7	1	-	-	2	9	9
194	2	5	7	7	9	7	3	1	1	-	-	2	9	7
196	2	7	7	7	9	3	5	7	1	-	-	2	9	7
197	2	5	7	5	9	7	5	7	1	-	-	3	9	7
198	2	5	7	7	9	7	7	7	1	-	-	2	9	7
199	2	7	7	7	1	5	5	7	1	-	-	1	1	7
200	2	7	7	5	9	7	3	3	1	-	-	2	1	9
201	2	7	7	5	9	5	5	3	1	-	-	3	9	9
205	2	7	7	5	9	3	5	7	1	-	-	7	1	7
206	2	7	7	5	9	5	3	7	1	-	-	1	1	9
207	2	3	7	5	9	7	3	7	1	-	-	3	9	5
209	2	5	7	3	1	3	3	3	1	-	-	2	9	7
210	2	5	5	3	9	5	3	7	1	-	-	2	9	5
213	2	5	7	7	9	5	7	7	1	-	-	3	9	7
214	2	7	7	7	9	7	3	7	1	-	-	2	1	7
215	2	7	7	7	9	7	3	7	1	-	-	2	9	9
216	2	5	5	5	9	5	3	7	1	-	-	1	1	9
217	2	5	7	3	9	7	3	7	1	-	-	2	9	9
218	2	5	7	3	9	7	3	7	1	-	-	1	1	7
220	2	5	7	7	9	5	5	7	1	-	-	2	9	9
221	2	3	7	5	9	5	5	7	1	-	-	7	1	9
222	2	7	7	5	9	3	3	7	1	-	-	2	9	7
223	2	7	7	3	1	5	5	7	1	-	-	3	9	5
224	2	5	7	3	9	5	5	7	1	-	-	3	9	7
233	2	7	7	7	9	7	3	9	1	-	-	1	1	7
234	2	7	7	5	9	7	3	7	1	-	-	3	9	9
235	2	5	7	3	9	3	5	7	1	-	-	2	1	9
236	2	7	7	5	9	5	5	7	1	-	-	7	9	9
238	2	5	5	5	9	5	5	7	1	-	-	3	9	9
239	2	3	5	3	9	7	3	7	1	-	-	2	1	9
240	2	7	7	5	9	3	5	9	1	-	-	2	1	9
242	2	7	7	7	9	5	5	7	1	-	-	7	1	9
243	2	7	7	5	1	5	5	7	1	-	-	2	1	7
244	2	7	7	7	9	5	5	7	1	-	-	7	9	7
245	2	5	7	5	9	5	5	7	1	-	-	2	9	9
248	2	7	7	5	9	5	5	5	1	-	-	1	1	9
249	2	7	7	5	9	5	3	7	1	-	-	3	9	9
252	2	7	7	7	9	5	3	7	1	-	-	1	1	9
254	2	5	5	5	9	5	5	7	1	-	-	2	9	5
255	2	3	7	3	9	5	5	7	1	-	-	2	1	7
256	2	5	5	3	9	5	5	3	1	-	-	2	1	5
260	2	7	7	7	9	5	3	7	1	-	-	2	1	7
263	2	7	7	7	9	5	3	7	1	-	-	3	9	9
264	2	7	7	5	9	5	5	7	1	-	-	3	9	7
270	2	7	7	7	9	7	5	7	1	-	-	2	1	7
271	2	5	5	5	9	5	5	7	1	-	-	2	9	9
Genotype number	**PGT**	**LA**	**LL**	**LW**	**IFN**	**FT**	**NFC**	**FC**	**PGN**	**GND**	**NS**	**FCS**	**FLS**	**MT**
273	2	7	5	3	9	5	5	7	1	-	-	1	1	7
277	2	5	7	3	9	7	3	3	1	-	-	2	9	7
278	2	5	7	7	1	5	5	7	1			3	9	7
280	2	7	5	7	9	7	7	7	1	-	-	3	9	7
281	2	5	5	5	9	7	3	7	1	-	-	2	9	9
283	2	5	7	7	9	5	5	7	1	-	-	2	9	9
284	2	5	7	7	1	5	5	7	1	-	-	3	9	9
286	2	7	5	5	9	7	5	7	1	-	-	7	1	9
287	2	5	7	5	9	5	5	7	1	-	-	3	9	7
288	2	7	7	7	9	5	7	7	1	-	-	3	9	7

290	2	5	7	7	1	5	3	7	1	-	-	1	1	7
291	2	5	7	5	9	7	5	7	1	-	-	3	9	9
293	2	5	7	3	9	5	3	7	1	-	-	3	9	9
299	2	5	7	7	9	5	3	7	1	-	-	7	9	9
302	2	3	5	7	9	5	5	7	1	-	-	3	9	9
304	2	5	7	3	9	5	7	7	1	-	-	7	9	9
306	2	5	7	3	9	7	5	7	1	-	-	3	9	7
312	2	5	5	5	9	5	3	7	1	-	-	7	9	5
315	2	5	7	7	9	7	3	7	1	-	-	1	1	9
316	2	5	5	7	9	5	3	7	1	-	-	1	1	7
319	2	5	7	7	9	3	7	7	1	-	-	3	9	9
320	2	3	5	7	9	7	7	7	1	-	-	3	9	9
322	2	7	7	7	9	5	3	3	1	-	-	2	1	9
323	2	5	5	5	9	7	7	7	1	-	-	2	1	9
325	2	5	7	5	9	7	3	9	1	-	-	1	1	9
326	2	7	5	5	9	7	3	3	1	-	-	7	1	9
327	2	5	7	5	9	5	5	3	1	-	-	2	1	9
328	2	7	7	5	9	5	5	7	1	-	-	2	1	9
331	2	3	7	3	9	5	5	7	1	-	-	7	1	5
334	1	5	7	5	9	7	5	7	1	-	-	2	1	9
337	2	5	7	5	9	7	5	7	1	-	-	3	9	5
340	2	5	3	3	9	5	5	7	1	-	-	7	9	5
345	2	5	7	5	9	5	3	7	1	-	-	2	9	3
350	2	3	5	3	9	5	3	7	1	-	-	3	9	5
351	2	5	7	5	9	5	3	7	1	-	-	1	1	7
361	2	7	5	3	9	5	3	7	1	-	-	3	9	5
362	2	5	7	7	9	5	3	7	1	-	-	3	9	7
371	1	3	3	5	1	5	3	7	1	-	-	3	9	9
377	2	5	7	5	9	5	7	9	1	-	-	1	1	7
379	2	5	7	5	9	5	5	7	1	-	-	2	1	9
383	2	3	5	5	9	7	3	7	1	-	-	2	9	9
384	2	5	5	5	1	7	3	7	1	-	-	2	1	9
386	2	3	5	5	1	7	3	9	1	-	-	2	9	7
391	2	5	7	5	9	5	5	1	1	-	-	1	1	7
392	2	5	7	5	9	5	5	5	1	-	-	1	1	7
393	2	5	7	7	9	5	5	9	1	-	-	2	1	9
395	2	3	5	3	9	7	3	5	9	5		3	9	5
396	2	5	5	3	9	5	5	1	1	-	-	3	9	7
398	2	3	7	5	9	5	5	7	1	-	-	3	1	7
399	2	5	7	5	9	7	5	7	1	-	-	3	9	9
401	2	5	7	7	9	5	7	9	1	-	-	4	1	5
407	2	3	5	3	9	7	3	7	1	-	-	1	1	9
408	2	5	7	5	9	7	3	7	1	-	-	3	9	9
409	2	3	7	5	9	7	3	7	1	-	-	7	9	9
411	2	3	5	5	9	7	3	7	1	-	-	1	1	9
413	2	5	7	5	9	7	3	5	1	-	-	2	9	9
416	2	5	5	3	9	5	7	9	1	-	-	3	9	3
418	2	5	7	5	9	5	5	3	1	-	-	3	9	5
422	2	5	5	7	9	5	5	7	1	-	-	2	1	9
423	2	5	7	3	1	7	5	7	1	-	-	2	1	3
424	2	5	5	5	9	5	7	9	1	-	-	1	1	5
427	2	5	5	5	9	7	5	5	1	-	-	2	1	9
Genotype number	**PGT**	**LA**	**LL**	**LW**	**IFN**	**FT**	**NFC**	**FC**	**PGN**	**GND**	**NS**	**FCS**	**FLS**	**MT**
430	2	5	7	7	9	5	3	3	1	-	-	**2**	**1**	9
433	2	5	7	5	9	5	5	7	1	-	-	2	1	9
437	2	7	7	7	9	3	3	3	1	-	-	2	9	5
439	2	5	5	5	9	5	3	5	1	-	-	2	1	7
440	2	5	5	3	9	7	3	5	1	-	-	2	1	7
441	2	7	7	7	1	5	3	5	1	-	-	3	9	9

442	2	5	5	5	1	7	5	7	1	-	-	7	9	7
446	1	3	5	5	9	7	3	7	1	-	-	1	1	7
447	2	3	3	3	9	7	3	5	1	-	-	2	1	9
450	2	7	7	7	9	5	5	7	1	-	-	3	9	9
453	2	5	7	7	9	5	5	3	1	-	-	2	1	7
455	2	5	7	3	9	7	5	7	1	-	-	2	1	7
461	2	3	7	3	9	5	3	7	1	-	-	1	1	9
462	2	3	7	5	9	5	5	7	1	-	-	7	1	7
463	2	5	5	7	9	7	3	7	1	-	-	3	9	7
464	2	3	5	3	9	7	3	7	1	-	-	1	1	7
465	2	3	7	3	9	7	7	7	1	-	-	2	1	5
466	2	5	7	7	9	7	3	7	1	-	-	2	1	9
467	2	5	5	3	9	7	3	7	1	-	-	2	9	9
469	2	5	7	5	9	5	5	7	1	-	-	7	9	7
471	2	5	7	7	9	7	5	7	1	-	-	2	1	9
480	2	5	7	7	9	7	5	7	1	-	-	7	9	5
483	2	5	7	7	9	7	5	7	1	-	-	1	1	5

PGT: Plant growth type: LA: Leaf attitude LL: Leaf length LW: Leaf width IFN: İnflorescence at first node FT: Flowering time NFC: Number of flowers per cluster FC: Fruit color PGN: Presence of green neck GND: Green neck density GNS: Green neck size FCS: Fruit cross section FLS: Fruit longitudinal section MT: Maturity time

Table 4. Fruit characteristics of tomato genotypes

Genotype number	FW	FS	PT	CN	SÇKM	AFW
2	71.88	55.77	6.30	3	4.20	198.33
5	61.60	53.20	7.64	3	3.88	125.00
10	57.78	62.84	6.49	4	4.09	153.70
14	82.15	62.09	8.59	4	4.46	239.57
16	68.85	57.01	6.00	3	4.05	148.00
19	62.54	50.59	6.75	3	4.17	123.33
20	82.39	60.07	5.62	6	3.73	298.33
22	99.50	64.29	6.97	6	3.92	338.83
25	83.71	68.04	8.30	3	4.15	269.00
27	77.92	55.44	6.05	4	3.33	177.20
30	84.47	63.50	8.27	5	4.04	276.00
34	75.94	56.90	6.46	5	4.00	207.71
35	60.79	51.26	9.25	2	4.30	138.50
36	73.65	57.02	8.42	4	5.05	205.00
37	64.44	68.45	7.33	3	4.15	194.00
41	69.87	52.98	6.53	4	3.13	166.67
43	62.49	66.38	7.73	2	4.90	160.40
44	67.86	58.83	7.62	3	4.26	140.17
45	72.32	55.09	7.79	4	4.15	204.50
50	75.13	60.64	7.59	4	3.75	194.33
52	64.09	71.27	7.84	3	3.61	186.86
54	64.02	78.21	9.50	4	3.55	225.00
55	56.73	78.86	8.58	4	4.97	199.67
56	71.98	66.94	7.67	3	4.63	209.17
60	62.09	65.03	5.48	5	4.70	172.75
61	85.88	55.26	6.28	6	3.97	239.00
64	81.83	62.25	8.36	4	3.22	243.50
66	76.53	60.41	7.63	3	2.15	196.00
67	81.77	62.33	8.79	3	4.20	244.00
69	66.65	67.40	8.36	6	3.73	231.71
Genotype number	FW	FS	PT	CN	SÇKM	AFW
70	58.24	92.05	7.34	6	3.65	262.25
71	65.40	89.86	6.52	6	4.10	288.75
72	69.93	60.31	7.70	4	3.20	204.44
73	65.54	56.30	7.05	3	4.25	138.00

74	77.36	70.38	8.38	4	4.28	271.20
76	73.70	66.34	9.49	4	4.41	229.11
78	64.40	60.05	4.91	2	4.05	153.33
79	69.14	74.04	8.72	4	3.87	258.30
82	68.31	64.55	8.42	3	3.72	174.00
83	59.04	67.62	5.68	2	3.90	159.50
85	64.29	56.35	6.32	3	2.95	138.33
86	56.32	81.19	7.34	6	3.50	197.00
88	62.83	50.40	6.50	4	3.97	148.00
89	62.16	64.02	6.85	5	3.61	180.25
90	63.27	61.69	5.96	3	3.83	164.93
91	79.85	74.71	8.29	5	7.69	297.11
93	64.71	68.67	5.21	5	3.63	198.00
96	67.32	69.31	7.50	4	4.20	218.80
98	71.67	56.93	5.10	4	5.10	175.50
99	70.80	64.79	8.92	3	3.68	185.11
100	54.05	67.06	4.17	4	3.13	124.40
101	67.27	67.89	7.93	2	4.00	194.00
102	60.95	54.25	6.36	2	4.90	118.66
103	80.12	72.24	9.38	4	3.82	272.00
108	46.30	55.91	7.23	2	5.07	74.83
109	61.40	53.57	7.93	2	4.63	120.00
110	78.76	61.04	8.28	3	3.50	250.83
112	72.18	64.92	7.51	3	4.03	184.33
114	66.08	101.48	8.12	7	4.12	396.20
116	77.00	58.37	7.54	3	4.27	223.33
117	67.52	62.33	7.28	3	3.87	179.33
118	70.40	60.50	5.46	5	3.73	553.11
119	70.80	64.79	9.45	3	3.68	248.00
120	56.11	59.90	8.58	2	4.53	111.75
121	74.73	63.18	8.91	3	3.75	222.25
122	73.57	61.82	9.29	3	4.13	200.85
126	90.76	68.06	7.80	6	3.33	400.71
128	66.70	70.04	8.18	5	3.92	229.29
129	78.37	64.66	8.63	3	4.00	242.50
130	58.63	62.61	5.97	2	4.78	145.40
131	74.80	61.04	6.80	4	3.89	209.25
132	80.58	71.16	9.87	4	4.20	306.38
134	66.74	72.87	6.42	3	3.42	180.20
136	58.34	63.80	8.46	3	3.48	163.90
138	77.35	54.27	4.86	5	4.16	193.20
139	69.22	62.29	7.11	3	3.41	192.83
140	83,39	63,09	7.14	4	3.85	225.00
143	71.97	60.73	8.14	3	3.93	185.33
145	73.99	67.78	7.74	4	3.81	233.86
146	69.44	64.81	7.50	2	3.92	185.19
147	69.46	74.77	6.78	5	4.17	247.33
149	78.63	73.27	6.56	7	3.68	295.33
150	66.98	61.70	6.97	3	3.67	176.67
151	85.28	60.01	6.38	5	3.42	294.27
152	66.74	69.19	7.59	3	3.79	199.88
153	66.77	65.86	10.06	2	3.75	185.00
155	74.23	59.24	7.87	4	4.14	206.97
158	65.33	61.31	8.31	2	4.73	155.00
159	57.55	70.24	6.96	3	4.77	168.67
160	82.22	63.45	8.88	4	3.63	273.00
161	74.00	67.55	7.68	4	3.59	232.00
Genotype number	FW	FS	PT	CN	SÇKM	AFW
163	65.69	57.37	7.22	2	4.25	148.71

164	65.18	67.37	8.04	4	3.98	190.60
165	65.49	96.82	8.27	5	3.98	360.00
166	90.40	63.77	7.32	6	4.40	293.89
167	55.91	78.22	5.70	5	4.67	190.00
169	66.30	76.92	6.39	2	3.85	222.00
171	66.65	55.98	8.20	3	3.80	130.50
172	67.60	53.71	7.25	5	4.50	156.17
173	61.57	61.22	6.72	2	3.95	146.00
174	79.86	64.53	6.54	5	4.55	262.50
180	69.53	65.53	6.36	3	3.77	191.22
181	62.71	68.89	5.89	5	4.15	194.50
182	73.97	66.36	7.37	3	3.95	225.18
183	60.70	76.65	8.59	4	3.81	223.18
184	69.16	59.40	8.05	4	3.87	171.50
185	59.12	84.31	8.28	4	3.49	242.86
186	71.67	61.11	8.08	3	4.28	161.06
188	61.72	51.15	5.61	2	3.55	115.00
193	68.98	63.20	5.78	5	4.01	244.11
194	67.08	61.70	8.36	3	4.06	160.33
196	77.65	57.75	6.90	4	3.60	208.50
197	56.65	75.98	6.85	5	4.60	164.00
198	65.43	69.33	7.88	4	3.83	213.44
199	60.55	40.42	6.07	4	4.00	260.00
200	68.47	77.66	8.39	5	3.97	263.67
201	67.80	71.14	7.52	4	3.95	156.75
205	61.94	62.41	6.88	2	4.77	134.67
206	64.97	89.54	7.59	4	4.23	306.50
207	57.16	46.68	4.87	3	4.30	84.50
209	90.63	66.51	6.21	6	3.20	115.19
210	81.34	60.28	8.11	5	4.09	244.11
213	67.96	65.77	7.84	4	3.73	191.56
214	70.68	66.95	6.76	5	3.92	208.00
215	73.94	84.44	8.92	5	4.05	322.17
216	81.85	71.20	7.68	5	4.40	303.67
217	61.62	78.08	5.31	6	3.95	230.50
218	74.88	56.05	8.73	4	4.44	180.67
220	68.90	57.82	7.64	3	3.73	188.50
221	74.77	63.45	8.45	3	3.76	212.20
222	72.19	67.66	7.83	5	3.83	229.71
223	65.71	62.06	7.43	2	4.72	156.54
224	66.50	58.66	7.88	3	4.30	147.86
233	98.43	75.27	9.41	5	3.50	455.00
234	73.33	57.71	8.58	3	4.61	198.11
235	69.01	55.77	9.17	2	3.32	171.40
236	73.77	62.68	8.20	3	3.87	206.33
238	58,67	50,03	4.65	3	3.50	121.67
239	70.04	64.82	5.83	4	4.28	201.00
240	62.45	72.88	7.37	4	4.09	193.33
242	69.91	62.44	7.70	2	3.95	178.75
243	72.37	63.94	7.71	3	3.98	217.36
244	66.26	56.76	7.75	2	4.43	145.92
245	74.17	56.17	8.11	4	4.03	186.64
248	72.77	54.06	7.93	3	3.95	170.00
249	66.97	59.15	8.16	4	4.47	162.67
252	54.03	70.96	7.35	3	4.63	175.25
254	72.50	58.09	9.31	3	4.20	181.25
255	86.81	64.23	8.39	5	4,3	292.33
256	92.37	53.29	4.93	7	4.47	246.67
260	78.97	60.52	8.08	3	3.95	241.91

263	66.74	57.00	6.32	2	4.35	155.50
Genotype number	FW	FS	PT	CN	SÇKM	AFW
264	75.82	63.98	8.00	3	3.89	218.88
270	72.97	66.64	10.66	2	4.10	196.50
271	66.25	89.92	7.04	6	3.88	319.17
273	73.96	56.57	8.26	4	3.50	183.60
277	63.11	61.00	6.99	4	3.91	150.62
278	64.03	45.56	6.54	3	3.83	130.00
280	67.08	73.04	7.57	2	3.88	196.25
281	72.23	58.00	7.18	3	4.22	206.80
283	81.09	63.40	8.66	4	4.28	240.00
284	70.96	60.36	8.18	3	3.72	183.33
286	57.39	58.39	6.09	2	4.20	103.00
287	70.25	59.91	7.53	3	3.56	183.63
288	68.74	59.88	7.25	2	3.95	167.25
290	80.82	59.95	7.86	3	2.85	230.50
291	65.54	56.30	7.05	3	4.25	138.00
293	69.30	57.48	7.14	4	3.90	171.00
299	54.66	66.31	6.13	4	4.30	147.00
302	63.97	55.84	8.59	3	4.12	131.00
304	55.25	57.76	6.69	2	4.37	108.33
306	78.23	70.45	7.79	3	4.18	247.75
312	72.31	61.30	9.50	2	4.33	196.22
315	70.99	64.85	8.53	3	4.31	213.43
316	76.95	63.26	6.30	7	4.40	256.00
319	73.71	60.14	9.05	4	3.48	194.75
320	74.41	88.22	6.83	5	4.08	340.00
322	85.69	60.78	6.47	5	3.43	264.00
323	72.02	73.57	7.14	5	4.18	265.50
325	74.90	66.83	8.30	3	4.47	228.71
326	77.11	65.26	7.15	3	3.75	231.00
327	62.90	61.24	6.33	3	3.35	162.17
328	74.65	55.09	8.51	4	3.60	164.50
331	61.83	62.06	7.17	2	4.53	144.33
334	76.69	64.01	8.78	3	3.38	219.00
337	60.41	67.62	7.20	2	3.87	160.33
340	53.54	60.75	4.18	4	3.93	134.25
345	59.17	84.55	8.45	4	4.77	241.33
350	55.19	58.34	7.71	2	4.65	109.40
351	58.24	92.05	7.34	6	3.65	262.25
361	65.51	64.01	7.47	2	4.00	165.00
362	70.80	81.32	5.63	4	4.20	283.00
371	51.84	62.97	6.38	3	3.45	113.00
377	56.73	70.14	6.40	4	3.10	174.50
379	73.44	69.98	7.57	5	4.11	270.11
383	59.76	71.42	7.12	4	3.65	186.00
384	63.80	69.52	7.44	4	3.92	191.67
386	60.60	74.15	6.06	3	4.67	76.80
391	70.68	103.45	8.01	5	3.95	377.00
392	72.71	49.90	5.62	5	4.03	154.33
393	64.78	92.18	8.66	4	4.54	334.80
395	72.94	54.55	8.34	4	4.67	196.38
396	65.74	61.08	7.32	5	4.55	214.50
398	62.44	62.70	8.15	3	5.00	159.50
399	64.12	61.13	7.95	2	4.90	177.30
401	51.87	47.93	6.33	2	5.47	110.86
407	53.39	81.24	6.57	5	4.18	204.00
408	66.70	68.47	6.66	4	4.04	197,2
409	69.19	60.05	7.31	4	3.50	173.33

411	56.34	86.80	6.92	5	4.60	223.50
413	66.26	65.78	8.54	4	4.20	191.60
416	58.90	54.13	8.20	2	3.33	148.25
418	68.14	60.00	4.11	4	4.33	174.33
Genotype number	FW	FS	PT	CN	SÇKM	AFW
422	60.05	70.27	7.69	3	4.08	172.00
423	75.27	54.07	6.71	4	3.90	179.29
424	53.15	76.45	6.11	3	4.55	190.25
427	67.71	59.62	6.59	3	3.62	162.00
430	63.19	66.99	6.85	6	4.03	283.10
433	70.21	72.25	7.72	5	4.26	233.71
437	64.66	73.72	7.60	5	4.75	226.13
439	56.73	80.30	6.25	5	4.37	211.67
440	53.52	84.46	7.46	6	3.55	220.00
441	62.34	74.48	6.26	3	4.15	153.00
442	70.03	76.46	7.68	4	4.79	250.13
446	47.62	77.21	5.43	6	3.95	174.50
447	79.62	62.95	11.09	4	3.60	300.00
450	68.47	55.18	7.62	2	4.30	164.75
455	85.87	65.22	7.87	6	3.85	188.67
453	62.73	62.61	5.23	6	3.92	190.00
461	57.32	81.54	4.95	6	4.10	289.00
462	59.37	69.86	7.28	3	4.50	176.60
463	61.05	73.25	9.94	3	3.97	186.67
464	93.33	64.04	8.30	6	3.80	317.00
465	79.12	55.94	8.93	5	3.53	135.20
466	68.58	69.18	5.68	5	3.75	224.00
467	58.71	67.44	6.62	3	4.23	159.00
469	64.57	53.37	7.77	3	3.30	138.67
471	68.49	59.09	7.60	3	4.82	171.33
480	66.22	62.70	5.94	2	4.40	134.50
483	59.82	65.39	5.29	5	3.80	250.00
Average	68.74	66.09	7.38	3.6	4.04	204.28

FW: Average fruit width (mm); FS: Average fruit size (mm); PT: Average Pericarp Thickness (mm); CN: Average Number of Carpels (pcs:); SÇKM Average Water-Soluble Dry Matter (Brix); FW: Average fruit weight (g)

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Beef Cattle Barn Design for Cold Climates; Case of Van Province

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HIGHLIGHTS

- The main consideration in the planning of beef cattle barns is to protect the animals from strong winds in winter and extreme heat in summer.
- Since 30% genetics and 70% environmental conditions are effective on animal productivity, animal barns should be planned to provide comfort areas for animals.

Abstract

Livestock operations generally deal with dairy or beef cattle. Although sufficient attention is paid in design and construction of dairy barns since modern technology, mechanization and labor are used intensively in dairy barns, beef cattle barns are still constructed in traditional styles. However, in cattle barns planned for meat production, which is one of the most important protein sources of human beings, desired conditions should be provided at optimum levels. Working conditions of the workers should be improved, physical, chemical and biological characteristics of animal rations should be improved and hygienic production environment should be provided. Therefore, beef cattle barns should be designed and constructed in accordance with modern technologies and technical standards and regional conditions should be taken into consideration. In this study, planning and design criteria of beef cattle barns for cold climate regions were determined and 100 and 200-head capacity beef cattle barns were developed and designed for Van Province conditions. The designed beef cattle barn project, was submitted to the Agricultural-Specialized Organized Industrial Zone Project Commission and it was deemed appropriate to carry out the exercise.

Keywords: Agricultural Structures; Barn; Beef Cattle; Biosystems; Cold Climate

1. Introduction

The primary objective of livestock operations is to increase profit margins. For this, quality product outputs must be achieved at the highest levels. Quality and quantity of animal products are largely

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designated by genetic structure of the animal at a rate of 30% and environmental conditions at a rate of 70% (Ekmekyapar 2001). Indoor environmental conditions play a great role in animal housings. Therefore, livestock operations should provide optimum environmental conditions for each species.

Animal housings are mostly constructed for cattle, ovine and poultry. Cattle operations usually deal with dairy or beef cattle. Small, medium and large business owners unfortunately do not pay a special attention on physical characteristics and environmental conditions of housing systems. Proper or optimum environmental conditions may reduce animal diseases by about 70% and increase animal performance and product quality (Zhong et al. 2020). In recent years, it has been seen that only large-scale enterprises give partial importance to housing design since construction of modern buildings is the primary criteria for state incentives provided to livestock operations. Such supports are limited only to dairy cattle barns. Small and medium-sized enterprises still house their animals in barns with traditional primitive conditions.

Beef cattle barns are built in 4 different styles as; closed fixed stall, closed free, semi-open free and open free barns. Open barns, semi-open barns and closed barns are built in regions where hot, warm and cold climatic conditions are prominent, respectively (Shouse et al. 2004). In closed cattle barns, animal stalls built in dairy barns may not be needed (Okuroğlu and Yağanoğlu 2015).

Beef cattle breeding is done for meat production and beef cattle barns should have features that will increase the yield and meat quality of beef cattle (Agus and Widi 2018). However, even in large-scale enterprises that have partially switched to modern barn planning, beef cattle barns are converted from barns designed by considering the planning criteria of dairy cattle barns.

In this research; beef cattle barns with a capacity of 100 and 200 heads were designed and presented for the beef cattle barns that are planned to be built in the region within the scope of the Agricultural-Specialized Organized Industrial Zone Project, which was approved and gained legal personality by the Ministry of Agriculture and Forestry in the province of Van. The developed beef cattle barn projects will also set an example and be applicable for the beef cattle enterprises that are planned to be built in other regions with cold climate conditions.

2. Materials and Methods

Present research area is located between Gürpınar, Edremit and Gevaş districts of Van province. Nearly 400 ha of approximately 1700 ha idle land of the province, most of which has been used as pasture for many years, were included in the scope of the Agricultural-Specialized Organized Industrial Zone Project under the leadership of Van Governorship (Anonymous 2019). The stakeholders of the project are Metropolitan Municipality, Chamber of Commerce and Industry, Eastern Anatolia Development Agency, Provincial Directorate of Agriculture and Forestry, Van Investment Monitoring and Coordination Department, Ipekyolu, Tusba, Gevaş, Gurpinar Municipalities, Van Commodity Exchange, Red Meat Producers and Cattle Breeders Associations. About 186 beef cattle enterprises with a capacity of 40 thousand cattle, industrial, treatment and biomethane facilities, emergency slaughter units and administrative and social units will be built within the scope of the project (Anonymous 2021). In Cattle Breeding units of the project, it was aimed to operate 100 enterprises (50 units of 100, 50 units of 200 heads) in the first stage, to raise a total of 11750 beef cattle and to produce 4000 tons of red meat annually. Another objective of the project is to reach out to livestock enterprises operating in the Central Districts of Van Province with the clustering model (VATSO 2013).

Livestock businesses operating in Central Districts of Van Province, plans and projects of 100 and 200-head beef cattle barns, which will serve as a model for beef cattle enterprises, one of the independent units of the facilities to be built in the region within the scope of the Agricultural-Specialized Organized Industrial Zone Project constituted the primary materials of the study.

2.1. Geographical and climate characteristics of the region where beef cattle enterprises will be built

Within the scope of the project, 4 alternatives were considered in the selection of places for the construction of beef cattle barns, taking into account criteria such as the presence of pasture in the region, topographic conditions, proximity and distance to settlements and climate characteristics. The characteristics of these places are given in Table 1 and Figure 1.

Table 1. Places where beef cattle barns will be built.

No	District	Neighborhood	Type	Total Area (Decare)
1	Gevas Gurpinar Edremit	Gundogan Sakalar Kopruler	Pasture	8620
2	Gurpinar	Kiziltas	Pasture + Government	1304
3	Tusba	Gollu/Tabanli	Pasture	2408
4	Tusba	Yumurtatepe/Yeni	Pasture	2515

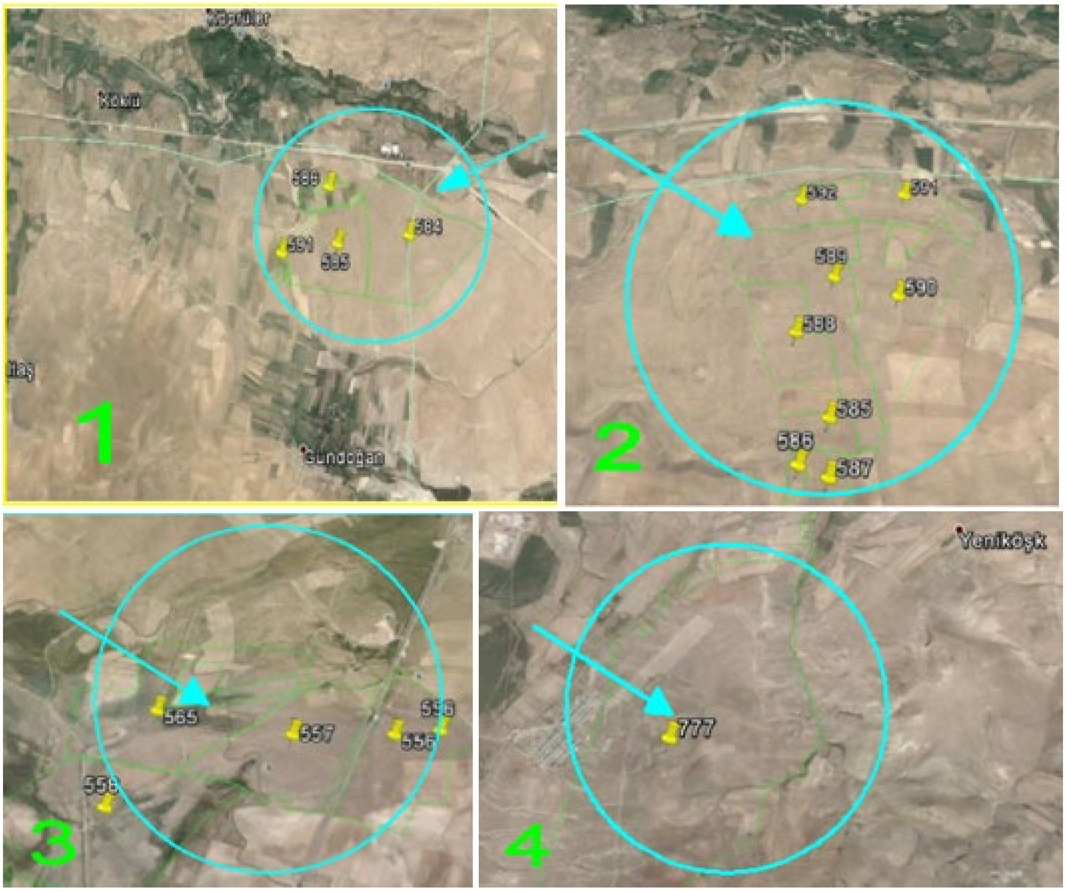


Figure 1. Topography of the places where the cattle barns will be built.

When the climate conditions of the regions where the lands are evaluated for the places where the cattle barns will be built are examined (Anonymous 2022), it was seen that they had the same climate characteristics since all of these alternatives are located in the same geographical area. These regions are classified as regions with cold climate conditions (Yılmaz and Çiçek 2018).

2.2. Livestock businesses operating in central districts of Van province

In the Central Districts of Van Province, there are a total of 1 567 livestock enterprises and 1 341 of them were actively operating. The enterprises mostly deal with small scale production with 20 heads on average. Animal assets of 124 enterprises with a capacity of 21 and above, which constitute 19% of cattle breeding establishments, have 81% of the total cattle stock (VATSO, 2013). All of these enterprises were breeding in barns with traditional primitive conditions (Figure 2).

Figure 2. Examples of beef cattle farms operating in Central District of Van Province.

2.3. Beef cattle barn planning criteria

Before the planning of cattle barns suitable for the region, the region where the barns will be built was examined on site in order for the study to serve the purpose. In this process; topographical conditions of the region, whether it is suitable for barn construction, soil characteristics, transportation and product marketing status were determined by considering climatic conditions.

Since cold climate conditions are dominant in Van Province, beef cattle barns were designed in closed fashion. To reduce construction costs and to have an economical production, animal stalls were not included and free style was adapted.

Present beef cattle barns are composed of a resting place, feeding and fodder storage area and a promenade. Providing the necessary space for each animal is one of the most important parameters when planning a resting place. The areas needed for each animal in closed free beef cattle barns are given in Table 2 (Koçak et al. 2015).

Table 2. Required area dimensions in closed free beef cattle barns.

Animal breed	Required area (m²/per animal)
Calf	2.0-2.5
Heifer	2.7-3.0
Cattle	3.0-3.6
Bull	4.0-6.0

Feeders are the most important equipment in beef cattle barns. It should be placed in the resting place and in the promenade. They can be made of wood or masonry and can be fixed or portable. Feeders should be considered as 45-55 cm for calves, 55-65 cm for beef cattle, 70-80 cm for adult cows and bull. The height of the part where the animal sticks its head and eats from the ground should not be higher than 45 cm for calves and 55 cm for adult animals (Achmad et al. 2019). It should have a width 120 cm for mangers feeding from both sides, lower width 45 cm, upper width 75 cm for one-sided ones. It is recommended that the lower part of portable mangers should be made at a height of 20-25 cm from the ground. The front of the mangers should be covered with 3-3.6 m wide and 10-15 cm wide concrete or 15 cm thick sand and gravel. The inclination of this part outward from the mangers by 6-8% makes it easier for animals to eat feed (Ekmekyapar 2001) (Figure 3).

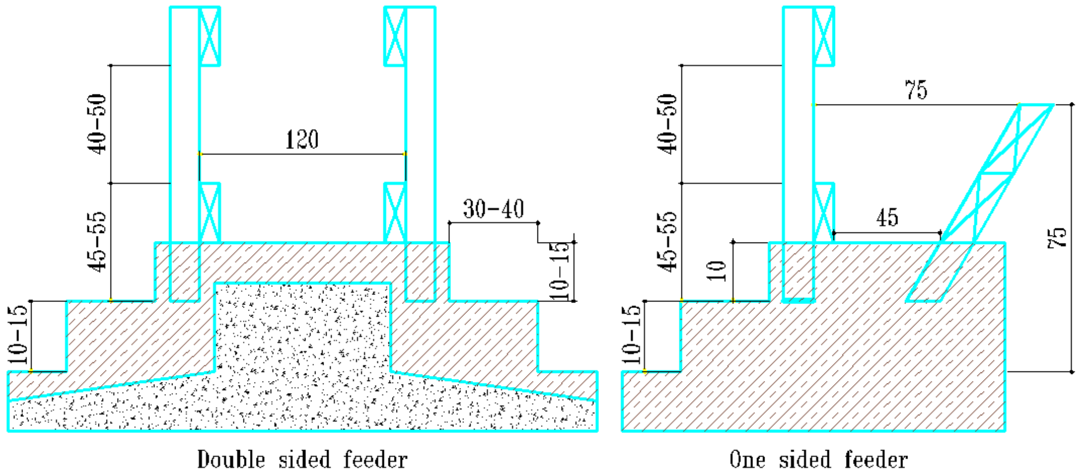


Figure 3. Single and double-sided fixed feeder dimensions.

In order to meet the water needs of the animals, automatically controlled water tanks should be built and precautions should be taken against the danger of freezing in winter (Karaman and Ekmekyapar 1996).

The resting place base can be concrete or compacted soil. It is considered appropriate to make the barn height between 2.8-3.6 m depending on the climate conditions in closed free barns applied in beef cattle breeding in Kapuinen (2001). The height of the beef cattle barns suggested in this study was 3.5 m.

Heat and moisture balance calculations for the developed beef barns were made in accordance with the principles specified in Ekmekyapar (2001), Beatty et al. (2006) and Okuroğlu and Yağanoğlu (2015). The amount of heat and moisture releases by the animals to the environment was taken from Okuroğlu and Yağanoğlu (2015). In the selection of structural members of the proposed closed free beef cattle barn, in the ground arrangement of the barns and in the design and preparation of sample barn plans, principles given in Rosina and Robison (2002), Shouse et al. (2004), Olgun (2016), Sirin and Kocaman (2016), Gozener and Sayili (2011), Ekmekyapar (2012) were used.

While designing agricultural structures, foundation members should be considered first. Foundations can be made in 4 different ways as under-wall, single, continuous and raft foundations. Foundation of single-story buildings is generally built as a single foundation using tie girders (Ekmekyapar 2012).

3. Results

Structural and technical features of closed free-breeding barns with a capacity of 100 and 200 head, which were thought to be suitable for cold climate conditions, and sections and plans showing these features were given as the results of this study.

A single foundation was chosen and it was suggested to be connected to each other with tie girders in order to prevent horizontal displacements (Figure 4). The foundation depth was taken as 0.90 m (KTMMOB 2009).

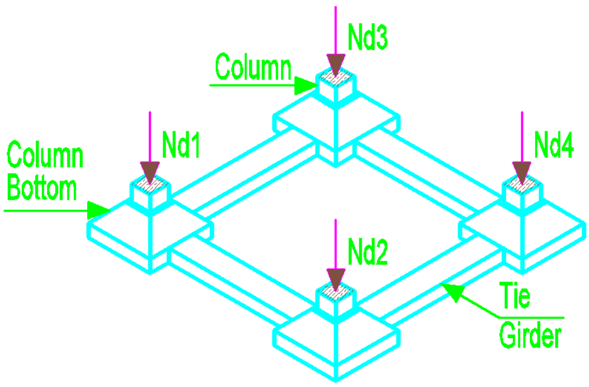


Figure 4. Single foundation section connected by tie girder.

To protect animals from northerly winds and rains, long axis of 100-head barns was closed with a wall and promenade was placed on the south face. The 200-head barn is formed by placing two separate 100-head barns facing each other. Width of the barn, except for the wall thicknesses, is 18.85 m, including 3.20 m service path, 0.75 m single-sided feeder, 12.50 m rest area, 2.40 m manure bed. The barn is composed of 8 paddocks, each of which is 8.00 m long and a 3.00 m service road left in the middle to facilitate herd management. Total length inside is 67.00 m excluding wall thicknesses. Paddocks are separated from each other by iron profiles with a diameter of 8.00 mm, a height of 120 cm from the floor. Each paddock (100.00 m2) is planned to accommodate 20 animals. Resting areas separated from feeders by manger irons was envisaged to planning of a 2% slope to facilitate maintenance and cleaning. A promenade of 8x20 m, with an area of 160.00 m2 and an outward slope of 5% is planned for each paddock in the barn. Shades have been made in the promenade to protect feeders and waterers from precipitations and for animals to be able to rest in the shade. A urine channel with a width of 30 cm and along the length of the barn, through which rain water and animal urine can be evacuated is considered in the promenade.

Masonry and wooden-framed walls are used in agricultural buildings. Masonry walls can be built with 5 different building elements: stone, brick, breeze-block, pumice block and adobe (Rosina and Robison 2002). In present beef cattle barns, it was deemed appropriate to be used long-lasting, easy to use, economical and light-weight pumice blocks. Side wall heights of the barns are designed as 3.5 m in order to provide adequate ventilation. No wall was considered on the long axis facing the promenade. Instead, a curtain wall with transparent insulation, which can be opened and closed, is planned separately for each paddock from the floor to the soffit level. Depending on the seasonal conditions, the curtain walls can be turned into semi-open barn models by opening fully and it can be turned into closed barn models with completely shut down. Or, since the curtain walls are adjustable, they can be left partially open as desired. Such a case allows animals to walk freely in the promenade without animal traffic. Desired level of ventilation can also be achieved and accumulation of harmful gases is prevented. Wall surfaces was covered with 2 cm thick interior and 3 cm thick exterior plaster and a light color paint should be applied on plasters.

About 10 cm blockage, 250 dose 15 cm lean-concrete, 10 cm floor covering and 3 cm screed-concrete should be placed on compacted soil on the floor of the barns. In order to increase the ventilation efficiency and reduce the cost, headlining is not considered.

Steel construction was chosen as the roof frame members. Insulated, corrugated sandwich panel was chosen as the covering material. A minimum slope of 33% was selected (Alptekin et al. 2014).

On the short sides of the barn; 3.00x3.20 m metal doors where tractors and other tools and equipment can enter have been placed at the start and end points of the service path and in the middle of its long walls. In addition, 1.60x1.20m portable doors that enable transition between paddocks and can be opened and closed in both directions were placed both in the resting areas and in the promenade. A total of 16 2.20x0.60m transom lightings were planned to be installed. Ventilation windows, 2 of which are in each paddock with the upper edge 60 cm below the soffit level on long pumice blocks wall were designed. A total of 8 1.00x0.60 m transom windows with the upper edge 60 cm below the soffit level (4 on each short walls) were also projected. No window was used on insulated transparent curtain wall.

Adjustable ventilation opening that can be opened and closed with automation system is built along the roof ridge of the beef cattle barns. In addition, to be used when the ventilation openings are closed, 8 ventilation chimneys with dimensions of 1.00x1.00 m were placed along the ridge and they were 50 cm high from the ridge (Şirin 2017).

4. Discussion

4.1. Barn height and barn building elements

The barn height is the clearance between the service road and the lower beam of the roof or roof truss (Ekmekyapar 2001). The height of the designed barn was determined as 3.5 m by considering the criteria for determining the barn height specified in Şirin and Kocaman (2016) and Olgun (2016).

The barn foundation floor must be able to safely bear the load coming on it through the foundation. Foundation width varies according to the foundation wall material to be used. Foundation depth varies between 80-120 cm (Okuroğlu and Yağanoğlu 2015). The barn was prepared with a foundation depth of 90 cm, which is in accordance with the foundation depth recommended in Okuroğlu and Yağanoğlu (2015).

It is proposed to use 30 cm wide bricks in the walls of the barn. The recommendation of Karaman and Ekmekyapar (1996) that "the wall thickness of barns planned in cold regions should be 1.5 bricks thick for brick walls" was taken into consideration.

The roof of the barn was planned as an asymmetric gable roof considering the barn roof planning principles stated in Alptekin et al. (2014).

According to Agus and Widi 2018, windows were placed on the long walls of the barns to ensure adequate lighting and ventilation.

4.2. Barn compartments

In beef barns, the barn compartments consist of a resting place, a feeding place and a promenade yard. In beef cattle barns, should be allocated an area of 2.0-2.5 m^2 for each calf, 2.7-3.0 m^2 for each heifers, and 3.0-3.6 m^2 for each beef cattle (Ekmekyapar 2012; Şirin and Kocaman 2016). In the planned beef cattle barn projects, the size of the resting area was adjusted according to these criteria.

The most important equipment needed in beef barns are feeders. The width of the feeder should be 120 cm for feeders with feeding from both sides, and the lower width of the feeder should be 45 cm and the upper width should be 75 cm for feeders with feeding from one side. Feeders placed in the promenade yard should be double-sided (Ekmekyapar 2001; Olgun 2016; Achmad et al. 2019). In the fattening cattle barn projects prepared, the planning of the feeders is in compliance with the specified rules. In order to meet the water needs of the animals, drinkers have been installed with precautions taken against the dangers of frost in winter.

The promenade yard is a place where animals can roam freely in order to benefit from daylight and sunlight. The promenade yard, which is at least the size of the resting place, is one of the most important compartments to be planned in beef cattle barns (Ekmekyapar 2012; Okuroğlu and Yağanoğlu 2015). It should be planned on the south side of the barn and at a slope of 1.5-2% towards the outside for easy cleaning (Karaman and Ekmekyapar 1996; Şirin and Kocaman 2016). The promenade yard compartments organized in the beef cattle barn projects meet the literature data. The beef cattle barn design projects proposed in our study set an example for the barn enterprises to be built in the region with these features.

5. Conclusions

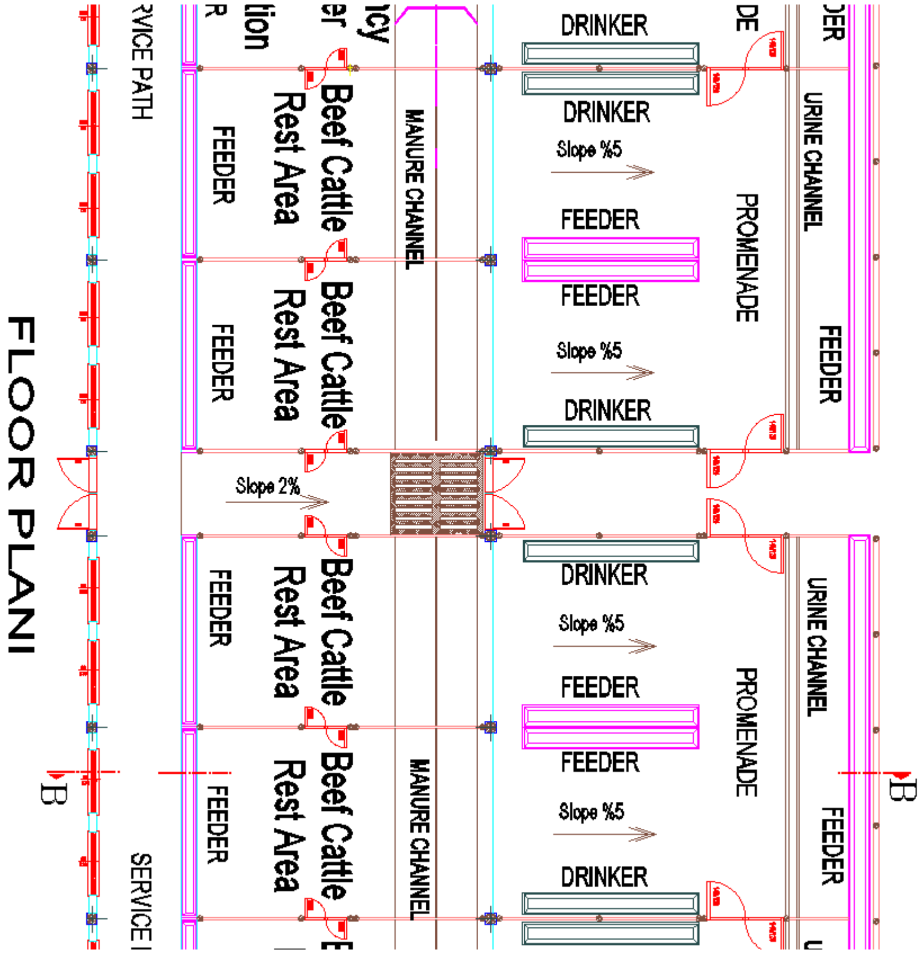
As a result; beef cattle barns do not need to be as protected and expensive as dairy barns. The main consideration in the planning of beef cattle barns is to protect the animals from strong winds in winter and extreme heat in summer. Different types of housing are applied in beef cattle breeding. However, plans and projects of beef cattle barns were developed according to the planning principles of closed barns, which are one of the forms of housing applied in beef cattle breeding, especially considering the trend in recent years and its suitability for local conditions.

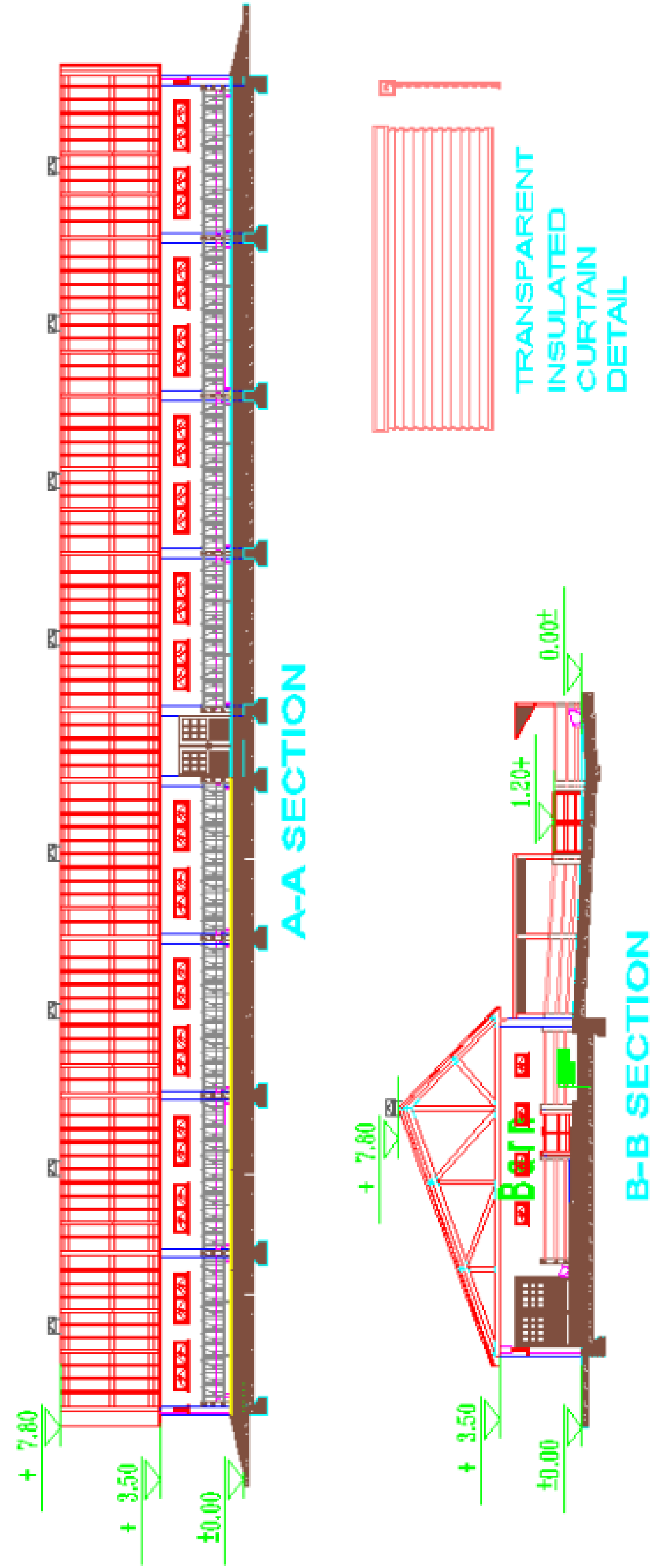
In this section, drawings of 100 and 200-head capacity closed free cattle barn projects prepared in accordance with cold climate conditions are included.

Author Contributions: The authors have an equal contribution. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: : The authors declare no conflict of interest.

5.1. 100-head capacity closed free cattle barn projects




A-A SECTION
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TRANSPARENT
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+ 7.80
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1.20+
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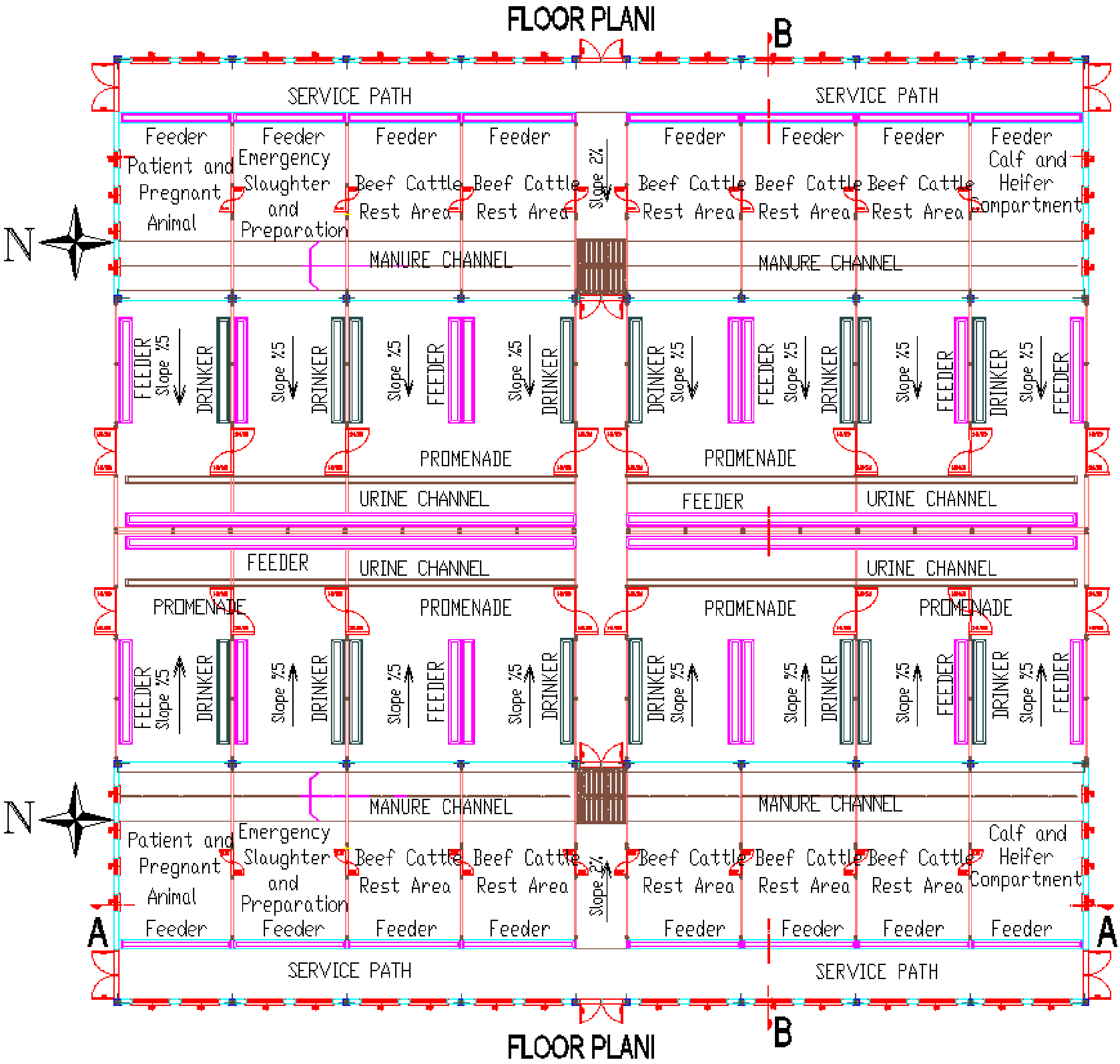
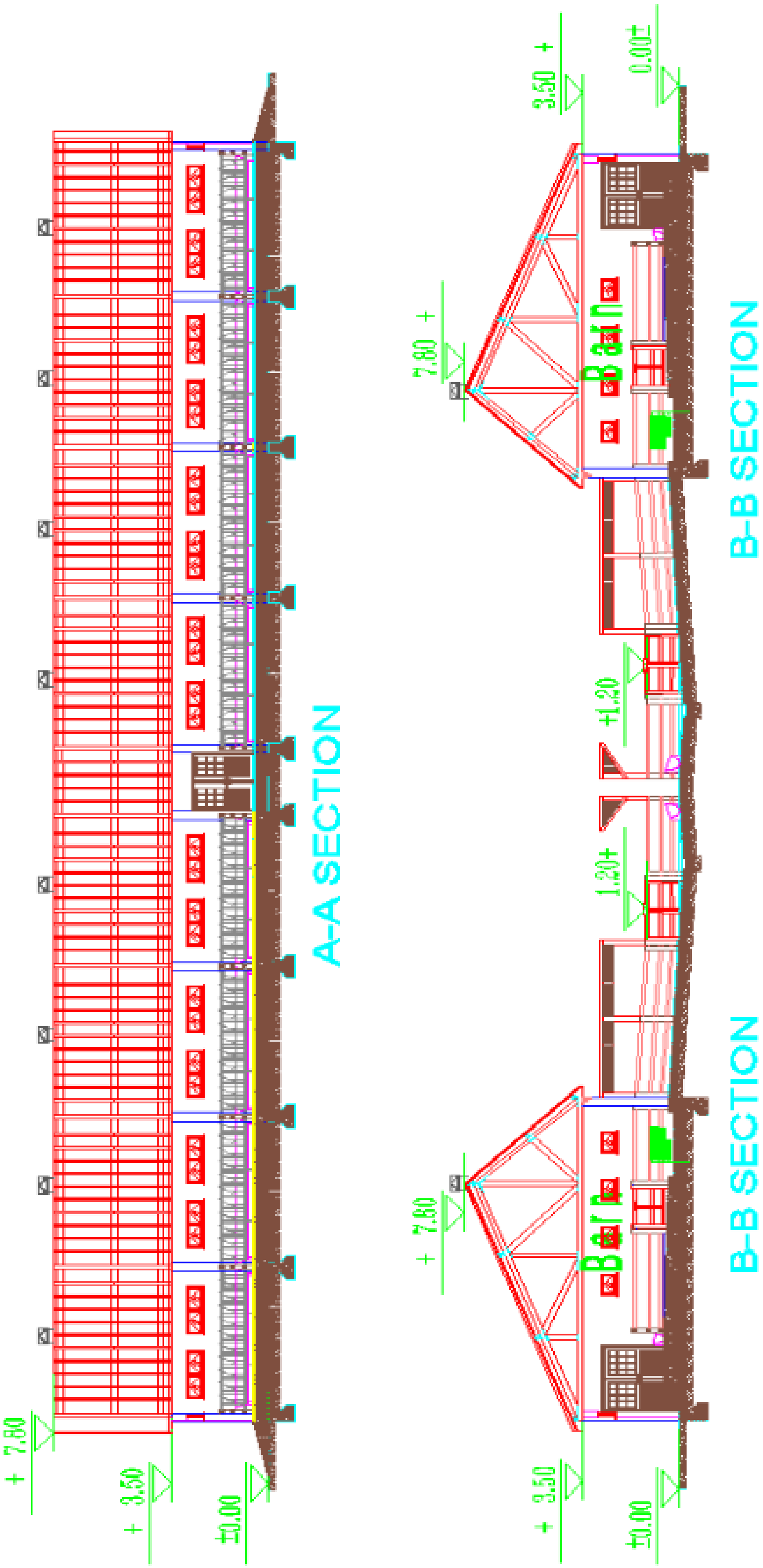
5.2. 200-head capacity closed free cattle barn projects




A-A SECTION
B-B SECTION
B-B SECTION
Barn
Barn
+ 7.80
+ 3.50
±0.00
+1.20
1.20+
3.50 +
0.00±

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Research Article

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The Stimulation of Chemical Male Sterility for F1 Hybrid Lettuce (*Lactuca Sativa* Var. Longifolia) Production

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HIGHLIGHTS

- Male sterility is utilized in the production of commercial F1 hybrid seeds of some vegetables.
- Türkiye seed market has not a native F1 hybrid lettuce variety.
- Procedures are not required to the detecting sterile and restorer lines and ensure the continuity of these lines in gametocyte-originated sterility.

Abstract

Male sterility is a unique application in the production of F1 hybrid seeds of some important species. Today, while F1 hybrid lettuce varieties of abroad origin take their place in the Türkiye seed market, unfortunately, we do not have a native F1 hybrid variety. Besides, F1 hybrid seed production has become a prestige for multi-national companies regardless of the type of vegetable. Within this perspective, the effects of some chemical hybridizing agents (CHAs) such as Ethyl 2-(4-fluoroanilino)-2-oxoacetate E4FO, 2-chloroethyl phosphonic acid (Ethrel) and GA_3 on male sterilizing activity in lettuce cultivars (Maylight352 F1, Presidential and Yedikule) were evaluated. Therefore, pollen presence in the early bud stage, seed formation and seed viability (germination) were examined. The applications had different effects on pollen presence, seed formation and seed germination, and thus male sterility. Ethrel was not effective at low doses, but at high doses, it caused flower bud deformation and growth retardation. E4FO is partially effective, but the application doses are low. Therefore, E4FO should be used at higher than 1500 ppm and 2000 ppm. GA_3 applications produced the best results in stimulating male sterility, and full sterility (% 100) was achieved from 200, 250 and 300 ppm in all cultivars. As a result, 200 ppm GA_3 was determined as the recommendable dose in the production of F_1 hybrid lettuce.

Key Words: Male Sterility, Gametocytes (CHAs), Lettuce

1. Introduction

Lettuce (*Lactuca sativa* L.) is at the forefront of the vegetable species (Eşiyok 2012) and its origin is considered to be Anatolia, Caucasus, Iran and Turkistan (Balkaya and Özgen 2019). They are grouped as curly leaves (*L. sativa* var. crispa), head (*L. sativa* var. capitata) and cos lettuce (*L. sativa* var. longifolia) concerning the leaf characteristics (Şalk et al. 2008). Curly lettuces show great diversity in

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terms of leaf size, shape, leaf color and texture (Karaağaç and Balkaya 2019). Lettuce can be grown all over the world due to its short vegetation period and the continuity of the lettuce's presence in the market is ensured by planting more than once a year.

Lettuce product shares vary considerably according to regions and segment groups. Availability of genetic material with variation is needed to achieve success in breeding efforts. Successful lettuce breeding requires observation, evaluation, selection and seeding stages by adjusting the conditions suitable for the plant's growing demands in a limited time. Breeding studies in salad group species are carried out in the world with classical methods (Prohens and Nuez 2008). In lettuce, inflorescences shoot bear 12-20 flowers and hermaphrodite flowering lettuces are obligate self-pollinated (Feráková 1977).

Due to the flower structure, the style is covered with pollen during elongation between the anthers and the external pollination rate remains very low (1%) (Thompson 1958). Lettuce pollen is heavy and sticky, making it difficult to transport by wind; the use of insects is unsuccessful and hand pollination is not feasible in large quantities. Thus, F1 hybrid seed production needs the support of some applications (Ryder 1999). The emasculation process for classical hybridization is based on the principle of washing the pollen by spraying water when the stigma of the female flower reaches the "V" shape (Nagata 1992).

Male sterility can be of cytoplasmic, genetic, and cytoplasmic-genetic origin, and sources are showing their use in lettuce breeding (Curtis et al. 1996; Goubara and Takasaki 2004; Takada et al. 2007; Hayashi et al. 2011; Ananthi et al. 2013; Michel and Soussin 2014). However, the materials containing the inducer ms genes are patented, and gametocyte applications are preferred for breeders who cannot obtain these materials (Eenink and Vereijken 1978). In addition, in gametocyte-originated sterility, procedures are not required to the detecting sterile and restorer lines and ensure the continuity of these lines.

Gametocytes are generally known as chemicals that cause deformations in pollen formation (in the meiosis), leading to the formation of pollen with lost germination ability and therefore inability to fertilize, and have been used successfully in F1 hybrid breeding in many species (Collantes et al. 1999; Colombo and Galmarini 2017; Hussain et al. 2018; Tinna 2019). A suitable gametocyte should not be mutagenic and non-toxic, does not limit the application dose and time, is environmentally friendly, has no negative effects on F1 hybrid seed, does not cause problems in seed setting, and is also applied cheaply and easily. In particular, some chemical hybridizing agents (CHAs) such as maleic hydrazide, gibberellins, dalapon, mendok, ethephone and ethyl oxanylates are used as male sterility-stimulating chemicals in vegetables. The first gametocyte applications to stimulate male sterility in lettuce were carried out by Eenink (1977) obtained male sterile lettuce plants with GA_3 applications in the early bud period in lettuce.

Most of the varieties cultivated are open-pollinated (OP) varieties in current lettuce production. Because F1 hybrid seed production is not possible with classical methods due to the flower structure, and it is imperative to benefit from the male sterility method used in species such as onion, carrot, cabbage and maize (Billore 2015). Although GMS (Genetically Male Sterility) in lettuce has been known since the 1960s, with the development of gene transfer techniques, GMS-derived F1 hybrid lettuce varieties have been put in the world seed market since 2002.

In this study, it was aimed to determine the effective chemical substance and dose, which reveals high male sterility (> 95%) and does not adversely affect plant and flower development (does not reduce seed setting and seed viability) by applying gametocytes at the beginning of flowering and early bud period. The objectives of the study were to find the appropriate concentration of E4FO, Ethrel and GA_3 to (1) the presence of pollen, (2) the seed setting, (3) the germination rate of the seeds (4) induce male sterility in lettuce.

2. Materials and Methods

The study was carried out in the Department of Horticulture, Faculty of Agriculture, Selcuk University, between April – August 2021. Field studies of the research were carried out in the greenhouse of Selçuk University.

2.1. Material

Mylight352 F1, Presidential and Yedikule cultivars with cos lettuce types were used in terms of leaf types (Figure 1).

Figure 1. The cultivars (Original)

2.2. Methods

2.2.1. Cultivation of Plants

Seeds of the cultivars were sown in plastic containers containing peat moss in the growth chamber, and the seedlings, which reached a certain size, were staggered into 45-mesh vials filled with peat moss-perlite (1:1 volume). Seedlings at the 3-4 leaves stage were planted at 0.7 x 0.4 m spacing and distances in the greenhouse, and then sap water was applied with the drip irrigation system. According to the soil analysis, the plants were fertilized by a drip irrigation system using Ammonium Sulphate (10.5 kg da^{-1}) and Potassium Sulphate (8.5 kg da^{-1}) fertilizers twice a week during their development period. Cultural practices such as weeding, hoeing, disease and pest management were implemented regularly. "Movento SC 100" was used against whitefly periodically, and "Flo-Captan 50 WP" was applied against mildew (*Bremia lactucae*). The plants were covered with a shade net to prevent early bolting of the lettuce at high light intensity and temperatures.

2.2.2. Determination of application time of gametocytes

For male sterility in lettuce, gametocytes must be applied in the early bud period. The optimum bud size was determined as 3-4 mm. Because this period is the first stage of meiosis and microspore mother cell formation and is accepted as the stage in which transformation into mature pollen can be prevented (Eenink and Vereijken 1978).

The buds in the blue and red areas are unsuitable and they were removed from the plants before gametocyte application. The buds in the yellow area are the most convenient size (Figure 2).

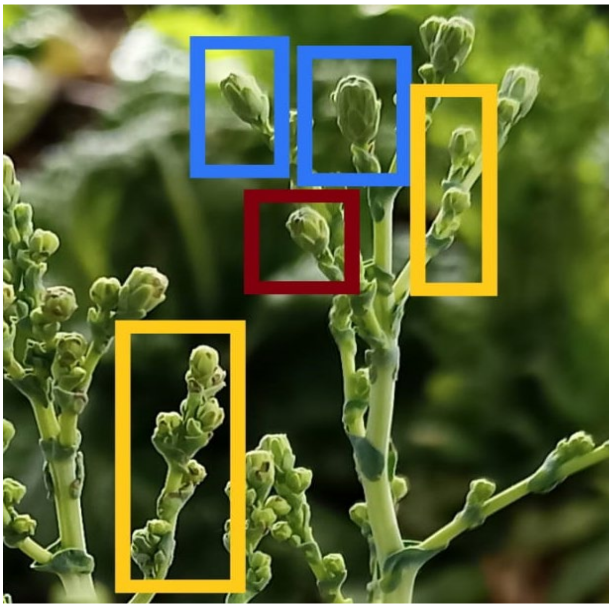

Figure 2. Flower buds (cv. Presidential) at different developmental stages

2.2.3. Gametocytes and applications

GA_3 (Gibberellic acid) was used in the commercial Berelex form, Ethrel (Ethephon) was applied in the commercial Maysal form, and E4FO [Ethyl 2-(4-fluoroanilino)-2-oxoacetate] was purchased from the ChemCruz company (Table 1).

Table 1. Gametocytes, chemical formulas and application doses.

Gametocyte	Formula	Dose (ppm)
GA_3 (Gibberellic acid)	$C_{19}H_{22}O_6$	50, 100, 150, 200, 250, 300
Ethyl 2-(4-fluoroanilino)-2-oxoacetate	$C_{10}H_{10}FNO_3$	1500, 2000
Ethrel (Ethephon)	$C_2H_6ClO_3P$	1000, 1500, 2000, 3000, 4000

Before the application, stock solutions of the chemicals were prepared and the final concentrations were created by taking appropriate amounts of these stock solutions before each application. Stock solutions were stored in the refrigerator at -18 ^oC.

Gametocyte applications to flower buds were made in the early morning at daylight or just after light. Applications were started in the cool and early times, as applied during the noon or hot hours would reduce the activity of the gametocyte. Before the application, inappropriate buds were discarded, and then gametocytes were applied to inflorescences.

To be able to apply in equal doses, each inflorescence was sprayed 3 times from the prepared gametocytes, and the inflorescences were closed from the back so that the applications would not reach other buds. After the first application, other applications were carried out 3 times at 3 days intervals to transmit the chemical to the flowers that may occur later in the inflorescence. After the first application, the buds were isolated with net sacs to avoid external pollen contamination, then labeled and recorded (Figure 3). Distilled water was only sprayed in control lots.

Figure 3. Gametocyte applications

2.2.4. Pollen and Seed Formation

After the applications, 5 flowers were gently removed from the inflorescences that bloomed 4 or 5 days after application. Then the flowers were cut longitudinally and the presence of pollen in the anthers was visualized under a light microscope at 10 x 10 magnifications. For the seed formation in inflorescences, 5 flower capsules were gently collected for each application, and the presence of seeds was examined under a light microscope at 10 x 10 magnifications.

Mature flower buds were carefully removed with the isolation sacs, and dried at room temperature for 2 weeks. The seeds were gently collected from dried capsules, cleaned from all other plant parts and 100 seed weight (g) was determined in randomly selected seeds for each genotype and each application

2.2.5. Germination tests

The seeds were subjected to a germination test in petri dishes with a diameter of 90 x 15 mm at 20 ± 1 ^{o}C. A double layer of Whatman No.1 filter paper had placed on the surface of Petri dishes, and filter papers had been moistened with a 5 ml carbendazole mixture (0.75 g lt^{-1}) to avoid fungal contamination. The germination tests were carried out in 3 replications and 100 seeds in each replication except for the Ethephon 1000 ppm treatment, due to the limited number of seeds could be obtained. The germination test was realized for 14 days and percentage germination were calculated at the end of the 14th day (ISTA 2009).

2.2.6. Data evaluation

The study was carried out with 3 replications for each application. However, in some applications, as no data could be obtained (at high doses of Ethephon), statistical analysis was not performed, and only standard deviations were presented.

3. Results and Discussion

3.1. Pollen Formation

Flower deformations were determined and pollen formation could not be examined at doses of 1500 ppm and above in Ethephon (Table 1). The plant growth inhibitory effect of Ethephon was reported in a male sterility study, and a shortening of the plant length was observed in 3 barley cultivars (Ma and Smith 1992). At the 1000 ppm Ethephon, a small number of flowers were obtained, but all of the flowers produced the pollen grains. These flowers then produced mature buds and seeds. The results revealed that Ethephon doses between 1000-1500 ppm should be examined or the range of applications should be arranged in further studies.

Table 1. Rate of pollen-containing flower (%).

Application	Dose (ppm)	Cultivar	PR (%)	Average
CONTROL	-	P	100	100
		Y	100	
		M	100	
ETHEPHON	1000	P	100	100
		Y	100	
		M	100	
	1500 - 4000	Could not evaluate due to floral deformation		
GA_3	50	P	100	100
		Y	100	
		M	100	
	100	P	100	100
		Y	100	
		M	100	
	150	P	93.3	91.1
		Y	86.7	
		M	93.3	
	200	P	93.3	91.1
		Y	93.3	
		M	86.7	
	250	P	20.0	24.5
		Y	26.7	
		M	26.7	
	300	P	0	0
		Y	0	
		M	0	
E4FO	1500	P	100	100
		Y	100	
		M	100	
	2000	P	66.7	71.1
		Y	73.3	
		M	73.3	

P: Presidential; **Y:** Yedikule; **M:** Maylight352
PR: Pollen-Containing Flower Rate

While pollen formation was observed in all the flowers of the cultivars at 50 and 100 ppm GA_3, pollen formation was in 91% of the flowers at 150 and 200 ppm, 24% at 250 ppm, and all the flowers were found to be sterile in the 300 ppm (Figure 4). Eenink and Vereijken (1978) stated that high doses of GA_3 did not produce pollen grains.

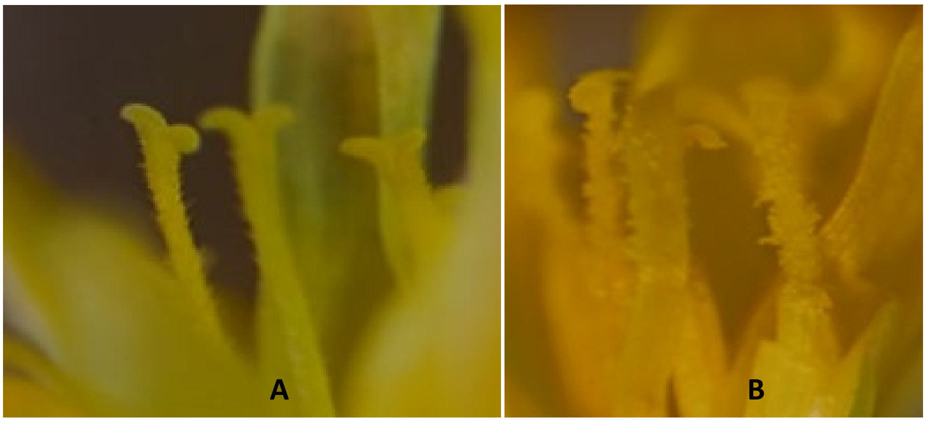

Figure 4. Sterile flower (A), and fertile flower with pollen grains (B).

Pollen was detected at 1500 ppm E4FO, and it was 71% in the 2000 ppm application. The results revealed that the doses of E4FO are also low and higher doses should be examined in further studies. Conversely, 1500 ppm E4FO induced 99.7% or 100% male sterility in rice (Ali et al. 1999) and wheat (Devakumar 2006). 2 mg l^{-1} E4FO provided complete pollen sterility (97% - 100%) and it had no adverse effects on female activity in sorghum (Amelework et al. 2016). In a study investigating the effects of Ethrel, acetic acid, E4FO and promaline (1.8% GA 47 – gibberellins A 4 +A 7 and 1.8% 6-BA-benzyl adenine) on male sterility in *Eragrostis*, 99.50% pollen sterility was achieved in the 1500 to 3000 ppm E4FO and 5000 ppm Ethrel (Ghebrehiwot et al. 2015).

All cultivars had similar responses to the applications, and no genotype differences were observed. This situation revealed that the recommended chemicals and doses for the induction of male sterility in lettuce will reveal sterility at a similar rate in all genotypes and it can be used safely.

3.2. Seed Formation

The number of seeds and 100 seed weight (g) were differentiated with CHAs and application doses (Table 2). Due to the deformation of the buds caused by Ethephon applications, either no seeds or very few seeds were detected. Only a limited number of buds were harvested in 1000 ppm Ethephon and a limited number of seeds were harvested compared to the control. While Ethrel was an effective gametocyte for wheat (Dotlacil and Apltauerová 1978), it also induced very high female sterility at the rates required for male sterility (Chakraborty and Devakumar 2006).

1500 and 2000 ppm E4FO and GA_3 up to 150 ppm produced similar results with the control lots and there was no negative effect on seed formation. Conversely, there was a noticeable decrease in seed weight at higher doses. It is suggested that even if seed formation occurs at 200 ppm and above GA_3 doses, these seeds may be relatively empty and nonviable (Figure 5). Treated once with 0.15% E4FO provided 99.8% male sterility without a significant reduction in total yield in wheat (Chakraborty and Devakumar 2006).

Although there is no statistical data, 200 ppm and above GA_3 applications cause elongation in flower stems. Likewise, GA_3 increased stem elongation in lettuce (Yılmaz et al. 2002) and transgenic tobacco plants treated with GA_3 had male sterility but developed undersized (Huang et al. 2003).

Since chemically induced male sterility was first introduced by Nelson and Rossman (1958) in maize, gibberellins have been successfully used in safflower (Baydar 2000), tobacco (Huang et al. 2003), sunflower (Duca et al. 2008; Yılmaz 2010) in barley (Altındal 2019).

Table 2. Number of seeds per 100 buds and 100 seeds weight (g)

Application	Dose (ppm)	Cultivar	SN	100 SW (g)
CONTROL	-	P	1238.0 ± 51.0	0.094 ± 0.008
		Y	1989.4 ± 57.6	0.076 ± 0.019
		M	1830.0 ± 45.2	0.050 ± 0.015
ETHEPHON	1000	P	142.0 ± 64.9	0.065 ± 0.009
		Y	642.4 ± 122.5	0.056 ± 0.044
		M	121.4 ± 98.0	0.042 ± 0.006
	1500 - 4000	Could not evaluate due to floral deformation		
GA_3	50	P	1348.6 ± 82.7	0.060 ± 0.018
		Y	1292.6 ± 76.3	0.054 ± 0.024
		M	1011.6 ± 61.1	0.049 ± 0.033
	100	P	1271.6 ± 80,0	0.037 ± 0.012
		Y	1198.2 ± 65,7	0.056 ± 0.023
		M	1003,5 ± 83,4	0.051 ± 0.029
	150	P	1102.4 ± 58.7	0.069 ± 0.047
		Y	1073.2 ± 76,3	0.071 ± 0.034
		M	971,9 ± 82,5	0.050 ± 0.042
	200	P	1189.4 ± 72.1	0.035 ± 0.014
		Y	1201.7 ± 86.6	0.039 ± 0.022
		M	1061.1 ± 49.4	0.032 ± 0.034
	250	P	1067.0 ± 61.1	0.031 ± 0.039
		Y	1101.6 ± 63.3	0.033 ± 0.031
		M	989.5 ± 42.2	0.027 ± 0.019
	300	P	1115.8 ± 54.8	0.034 ± 0.011
		Y	1001.1 ± 88.3	0.031 ± 0.027
		M	865.4 ± 55.5	0.029 ± 0.051
E4FO	1500	P	961.4 ± 79.7	0.078 ± 0.036
		Y	1014.1 ± 48.3	0.079 ± 0.025
		M	909.4 ± 75.3	0.064 ± 0.030
	2000	P	953.3 ± 30.7	0.070 ± 0.029
		Y	869.0 ± 72.9	0.065 ± 0.022
		M	963.0 ± 38.5	0.077 ± 0.034

P: Presidential; **Y:** Yedikule; **M:** Maylight352; **SN:** Seed Number; **SW:** Seed Weight

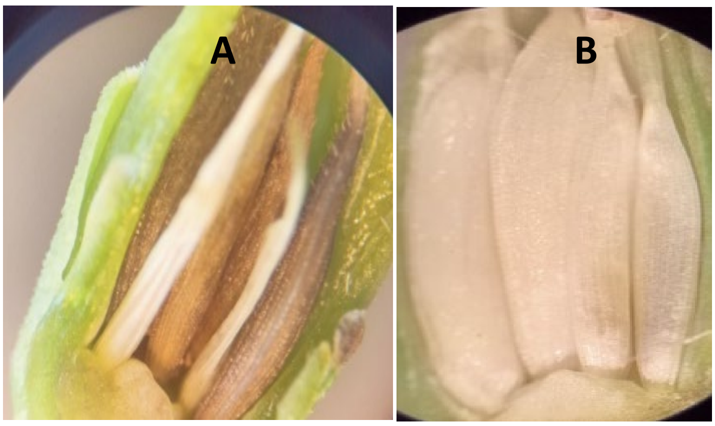

Figure 5. The buds with full seeds (A) and empty seeds (B).

3.3. Seed Germination

Due to excessive bud deformations, the seeds could not be obtained and therefore germination tests could not be evaluated at Ethephon doses higher than 1000 ppm (Table 3). 1000 ppm Ethephon had not any effect on sterility and the seeds produced similar germination with the control lots. Similar effects were also determined at 1500 and 2000 ppm E4FO. This revealed that between 1000-1500 ppm of Ethephon should be examined or the range of applications should be arranged and doses of E4FO above 2000 ppm should be evaluated in lettuce.

Seed germination decreased below 50% in 50 ppm, and it was 10% and 3% in 100 ppm and 150 ppm GA_3, respectively. The germinating seeds could not be detected and complete sterility was achieved in 200, 250 and 300 ppm applications. Our results are similar to the Khatib et al. (2016) findings and the 200 ppm GA_3 was the recommendable dose.

Table 3. Effects of CHAs on seed germination (%) of lettuce cultivars

Treatments	Dose (ppm)	Cultivar	G (%)
CONTROL	-	P	95.7 ± 1.2
		Y	97.4 ± 0.6
		M	98.0 ± 2.0
ETHEPHON	1000	P	96.0 ± 2.1
		Y	95.4 ± 1.2
		M	97.2 ± 2.7
	1500 - 4000	Could not evaluate due to floral deformation	
GA_3	50	P	48.3 ± 15.2
		Y	44.1 ± 11.7
		M	46.9 ± 9.6
	100	P	13.0 ± 4.2
		Y	15.5 ± 6.7
		M	12.0 ± 4.2
	150	P	2.7 ± 0.4
		Y	3.5 ± 1.1
		M	2.3 ± 0.9
	200	P	0.0
		Y	0.0
		M	0.0
	250	P	0.0
		Y	0.0
		M	0.0
	300	P	0.0
		Y	0.0
		M	0.0
E4FO	1500	P	95.7 ± 2.6
		Y	95.0 ± 3.0
		M	96.0 ± 2.0
	2000	P	95.4 ± 2.4
		Y	94.7 ± 7.7
		M	96.0 ± 1.0

P: Presidential; **Y:** Yedikule; **M:** Maylight352
G: Germination

4. Conclusion

It is imperative to produce male sterile lines, especially in species where emasculation is impractical due to their flower structure and morphology and it is imperative to utilize the male sterility mechanism to develop new F1 hybrid varieties. Our findings indicated that;

1) Gibberellic acid did not show any effect at low doses, and the most effective dose was 200 ppm. All cultivars produced similar results.

2) Flower and bud formation was occurred at 1000 ppm Ethephon, while 1500 ppm and upper doses caused deformations in the inflorescences. Thus, Ethephon dosses between 1000-1500 ppm should be examined.

3) The 1500 and 2000 ppm E4FO were also not effective on male sterility and produced similar results with the control. It would be beneficial to study higher E4FO doses in further lettuce breeding programs.

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Conflicts of Interest: The authors declare no conflict of interest.

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Research Article

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Analysis of Resource Use Efficiency and Profitability of Maize Seed Production in the Rolpa District of Nepal

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HIGHLIGHTS

- Female farmers are increasing due to male out-migration, and youth engagement in agriculture is low.
- Maize production is financially viable despite high labor costs.
- Farmyard manure and tillage are overused, while seed, fertilizer, and management inputs are underutilized.
- Maize farmers face challenges like insect infestations, disease spread, and inadequate irrigation resources.

Abstract

This study aimed to assess the profitability and resource use efficiency of maize seed production in the Rolpa district of Nepal. Primary data were collected from a sample of 67 maize growing farmers involved in maize seed production, selected randomly from the sampling frame with a confidence level of 95% and a margin of error of 5%, using Raosoft. Additionally, secondary information was obtained through a review of relevant literature. Descriptive statistics and the Cobb-Douglas production function were employed for data analysis. The results indicated that maize seed production in the study area was profitable, evidenced by a gross margin of 17,160.6 NRs/ha and a benefit-cost ratio of 1.12. The productivity level was estimated at 15.46 quintal/ha. Moreover, the return to scale of maize seed production was calculated to be 0.79, suggesting a decreasing return to scale. The analysis of allocative efficiency indices highlighted the need for optimizing resource allocation. Specifically, increasing costs on seed, chemical fertilizers, and management by 94.03%, 99.30%, and 60.25% respectively would lead to optimal resource allocation. Conversely, costs related to human labor, farmyard manure (FYM), and tillage should be reduced. This research contributes to a better understanding of the profitability and resource utilization in maize seed production in the Rolpa district of Nepal. The findings provide valuable insights for farmers and policymakers to make informed decisions regarding resource allocation and enhance the overall efficiency and sustainability of maize seed production in the region.

Keywords: Cost Estimation, Efficiency, Gross return, Geometric Mean, MVP, Regression Coefficient

1. Introduction

Agricultural production must be more profitable as it directly contributes to economic growth. The necessary framework to strengthen the agricultural value chain must be created to realize profitable

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production. *Zea mays*, also known as maize, is one of the world's most widely grown cereal crops and has a wide range of adaptability to different agro-climatic conditions. Non-waterlogged soil, like sandy loam or loamy soil, is the best soil for growing maize. 28 to 34 degrees Celsius is the ideal temperature range for maize cultivation. Higher yields depend on timely sowing (Biswas, et al., 2022). Among cereal crops, maize has emerged as one of the most significant modern staple food crops, and with the highest genetic yield potential (ALABUJA et al. 2022; Adesina and Omonona 2019). Regarding global importance, maize came in third place behind wheat and rice. However, maize is known as the world's top source of calories, providing 19.5% more than either wheat (16.5%) or rice (15.0%) (World Atlas 2017; Adesina and Omonona 2019). It serves as a crucial raw material for the industrial production of fuel, starch, medications, and food sweeteners. Levulinic acid, a chemical derived from maize, replace of hazardous petroleum-based ingredients in anti-freeze products. Maize-derived ethanol is used as a biomass fuel. In order to heat furnaces in homes, maize straw is used as a cheap energy source. A common primary ingredient in fish and poultry feed is maize. Additionally, maize is used for direct human consumption (Adesina and Omonona 2019; Biswas et al. 2022).

Maize (*Zea Mays*) is the world’s second most cultivated crop with 197 million ha of land cultivation (Erenstein et al. 2022). In Nepal, it is the second most important crop with a production area of 979,776 ha and production of 2,997,733 Mt indicating a productivity of 3.06 Mt/ha (MoALD, 2021). Maize is grown in various agro-regions of Nepal and contributes to over 26% of food requirements in the hills and mountains (Sapkota and Pokhrel 2013). Mid-hill region of Nepal which covers 43% of the land has a significant variation in maize production and productivity (Dhakal et. al. 2022). The yield shown by different research in hills is about 1.98 MT/ ha in Sindhuli (Dahal and Rijal 2019) and 2.30 tons/ ha in Rolpa (Agriculture Knowledge Center, Rolpa 2020). Maize food and feed demand grows by 5% and 11% respectively per annum (Sapkota and Pokhrel 2013). However, Nepalese production has not meet this increasing demand, thus a large amount of Maize is imported from India. The yield and profit of a crop rely on the inputs and their efficient use. Low-quality seeds, low soil fertility and lack of an appropriate crop management system (Karki et al. 2015), disease infestation, and labor shortage cause lower yield of maize in Nepal. Overusing or underusing of any input resource can thus lead to a waste of money and time, leading to an economic loss for the farmers (Băşa et al. 2016). Thus, generating an idea of resource use efficiency can directly impact the farmers. Rolpa, the mid-hill district of Nepal, in the Lumbini Province, mainly relies on agriculture (Pokhrel 2019). Among 31496 hectares of Rolpa’s cultivable land, Maize alone covers 12660 ha with an annual production of about 29150 metric tons (DADO 2016). Most of the Maize Production area of Rolpo is concentrated in the Uplands, where the plant is grown from April to October in rain-fed conditions (Pokhrel et al. 2018). After the establishment of the PMAMP Maize Zone in Rolpa, government sector is encouraging farmers towards Maize Seed Production (Ghimire et al. 2019). Even then, the economic status of Maize Seed Farmers in Rolpo has not improved significantly (Sapkota et al. 2018). Hence, evaluating the social features and the cost/ profit of Maize Seed Production in Rolpa is important.

2. Materials and Methods

2.1 Study Area

The study was carried out in the Rolpa district of Lumbini province with its headquarters at Libang, situated in the Western Development Region of Nepal, at 28.8° to 28.38° N latitude and 83.10° E to 83.90° longitude. It is at a height of 701 m to 3669 m from the sea level. The population of this district is 224506. In Ropla, Maize Seed Production has been a major focus of PMAMP (Prime Minister Agriculture Modernization Project). Rolpa municipality is the only Municipality of the district that is declared as a Maize zone area under PMAMP.

2.2 Sampling Procedure and Data Collection

Wards 4, 7, 9, and 10 were selected purposively as survey zones as major seed-producing cooperatives and Farmer's groups are located in these wards. There were about 80 farmers from 4 cooperatives and 1 farmer group involved in maize seed production under the study area. The sample size was determined from the Raosoft software (Raosoft 2014). The required sample size at a 95% confidence level and a 5% margin of error using Raosoft is 67. Hence, a random sampling of altogether 67 farmers was done.

The questionnaire was Pre-tested with 10 respondents (5.5 percent of the sample size) from different wards. A pre-tested questionnaire was used for the data collection between March to July 2023. Key Informant Interview was done with 4 cooperative members at the PMAMP zone office. Chairpersons of cooperatives and farmer groups; and progressive farmers were selected for Key Informant Interviews. Also, Focus group discussions (FGD) among cooperative members and farmers' group was done to verify the responses obtained. Furthermore, different data collected from the government and non-governmental organizations over a period of time was considered secondary data.

2.3 Methods and Techniques of Data Analysis

Statistical Package for Social Science (SPSS) and Microsoft Excel (MS Excel) were used for data entry and analysis.

2.4 Socio-Demographic and Economic Variables

Calculation of socio-demographic variables such as the education of the sample, population distribution, family size, income level, and land holding will be done with the use of descriptive statistics. Model as percentage, frequencies, means, and standard deviation shall be used.

2.5 Gross Margin

The gross margin was calculated by deducting the total variable cost from the gross return. Gross margin calculation was done to estimate of the difference between the gross return and variable costs.

$Gross\ Margin\ (NRs/ha) = Gross\ return\left(\frac{NRs}{ha}\right) - Total\ Variable\ Cost(\frac{NRs}{ha})$ Olukosi et al. (2006)

2.6 Resource use efficiency

Cobb-Douglas production function together with SPSS25 and Excel shall be used to analyze the Resource use efficiency. Cobb-Douglas production indicates the formula below.

$Y=aX1^{b1}\ X2^{b2}\ X3^{b3}\ X4^{b4}\ X5^{b5}\ e^{u}$ (Sapkota et al. 2018)

Where Y=income of maize production in ha (Nrs),

X1=cost of maize seed per ha

X2=cost of labor per ha,

X3=cost of FYM per ha,

X4=Cost of chemical fertilizer per ha,

X5=Management cost per ha.

e is error term and b1 to b5 is coefficient to be estimated.

The above mentioned equation is linearized in logarithmic function.

lnY= lna+ b1lnX1+ b2lnX2+ b3lnX3+b4lnX4+b5lnX5+u

Where, ln= natural logarithm, a= constant and u is random disturbance.

The efficiency ratio (r) was computed using the formula

$r = \frac{MVP}{MFC}$ (Sapkota et al. 2018)

where, MFC= Marginal factor cost

MVP= Marginal value product,

The marginal value product was computed by using formula:

MVPi = bi× Y/XI

Where, bi = Estimated regression coefficients

Y and Xi are the values from geometric mean.

Efficiency estimation r = 1 indicate the efficient use of resource r < 1 indicate overused of resource r > 1 indicate underuse of resource

Again, the MVP relative % change of indivisual resource was calculated by the use of following formula,

D= (1- MFC/MVP) ×100 Or, D= (1-1/r) ×100 (Sapkota, et al., 2018)

Where, D= Absolute value of percentage change in MVP of each resource

2.7 Economic Analysis

Cost-benefit analysis was done after the calculation of total cost and gross return. Cost of production included all variables cost items as the investment done for Seed, Labor, FYM, fertilizer, and Management practices such as sowing, weeding, harvesting and marketing. Similarly, the income was obtained from the sales of maize seed and maize grain.

Then, benefit-cost analysis was done with the following formula: (Dhakal et al. 2015; Subedi et al. 2019).

$$Benefit\ Cost\ Ratio = {}^{Gross\ Return}/_{Total\ Variable\ Cost}$$

$$Gross\ return = Total\ quantity\ of\ seed\ sold\ (kg) * Price\ per\ unit\ of\ maize\ seed (NRs) + Total\ quantity\ of\ grain\ produced\ (kg) * Price\ per\ unit\ of\ maize\ grain\ (NRs)$$

Total variable cost = seed cost + Bullock cost + Labor cost + seed cost of mixed crop

2.6 Problem ranking

With the use of qualitative data, the index values were calculated. The rank received by each problem given by the individual respondent, we get the final index value to show the severity of each of the farmer's problems.

Five major problems of the study site were ranked according to the severity that farmers have experienced. The problems included the incidence of disease and pests, lack of irrigation, Natural Hazards, Lack of technical services, and labor shortage.

3. Results

3.1 Socio-economic Characters

The majority of farmers from Rolpa municipality were from medium-size families with 4 to 7 family members. Most of those farmers were female i.e. 62.7% and few were male. The number of female farmers in agriculture sector of Rolpo has increased after male members of family started out-migrating to larger cities or foreign countries in search of better economic conditions (Tamang et al. 2014). 41.8% of the family head were in the age group 30 to 40 years. Engagement of young adults in agriculture was seen significantly less in Rolpa. As per a research, the lower number of young adults i.e. 20 to 30 years old is due to parental pressure for alternative job and problems in agriculture like crop loss, lack of resources and less access to technical and financial support (Pelzom and Katel 2017). Agriculture Extension programs and engagement of, the government as well as private sector, beholds great importance in the increase of Maize seed production and higher resource use efficiency in Rolpa. 67% of farmers are engaged in farmers group and cooperative. Facts indicates that these cooperatives help farmers with better accessibility of Farm Inputs and subsidy, thus improving the overall farming practices.The engagement of farmers' cooperative also increased their exposure to different agriculture training (Neupane et al. 2018). 46.3% of the farmers in the area received training about appropriate management practices. However, another 53.7% of farmers who do not have excess to such training program lack knowledge of modern agriculture practices and resource use efficiency. The role of cooperative in Rolpa is not limited to extension and training, but also play a vital role in accessing credit facility to the farmers have been remarkable. However, only 25.4% of farmers have accesss to credit facilities. This explains the lack of capital for large-scale farming and technology adoption in Rolpa municipality.

Table 1: Socio-economic characteristics of the respondents

S.N.	Socio-economic characters	Frequency	Percent
1	Family Size		
	Large	6	9.0
	Medium	36	53.7
	Small	25	37.3
2	Gender		
	Male	25	37.3
	Female	42	62.7
	Other	0	0.0
3	Age of household head		
	Young adults	6	9
	Adults in thirties	13	19.3
	Adults in forties	28	41.8
	Middle-aged adults	14	20.9
	Other adults	6	9.0
4	Training Received		
	Received	31	46.3
	Not received	36	53.7
5	Access to credit		
	Yes	17	25.4
	No	50	74.6
6	Seed Variety		
	Manakamana	27	40.3
	Deuti	40	59.7
7	Membership in farmers group		
	Member	67	100.0

Source: Field survey (2023)

3.2 Cost of Maize Production

The overall expense of maize production was determined to be NRs 134062.6. The largest portion of the total cost was attributed to labor expenses, accounting for 45.61% of the total cost, followed by FYM at 39.27% and tillage at 7.03%. The labor force is employed in various tasks related to maize production, including nursery bed preparation, sowing, weeding, applying weedicides, insecticides, and pesticides, as well as harvesting.

Table 2: Summary of resource use and production metrics in maize seed production

Metrics	Minimum	Maximum	Mean
Total Area (ha)	0.153	1.224	0.592
Cultivated Area (ha)	0.051	0.714	0.244
Seed Quantity (kg)	19.607	39.215	34.829
Seed Quantity Produced (100 kg)	9.803	19.607	15.460

Source: Field survey (2023)

Table 3: Average cost of Maize production

Variables	Minimum cost	Maximum cost	Mean	Percentage
Labor cost	549.01	117647.10	61154.73	45.62
FYM Cost	39215.69	58823.53	52648.52	39.27
Tillage cost	5392.15	11764.71	9426.05	7.04
Management cost	1426.02	21568.63	4186.73	3.12
Seed cost	1960.78	3921.56	3482.95	2.59
Fertilizer cost	0	7843.13	2575.35	1.92
Other costs	588.23	588.23	588.23	0.44
Total variable cost	49131.88	222156.89	134062.60	100.00

Source: Field survey (2023)

3.3 Returns from Maize Production

A total of NRs. 134062.60 was determined as the cost of producing maize. The main cost component with the highest percentage of the total cost was labor costs (45.62%), followed by farm yard manure (39.27%) and tillage operation (7.04%).

Table 4: Average returns from maize production

Return items	Mean (NRs/ha)	Percentage
Seed of maize	92995.30	61.25
Grains of maize		38.75
Total returns	151832.92	100

Source: Field survey (2023)

It was determined that the average total returns from maize production were NRs 151832.92. While grains make up 38.75% of the total, maize seeds account for 61.25%.

3.4 Financial Indicators

Table 5: Financial indicators of maize seed production in the study area

Indicators	Average value (NRs/ha)
a. Total fixed cost	609.74
b. Total variable cost	134062.58
c. Total cost (a+b)	134672.32
d. Gross returns	151832.92
e. Gross margin (d-c)	17160.60
f. Benefit-cost ratio (d/c)	1.127

Source: Field survey (2023)

The gross margin of maize production in the study area was calculated NRs. 17160.60. The benefit- cost ratio was estimated 1.127 which means if 1 rupee is invested it will give 1.127 rupees returns. The positive value of BC Ratio being greater than 1 indicates the financial viability. The calculations mentioned above revealed that maize production is profitable in the research area.

Table 6: Estimated value of coefficients and related statistics of Cobb-Douglas production function of maize seed production

Variables	Unstandardized Coefficients		Standardized Coefficients	t-value	Sig.
	B	Std. Error	Beta		
(Constant)	5.055	4.740		1.066	0.291
Seed cost	0.485	0.122	0.442	3.967	0.000
Labor cost	-0.010	0.026	-0.045	-0.401	0.690
FYM cost	0.182	0.444	0.248	0.410	0.684
Fertilizer cost	0.023	0.019	0.711	1.218	0.228
Tillage cost	0.031	0.059	0.068	0.528	0.599
Management cost	0.084	0.058	0.155	1.447	0.153

Model summary statistics	
Number of sample (N)	67
R	0.593
R square	0.352
Adjusted R square	0.287
Std. error of estimate	0.115
R square change	0.352
F change	5.423
Sig. f change	0.000
Durbin-Watson	2.021

Source: Field survey (2023)

The value of the coefficient of multiple determination R square (R^2) was estimated 0.35 which indicated that 35% of the variation in the total maize income was explained by the explanatory variables in the model. Six independent variables are included in the model, one variable have statistically significant i.e. seed cost (1% level of significance). With an increase in the seed cost, the income from maize production increases by 48%. Increase in the cost of seed was in accordance with the findings of Dhakal et al. (2015); Ghimire and Dhakal (2014); Sharma (2009); Gani and Omomona (2009); Ojo, Salami and Mohammed (2008).

3.5 Input Use Efficiency in Maize Production

The resource use efficiency (r) was calculated as the ratio of the marginal value products (MVP) to the corresponding marginal factor costs (MFC). The marginal value products (MVP) of the maize farmers were calculated using the estimated coefficients of the exogenous variables, and the marginal factor cost (MFC) was determined using the current inputs unit market price (Adesina & Omonona, 2019).

Table 7. Resource use efficiency analysis of maize seed production in the study area

Input	G.M (Geometric Mean)	Coefficient	MVP	MFC	r (MVP/MFC)	Efficiency (Decision rule)	%Adjustment req.
Seed cost	3457.95	0.485	16.77	1	16.77	Under-utilized	94.03
Labor cost	57471.6	-0.01	-0.020	1	-0.020	Over-utilized	4905.29
FYM cost	51803.4	0.182	0.420	1	0.420	Over-utilized	-137.98
Fertilizer cost	19	0.023	144.77	1	144.77	Under-utilized	99.30
Tillage cost	9048.48	0.031	0.409	1	0.409	Over-utilized	-144.05
Management cost	3992.67	0.084	2.516	1	2.516	Under-utilized	60.25

Source: Field survey (2023)

The ratio of MVP and MFC of Farm Yard Manure and tillage for maize production was positive and less than one, which indicated that in the study area farm yard manure and tillage for maize cultivation was over-utilized. Therefore, farmers should decrease the use of farm yard manure and tillage to attain an efficiency level.

The ratio of MVP and MFC of seed, fertilizer, and management was found to be 16.77,144.77 and 2.516 for maize cultivation, positive and more than one which indicated that in the study area, the use of seed, fertilizer, and management for maize production was under-utilized. Therefore, the farmers should increase the use of seed, fertilizer, and management to attain efficiency in maize cultivation.

The ratio of MVP and MFC of labor was found to be (-0.020) for maize cultivation was negative and less than one, which indicated that in the study area, the use of labor for maize production is over-utilized. Therefore, farmers should decrease the use of labor to attain efficiency considerably

3.6 Constraints Faced by Farmers

Table 8. Problems associated with maize seed production in the study area

Problems	Most serious	Serious	Moderate	Less serious	Least serious	Index value	Score
Disease/Insect incidence	40	16	5	4	2	0.86	I
Lack of Irrigation Facility	15	30	10	7	5	0.70	II
Natural Hazards	7	18	20	16	6	0.61	III
Lack of technical services	1	15	22	10	19	0.50	IV
Labor Shortage	4	20	11	18	14	0.33	V

Source: Field survey (2023)

The information provided highlights the difficulties faced by producers of maize seeds. To examine the issues facing farmers from each perspective, five key issues were identified. Infestations with insects and the spread of diseases are the main, serious issues with maize seed production. Lack of irrigation resources is a second major issue. Learning how to manage insect pest incidents is crucial for farmers.

4. Conclusions

The findings of this study demonstrate that maize seed production exhibits financial viability and represents a profitable enterprise. Moreover, the Rolpa district has been identified as a highly productive and promising area for maize seed production. However, the research reveals that maize seed production is used inefficiently. In light of the data obtained, it is predicted that in order to ensure optimal allocation of resources in the research area, reducing the costs of human labor, farmyard manure (FYM), and tillage, and increasing expenditures on seeds, chemical fertilizers, and management costs will provide a more effective production. However, the study reveals that the inputs employed in maize seed production are being inefficiently utilized. To achieve optimal allocation of resources, it is recommended to increase expenditure on seed, chemical fertilizers, and management costs, while reducing costs associated with human labor, farmyard manure (FYM), and tillage. By ensuring rational utilization of resources, maize seed production can evolve into a commercially more viable venture with enhanced profitability and improved food availability. Further research and implementation of efficient resource management strategies are warranted to fully realize the economic potential of maize seed production in the study area.

Annex

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.433	6	.072	5.423	.000[b]
	Residual	.798	60	.013		
	Total	1.231	66			

a. Dependent Variable: ln Total return

b. Predictors: (Constant), ln Management cost, ln labor cost, ln seed cost, ln Tillage cost, ln Fertilizer cost, ln FYM cost

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	11.734075546264648	12.119148254394531	11.921361756345663	.080980951008126	67
Residual	-.200426161289215	.354093074798584	.000000000000002	.109964822661660	67
Std. Predicted Value	-2.313	2.442	.000	1.000	67
Std. Residual	-1.738	3.070	.000	.953	67

a. Dependent Variable: ln Total return

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The Effect of Different Priming Applications on The Germination of Seeds of Some Hazelnut Cultivars

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HIGHLIGHTS

- Different priming applications on seeds of hazelnut cultivars.
- Different parameters on germination were examined and it was determined that the effects of primer applications showed different effects.

Abstract

Priming is a simple operation that partially hydrates seed in a controlled environment, followed by seed drying, allowing germination processes to commence without the development of radicles. In this study, the germination of shelled and kernel seeds of the Kara and Tombul cultivars was examined in relation to the effects of hormonal and hydro-priming treatments. According to the results, the control group produced the best shelled seed of the Kara cultivar in terms of germination rate, root length, root fresh and dry weight, plant fresh and dry weight, and number of leaves. The seeds of the Kara cultivar kernel, 60 minutes of Perlan + 2 days of soaking in water in the control group (56.0 pcs), and the kernel seeds of the Kara cultivar, respectively, and the seeds with shelled of the best Tombul cultivar (53.6 pcs), produced the best results when the root diameters were measured. The application of 30 minutes of Perlan + 2 days of soaking in water to the seeds kernel of the Kara cultivar produced the best results in terms of leaf area, and the control group produced the best results for the chlorophyll value in the seeds kernel of the Tombul and Kara cultivars.

Keywords: Coryllus avellana L.; germination priming; stratification

1. Introduction

One of the most significant species in the genus Corylus of the Betulaceae family is the European hazelnut (Coryllus avellana L.) (Serdar and Akyuz 2017). The demand for hazelnuts in the food business is rising, and there is a constant rise in production due to its high nutritional value worldwide. Turkey is the leader in the world with 665.000 tons of hazelnut production in an area of 735 thousand hectares. (Anonymous 2023).

Hazelnuts have been used for nearly 5000 years and are employed in numerous products, from their fruit to their wood (Silvestri et al. 2021). Although it thrives in cool, mid-altitude areas, the hazelnut tree can adapt to different environmental conditions. It is a type of fruit that grows in the form of a hazelnut bush, can be reproduced by bottom shoot, dip and grafting, and it has a monoic flower state. Fruit trees are typically reproduced vegetatively because of their heterozygous structure. It has been observed that propagation by tissue culture or by cutting of hazelnut plants have successful outcomes, however some other researches are

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still required to improve the production of hazelnut saplings (İslam et al. 2019; Kaplan et al. 2020; Rahemi et al. 2016). Hazelnut bush cultivation system is widespread, particularly in Turkey and the Mediterranean region (Silvestri et al. 2021) while single-stem cultivation system has recently been discovered to produce greater yield and fruit qualities due to less labor. On the other hand bush cultivation system has long been preferred since it renews itself with orchard bottom shoots and prevents erosion (İslam et al. 2019; Karata et al. 2017).

Studies on rootstock breeding began in the United States in the 1940s and are still going strong today because Corylus colurna L. has little to no tendency to shoots from the bottom. Grafting allows for the quick development of new species without bottom shoots (Wilkinson 2005) and also makes it possible to take advantage of rootstocks' outstanding qualities (Foidl and Paull 2008). Seedling and clone rootstocks are the two categories of rootstocks. Compared to clone rootstocks, seedling rootstocks produce disease-free individuals with a solid root system (Rahemi et al. 2016).

Because of their dormancy and pericarp structure, seeds with hard-shelled are challenging to germinate (Ulu 2022). Due to this circumstance, both the workforce and the sapling production period are extended. As with other fruit seeds, some pretreatments are made to hazelnut seeds to speed up germination. By folding, dormancy brought on by the embryo is overcome (Alkan et al. 2015). By eliminating the seed coat or surrounding tissues, physiological variables in the breaking of dormancy can be eliminated (Abbasi 2020). Plant growth regulators like auxin, cytokinin, gibberellin, abscisic acid, salicylic acid, 6-Benzyladenine, and Perlan, which encourage germination using the hormonal priming technique, are another way to overcome dormancy (Adkins and Bellairs 2000; Baskin et al. 2000; Keshtkar et al. 2008). Another pre-germination treatment is called "hydro-priming," which introduces very little water into the seeds during the initial stage of water uptake in an environment with 100% relative humidity (Karakurt et al. 2010). Additionally, it is well recognized that soaking techniques have a substantial impact on germination by allowing the inhibitors that cause dormancy to dissipate or move away from the seed by weakening the impermeable testa (Akın 2004; Edwards 1968; Güncan 1975).

To better understand how Perlan (18.5% GA4+7 + 18.8% 6-Benzyladenine) and hydro-priming applications, which have an increasing impact on germination, affect the germination of the widely produced Tombul and Kara hazelnut varieties in our nation.

2. Materials and Methods

The study was carried out in the climate cabinet of the Central laboratory of Bilecik Şeyh Edebali University during 2022 and 2023. In the study, 'Tombul' and 'Kara' hazelnut varieties, which have high economic value in hazelnut production,were used as material, The seeds of the two hazelnut cultivars were obtained from the producer's garden in Ordu province in 2022. The world's highest grade hazelnut cultivar, Tombul, is a very productive type with round-shaped fruits and excellent oil, protein, and whitening rates. The Kara cultivar is characterized by thick-shelled, dark-colored, big, low-fat hazelnuts. Perlan commercial preparation is a plant growth regulator produced by Fine Agrochemicals Limited, containing (18.5% GA4+7 + 18.8% 6-Benzyladenine.

Before the application, the seeds were soaked in 3% sodium hypochlorite solution for surface sterilization for 10 minutes, washed several times with distilled water and mixed into perlite placed and folded at +4 ºC for 3 months. Some pre-germination treatments were applied to the shelled and kernel seeds of both cultivars, after removing from stratification conditions (Table 1).

Table 1. Types and applications used in the experiment

Varieties	Tombul		Kara	
Seed shape	Shelled	Kernel	Shelled	Kernel
1. Application	Control		Control	
2. Application	30 min Perlan + 2 days soaking		30 min Perlan + 2 days soaking	
3. Application	60 min Perlan + 2 days soaking		60 min Perlan + 2 days soaking	

The collected data were subjected to analysis of variance at the 5% significant level by using the SPSS 23 package program.

3. Results

Tables 2 and 3 illustrate the effects of soaking in water for specific periods (30 and 60 minutes) of seeds treated with commercial preparation Perlan on germination.

The results showed that the pre-germination treatments were statistically significant (P<0.05) when seed germination rates were considered. In the control group, germination rate of shelled seeds of Kara cultivar was 80%, while germination rate was 20% in shelled seeds of Tombul and Kara cultivars treated with 60 minutes of Perlan and 2 days of soaking in water (Table 2).

Table 2. The effect of different applications on the parameters examined in the research

Application	Germination percentage (%)	Root length (mm)	Root diameter (mm)	Hypocotyl length (mm)	Hypocotyl diameter (mm)	Number of lateral root (number)
TOMBUL- Shelled						
Control	60,00ab	70,5ab	0,78bc	81,2abc	2,33d	56,0a
30 min PERLAN +2 days soaking	46,6 ab	57,3b	0,95bc	103,5abc	1,38d	29,6abc
60 min PERLAN +2 days soaking	20,0c	64,0b	1,40a	60,1bcd	2,10a	46,0ab
TOMBUL – Kernel						
Control	53,3abc	70,0ab	0,76bc	77,0bc	1,77cd	31,6abc
30 min PERLAN +2 days soaking	33,3bc	54,1b	0,68bc	90,4abc	1,60cd	44,6ab
60 min PERLAN +2 days soaking	40,0bc	55,0b	0,60c	61,4bcd	1,80cd	42,6abc
KARA- Shelled						
Control	80a	113,9a	0,59c	124,4a	1,75cd	36,0abc
30 min PERLAN +2 days soaking	26,6bc	55,1b	0,16d	0,0e	1,10e	31,3abc
60 min PERLAN +2 days soaking	20,0c	65,8b	0,83bc	47,4cde	1,30d	26,0abc
KARA- Kernel						
Control	53,3bc	77,2ab	0,77bc	109,9ab	1,39d	17,6c
30 min PERLAN +2 days soaking	40,0bc	52,3b	1,00b	89,0abc	3,10b	42,3abc
60 min PERLAN +2 days soaking	33,3bc	84,0ab	1,46a	24,3de	1,36d	53,6a

The difference of root length and root diameter was found to be statistically significant in our study. The best root length of the Kara cultivar shelled seeds in the control group was 113.99 mm, while the lowest was obtained after 30 minutes of Perlan + 2 days of soaking in water of the Kara cultivar seeds (52.3 mm) (Table 2). Rostamikia et al. (2018) reported that obtained from the kernel after four months of stratification period. The difference of root diameters by the aplications was also statistically significant in our study at P<0.05 level. . The highest root diameter values were obtained from kernel seeds (1.46 mm) of the Kara cultivar treated with 60 min Perlan + 2 days soaking. It is well recognized that hydro-priming and hormonal priming have beneficial effects on seeds, and our findings support this (Ceritoğlu et al. 2021).

The number of lateral roots was found to be statistically insignificant. The best number of lateral roots was determined to be 56.0 pcs in the control group shelled hazelnut seeds of the Tombul cultivar, 53.6 pcs in the 60 minutes Perlan+2 days soaking application applied to the kernel seeds in the Kara cultivar, and the least lateral root number was 17.6 pcs in the control group kernel seeds of the Kara cultivar. It turned out to be statistically significant (P<0.05) when the root fresh weights were analyzed (Table 3). The control group of shelled seeds of Kara cultivar had the highest fresh weight (2.95 g), followed by kernel seeds of Tombul cultivar (1.73 g) treated with 30 min Perlan + 2 days soaking. In terms of root dry weights, the control treatment in the shelled seeds of the Kara cultivar yielded 0.33 g, while the 30 minutes Perlan + 2 days soaking application in the kernel seeds of the Tombul cultivar yielded 0.32 g (Table 3). Table 3 shows the fresh and dry weights of the plants. The fresh weights of plants were found to be statistically significant (P<0.05). The shelled seeds of the control group Kara cultivar yielded the best plant fresh weight (1.42 g). The number of leaves data obtained was judged to be statistically significant ($P<0.05$). The greatest number of leaves were obtained in the control application of seeds with shelled from the land cultivar and the application of 60 minutes Perlan + 2 days of soaking in kernel seeds from the Tombul cultivar. The optimal leaf numbers were found to be 6.08 pcs and 6.00 pcs (Table 3).

Table 3 shows the results of utilizing Adobe Photoshop CS6 Extend software (İpek et al. 2014) to compute the leaf area in cm2 based on the pixel value of the images generated by scanning the leaves with a gauge (ruler) using a scanner. It was obtained by soaking treated seeds of the Kara cultivar with the maximum leaf area, minus the shelled, in water for 30 minutes Perlan + 2 days. Table 3 provides the chlorophyll values (SPAD) on the germination of seeds from several pre-treated hazelnut cultivars. The control application Tombul and Kara cultivars kernel, yielded the highest concentration of chlorophyll. They discovered that there was no change in terms of chlorophyll indices in a study on the germination of Guava (Sidium guajava L.) seeds, which is similar to like our own conclusion (Nafiye et al. 2019).

Table 3. The effect of different applications on the parameters examined in the research

Application	Root fresh weight (g)	Root dry weight (g)	Plant fresh weight (g)	Plant dry weight (g)	Leaf number (number)	Leaf field (cm^2)	Amount of chlorophyll (SPAD)
TOMBUL- Shelled							
Control	1,20bc	0,12abc	0,82ab	0,32bc	5,00ab	28,2a	24,0b
30 min PERLAN +2 days soaking	1,73b	0,05bc	0,27b	0,14cde	2,66b	23,4ab	23,6b
60 min PERLAN +2 days soaking	0,18d	0,02c	0,27b	0,065de	1,20c	17,4ab	26,4abb
TOMBUL – Kernel							
Control	1,27bc	0,12abc	0,46b	0,29bcde	5,00ab	26,3ab	27,8a
30 min PERLAN +2 days soaking	0,19d	0,26abc	0,27b	0,31bcd	4,33ab	26,6ab	25,6ab
60 min PERLAN +2 days soaking	0,19d	0,32ab	0,46b	0,44ab	6,00a	19,2ab	25,0ab
KARA- Shelled							
Control	2,95a	0,33a	1,42a	0,62a	6,08a	24,4ab	26,9a
30 min PERLAN +2 days soaking	0,43cd	0,11abc	0,86ab	0,14cde	1,12c	29,5a	23,6b
60 min PERLAN +2 days soaking	0,45cd	0,080abc	0,21b	0,05e	2,66b	8,43b	25,9ab
KARA- Kernel							
Control	1,12bcd	0,16abc	0,45b	0,11cde	4,60ab	32,1a	27,8a
30 min PERLAN +2 days soaking	0,48cd	0,21abc	0,98ab	0,15cde	5,33ab	33,02a	25,3ab
60 min PERLAN +2 days soaking	1,46	0,16abc	0,20b	0,17cde	4,33ab	18,2ab	25,3ab

4. Discussion

Beyhan et al. (1999) the germination rate of seeds treated to stratification between 12.3% and 39.5% was determined in their study on seed germination and seedling growth of varied stratification and GA3 dose treatments in hazelnut cultivars. The maximum germination rate was discovered to be 64.17% in the Çakıldak cultivar and 37.08% in the Kalınkara cultivar in the study examining the effects of potassium humate on the germination of hazelnut kernels of various hazelnut varieties (Bostan et al. 2000). As the GA3 dose increased, the germination percentage increased, but after a certain level, the best germination was obtained at 75 ppm GA3 concentration, according to a study looking at the effects of various doses of GA3, and water applications on germination in Turkish hazelnut seeds (Aygun et al. 2008).

Yıldırım et al. (2009) in a study on the germination of Chamaecytisus drepanolobus (Boiss.) Rothm species seeds, it was shown that seeds kept in clean water for 48 hours had no germination whereas seeds kept in 10 ppm GA3 had the best germination. In a study where Ceratonia siliqua seeds were subjected to various chemical etching treatments before germination, it was found that 30 minutes of H2SO4 + soaking in water for 2 days followed by 30 minutes in H2SO4 solution gave the best results. (Gübbük et al. 2012). Carob seeds were immersed in water after being exposed to sulfuric acid or mechanical abrasion, and germination rates ranged from 10% to 80% (Kleynhans et al. 2016). Rostamikia et al. (2018). They evaluated the effects of folding in hazelnut genotypes on germination in seeds with and without a shelled in their study, and they discovered that kernel seeds had the highest germination rate of 51.66%. The maximum germination rate was found from the 60-day folding treatment without chemical application in the study where varied folding and chemical applications were done on the seeds of terebinth before germination (Hashim et al., 2018). Researchers reported that gibberellic acid had no influence on germination rate in elderberry seeds that were not treated with sulfuric acid, and that the germination rate remained constant at 67.50% with increasing doses of gibberellic acid (Odabas et al. 2020). When the germination rate achieved in our study is compared to other studies, it is shown that many studies obtain comparable or superior findings. The researchers discovered that soaking the elderberry plant seeds in sulfuric acid (15 minutes) and 500 ppm gibberellic acid solution (24 hours) boosted root length by 22.6% compared to control seeds. (Odabas et al. 2020). Okatan (2017) discovered that when abrasion and soaking in GA3 solution were applied to elderberry seeds, the rate of hypocotyl rose by 143%. The best hypocotyl diameter of 3.10 cm was obtained from kernel seeds of Kara cultivar after 30 minutes of Perlan + 2 days of soaking in water. They reported 6.2 mm in the control application as a result of the development status of the seedlings planted in the seedling plots by applying GA3 (0-50-100-200 ppm) at different rates without folding in the hazelnut seeds (Beyhan et al. 1999).

In a study on Malta plum seeds, the lowest root wet weight was 0.04 g after pre-treatment with GA3, whereas the greatest root weight was 0.09 g after 300 ppm GA3 application (Okatan 2017). In a study conducted on hazelnut seeds, they reported that the highest rate of root dry weight was obtained from the control group (56%) (Beyhan et al., 1999). For 24 hours, different concentrations of GA3 (control, 100, 200, and 300 ppm) were treated to Malta plum, and the root dry weight was 0.02 g in the control group and 0.05 g in the highest 300 ppm treatment (Okatan 2017).

They discovered that applying nitric and gibberellic acid to black elderberry seeds at different intervals had a detrimental influence on plumule fresh weight, resulting in a considerable drop (Odabas et al. 2020). Plant dry weights were statistically significant ($P<0.05$). The control group yielded the best plant dry weight (0.62 g) in the shelled seeds of the Kara cultivar. This was followed by the plant dry weight (0.44 g) of 60 minutes Perlan + 2 days of soaking.

5. Conclusions

Because of dormancy and the hard shell, germination takes a lengthy time in hard-shelled fruit species. Both breeding experiments and rootstock production require rapid and smooth germination. In this study, the responses of pre-germinated hazelnut seeds of Kara and Tombul cultivars to Perlan and soaking applications were determined. The control application of shelled seeds of the Kara cultivar produced the best results in many metrics tested, including germination percentage. Three months of cold folding was found to be advantageous for the cultivation of shelled seeds by direct sowing in nurseries and forest regions. Perlan application and soaking applications, in addition to folding, have a favorable influence on several parameters. As a result, it is suggested that the study should be expanded in future studies by increasing the number of species and applications.

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Corn Oil Oleogel Structured with Chicken Skin as A Potential Fat Replacer in Meat Batters

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HIGHLIGHTS

- Beef fat was substituted by corn oil oleogel based on chicken skin in meat batters.
- Microstructure, color, and texture were considerably affected by oleogel utilization.
- Meat batters formulated with oleogel had healthier lipid composition and improved nutritional ratios.
- Water holding capacity and cooking yield improved by the addition of oleogel.
- Reformulated samples had better oxidative stability.

Abstract

This study investigated the effects of replacing beef fat with corn oil chicken skin oleogel on the quality parameters of model meat batters. Four different batches were prepared with varying amounts of oleogel (50% (O50), 75% (O75), 100% (O100)) as a fat substitute, while a control group was prepared with 100% (C) beef fat. Chemical composition, technological properties, color, texture, fatty acid composition, and oxidative changes were evaluated. The results showed that oleogel addition increased moisture and decreased protein content. Water holding capacity and cooking yield improved by the addition of oleogel. Replacing beef fat with oleogel increased lightness and yellowness, and reduced redness compared to control samples. The hardness, gumminess, springiness, and chewiness of the control were significantly higher than the samples formulated with oleogel. The incorporation of oleogel resulted in a reduction in saturated fatty acids (SFA) and an increase in polyunsaturated fatty acids (PUFA). As the level of oleogel increased, the amount of linolenic acid also increased significantly. Replacing beef fat with oleogel resulted in significant reductions in the atherogenicity (IA) and thrombogenicity (IT) indexes. At the end of the storage, the highest TBARS value was recorded in the control sample formulated with 100% beef fat.

Keywords: Fatty acids, Edible oils, Chicken skin, Meat products, Texture, Oxidation

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1. Introduction

Therefore, it is essential to select appropriate fat sources and levels to optimize the sensory and technological quality of meat products while keeping in mind the potential health impacts of excessive fat consumption (Serdaroğlu et al. 2016). Nevertheless, animal fat replacement by vegetable oils resulted in unfavorable technological and sensory changes and decreased oxidative stability. Therefore, incorporating vegetable oils into meat products requires the use of structured emulsion systems like emulsion gels, hydrogels, or oleogels (Serdaroğlu et al. 2017). In particular, the utilization of oleogels as a substitute for fat in meat product formulations has become increasingly popular in recent times. Oleogel systems, formed by gelling vegetable oil with an oleogelator, provide a viable alternative to animal fats high in saturated fatty acids (Martins et al. 2020). These heat-reversible oleogels exhibit solid-like properties and can mimic the behavior of fats, even with a high proportion of unsaturated fatty acids. By reducing overall fat content while maintaining texture and mouthfeel, oleogels offer an appealing option in meat product formulations (Silva et al. 2021). They help control oil relocation, prevent oxidative changes, and improve technological properties while preserving a healthier fatty acid profile (Serdaroğlu et al. 2017). Numerous studies have successfully used oleogels as fat replacers in various meat products, including sausages, patties, and pâtés (Panagiotopoulou et al. 2016; Barbut and Marangoni 2016; Wolfer et al. 2018; Silva et al. 2019). These studies have shown that incorporating oleogels enhances sensory properties, such as texture and juiciness, without compromising nutritional value. Furthermore, oleogels can be tailored to possess functional properties, enabling control over flavor release and the delivery of active compounds, which can be advantageous in meat products. Researchers have utilized various vegetable oils, such as olive, corn, flaxseed, and sunflower oils, to create oleogels as alternatives to animal fat in meat products (Silva et al. 2019; Martins et al. 2020).

Corn oil, in particular, is a popular choice due to its wide availability, cost-effectiveness, and favorable fatty acid composition. It contains high levels of linoleic acid (C18:2n-6) and consists of 54% polyunsaturated fatty acids, 33% monounsaturated fatty acids, and 13% saturated fatty acids, making it a nutritionally valuable option (Wagner et al. 2001). The structure of corn oil oleogels can be achieved using different structurants, including waxes, polymers, proteins, and by-products like pork skin. Researchers have explored various oleogelators to form the structure of corn oil, such as ethyl cellulose, sorbitan monostearate, soy protein concentrate, γ-oryzanol, β-sitosterol, beeswax, and rice wax (Panagiotopoulou et al. 2016; Wolfer et al. 2018; Silva et al. 2019). However, there is currently no specific study on the use of corn oil oleogel as a fat replacer, nor has the potential utilization of chicken skin as a structurant in oleogels been extensively investigated, despite its collagen and fat content, which could contribute to its suitability (Xia et al. 2022). To explore the development of healthier meat products with reduced fat content, one approach is to use oleogels. Oleogels are semi-solid systems that consist of liquid oil structured to provide a solid-like texture. In this study, corn oil oleogels structured with chicken skin were investigated as a potential fat replacer in meat batters. The aim was to assess how different concentrations of chicken skin affect the physicochemical and rheological properties of the oleogels, as well as their performance in meat batters. The research aims to provide valuable insights into the creation of healthier meat products with lower fat content.

2. Materials and Methods

Lean beef (M. semitendinosus; 72.82% moisture, 20.82% protein; 3.12 % fat, 1.6% ash), beef fat, and chicken skin were obtained from a supermarket (İzmir, Turkey). Corn oil (Sırma, Turkey), palmitic acid, 9.6%; palmitoleic acid, 0.9%; stearic acid, 4.6%; oleic acid, 31.5%; linoleic acid, 48.4%; linolenic acid, 4.1%; and arachidic acid, 0.8%).

2.1. Preparation of oleogel

Oleogel was prepared with slight modifications based on the procedures described by Silva et al. (2019). Chicken skin is chopped into small pieces (2 cm x 2 cm) using a knife and then placed in a glass jar. The jar is then subjected to heat treatment by immersing it in a water bath at 90°C for 15 minutes. after the chicken skin was heat-treated and cooled to room temperature, it was mixed with water and corn oil in a ratio of 1:5:5

(chicken skin: water: corn oil). The mixture was then homogenized with a blender (Sinbo, Turkey) for 5 minutes, which helps to break up the chicken skin and create a smooth, uniform mixture. The resulting mixture was then stored at +4°C overnight to allow for gelation, which is the process by which the mixture forms a solid gel-like structure. The final product is an oleogel, which is a semi-solid gel-like substance made up of oil and a gelling agent (in this case, collagen from the chicken skin).

2.2. *Experimental design and preparation of meat batters*

Meat batters were prepared according to the method of Serdaroğlu et al. (2021) the preparation steps are given in Fig 1. Four batches (as shown in Table 1) were prepared for the experiment. Control samples (C) were formulated using 100% beef fat. In the reformulated treatments, the beef fat was replaced with oleogel at different levels; 50% (O50), 75% (O75), and 100% (O100). To prepare the meat batters (as shown in Fig. 1), the lean beef and beef fat were ground separately using a 3 mm plate meat grinder (Arnica, Turkey). The ground meat was then homogenized for 1 minute at 500 rpm using a Thermomix (Vorwerk, Wuppertal, Germany). NaCl and STPP (sodium tripolyphosphate) were added, followed by iced water, and the mixture was homogenized for an additional 3 minutes at 500 rpm. After the initial homogenization step, beef fat and/or oleogel and the remaining iced water were added to the mixture. The emulsification process was then continued at 1100 rpm for 3 minutes, followed by 2500 rpm for 2 minutes. Throughout the process, the temperature was kept below 12°C to prevent any undesirable changes to the meat batter. To ensure uniformity in the final product, the meat batters were filled into 50 ml centrifuge tubes and centrifuged at 2500 rpm for 1 minute (using a Nüve NF 400 centrifuge, Turkey) to remove any air bubbles. The tubes were then transferred to a water bath (using a Nüve water bath, Turkey) and heat-treated at 70°C for 30 minutes. After the heat treatment, the samples were immediately cooled in a cold water bath (+1°C) and stored at +4°C for 7 days. During this storage period, lipid oxidation analyses were performed in triplicate at 0, 3, 5, and 7 days to assess the oxidative stability of the samples. All other analyses were conducted within 72 hours of production to ensure the freshness.

2.3. *Analysis of oleogel*

pH meter (WTW pH 330i/SET) equipped with a probe electrode was used to measure the pH of oleogel samples, four readings were taken at four different points. To determine the color parameters, a CR-200 portable colorimeter (Konica Minolta, Japan) equipped with a ten-degree observer angle and D65 illuminant was utilized. Prior to conducting measurements, the colorimeter underwent calibration using traditional white and black plates. CIE L*, CIE a*, and CIE b* values were measured. Oil binding capacity (OBC) was assessed using a modified method described by Fayaz et al. (2017). A plastic centrifuge tube containing 5 g of oleogel sample was weighted and centrifuged for 15 min at 10,000 rpm (Universal 320 centrifuge Hettich, Germany), and the supernatant (oil) was weighed. The following equation was used to calculate the OBC:

$$\%Oil\ released = \frac{Mass\ of\ expressed\ oil\ (g)}{Total\ mass\ of\ sample\ (g)} x\ 100$$

$$OBC = 100 - \%Oil\ released$$

2.4. *Proximate composition and pH values of the emulsions*

Moisture (AOAC-925.10) and ash (AOAC-923.03) contents were determined according to the Association of Official Analytical Chemists AOAC (2003) methods, chloroform/methanol extraction method was used for fat determination (Flynn and Bramblett 1975). Dumas combustion method was used to analyze protein content (LECO, FP-528, USA).

2.5. *Water holding capacity and emulsion stability*

The water holding capacity (WHC) was measured using the method described by Hughes et al. (1997). First, 10 g of batter was weighed (W1), transferred to a glass tube, and heated in a 90°C water bath for 10 minutes. After cooling to room temperature, the sample was wrapped with cotton cheesecloth, centrifuged at 1400 rpm for 15 minutes, and weighed again (W2). The WHC was calculated using the following equation:

$$\%WHC = 1 - T/M \times 100 = 1 - (W1 - W2)/M \times 100$$

$T: water\ loss\ after\ heating\ and\ centrifugation$

$M: sample's\ total\ moisture\ content$

The emulsion stability, total expressible fat, and total expressible moisture of meat batters were determined by the following method: 25 g of raw meat batter was placed in a centrifuge tube and centrifuged for 15 min at 6000 rpm. The pellet obtained was heated in a water bath at 70°C for 30 min and then centrifuged again at 6000 rpm for 20 minutes. Afterward, the pellet was weighed, and the supernatant was poured into pre-weighed crucibles and dried overnight at 100°C. The total expressible fluid (TEF) volume and total expressible fat (EFAT) were calculated using the equations outlined by Hughes et al. (1997).

$$TEF = (WCT + WS) - (WT + WP)$$

$WCT: Weight\ of\ centrifuge\ tube$

$WS: Weight\ of\ sample$

$WP: Weight\ of\ pellet$

$$TEF(\%) = (\frac{TEF}{WS}) \times 100$$

$$EFAT(\%) = [(WC + WDS) - (WCT + WS)]/TEF \times 100$$

$WC: Weight\ of\ crucible$

$WDS: weight\ of\ dried\ supernatant$

$WCT: Weight\ of\ centrifuge\ tube$

$WS: Weight\ of\ sample$

2.6. Cooking yield

Weight of meat batter before and after cooking was recorded and the cooking loss was expressed as a percentage difference between the raw and cooked weights.

2.7. Fatty acid composition

The procedure for lipid extraction followed Flynn and Bramblett's (1975) method, and the resulting lipid phase was subjected to methylation using the procedure described by Anon (1987). To analyze the fatty acid methyl esters (FAME), gas chromatography (GC 2010 Plus, Shimadzu Corp., Kyoto, Japan) was employed, with a silica capillary column (SUPELCO SP TM-2560; 100 m × 0.25 mm id., 0.20 μm/m film thickness). After setting the helium injector and detector (FID) at 140°C, they were kept constant at that temperature. The oven temperature was then increased from 140°C to 250°C at a rate of 4°C/min and held at 240°C for 10 minutes. To calculate the atherogenicity (IA) and thrombogenicity (IT) indexes, the equations proposed by Ulbricht and Southgate (1991) were used.

$$IA = [C12{:}0 + (4 \times C14{:}0) + C16{:}0]/\Sigma UFA$$

$$IT = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n - 6\ PUFA) + (3 \times \Sigma n - 3\ PUFA) + (n - 3\ /\ n - 6)]$$

2.8. Texture evaluation

To evaluate the texture (TPA), a texture analyzer (TA-XT2, Stable Micro Systems, UK) equipped with a 30 kg load cell was used. The following texture parameters were determined from the force and time curves: hardness (N), springiness, cohesiveness, gumminess (N), and chewiness (N.mm). Using an aluminum cylindrical probe with a diameter of 36 mm and a load cell set to 5 kg, cooked meat batters measuring 1 cm x 1 cm x 1 cm were compressed to 50% of their original height. The post-test speed, crosshead speed, and test speed were set to 2 mm/s, 1 mm/s, and 1 mm/s, respectively.

2.9. Microstructure

To assess the microstructure of the samples, scanning electron microscopy (Thermo Scientific Apreo 2, Waltham, MA) was utilized. The meat batters were dried to obtain a powder, which was then filled into the

SEM unit, coated with gold in a vacuum evaporator, and examined under high vacuum. Micrographs were created as a result of the sample's interaction with the electron beam.

2.10. Color parameters

The color of the samples was evaluated using a portable colorimeter (CR-200, Konica Minolta, Japan) equipped with a ten-degree observer angle and D65 illuminant. Color parameters of CIE L*, a* and b* values were measured at four different points of each sample.

2.11. Lipid oxidation

Primary oxidation products were determined by peroxide value (PV) according to AOAC (1990) procedure; results were expressed per mEqO2 (milliequivalent peroxide oxygen)/kg sample. TBARS analysis was performed according to Witte et al. (1970) to detect lipid oxidation second products. Results were expressed as mg malonaldehyde/kg sample.

2.12. Statistical analysis

The entire procedure for the meat batter production was replicated three times (three individual batches) on different days and measurements of related traits were conducted in triplicate for each batch. Within one replication, totally four different meat batter formulations (C, O50, O75 and O100) were prepared. Mean values for measured parameters were calculated by using the SPSS software for windows (SPSS 21.0 for Windows; SPSS Inc. Chicago, IL, USA). Production batches were expressed as a random variable, and each treatment was recognized as a fixed variable. To evaluate the effects of fat reduction and/or oleogel, one-way ANOVA was applied. Means were further compared using the Duncan test. Duncan's multiple range test was employed to compare group values with a significance level of $p<0.05$.

3. Results and Discussions

3.1. Characterization of oleogel

The pH value of oleogel was recorded as 6.16. The obtained result for pH in the present study was higher than the pH of oleogel formulated with pork skin (pH 5.80) (Silva et al. 2019). Lightness, redness, and yellowness values were measured as 69.79, 0.70 and 18.85, respectively. The color of oleogel depends on the color of oil and oleogelator used in the formulation. The pale-yellow corn oil and white chicken skin utilized in the oleogel formulation could be the reason for the high L* value in the present study. The amount of oil entrapment in the 3D network by the gelator is indicated by the OBC, a crucial characteristic of oleogels (Pandolsook and Kupongsak 2017). Although the OBC value for the oleogel was not high (73.96%), this value is in the appropriate range with the literature data (Thakur et al. 2022).

3.2. Chemical composition and pH

Table 2 provides a summary of the chemical composition of cooked meat batters. The substitution level significantly affected the moisture, protein, fat, and pH content ($p<0.05$). Substituting 75% and 100% of beef fat with oleogel increased the moisture content ($p<0.05$), which is consistent with findings from a study on hamburgers by Moghtadaei et al. (2018) using sesame oil-beeswax oleogel. The protein content in the samples ranged from 20.41% to 23.25%. The incorporation of oleogel led to a significant reduction in protein content ($p<0.05$), with the C treatment exhibiting the highest protein content and the O50 treatment having the lowest ($p<0.05$). Similar observations were made in pâté samples by Martins et al. (2020). Regarding fat content, the O100 samples had the highest percentage (13.97%), primarily due to the fat content of chicken skin in the oleogel formulation. However, there were no significant differences observed among the other treatments. The ash content did not show significant differences among the treatments ($p>0.05$), which is consistent with studies using oleogel as a fat replacer (Gómez-Estaca et al. 2019). The replacement of beef fat with oleogel led to an increase in pH values, regardless of the level of incorporation ($p<0.05$). The high pH of the oleogel may account for this increase. Similar results were reported by Gómez-Estaca et al. (2019) in pâté samples using ethyl cellulose and beeswax as fat substitutes.

3.3. Techno-functional quality

Table 3 presents the techno-functional properties of meat batters based on the quantity of oleogel incorporated. Water holding capacity (WHC), which refers to the meat's ability to retain moisture, was significantly affected by the replacement of beef fat with corn oil oleogel ($p<0.05$). The WHC of the control treatment was lower than that of the reformulated treatments ($p<0.05$), and the highest WHC was observed in the O100 treatment ($p<0.05$). The addition of chicken skin, which contains collagen, has been shown to improve the WHC of meat emulsions in previous studies. The cooking yield, which indicates the amount of moisture retained during cooking, ranged from 95.34% to 98.72%. The addition of oleogel significantly increased the cooking yield ($p<0.05$). The thermal gelation of collagen in the chicken skin oleogel may have contributed to the improved cooking yield by increasing water retention. Similar findings have been reported in studies where the substitution of animal fat with plant oils improved the cooking yield of sausages (Wolfer et al. 2018). Emulsion stability, which refers to the ability of the emulsion to resist separation, was significantly influenced by the addition of oleogel ($p<0.05$). The addition of oleogel led to a significant decrease in the total amount of expressible water ($p<0.05$), indicating improved emulsion stability. The water-binding ability of collagen present in chicken skin may contribute to this effect. However, there was no significant difference in the total amount of expressible oil when oleogel was added to the formulation ($p>0.05$). Overall, the incorporation of oleogel in meat batters improved water holding capacity, cooking yield, and emulsion stability, indicating its potential as a functional ingredient in meat product formulations.

3.4. Fatty Acid Profile and Health Indices

Reducing fat content in meat products is a challenge due to its impact on technological and sensory properties. High intake of saturated fat is associated with adverse health effects (WHO, 2021). To address this, replacing animal fat with healthy plant oils like gelled emulsions or oleogels is gaining importance. The fatty acid profile was affected by the corn oil-chicken skin oleogel and its addition level ($p<0.05$) and increasing oleogel levels led to higher amounts of linolenic and oleic acid. This may be due to the high oleic acid content (43%) in chicken skin. These findings align with studies on reduced-fat beef burgers using olive oil-based oleogel substitutes (Özer and Çelegen 2020). Treatment with PUFA O50, O75, and O100 showed increased levels of polyunsaturated fatty acids (PUFAs) compared to the control. SFA levels in 050, 075 and O100 samples decreased by 30.9, 57.4 and 71.9 % respectively compared to the control samples. Therefore, oleogel added samples can be classified as "reduced in saturated fat" according to the European Parliament's definition, as they achieved a reduction in SFA content of over 30% (European Parliement 2006). Compared to the control, the treatments of PUFA O50, O75, and O100 have shown an increase of 26.34%, 32.05%, and 32.63%, respectively.

Table 4 presents the health indices of the meat batters, with the PUFA/SFA ratio being a commonly used measure of nutritional quality. The control samples had a ratio of 0.06, while the O100 treatment significantly increased it to 1.68. The addition of oleogel reduced the palmitic acid ratio compared to beef fat ($p<0.05$). Nutritional recommendations suggest a PUFA/SFA ratio above 0.45 in the human diet (HMSO 1994). Meat batters with oleogel exhibited significant increases in their PUFA/SFA ratios as oleogel levels increased ($p<0.05$). This suggests that using oleogel can enhance the nutritional quality of meat products, potentially benefiting consumers' health. The USDA (2015) and WHO (2021) recommend a daily intake of up to 3% of energy from PUFAs and 1.4-2.5 g of n-3 PUFAs, while also considering the PUFA/SFA ratio. Incorporating oleogel made from chicken skin and corn oil into meat batters led to a significant decrease in saturated fatty acids, along with increased PUFA/SFA and n-6/n-3 ratios ($p<0.05$). These health indicators are important as high n-6/n-3 ratios in diets can contribute to various illnesses, while higher n-3 content has a suppressive effect. Replacing beef fat with oleogel resulted in a notable decrease in the n-6/n-3 ratio, aligning with dietary guidelines. Utilizing innovative lipid materials such as oleogels made from plant oils with a favorable fatty acid profile enabled an increase in the PUFA/SFA ratio (from 0.06 to 1.68) and a reduction in the n-6/n-3 ratio towards recommended values. Similarly, in a different study, replacing pork back fat with beeswax oleogel led to an increased PUFA/SFA ratio and decreased n-6/n-3 ratio (Gómez-Estaca et al. 2019). Furthermore, replacing beef fat with oleogel resulted in significant reductions in the atherogenicity (IA) and thrombogenicity (IT) indexes, indicating higher levels of anti-atherogenic fatty acids that can help prevent coronary diseases. The IA and IT indexes were significantly decreased ($p<0.05$) by 73% and 84%, respectively, in the O100 treatment compared to the control samples. Similar reductions in IA and IT indexes were observed when replacing 75% and 100% of pork back fat with oleogel in a previous study (Silva et al. 2019).

3.5. Instrumental quality

Color is a crucial factor influencing consumer preference when it comes to meat products. Reformulation efforts have led to significant changes in color parameters. The L*, a*, and b* values of meat batters are presented in Table 5, ranging from 42.12 to 55.50, 11.76 to 6.81, and 11.64 to 14.42, respectively. Incorporating oleogel in the formulation resulted in higher L* and b* values and lower a* values compared to the control samples ($p<0.05$). The addition of oleogel can contribute to improved homogeneity and compactness of the meat batters' structure, leading to increased L* values. The enhanced L* values may be attributed to the more uniform distribution of the oil phase within the protein matrix, as liquid oil tends to disperse more evenly compared to animal fat. Similar findings have been reported in studies on the use of oleogels as animal fat replacers in breakfast sausages and frankfurters (Barbut et al. 2016). However, variations in oil composition, oleogelator composition, and raw materials can influence the results observed in different studies. Lower redness levels were observed in samples with oleogel compared to the control ($p<0.05$), likely due to color differences between beef fat and oleogel. These results align with the findings of previous studies that demonstrated lower redness values with the addition of oil gel emulsions in emulsion-type sausages (Nacak et al. 2021). All treatments, except for the control group, exhibited an increase in the b* value ($p<0.05$), with no significant difference among the treatments with oleogel ($p>0.05$). The disparity in color between beef fat (creamy white) and corn oil (pale yellow) could account for this observation. Consequently, the b* value of all other treatments is higher than that of the control group. These findings are consistent with similar results reported in studies focusing on b* values (Jimenez-Colmenero et al. 2010; Serdaroğlu et al. 2016).

The reduction and modification of fat significantly impact the texture properties (hardness, cohesiveness, gumminess, springiness, and chewiness) of meat products (Table 5). The texture parameters of the control samples were significantly higher than those of the samples formulated with oleogel, indicating the effect of fat substitution on texture ($p<0.05$). This effect can be attributed to changes in the protein structure of the meat and the emulsion stability of the oleogel. The type and properties of the lipid source and its behavior in the system play a major role in determining the textural quality of fat-substituted meat systems. Similar findings were reported in reduced-fat beef burgers where animal fat was partially replaced with olive oil oleogel-based emulsion (Özer and Çelegen 2020). An increase in moisture retention caused by soybean protein was found to decrease the hardness and chewiness of patties, which may have influenced the results of this research. The hardness value was also observed to decrease in beef burgers produced with olive oil oleogel-based emulsions. Texture parameters play a crucial role in determining the overall quality and sensory properties of meat products. The substitution of animal fat with sesame oil-beeswax oleogel resulted in reduced gumminess and chewiness in burgers (Moghtadaei et al. 2018). The springiness values of meat batters decreased, and the lowest value was observed in the O50 treatment. Reduced springiness was also observed in Frankfurter-type sausages formulated by replacing lard with rice bran wax oleogels. These results can be attributed to the more elastic properties of animal fats. The reduced gumminess of samples containing beef fat substituted with oleogel could be due to weaker internal bonds and a softer network. Cohesiveness values ranged from 0.30 (O100) to 0.39 (C), while gumminess values decreased from 14.57 (C) to 3.22 in the O100 treatment ($p<0.05$).

Chewiness values ranged from 1.07 (O100) to 6.83 (C), with a significant decrease observed as the oleogel ratio increased ($p<0.05$). Hardness values are generally correlated with gumminess and chewiness values.

3.6. Microstructure

The SEM images of beef emulsion samples (1000x magnification) have been shown in Fig 2. Treatments formulated with oleogel had a coarse microstructure. As the amount of beef fat was reduced, the product structure appeared to become more compact. Microstructural impacts of oleogel addition are markedly visible. Control samples formulated with only beef fat had smaller fat globules. Increasing oleogel in formulation resulted more integrated and homogenous sturcture. A study using the bigel (mixing hydrogel and oleogel) made of starch and ethylcellulose as a fat replacer showed that control samples had microstructures with greater gaps (Ghiasi and Golmakani 2022).

3.7. Fat oxidation products: Peroxidases and TBARS

The measurement of peroxide value is crucial in assessing the oxidation status of muscle foods, as hydroperoxides are primary oxidation products associated with quality deterioration. Fig 3A presents the peroxide values of the treatments. Initial peroxide values ranged from 1.86 (O75) to 3.04 (O50) meqO^2/kg. The storage period and fat formulation were found to have a significant effect on peroxide values ($p<0.05$). On the 3rd day, all samples exhibited an increase in peroxide value, with the highest value observed in O75 (4.43 meqO^2/kg) and the lowest in O100 (2.54 meqO^2/kg) samples ($p<0.05$). In the C and O50 samples, peroxide values continued to increase until the 5th day, followed by a decrease on the 7th day. Over time, peroxide values tend to decrease as hydroperoxides break down into secondary oxidation products. At the end of storage, peroxide values were 2.66, 3.54, 2.61, and 2.76 meqO2/kg in the C, O50, O75, and O100 samples, respectively. Some studies have shown that samples containing added oleogel had lower peroxide values than the control on the 5th day (Tabibiazar et al. 2020). The structure of the oleogel formulation, with its solid-like consistency, can offer protection against oxidation. However, there are also studies that have reported higher peroxide values in meat products formulated with oleogel compared to the control (Moghtadaei et al. 2018; Tabibiazar et al. 2020). The variations in oil sources, product formulations, and storage parameters can contribute to these different outcomes. Throughout the storage period, all samples had peroxide values significantly lower than the acceptable limit (25 meqO^2/kg) established by Evranuz (1993).

Fig 3B illustrates the TBARS values. The incorporation of corn oil oleogel did not have an adverse effect on the TBARS values of the meat batters ($p>0.05$). In fact, the addition of oleogel resulted in a significant reduction in TBARS values compared to the control treatment ($p<0.05$). Initially, there was no significant difference in TBARS values between the C and O50 samples. During storage, the control group showed a significant increase in TBARS value ($p<0.05$), while the other treatments experienced minor fluctuations. The lowest TBARS values were observed in the O75 and O100 treatments on the 5th day of storage ($p<0.05$). The control sample formulated with 100% beef fat had the highest TBARS (2.87 mg MDA/kg) value at the end of storage. Most groups, except the control, had TBARS values within the limit of 2 mg MDA/kg (Witte et al. 1970). The variation in TBARS values can be influenced by the fatty acid composition of the vegetable oil used and the antioxidants in the formulation. These findings align with previous studies on meat products formulated with oleogel (Wolfer et al. 2018; Gómez-Estaca et al. 2019). The increase in TBARS values may be attributed to a higher formation ratio of malonaldehydes compared to their disappearance, but over time, the disappearance ratio may surpass the formation ratio, leading to a decrease in TBARS values.

4. Conclusions

Overall, it appears that the addition of corn oil chicken skin oleogel had a positive impact on the technological attributes and textural parameters of the meat batter. Also, beef fat replacement of oleogel positively enhanced the quality attributes of meat batters by higher water holding capacity and cooking yield.

The incorporation of chicken skin into the oleogel matrix can provide a sustainable and cost-effective approach for reducing the amount of saturated and trans fats in meat products. However, it is important to consider the potential effects of any color alterations that may have occurred. Further research is needed to optimize the formulation and processing conditions of chicken skin-based oleogels and evaluate their sensory and nutritional properties in food products. However, more research is needed to optimize the formulation and processing conditions of chicken skin-based oleogels, as well as evaluate their nutritional and sensory properties in various meat products.

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Research Article

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Effects of Fermentation Temperature and Time on Chickpea-Initiated Sourdough Production

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HIGHLIGHTS

- Fermentation temperature and time affect the production of chickpea-initiated sourdough production
- Chickpea particle size and water-chickpea ratio had rather limited effects on the FCL and CY properties

Abstract

Chickpea is a nutritious staple pulse with numerous consumption patterns. It is also used in bakery products, especially in bread, in the forms of chickpea flour, fermented-chickpea liquor (FCL), and chickpea yeast (FCL-fermented sourdough) in various countries. However, a large variation exists in the traditional fermentation of FCL and chickpea yeast (CY). In this study, fermentation parameters (cracked-chickpea size, water-chickpea ratio, fermentation temperature, and fermentation time) for the production of FCL and CY were sequentially optimized through separate RSM designs. Therefore, this study aimed at optimization of the fermentation conditions for the preparations of both FCL and CY. First, FCL production parameters of fermentation temperature, time, chickpea particle size, and water-chickpea ratio were optimized and determined to be 40°C, 26 h, 2-6 mm, and 4:1 ratio, respectively. Secondly, the CY process parameters (fermentation temperature and time) were optimized using the optimized FCL conditions and determined to be 38°C and 9 h. The validation studies proved that there is no statistical difference ($p>0.05$) between the RSM-model predicted and experimental responses. At the optimized fermentation conditions, the FCL and CY had pH values of 4.44 and 4.31, and LAB counts of 9.87 and 9.08 log cfu g-1. The optimum fermentation conditions determined in this study are somewhat comparable to those commonly employed in the traditional preparations of both FCL and CY. Such optimization could lead to the better utilization of FCL and CY in the food industry and improved consumer health outcomes.

Keywords: Sourdough; fermented-chickpea liquor; chickpea yeast; fermentation conditions; lactic acid bacteria

1. Introduction

Chickpea (*Cicer arietinum*) is a leguminous grain rich in proteins, dietary fiber, minerals, and health-promoting phytochemicals (Foschia et al. 2017). It is processed into various foods through decortications/dehulling, soaking, sprouting, fermenting, boiling, mashing, steaming, frying, and roasting

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(Deshpande and Damodaran 1990). Furthermore, chickpea is used in bakery products, especially in bread, in the forms of chickpea flour, fermented-chickpea liquor (FCL), and FCL-initiated sourdough (chickpea yeast - CY) in certain localities of Türkiye, Greece, Bulgaria, and Macedonia (Hatzikamari et al. 2007a; Sayaslan and Şahin 2018). The CY, also known as sweet dough to differentiate it from the conventional sourdough, is a traditional leavening practice to improve the flavor, texture, and nutritional properties of bakery products (Sayaslan and Şahin 2018; Durmaz et al. 2023).

Extensive investigations on the sourdough process (Crowley et al. 2002; Dal Bello et al. 2007; Moroni et al. 2009; Durmaz et al. 2023) revealed that sourdough usage improved dough processability, retarded staling and molding, enhanced taste and aroma, and improved nutritional quality of bakery foods. Considered a variant of the conventional sourdough practice, the FCL and CY were also shown to provide similar benefits (Hancıoğlu-Sıkılı 2003; Baykara 2006; Kefalas et al. 2009; Narlıoğlu 2013; Çebi 2014; Kasım 2014; Saad et al. 2015;
Hendek-Ertop and Coşkun 2018; Hendek-Ertop and Şeker 2018). Microbiological, enzymatic and hydrolytic reactions occur in the fermentation stages of the FCL and CY preparations (Hatzikamari et al. 2007), all of which positively contribute to the dough rheology and sensory properties of baked goods. During chickpea fermentation, mostly lactic acid bacteria (LAB) and, to a lesser extent, certain nonpathogenic species of *Bacillus* and *Clostridium* were found to increase in the fermentation medium (Katsaboxakis and Mallidis 1996; Hatzikamari et al. 2007a, b; Çebi 2009; 2014). Additionally, the activities of hydrolytic enzymes, including amylases, cellulase, α-galactosidase, invertase, and proteases; and the amounts of such hydrolysis products as free fatty acids, reducing sugars, and free amino acids were also elevated (Hatzikamari et al. 2007a). Furthermore, a vast number of taste and aroma compounds were generated during the chickpea fermentation (Hancıoğlu-Sıkılı 2003).

In general, the FCL is traditionally prepared using coarsely ground or cracked chickpea. The cracked chickpea is then spontaneously fermented in several folds of water at 30-40°C for 15-20 h. The development of a foamy structure atop the fermentation medium is regarded as a sign of successful fermentation (Hatzikamari et al. 2007a; Sayaslan and Şahin 2018). Upon completion of the fermentation, the foamy FCL is sieved and used in the bakery formulations either directly (Kefalas et al. 2009; Kasım, 2014; Sayaslan and Şahin 2018, Şahin et al. 2018; Durmaz et al. 2023) or in the form of FCL-fermented sourdough, i.e., chickpea yeast (Hancıoğlu-Sıkılı 2003; Baykara 2006; Narlıoğlu 2013; Çebi 2009; 2014; Hendek-Ertop and Coşkun 2018; Hendek-Ertop and Şeker 2018). Although numerous preparation methods for the FCL and CY were reported in the literature, all extensively varied in the fermentation conditions. For instance, Kefalas et al. (2009) used coarsely-ground chickpea (>1.5 mm) for the FCL preparation, in which the crushed chickpea (100 g) was fermented in 300 ml of water at 35°C overnight. Kasım (2014) utilized 100 g of coarsely-ground chickpea (2-3 mm) and 1.5 g of salt to obtain the FCL through the fermentation in 550 ml of water at 42°C for 16 h. Sayaslan and Şahin (2018) and Şahin et al. (2018) also used coarsely-ground chickpea (>2.0 mm). The chickpea (100 g) together with 1.0 g of salt were fermented in 350 ml of boiled and cooled water at 40°C for 16 h. In those studies, the FCL was directly added to the bakery formulations in exchange of water. In the subsequent studies, however, CY was utilized in the bakery products instead of FCL. In this respect, Baykara (2006) and Narlıoğlu (2013) used 100 g of blender-ground chickpea (no mention of size distribution) and fermented it in 500 ml of salt-containing (1%) water at 35°C for 24 h. Çebi (2009) and Çebi (2014) also utilized coarsely-ground chickpea (100 g) together with 1.0 g of salt and fermented it in 350 ml of boiled and cooled water at 40°C for 16 h. Hendek Ertop and Coşkun (2018) and Hendek Ertop and Şeker (2018) followed somewhat a different FCL preparation approach. The process for preparing the chickpea mixture involved soaking 100g of whole chickpeas in 100ml of water for 20 hours. The mixture was blended until it reached a uniform consistency. Following this, 220ml of water, 2g of salt, 10g of sugar, and 50g of wheat flour were added, and the mixture was left to ferment for 12 hours at 26°C. The fermented slurry was then filtered through a fine screen (no mention of size) to obtain the FCL. The FCL was further fermented with wheat flour to produce CY. Finally, the CY was dried and incorporated into bread formulations.

The above-discussed literature on the preparations of FCL and CY indicates that rather a large variation exists in such fermentation parameters as cracked-chickpea size, water-chickpea ratio, fermentation temperature, and fermentation time. Therefore, this study aimed at optimization of the fermentation conditions for the preparations of both FCL and CY.

2. Materials and Methods

The chickpea (variety Koçbaşı), straight-grade wheat flour, and non-iodized table salt were purchased from local suppliers in Karaman, Turkey. The de Man, Rogosa and Sharpe (MRS) agar and Anaerocult®A for anaerobic conditions were from Merck (Darmstadt, Germany). Other chemicals used in the analyses were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Grinding and size classification of chickpea

The chickpea sample was coarsely ground using a pilot-scale custom-made hammer mill equipped with an 8.0-mm screen. The ground chickpea was sieved using a stack of sieves with successive apertures of 6.0, 4.0, 2.0, and 0.15 mm. The overs of sieves were utilized in the study as per the experimental design (Table 1).

Table 1. Independent and dependent variables of RSM design used in optimization of fermented-chickpea liquor (FCL) production

Run	Independent variable				Dependent variable (experimental response)			
	Temperature (°C)	Time (h)	Water-chickpea ratio	Chickpea particle size (mm)	Dry-matter content** (%)	Foam height (mm)*	pH**	LAB count (log cfu g^{-1})*
1	37.5	10	5	2-4	3.5	27	5.1	10.06
2	30.0	10	4	2-4	4.3	10	6.0	9.40
3	37.5	10	3	2-4	6.6	17	5.0	10.61
4	30.0	20	4	0.15-2	4.9	27	5.0	10.26
5	37.5	20	4	2-4	4.5	40	4.8	10.18
6	37.5	30	3	2-4	7.0	33	4.8	10.78
7	37.5	20	4	2-4	4.4	40	4.9	10.13
8	37.5	20	5	0.15-2	4.3	27	4.6	10.06
9	37.5	20	4	2-4	4.6	37	4.7	10.28
10	37.5	30	4	4-6	4.6	33	4.5	10.84
11	37.5	20	4	2-4	5.1	40	4.6	10.29
12	30.0	20	4	4-6	3.9	13	5.1	9.73
13	30.0	20	3	2-4	6.2	41	5.6	10.50
14	45.0	20	3	2-4	7.1	33	4.6	9.80
15	37.5	20	3	4-6	5.8	27	4.8	10.14
16	37.5	20	4	2-4	4.6	43	4.7	10.29
17	37.5	10	4	4-6	3.9	7	4.9	10.26
18	37.5	20	5	4-6	3.2	13	4.6	10.00
19	45.0	20	4	4-6	4.8	33	4.7	10.20
20	45.0	30	4	2-4	4.7	20	4.5	10.21
21	37.5	30	4	0.15-2	5.4	13	4.8	10.85
22	37.5	30	5	2-4	3.7	43	4.7	10.88
23	45.0	10	4	2-4	4.2	27	5.1	10.33
24	45.0	20	5	2-4	3.7	33	4.5	10.00
25	30.0	20	5	2-4	2.6	30	5.7	9.00
26	37.5	20	3	0.15-2	7.7	13	4.7	10.34
27	45.0	20	4	0.15-2	5.4	13	4.9	9.96
28	37.5	10	4	0.15-2	5.5	7	5.0	10.61
29	30.0	30	4	2-4	4.6	47	5.0	10.63

*Results were given as mean of dublicate measurements.** Results were given as mean of triplicate measurements

Preparation and optimization of fermented-chickpea liquor (FCL)

In the first stage of the study, important fermentation variables in the FCL production stage were optimized through the response surface methodology (RSM) with a four-factor and three-level Box-Behnken design (Table 1) (Montgomery 2017). The independent variables of the design were fermentation temperature (30, 37.5, 45°C), fermentation time (10, 20, 30 h), chickpea particle size (0.15-2.0, 2.0-4.0, 4.0-6.0 mm), and water-chickpea ratio (3:1, 4:1, 5:1) (Table 1). The dependents variables (responses) of the RSM design were soluble plus suspended solids (dry-matter), foam height, pH, and LAB count in the FCL.

The fermentation process for the coarsely-ground chickpea was adapted from Sayaslan and Şahin (2018). For this purpose, the ground chickpea (100 g), salt (1.0 g) and boiled-cooled warm (about 50°C) distilled water (amount as per the experimental design) were placed in a 1-liter graduated glass bottle with an air-tight cap. The bottle content was subjected to spontaneous fermentation in an incubator (WiseCube, Daihan Scientific, Seoul, South Korea) without shaking (temperature and time as per the experimental design). Once the fermentation was over, the height of the foam atop the fermentation medium was recorded. The content of the bottle, including the foam, was then sieved through a 0.2-mm screen to obtain the FCL. The FCL was sampled and used for the determination of dry-matter, pH, and LAB count.

Preparation and optimization of chickpea yeast (FCL-fermented sourdough)

In the second stage of the study, the best fermentation conditions for the CY production were studied through the RSM Central Composite Design with two independent variables at three levels (fermentation temperatures of 30, 35, 40°C; fermentation times of 3, 6, 9 h). The CY was prepared using the FCL at the optimized conditions (40°C, 26 h, 2-6 mm of chickpea size, 4:1 water-chickpea ratio) that were determined in the first stage of the study. For this purpose, the FCL (100 g) was mixed with 100 g of wheat flour at 200 rpm for 2 min using a mixer (RW20, IKA GmbH, Staufen, Germany) to give a slack dough with a yield of 200% (Chavan and Chavan 2011). A certain amount of that dough (150 g) was then placed in a 1-liter glass bottle with an air-tight cap fitted with a monometer and subjected to fermentation as per the RSM experimental design (Table 2). At each h of the fermentation, the generated pressure (gassing power) was recorded, the system was zeroed, and restarted for the regeneration of the gas. When the fermentation was over, the CY was sampled for pH measurement and LAB count.

Table 2. Independent and dependent variables of RSM design used in optimization of chickpea yeast (CY) production

Run	Independent variable				Dependent variable (experimental response)		
	Coded value		Actual value				
	Factor-1	Factor-2	Temperature (°C)	Time (h)	pH**	Gassing power (mmHg)*	LAB count (cfu g-1)*
1	0	0	35	6	4.69	102	3.1x10^8
1	0	0	35	6	4.69	102	3.1x10^8
2	1	-1	40	3	4.83	78	6.0×10^8
3	0	1.41	35	10.2	4.26	130	2.9×10^8
4	0	0	35	6	4.70	89	6.7×10^8
5	-1.41	0	27.9	6	4.89	38	1.4×10^9
6	1.41	0	42.1	6	4.65	152	1.8×10^9
7	0	0	35	6	4.72	92	1.1×10^8
8	0	0	35	6	4.7	86	6.6×10^8
9	-1	-1	30	3	5.21	42	3.5×10^8
1	-1	1	30	9	4.65	75	8.4×10^8
11	0	-1.41	35	1.8	5.28	58	3.3×10^7
12	0	0	35	6	4.78	92	8.3×10^8
13	-1	1	40	9	4.32	125	1.2×10^9

*Results were given as mean of dublicate measurements.** Results were given as mean of triplicate measurements

Chemical and microbiological analysis

The moisture content of the wheat flour was determined using a moisture analyzer (ATS-120, Axis, Gdansk, Poland). The height of the foam (foam height) atop the FCL container was manually measured. The dry-matter (solubles plus suspended solids) content of the FCL was measured on a refractometer (RA-600, Kyoto Electronics, Tokyo, Japan). The pH of the FCL was read directly, while that of the CY was measured upon homogenization of the CY (10 g) in 90 ml of distilled water for 1 min (Çebi 2009). The gassing power (gas production capacity) of the CY samples was measured in a 1-liter glass bottle with an air-tight cap fitted with a monometer during fermentation. For the LAB count, the FCL was sampled and used directly for inoculation. In the case of CY, however, the CY (25 g) was first homogenized in the physiological saline water (225 ml) for 1 min and used for inoculation at appropriate dilutions. The LAB count was performed using the De Man, Rogosa and Sharpe (MRS) agar as described by Çebi (2009).

Data analysis and model fitting

The experimental data collected through the RSM designs were analyzed using the Design-Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA). Appropriate models were selected for each response using the degree of significance (p), regression coefficient (R^2), and lack of fit test.

3. Results

3.1. Optimization of fermented-chickpea liquor (FCL) production

The optimization process for the FCL aimed to maximize foam height and LAB count while minimizing pH. Table 1 presents the responses to the independent variable combinations used in the FCL optimization. The experimental design resulted in different dry-matter contents for the FCL treatments, ranging from 2.63% to 7.70%, and foam heights varying between 7 and 43 mm. The pH values and LAB counts of the FCL samples ranged from 4.5 to 6.0 and from 9.00 to 10.88 log cfu g-1, respectively. The quadratic models were well-suited for predicting all responses, including dry-matter content, foam height, pH, and LAB count, as indicated by the ANOVA results presented in Table 3. All models were significant ($p<0.05$), and their lack of fit tests was insignificant ($p>0.05$), indicating their soundness and validity for predicting the responses.

Table 3. Model type and significance (*p*) values of RSM optimization for fermented-chickpea liquor (FCL) production*

Models type and terms		Significance (*p*) value by response			
		Dry-matter content (%)	**Foam height (mm)**	**pH**	**LAB count (log cfu g-1)**
Model type	Quadratic	0.0075	<0.0001	0.0035	< 0.0001
Lack of fit		0.3691**	0.0507**	0.1246**	0.0691**
Model		< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Terms	A	0.0028*	0.5554	< 0.0001*	0.0733
	B	0.0535	< 0.0001*	0.0004	< 0.0001*
	C	< 0.0001*	0.5554	0.6903	0.0007*
	D	< 0.0001*	0.1028	0.5961	0.0943
	AB	0.7798	0.0001	0.3634	0.0004*
	AC	0.7798	0.2217	0.6458	< 0.0001
	AD	0.5134	0.0016*	0.4926	0.0183*
	BC	0.7424	1.0000	0.6458	0.0408
	BD	0.1858	0.0447*	0.6458	0.2582
	CD	0.2012	0.0070*	0.8177	0.6350
	A^2	0.1589	0.0521	0.0008*	< 0.0001*
	B^2	0.7806	0.0002*	0.1422	< 0.0001*
	C^2	0.0003*	0.1887	0.6191	0.2190
	D^2	0.0529	< 0.0001*	0.2748	0.2908

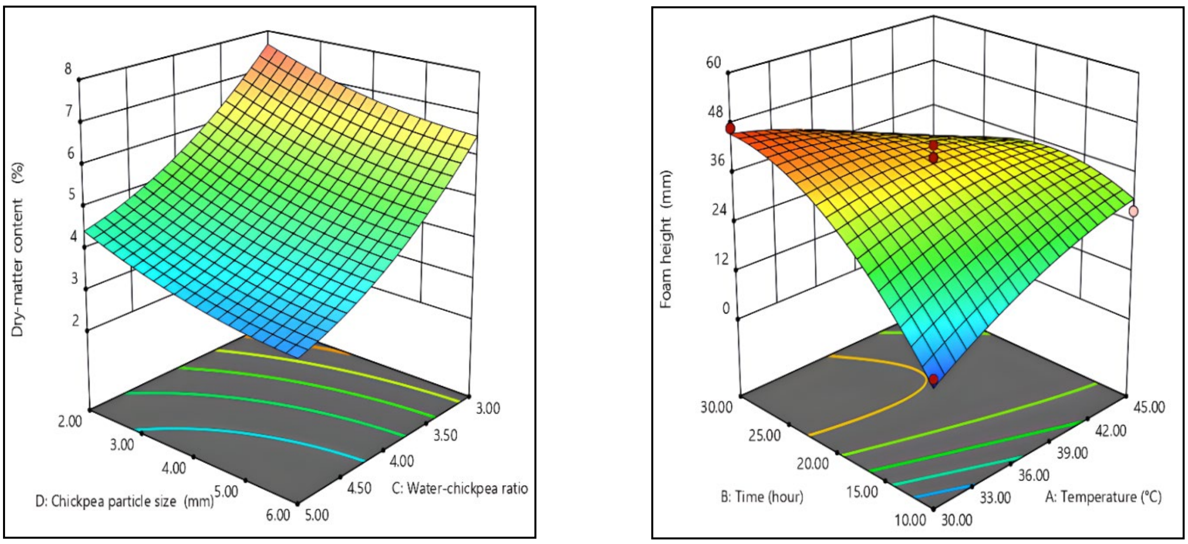
A: Fermentation temperature, B: Fermentation time, C: Water-chickpea ratio, D: Chickpea particle size *p<0.05; significant,**Lack of fit should be non-significant at p<0.05

The dry-matter contents of the FCL samples were significantly impacted by the water-chickpea ratio and chickpea particle size, as evidenced by Table 3. A 3D-contour plot in Figure 1a illustrates the correlation between the water-chickpea ratio and chickpea particle size to the dry-matter contents of the FCL. Increasing water content and chickpea particle size reduced the amount of solid substances transferred to the water. However, as temperature increased, more chickpea components were dissolved, increasing the amount of water-soluble substances transferred to the water. A significant success criterion in the fermentation process is generating a considerable amount of foam atop the chickpea liquor's surface, as Hatzikamari *et al.* (2007a) and Sayaslan and Şahin (2018) noted. The ANOVA results in Table 3 indicated that time and temperature-time interaction had a significant impact on foam height, as shown in Figure 1b. The chickpea is a source of saponins (Kerem et al. 2005), which are amphiphilic in nature, containing lipid-soluble glycone and water-soluble sugar chains. They act as surface-active compounds possessing wetting, emulsifying, and foaming properties (Güçlü-Üstündağ and Mazza 2007). According to Çabuk et al. (2018), foaming capacity is related to pH and fermentation time. Shi et al. (2015) soaked various pulses at room temperature for 12 to 18 hours to release saponins by simple diffusion. They discovered that saponin levels in the slurry increased with extended soaking time and soaking-cooking combinations. Our study revealed that chickpea particle size had a positive interaction with fermentation temperature and time but a negative interaction with the water-chickpea ratio, as shown in Table 4. The negative quadratic terms indicated an excessive increase in those variables reduced foam height. During fermentation, as the amount of chickpea remains constant, increasing the water-chickpea ratio leads to reduced concentrations of saponins in the water, restricting foam formation. Furthermore, the temperature-time interaction was negatively correlated with foam height because high-temperature short-time treatments promote the degradation of saponins. In contrast, low-temperature long-time treatments have a limited impact (Shi et al. 2004).

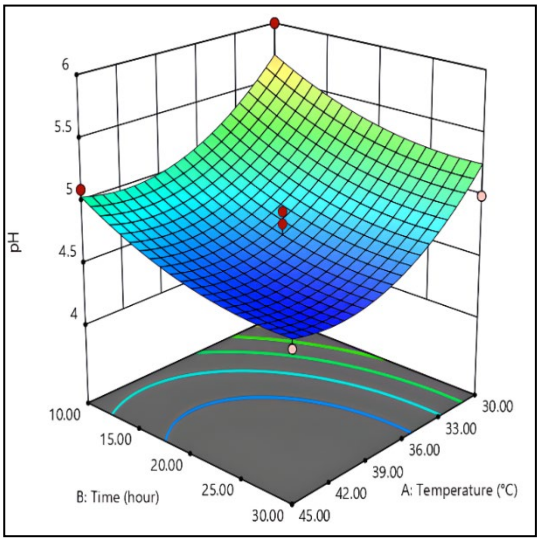
Table 4. Reduced best models for fermented-chickpea liquor (FCL) production and their coefficient of variation (R^2) values

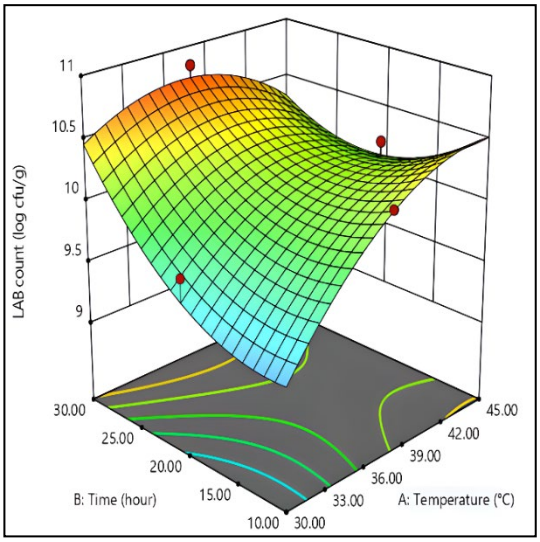
Response	Reduced best model	R^2	Adjusted R^2	Predicted R^2	Adequate precision*
Dry-matter Content (%)	$4.68+0.2833\times A-1.61\times C-0.5858\times D+0.4383\times C^2$	0.94	0.93	0.90	32.36
Foam height (mm)	$36.77+7.83\times B-11\times A\times B+8.5\times A\times D+5\times B\times D-7\times C\times D-8.02\times B^2-15.77\times D^2$	0.89	0.85	0.76	19.08
pH	$4.78-0.3417\times A-0.2333\times B+0.2819\times A^2$	0.73	0.70	0.61	14.86
LAB count (log cfu g-1)	$10.23+0.0817\times A+0.2433\times B-0.1808\times C-0.0758\times D-0.3375\times A\times B+0.425\times A\times C+0.1925\times A\times D+0.1625\times B\times C-0.3399A^2+0.3401\times B^2$	0.92	0.87	0.72	18.41

A: Fermentation temperature, B: Fermentation time, C: Water-chickpea ratio, D: Chickpea particle size *A ratio greater than 4 is desirable



(a) **(b)**





(c) **(d)**

Figure 1. 3D-contour plots for optimization of FCL production - effects of chickpea particle size x water-chickpea ratio on dry matter content (a), chickpea particle size x fermentation time on foam height (b), fermentation time x fermentation temperature on pH (c), and fermentation time x fermentation temperature on LAB count (d)

The production of FCL is subject to pH changes, which have been found to be negatively correlated with temperature and time, as indicated in Table 3 and Figure 1c. Specifically, the first-order coefficients for temperature and time are negative, signifying that pH levels decrease as these variables increase. However, the positive quadratic terms suggest that extreme increases in these variables may actually enhance pH levels during FCL production. Based on our data analysis, the pH changes during FCL production are not significantly influenced by variations in the water-chickpea ratio or the particle size of chickpeas. During the fermentation of chickpea liquor, various bacteria and yeast develop over time, leading to the generation of a number of biochemical substances including ethanol, hydrogen peroxide, lactic acid, acetic acid, and CO_2. These substances are produced as a result of hydrolytic enzymes such as cellulase, galactosidase, invertase, amylase, and proteinase. In turn, the presence of these enzymes is likely to reduce pH levels in the FCL. It is worth noting that multiple instances of invertase and amylase were observed throughout fermentation, which could further contribute to decreased pH levels (Galal et al. 1978; Hancıoğlu-Sıkılı 2006; Hatzikamari et al. 2007b).

The LAB count in FCL samples was found to be significantly affected by the fermentation temperature and time, as indicated by the linear and quadratic effects observed in Table 3, Table 4, and Figure 1d. Additionally, the water-chickpea ratio and chickpea particle size were found to have negative linear effects on the LAB count, with increasing values of these variables leading to decreased LAB counts. These findings suggest that using medium-sized chickpea particles (2-6 mm) and a water-to-chickpea ratio of approximately four-fold could maximize the LAB count. Moreover, it was observed that temperature and water-chickpea ratio, temperature and chickpea particle size, and time and water-chickpea ratio showed positive correlations, except for the temperature and time interaction. Previous studies (Hancıoğlu-Sıkılı 2006; Hatzikamari et al. 2007b; Çebi 2009) have reported the development of various bacteria and yeast during chickpea fermentation, including LAB such as *Lb. plantarum, Lb. pentosus, Lb. bifermantans, Str. thermophilus, Lc. ssp. lactis, Lb. brevis, Lb. plantarum, Lb. pentosus, Weisella confuse*, and the yeast *S. cerevisiae*. However, nonpathogenic *Bacillus spp.* (specifically *B. cereus, B. thuringiensis*, and *B. licheniformis*) and *Clostridium spp*. (especially *Cl. Perfringens* and *Cl. beijerinckii*) were also identified in FCL prepared in Greece (Hatzikamari et al. 2007b). These findings highlight the importance of carefully selecting fermentation conditions to achieve optimal LAB count in FCL samples. Selecting the appropriate fermentation temperature, time, water-chickpea ratio, and chickpea particle size may increase the LAB count and produce high-quality FCL.

Table 4 displays the coded equations, or reduced models, derived from the RSM design. These models serve as a valuable tool for identifying the relative impacts of the factors under analysis by comparing the factor coefficients. After removing nonsignificant terms (p>0.05) from the equations, the reduced models (Table 4) accurately describe the effects of significant process variables on the responses. The values of R2, adjusted R2, predicted R2, and adequate precision for the responses range between 0.69-0.94, 0.66-0.93, 0.59-0.90, and 13.99-32.16, respectively. The difference between the predicted and adjusted R^2 values is suggested to be less than 0.2. Table 4 shows that all coefficients are reasonable; thus, the RSM models are deemed acceptable. Generally, a model can be considered significant if the lack of fit test is insignificant, there is a satisfactory agreement between the adjusted and predicted R2, the adequate precision is over four, and the residuals are suitably distributed. In these cases, the model is considered a good predictor of the responses, and the desirability value of the model is closest to 1.0 (Anonymous 2007).

This study aimed to determine the optimal conditions for producing FCL with maximum foam height, LAB count, and minimum pH. Experimentation showed that the highest desirability value of 0.92 was achieved at a fermentation temperature of 40 °C for 26 hours, with a four-fold water-chickpea ratio and 2-6mm chickpea particle size. Validation studies showed no statistical difference between the experimentally obtained values and those predicted by the RSM model (p > 0.05). At the optimized fermentation conditions, the FCL had a pH value of 4.44 and a LAB count of 9.87 log cfu g-1 (Table 5). These results demonstrate the successful optimization of FCL production under specific conditions and may have important implications for future research.

Table 5. Verification of RSM-optimized model for fermented-chickpea liquor (FCL) production

Response	Fermentation parameter				RSM-model predicted value	Experimental value	Error (%)
	Temperature (°C)	Time (h)	Water-chickpea ratio	Chickpea particle size (mm)			
Dry-matter content (%)	40	26	4:1	2-6	4.41	4.57	3.63
Foam height (mm)					29.81	33.00	10.70
pH					4.54	4.44	2.20
LAB count (log cfu g-1)					10.41	9.87	5.19
Desirability value of model 0.92							

3.2. Optimization of chickpea yeast (CY) production

The second stage of the study involved optimizing the process conditions that resulted in the highest LAB count and gas production capacity, with the lowest pH in the CY, based on the optimized FCL conditions. The RSM design consisted of two independent variables (fermentation temperature and time) with five levels each. The responses measured were pH, gassing power, and LAB count. Based on the experimental runs, the pH and gassing power of the CY samples varied from 4.32 to 5.28 and from 38 to 152 mmHg, respectively. The LAB counts of the samples ranged from 3.3x10^7 to 1.8x10^9 cfu g-1. Linear models were found to be the best fit (p<0.01) for predicting the pH and gassing power of the CY, while a quadratic model was appropriate (p<0.01) for the LAB count. All models developed for the responses of pH, gassing power, and LAB count passed the lack-of-fit tests (p>0.05) with acceptable R2 (0.86-0.95), adjusted R2 (0.79-0.94) and predicted R2 (0.69-0.90) values, as shown in Table 6.

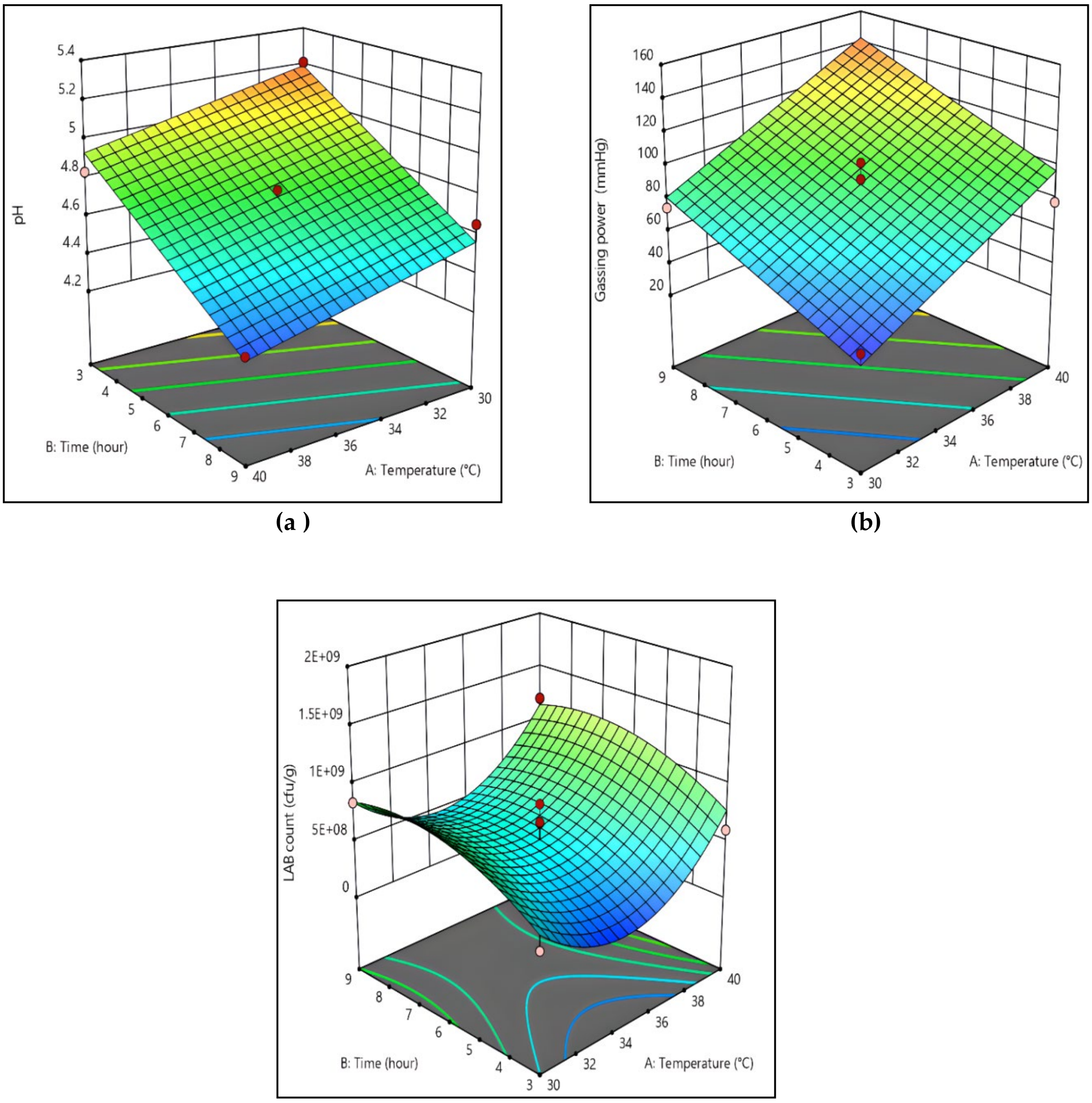
The findings of the ANOVA test, as presented in Table 6, indicate that temperature and time exhibit significant negative linear effects on the pH of the CY. The observed negative coefficient suggests a negative relationship between the two variables. The pH reduction due to the increased LAB count is a critical quality parameter for the CY, as with the FCL. Previous research studies (Chavan and Chavan 2011; Şahin et al. 2018) have reported the pH values of fermented dough/sourdough and FCL/CY in the 4.0-4.5 and 4.5-5.0 range, respectively. Our present study confirms that fermentation temperature and time influence pH reduction in FCL and CY, as illustrated in Table 6. The linear decline in pH from 5.2 to 4.2 of CY with an increase in fermentation temperature and time is evident from Figure 2a. The pH range of traditional sourdoughs is typically between 3.5 and 4.3, which satisfies the growth requirements of the dominant sourdough microorganisms (*30*). Among the *Lactobacillus spp., L. plantarum's* growth during fermentation produces weak acids that lower pH from 7.5 to 4.3 in 11 hours of fermentation (Chandra-Hioe et al. 2016; Çabuk et al. 2018).

Table 6. Model types, significance (*p*) values and reduced best models of the RSM optimization for chickpea yeast (CY) production

Response	Model type and terms	F value	p value	R^2	Adjusted R^2	Predicted R^2
pH	Linear	96.02	<0.0001	0.96	0.95	0.90
	A	28.53	0.0003			
	B	163.51	<0.0001			
	Lack of fit	5.42	0.062			
Reduced best model	pH = 4.74 - 0.131×A - 0.315×B					
Gassing power (mmHg)	Linear	40.28	<0.0001	0.89	0.87	0.77
	A	52.50	<0.0001			
	B	28.06	0.0003			
	Lack of fit	5.70	0.0569			
Reduced best model	Gassing power (mmHg) = 89.05+30.92×A+22.77×B					
LAB count (cfu g-1)	Quadratic	12.01	0.0018	0.86	0.79	0.69
	A	3.04	0.1194			
	B	4.84	0.059			
	A^2	30.94	0.0005			
	B^2	5.31	0.0475			
	Lack of fit	0.26	0.8755			
Reduced best model	LAB count (cfu g-1) = $5.154 \times 10^8 + 1.444 \times 10^8 \times A + 1.835 \times 10^8 \times B + 4.916 \times 10^8 \times A^2 - 2.082 \times 10^8 \times B^2$					

*A: Fermentation temperature, B: Fermentation time -Lack of fit should be non-significant at p<0.05

An increase in fermentation temperature and time has been found to correspond with an increase in the gassing power of CY, as demonstrated by Table 6 and Figure 2b. During the processing of FCL and CY, the yeast *S. cerevisiae* produces CO_2 gas naturally within the fermentation medium, thereby supporting the leavening of dough. This is in accordance with previous studies by Chavan and Chavan (*29*), Hendek Ertop and Coşkun (2018), and Sayaslan and Şahin (2018). Additionally, *Lactobacillus brevis* and *Weisella confuse*, both gas-producing heterofermentative LAB, were identified in the FCL and CY and were determined to be responsible for dough leavening (Çebi 2009). It is widely accepted that yeast and heterofermentative LAB synergistically contribute to the leavening of dough in FCL and CY (Çebi 2009; Hendek-Ertop and Şeker 2018). The findings of this study indicate that fermentation temperature and time are important parameters in determining the LAB count of CY, as evidenced by a quadratic model (Table 6 and Figure 2c). Previous studies have also demonstrated that traditional wheat sourdough's breadmaking performance is closely related to sourdough incubation temperature and time, inoculum level, and proof time (Göçmen et al. 2007; Minervini et al. 2014). The LAB count in a typical sourdough and CY is expected to be around 10^7-10^8 cfu g-1 (De Vuyst and Neysens 2005; Sayaslan and Şahin 2018). In this study, the LAB count of the CY at the optimized conditions was found to be at an adequate level of 9.08 log cfu g-1.



(c)

Figure 2. 3D-contour plots for optimization of chickpea yeast production - effects of fermentation temperature x fermentation time on pH (a), gassing power (b), and LAB count (c)

The results of the validation study conducted on the CY production under optimized conditions of 38 °C and 9 hours indicated that the RSM model's predicted values, including pH at 4.35, gassing power at 130.4 mmHg, and LAB count at 8.87 log cfu g-1, were not statistically different from the experimental data. The measured values, including pH at 4.31, gassing power at 136 mmHg, and LAB count at 9.08 log cfu g-1, were in line with the model's predictions. These findings suggest that the optimized conditions are effective for producing CY that the RSM model accurately predicts the values of key parameters.

4. Conclusions

The study's results have indicated that the production of FCL and CY is predominantly influenced by the fermentation temperature and duration, with the chickpea particle size and water-chickpea ratio having only minor contributions. The best temperature and duration for making FCL are 40°C and 26 hours, with chickpea particles that are 2-6 mm in size and a water-chickpea ratio of 4:1. For making CY, the best temperature is 38°C, and the best duration is 9 hours. When made under these conditions, FCL has a pH of

4.44 and LAB counts of 9.87 log cfu g-1, while CY has a pH of 4.31 and LAB counts of 9.08 log cfu g-1. These results are similar to traditional FCL and CY productions. This study underscores the potential to optimize fermentation conditions for producing FCL and CY with consistent quality and nutritional value. Such optimization could lead to the better utilization of CY in the food industry and improved consumer health outcomes.

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Assessment The Effects of Psyllium And Hydrocolloids in Gluten-Free Noodle

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HIGHLIGHTS

- Psyllium utilization was evaluated accompanied with two different gums.
- Combination of psyllium with other two gums resulted in higher cooking quality.
- Textural analysis showed consistency with cooking quality results.

Abstract

This study was performed to examine the effects of individual and combined usage of psyllium husk powder (PHP) with guar gum (GG) or locust bean gum (LBG) on the production of gluten-free Turkish-type noodles. For this purpose, six different formulations were created with the total amount of variables being 3%, and samples' physical, chemical, cooking, textural and sensorial properties were performed. The results revealed no significant decrease or increase in moisture, ash, protein and fat contents. On the other hand, although individual usage of psyllium caused a slight decrease in carbohydrate content, this did not reflect energy values. The lowest L^* and hue and the highest a^* values were observed in the samples where only psyllium was used, and there was no significant difference in b^* and saturation index values. Regarding cooking quality and textural analysis, the results from these two analyses supported each other, and there is a significant difference between the samples. An additional disliking that can be considered significant was not determined by using psyllium with or without other gums in noodle formulation. As a result, the dual combined psyllium with other gums in gluten-free noodle production could be appropriate without adversely affecting the quality of the product.

Keywords: gluten-free; guar gum; locust bean gum; noodle; psyllium husk.

1. Introduction

Coeliac disease (CD) is a well-known food intolerance affecting approximately 1% of the world population (Lionetti et al. 2015). This disease, also known as gluten enteropathy, is triggered by consuming wheat, rye and barley protein (Foschia et al. 2016; Dahal et al. 2021). After consuming these cereals by genetically susceptible individuals, small intestinal damage emerges, which causes digestion problems and

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malabsorption of nutrients (Allen and Orfila 2018; Kastin and Buchman 2002). Since it is a lifelong intolerance, CD patients should avoid gluten and products that include gluten (Yalcin and Basman 2008a). Therefore, gluten-free or modified cereal products were developed targeting these patients.

Turkish noodle, locally called 'erişte', is a traditional cereal-based food including egg, wheat flour and salt (Ozkaya et al. 2004). In recent years, the fortification of erişte was investigated by enrichment with different food components such as dairy by-products, apple fibre and chia flour (Aktaş and Türker 2015; Yuksel and Gurbuz 2019; Levent 2017) to increase the health benefits and functionality. Some of these fortification studies focused on people with gluten sensitivity or CD; thus, noodle formulation was modified with different gluten-free cereals (corn, rice), pseudocereals and legumes (Bilgicli 2008; Bilgicli 2013; Yalcin and Basman 2008a). However, replacing gluten with other ingredients is crucial for gluten-free products since gluten provides the structure, rheological and organoleptic features in dough systems. Mainly, hydrocolloids such as hydroxypropyl methylcellulose (HPMC), carrageenan, xanthan gum, locust bean gum (LBG) and guar gum (GG) can be used for this purpose (Allen and Orfila 2018; Belorio et al. 2020). Owing to their water-holding, gelling and thickening properties, they can help to form intermolecular networks; thus, they improve the structure and quality of the products (Dahal et al. 2021). In gluten-free pasta and noodle formulations, hydrocolloids affect the dough properties, and they change the colour, firmness and hardness of the pasta by increasing the firmness of the protein-starch matrix. Therefore, the cooking quality, mouthfeel, overall acceptability and dough kneading in noodles may also be enhanced (Culetu et al. 2021). Furthermore, these gums are also known as 'dietary fibre'; thus, adding these gums into gluten-free cereal formulas improves the nutritional quality of products while potentially reducing the glycaemic index of the foods (Brennan and Tudorica 2007). However, increasing interest in dietary fibre emerged in the inquiry of new fibre sources to enhance its capability and utilisation in the food industry. Thus, new food design can be accomplished, and the textural and sensory quality of products can be improved while increasing the fibre intake (Noguerol et al. 2022).

Psyllium is a soluble polysaccharide and natural fibre obtained from the seed of *Plantago ovato* (Belorio et al. 2020). It mainly consists of arabinose, xylose and other sugars such as galactose, rhamnose, glucose, and mannose (Zhang et al. 2019). It is also highly branched in the xylan backbone, which leads to high water-binding capacity and high viscosity in food systems. Therefore, it can be used as a thickening agent, emulsifier and stabiliser in food applications (Beikzadeh et al. 2016). Moreover, this viscous polysaccharide can form gels, enabling its usage in processed foods while lowering the glycaemic index of the food thanks to its dietary fibre content (Fradinho et al. 2020). The usage of psyllium in cereal and gluten-free bakery products has previously been suggested and investigated. In these researches, its effect has been investigated as a gluten substitute (Zandonadi et al. 2009), as a hydrocolloid substitute (Ziemichód et al. 2019), as a fat replacer (Belorio et al. 2019) and as combined with other gums (Mancebo et al. 2015) in food formulas. As it is used as a hydrocolloid, psyllium also improves the bread structure, volume and texture while reducing the glycaemic index in gluten-free bread. Furthermore, psyllium is a fibre source in bakery products, increasing the dietary fibre content of noodles (Culetu et al. 2021).

This study investigated the influence of a hydrocolloid of psyllium husk (PHP) in Turkish gluten-free noodle production and its effect on the end product's physicochemical, sensory and cooking quality. In this study, the two most used hydrocolloids, LBG and GG, were also used to compare the effect of psyllium in noodle formulation. Furthermore, these two gums are also paired with psyllium to characterise the physicochemical, textural and sensory qualities of the Turkish gluten-free noodle.

2. Materials and Methods

2.1. Materials

For gluten-free noodle production, commercial rice flour, corn starch, whole egg and salt were purchased from local markets from Karaman, Türkiye. Psyllium husk powder were obtained from Doğavera Gıda Co. (İstanbul, Türkiye). Guar gum and locust been gum were purchased from Sigma-Aldrich (USA).

2.2. Noodle preparation

Noodle samples were produced by adjusting the method of Levent (2017). Noodles was prepared with 100 g gluten-free flour mix (50% rice starch, 50% corn starch), 0.5% salt, 30% egg. The hydrocolloids; guar gum, locust bean gum and psyllium husk were added into the formulations according to Table 1. The amount of water was variable. The ingredients given in Table 1 were blended and mixed with a laboratory type mixer (Kitchen Aid Artisan Series Mixer; Kitchen Aid, St. Joseph, MI, USA) for 5 min. Before processing the noodle dough as sheets (2mm thick), it was divided into three pieces and rested at room temperature for 15 min in polyethylene bags. After that, the sheets were cut as 6mm wide long stripes with using a noodle machine (Vitalia Pasta Machine, İzmir, Turkey). Following the cutting process (4.5 cm long pieces) of these long stripes, the noodle samples were dried at ambient conditions till the moisture content of end product no more than 10%.

Table 1. Formulations of gluten-free noodle samples (g)

Samples	Gluten-free flour mix	Whole egg	Salt	PHP	GG	LBG
S1	100	30	0.5	3	-	-
S2	100	30	0.5	-	3	-
S3	100	30	0.5	-	-	3
S4	100	30	0.5	1.5	1.5	-
S5	100	30	0.5	1.5	-	1.5
S6	100	30	0.5	1	1	1

2.3. Chemical analysis

Noodle samples were analysed for their moisture (method 44-19), ash (method 08-01), fat (method 30-25) and protein (method 46-12) contents (AACC, 1990). The moisture content was determined using the drying norm at 135 °C. For the determination of ash content, the samples were burned at 550 °C. Protein determination was made by the Kjeldahl method and a multiplication factor of 6.25 was used in the calculation. For fat determination, the samples were extracted with hexane using a soxhelet device and then the solvent was removed.

2.4. Colour Analysis

The colour of noodle samples was measured using Hunter Lab Chroma Meter (Minolta CR-400, Osaka, Japan) in terms of the Hunter *L** (white; black), *a** (red; green) and *b** (yellow; blue) values. Besides, saturation index (SI) – Chroma, and Hue angle values were calculated according to equations below:

SI: $(a^{*2} + b^{*2})^{1/2})$ (1)

Hue: Arctan $[b^* a^{*-1}]) + 180$ where a<0 and b>0 (2)

Hue: Arctan [b* a*-1] where a>0 and b>0 (3)

For colour measurement, the samples were grounded with a blender (Sinbo SCM-2934; Sinbo, Istanbul, Turkey), sieved from 500 µm opening screen and the samples' colour were measured with the granulated material in a glass container. The measurements were done as triplicate and the average value of these readings were given.

2.5. Cooking Quality of Noodle Samples

For determination of cooking quality of samples, weight increase (WI), volume increase (VI) and cooking loss (CL) were monitored according to method of Özkaya and Kahveci (2005). 10 g noodle sample was boiled for 18 minutes in 250 ml distilled water and drained. Weight and volume of the samples were specified and recorded before cooking and after draining. A precision scales and a graduated cylinder filled with distilled water were used for determination of WI and VI respectively. The weight difference and water overflow in cylinder were used for calculation WI (%) and VI (%) respectively. On the other hand, for CL, the cooking water was evaporated to constant weight and total solids were expressed as percentage.

2.6. Texture Analysis

The firmness values of cooked gluten-free noodle samples were measured using a texture analyser model TAXT Plus Texture Analyser (Stable Micro Systems, Surrey, UK). The samples were sheared with A/LKB-F probe and the measurements were performed with load cell of 5 kg. The tests were carried out in duplicate and the average values were stated. Results were given as g (AACC 2000).

2.7. Sensory analysis

For sensorial evaluation, 100g of noodle samples were cooked in 500 ml boiling distilled water with 2.5 g salt up to optimum cooking time and drained in a colander. A laboratory panel was installed in an area far from the work area under daylight room conditions. The noodle samples with randomized order on a plate were presented to 7 panellists. They evaluated the samples regarding colour, cohesiveness, chewiness, taste, odour and overall acceptability by using a 7-point hedonic scale (1 = dislike extremely, 4 = acceptable and 7 = like extremely).

2.8. Statistical analyses

The results were displayed as mean ± standard deviation. SPSS statistics software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The differences obtained from results was assessed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test at 5% confidence interval.

3. Results

3.1. Chemical Analysis

The results of fat, protein, moisture, ash and carbohydrate are given in Table 2. According to this table, the differences between the samples were insignificant. However, different hydrocolloids showed a slight effect on the moisture of the glute-free noodles. Thus, the moisture content of sample S2 had the lowest moisture content and this data was consistent with Sutheeves et al. (2020).

Table 2. Proximate composition of noodle samples

	Moisture (g)	Ash (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (g)
S1	9.45±0.21[a]	0.80±0.09[a]	7.38±0.21[a]	3.64±0.12[a]	78.75±0.21[b]	377.20±1.08[a]
S2	9.11±0.06[a]	0.78±0.01[a]	7.12±0.23[a]	3.30±0.08[a]	79.71±0.10[ab]	376.96±0.16[a]
S3	9.27±0.21[a]	0.78±0.06[a]	7.08±0.04[a]	3.36±0.08[a]	79.52±0.40[ab]	376.64±0.65[a]
S4	9.44±0.10[a]	0.69±0.07[a]	7.06±0.18[a]	3.76±0.32[a]	79.06±0.33[ab]	378.26±2.27[a]
S5	9.14±0.25[a]	0.82±0.08[a]	6.81±0.08[a]	3.72±0.17[a]	79.52±0.08[ab]	378.78±1.56[a]
S6	9.34±0.26[a]	0.79±0.04[a]	6.71±0.19[a]	3.32±0.28[a]	79.86±0.38[a]	376.10±0.19[a]

* Means followed by different letters in the same column are significantly different ($p<0.05$).

On the other hand, samples S1 and S4 were higher than others, indicating the effect of PHP's high water-holding capacity. A similar effect was observed by Pejcz et al. (2018) when psyllium was added to the gluten-free bread formula. The ash contents of the samples were found to be almost at the same values since the addition of the gums was very low; there were not any significant differences among the samples. Considering protein, S1 had the highest protein ratio among all the samples. This situation showed that adding only PHP into the formula as a hydrocolloid resulted in the highest protein amount since PHP includes approximately 2.5% of protein (Aldughpassi et al. 2021). Therefore, adding 3% of PHP resulted in a slight increment in protein ratio, which also agreed with other research (Fradinho et al. 2020). On the other hand, although individual usage of psyllium (S1) caused a slight decrease in carbohydrate content, this did not reflect in energy values.

3.2. Colour analysis

The colour parameters (L^*, a^*, b^*, and colour intensity) of the uncooked noodle samples are presented in Table 3. As it can be seen, the colour of the gums slightly affected the colour and brightness of the noodle samples. All the samples had higher L^* values, which might increase the tendency of consumers to consumption of them since bright colours of noodles are generally desirable by consumers (Srikaeo et al. 2018). However, when the samples were examined individually, S1 (%3 PHP) was recorded with the lowest value of *L** colour (less lightness), which was significantly different from the other samples. On the other hand, redness (*a**) was also increased for the same sample while lightness decreased. The same observation was noted with Peressini et al. (2020) and Renoldi et al. (2021), where *a** increased with the increment of PHP ratio in the pasta formula. Ziemichód et al. (2019) expressed that PHP addition to the gluten-free bread as a hydrocolloid darkened the crumb colour owing to its phenolic component. In terms of yellowness, there was no significant differences between the samples. The addition of locust bean gum increased the yellowness of noodles, and Yalcin and Basman (2008b) also obtained similar results when transglutaminase and locust bean gum were used together in noodle formulations.

Table 3. Colour measurements of noodle samples

Samples	L^*	a^*	b^*	Chroma	*Hue*
S1	$91.08±0.14^{b}$	$2.12±0.03^{a}$	$11.96±0.28^{a}$	$12.15±0.29^{a}$	$79.99±0.11^{d}$
S2	$91.65±0.07^{a}$	$1.89±0.01^{bc}$	$11.88±0.04^{a}$	$12.03±0.05^{a}$	$81.00±0.03^{b}$
S3	$91.85±0.27^{a}$	$1.83±0.05^{c}$	$12.20±0.39^{a}$	$12.33±0.40^{a}$	$81.48±0.05^{a}$
S4	$91.71±0.08^{a}$	$1.93±0.01^{bc}$	$11.53±0.00^{a}$	$11.70±0.01^{a}$	$80.51±0.03^{c}$
S5	$91.97±0.04^{a}$	$1.93±0.04^{bc}$	$11.61±0.12^{a}$	$11.77±0.13^{a}$	$80.57±0.07^{c}$
S6	$91.80±0.01^{a}$	$2.00±0.03^{ab}$	$11.93±0.05^{a}$	$12.09±0.06^{a}$	$80.52±0.09^{c}$

* Means followed by different letters in the same column are significantly different ($p<0.05$).

3.3. Cooking Quality of Noodle Samples

The cooking quality of noodles is an essential parameter for consumer acceptance and industry, which is generally assessed by measurement of weight increase (WI), volume increase(VI) and cooking loss (CL). Since low CL and high volume and weight increase are preferred and evaluated as high cooking quality, these features are examined for the noodle and pasta samples (Zhou et al. 2013). According to Table 4, all noodle samples had a significant effect ($p < 0.05$) on WI, VI, and CL of all the samples. WI and VI values of all noodles were found to be 88.24- 113.56% and 105.00- 141.22%, respectively. Among the samples, S3 showed the highest volume, weight increase, and lower cooking loss after cooking. Tan and her co-workers also examined the similar effect of LBG when they added 1.5% LGB into salt-free noodles (Tan et al. 2018). This lowest cooking loss effect of LBG was associated with gum's ability to help gel formation of the noodle network; thus, noodles were stable during the cooking process (Han et al. 2011).

On the other hand, the lowest WI, VI and highest CL were found for S6, which was a combination of all three hydrocolloids. These results showed that %1 addition of each hydrocolloid might not be applicable for gluten-free noodle production, although individual usage of each hydrocolloid had better values than combinations. Since noodles' preferred cooking loss is at most 12%, S6 might not be evaluated as good quality noodles (Ugarcic-Hardi et al. 2007). Moreover, this CL value of S6 might indicate the lower structural strength of noodles and this was correlated with firmness results (refer to Fig 1.) (Sutheeves et al. 2020). On the other hand, the CL value of S1 was the second highest value, which shows that PHP might not be an alternative for LBG or GG. Although its swelling capability and thickening ability was stated higher in dough systems, it did not present the WI and VI values in noodle as high as with LBG and GG. However, WI and VI values of S5, which was higher than S1, pointed out that combining PHP with LBG enhanced the cooking quality of noodles.

Table 4. Cooking properties of gluten-free noodles

	Weight Increase WI (%)	Volume Increase VI (%)	Cooking Loss CL(%)
S1	$93.98±0.82^{bc}$	$111.25±1.77^{bc}$	$11.63±0.66^{ab}$
S2	$97.87±0.60^{bc}$	$123.75±1.77^{abc}$	$8.51±0.36^{bc}$
S3	$113.56±5.97^{a}$	$141.22±7.69^{a}$	$7.64±0.70^{c}$
S4	$104.92±1.34^{b}$	$105.83±0.60^{c}$	$9.79±0.76^{bc}$
S5	$112.80±1.44^{a}$	$131.25±8.84^{ab}$	$8.23±0.01^{c}$
S6	$88.24±3.44^{c}$	$105.00±7.07^{c}$	$14.56±1.44^{a}$

* Means followed by different letters in the same column are significantly different ($p<0.05$).

3.4. Texture Analysis

In addition to less cooking loss and high cooking yield values, the cooking quality of noodles is generally assessed with high firmness (Marti et al. 2013). As presented in Figure 1, the firmness of noodles was significantly ($p < 0.05$) affected by hydrocolloid type and features used for the formula. The amount and type of hydrocolloids influence starch gelatinisation and protein-starch network in noodles; thus, the structure of noodles and the release of solid components in cooking water are varied (Gasparre and Rossel 2019). Furthermore, it can be noted that the firmness results were compatible with cooking quality results, where the highest score was obtained for the sample containing 3% LBG (S3). However, adding PHP in gluten-free noodle formulation lowered the firmness results, which Fradinho and her co-workers previously observed while examining the effect of PHP in gelatinised and non-gelatinised noodle production (Fradinho et al. 2020). A combination of GG and PHP (S2) in the noodle showed higher firmness than PHP alone (S1). Kaur et al. (2015) examined higher firmness values for GG than xanthan gum when these gums were added to corn noodles. However, in this study, LBG addition presented better values than GG.

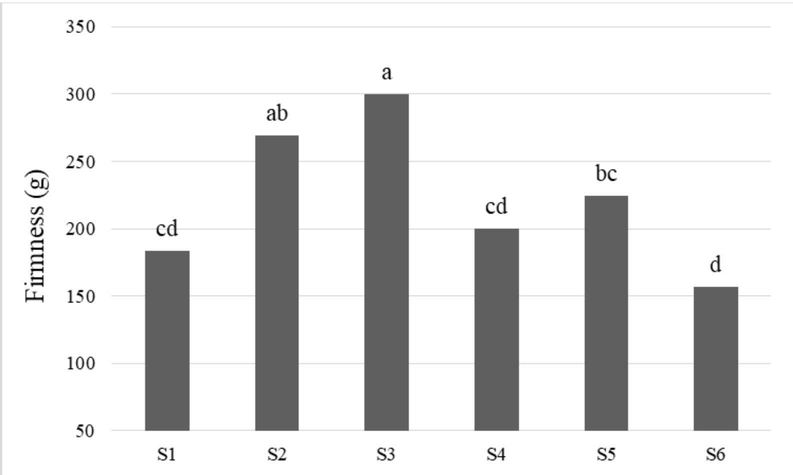

Figure 1. Firmness of noodle samples

On the contrary, the triple combination of LBG, GG and PHP decreased the firmness of sample S6, which has already been observed in cooking quality results. Therefore, this analysis confirmed that the combination of these three hydrocolloids did not synergistically impact of noodle quality. However, instead of applying LBG and PHP together in gluten-free noodle production might be more suitable than just PHP addition as a hydrocolloid in the formula. Thus, this implementation may increase the dietary fibre and amino acid quality by lowering the glycaemic index of the gluten-free noodles. On the other hand, Renoldi et al. (2021) obtained higher firmness values of spaghetti when the PHP ratio in the formula was increased. It is noteworthy that the cooking quality might improve if the PHP addition rate increases in the noodle formula.

3.5. Sensory analysis

The sensory analysis was applied to all formulations with different combinations of the hydrocolloids and PHP regarding colour, cohesiveness, chewiness, taste, odour and overall acceptability. As shown in Figure 2, samples were scored close to each other and the sensory analysis was insignificantly affected by different formulations. There was not a significant difference in all sensory criteria. The colour, cohesiveness, chewiness, taste, odour and overall acceptability scores ranged between 6.6-6.9, 4.5-5.3, 5.9-6.6, 5.5-6.5, 5.8-6.3, 5.5-6.3 respectively. These values were expected since hydrocolloids and PHP do not have a distinct colour, odour and taste. Beikzadeh et al. (2016) that the overall acceptability values were between 3.68 - 4.76 when the PHP level reached a 15% maximum. Furthermore, in that study, it was stated reported that there were no differences between PHP added and control cake regarding all sensory attributes.

On the other hand, Zandonadi et al. (2009) pointed out that PHP addition to gluten-free bread did not change the consumer choice for accepting the new product. Moreover, they found that this gluten-free bread with PHP was not preferred by CD patients when it was compared with the commercial gluten-free products.

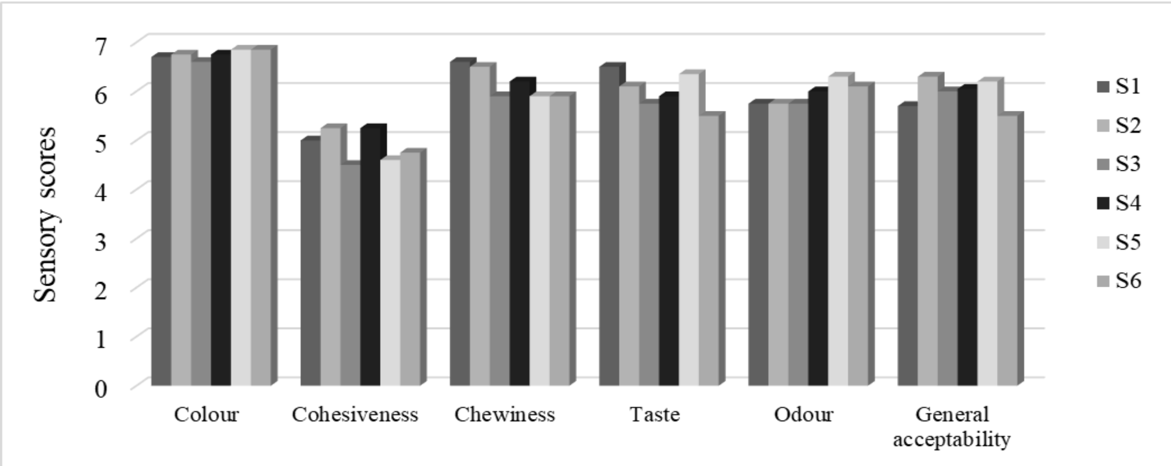

Figure 2. Sensory evaluation of noodle samples

4. Conclusions

This study revealed the effects of PHP utilisation combined with or without GG and LBG on some properties of Turkish gluten-free noodle samples. The results showed significant changes in cooking and textural properties rather than chemical, physical and sensory properties. Data obtained from the cooking quality and textural analyses had consistency and suggested that either a combination of LBG–PHP or GG-PHP might be used in the formulations. As a result, it was seen that the combination of PHP and LBG or GG in gluten-free noodle production could be appropriate without adversely affecting the quality of the product. We hope our findings about PHP offer an alternative to industrial gums such as guar gum and locust bean gum. Future studies can be carried out to investigate the usage of PHP in different forms and ratios in order to increase the usage rate of PHP in Turkish-type noodle samples. In addition, its application with other gums other than guar and locust bean gums in gluten-free noodle production may be investigated accompanied by advanced techniques.

Author Contributions: The authors have an equal contribution. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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Feed Quality of Sweet Sorghum Grains

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HIGHLIGHTS

- Considering global climate change, sorghum has become an increasingly important crop in terms of tolerance to drought, high temperatures and salinity.
- While sorghum herbage constitutes the roughage source of ruminant animals in many countries of the world, its grain is included in both human nutrition and concentrated feed rations of animals.
- In this study, we tried to determine the nutritional properties of grains of some sweet sorghum varieties.

Abstract

Sweet sorghum crop produces grains at its ear-head which can be utilised as food and feed, produces stalks for the production of syrup, bio-ethanol, bio-diesel, bagasse and green foliage for use as organic fertiliser and excellent animal fodder. Approximately 4000 sweet sorghum cultivars are spread over the globe, providing a broad and varied genetic foundation for the development of highly productive, region-specific cultivars. A study was conducted to assess the grains of selected 10 different sweet sorghum varieties for the feed quality characters. For this aim, ADF, NDF, ADL, DMD, DMI, TDN, RFV, DE and ME values of sweet sorghum grains were determined. As a result of the study, crude ash, CP, ADF, NDF, ADL, DMD, DMI, RFV, TDN, DE and ME values were determined as 0.19-3.74%; 9.03-11.05%; 10.0-24.6%; 24.3-38.0%; 1.07-9.13%; 69.69-81.09%; 3.16-4.94%; 170.5-309.5%; 63.85-74.84%; 3.25-3.74 MJ/kg and 9.85-11.78 Mcal/kg, respectively. Variety "Smith" stands out with its ADF, NDF and ADL characteristics. Gülşeker, USDA-Taiwan and Erdurmuş varieties were significant in terms of CP. Ulusoy variety stands out with DMI, TDN, RFV, DE and ME.

Keywords: Sweet sorghum; *Sorghum bicolor*; grain quality.

1. Introduction

Warm-season tropical grass *Sorghum bicolor* [L.] Moench is the most well adapted species of cereal grass that survive in arid climates. Sorghum uses more water efficiently and grows longer, denser, and longer roots to sustain a physiological activity that is similar to that of plants with enough moisture in the soil. Sweet

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sorghum (*Sorghum bicolor* [L.] Moench) yields less grain than other sorghums, but the stem of this plant has a significant amount of easily fermentable sugars. In comparison to maize, sweet sorghums yield 23% more fermentable carbohydrates, require 37% less nitrogen fertiliser, use 17% less irrigation water, and may produce more ethanol in a dry year. Approximately 4000 sweet sorghum cultivars are spread over the globe, providing a broad and varied genetic foundation for the development of highly productive, region-specific cultivars (Rutto et al. 2013). Sorghum is a significant grain crop that is fed to cattle, humans, and ethanol plants to produce biofuel. Sorghum's ability to withstand high temperatures, droughts, and salt will probably make it increasingly significant in the context of global climate change. Sorghum is an intriguing feed option for salty soils in arid and semi-arid locations due to its resistance to salt and drought (Fahmy et al. 2010). In addition to grain as food for human and feed for non-ruminant and ruminant livestock, sorghum stover is an important source of roughage for ruminants in many developing countries of the world (Singh et al. 2017).

Similar to grain sorghum, sweet sorghum has stalks that are high in sugar. It is a crop with many uses that can be grown in tandem to produce grains from its ear-head for use as food and feed, sugary juice from its stalks for the production of syrup, bio-ethanol, and bio-diesel, and bagasse and green foliage for use as organic fertiliser, paper manufacturing, and excellent animal fodder (Ray and Behera 2011). According to Almodares and Hadi (2009), sweet sorghum yields 3-7 tonnes of grain and 54-69 tonnes of stalk per hectare.

Some of the many positive traits of sweet sorghum include resilience to salinity, drought, and waterlodging, as well as a high biomass output. Sweet sorghum is a C_4 crop that has a good photosynthetic efficiency as well. Therefore, the advancement of sweet sorghum will be crucial to the growth of animal husbandry, agricultural output, and energy sources. Sweet sorghum's primary nonstructural carbohydrate in the grain is starch, whereas the primary carbohydrate in the stalk-which is the predominant form carried within the plant-is sucrose. The best seeds for producing starch are white or pale yellow. The main sugars found in sweet sorghum grains include maltose, sucrose, raffinose, glucose, and fructose. Studies on sweet sorghum grain plus stem have demonstrated higher yields of fermentable carbohydrates compared to other fuel crops (Murray et al. 2008b). Furthermore, high fructose syrup and animal feed can be made from the grain (Ebadi et al. 1997). Sorghum grain is a vital cereal to grow in tropical and subtropical regions. There might be less demand for imported maize if its cultivation is expanded and used in the diets of animals and poultry (Azarfa et al. 1998).

To ascertain the nutritional value of sorghum, 36 sorghum grain (SG) cultivars and one type of maize were grown at the same location in the study carried out by Ebadi et al. (1997). The nutritional makeup of the grains was examined. The SG's ash, crude protein (CP), phosphorous (P), and acid detergent fibre (ADF) contents were 1.72±0.54, 11.6±1.18, 0.34±0.03, and 8.35±3.93, respectively, according to the results of proximate analyses. SG's ether extract (EE) was lower than that of imported and Iranian corns. The quantities of CP (10.7%), EE (5.9%), and CF (3.3%) in Iranian maize were higher than those in imported maize (7.8, 4.2, and 2.25). The levels of tannin in the high and low SG cultivars were 0.998% and 0.021%, respectively. The increase in tannin levels resulted in a decrease in the apparent and true metabolizable energy (AME and TME) of SG. Significant variations were seen in the levels of TMEn (3853, 3771, and 3213 Kcal/kg) between the low, medium, and high tannin sorghum (LTS, MTS, and HTS) cultivars. Nonetheless, the maize showed greater TME levels (3853, 3771, and 3213 VS. 3947 Kcal/kg) than the SG. Of all the cereals, LTS had the highest AME (3453 Kcal/kg), while MTS (3458 Kcal/kg) and the two maize grains (3406 Kcal/kg) did not differ significantly from one another.

The objective of this study was to assess the grains of selected sweet sorghum varieties for the feed quality characters.

2. Materials and Methods

The Seeds of sweet sorghum (*Sorghum bicolor* [L.] Moench) varieties (Ulusoy, Sorge, Gülşeker, USDA-Tayvan, Smith, Cowley, Tracy, Uzun, MSI-E, Erdurmuş) were obtained from XXX University, Faculty of Agriculture, Department of Field Crops (XXX, Turkiye).

Seeds of the sweet sorghum varieties were ground in a mill and passed through 1 mm for chemical analysis. Crude ash ratio of sweet sorghum grain samples was determined by burning at 550 °C for 8 hours. Crude protein analyses were performed by the methods specified in AOAC (1990). The ADF, NDF and ADL constituting the cell wall were performed by the method specified in Van Soest (1963) and Van Soest and Wine (1967). Relative feed value (RFV), dry matter digestibility (DDM) and dry matter intake (DMI) of sweet sorghum grains were calculated according to the following formulas (Rohweder et al. 1978).

DMD % = 88.9 - (0.779 x ADF %);

DMI % of BW = 120 / NDF %;

RFV = (DDM % x DMI %) /1.29

The TDN and ME values of the seeds of sweet sorghum cultivars were determined according to the method specified by Moore and Undersander (2002), and the DE value was determined by Fonnesbeck et al. (1984) according to the method specified.

The data obtained from the features examined in the study were analyzed in the Jump-Pro13 statistical package program according to the randomized block trial design, and the differences between the averages were compared according to the LSD (0.05) test. Scatterplot biplot and scatterplot matrix graphics were generated to determine the relationship between features were obtained from the Genstat 12th (Genstat 2009; VSN international 2021) program, and the correlation table was obtained from the Jump-Pro13 package program. Interpretations of the graphs were made according to Yan and Tinker (2006).

3. Results and discussion

Crude ash, crude protein, ADF, NDF, ADL, DMD, DMI and TDN ratios and RFV, DE and ME values determined in the seeds of some sweet sorghum cultivars were found to be statistically significant at the 1% level (Table 1).

Table 1. Means of the examined characteristics

Varieties	CA (%)	CP (%)	ADF (%)	NDF (%)	ADL (%)	DMD (%)	DMI (%)	RFV	TDN (%)	DE (MJ/kg)	ME (Mcal/kg)
Ulusoy	2.07 d	9.72 d	10.8 ef	25.9 de	1.96 c	80.44 ab	4.94 a	288.6 bc	74.21 ab	3.71 ab	11.78 a
Sorge	1.55 e	9.03 f	10.0 f	24.8 ef	1.23 e	81.09 a	4.83 ab	303.9 ab	74.84 a	3.74 a	11.78 a
Gülşeker	1.56 e	11.05 a	10.4 ef	24.3 f	1.56 d	80.77 ab	4.63 bc	309.5 a	74.54 ab	3.73 ab	11.73 ab
USDA-Tayvan	2.24 c	10.67 b	12.0 de	27.5 cd	1.16 e	79.53 bc	3.16 g	269.1 cd	73.34 bc	3.67 bc	11.67 ab
Smith	2.46 b	9.72 d	24.6 a	38.0 a	9.13 a	69.69 f	4.29 e	170.5 f	63.85 f	3.25 f	11.52 bc
Cowley	0.19 g	9.74 d	10.0 f	26.3 de	1.43 d	81.08 a	4.36 de	286.8 bc	74.84 a	3.74 a	11.52 bc
Tracy	1.63 e	10.03 c	13.7 c	31.6 b	2.67 b	78.21 d	3.71 f	230.0 e	72.06 d	3.62 d	11.32 cd
Uzun	0.88 f	9.46 e	15.5 b	32.3 b	1.44 d	76.80 e	4.27 e	220.9 e	70.71 e	3.56 e	11.30 d
MSI-E	0.97 f	10.05 c	13.5 cd	28.1 c	2.06 c	78.36 cd	3.79 f	259.4 d	72.21 cd	3.62 cd	11.06 e
Erdurmuş	3.74 a	10.57 b	12.0 de	25.9 de	1.07 e	79.55 bc	4.56 cd	264.9 d	73.36 bc	3.67 bc	9.85 f
Mean	**1.73**	**10.01**	**13.2**	**28.7**	**2.37**	**78.55**	**4.25**	**260.3**	**72.40**	**3.63**	**11.35**
LSD (0.05)	**0.081****	**0.086****	**0.777****	**0.783****	**0.082****	**0.605****	**0.124****	**9.678****	**0.584****	**0.025****	**0.102****
CV (%)	**5.232**	**1.050**	**7.153**	**3.308**	**4.219**	**0.942**	**3.529**	**4.551**	**0.980**	**0.826**	**1.057**

**; significant at the P≤0.01 level. There is no statistical difference between the averages shown with the same letter.

The crude ash and crude protein ratios of the seeds of sweet sorghum cultivars varied between 0.19-3.74% and 9.03-11.05%, respectively. Crude ash and crude protein ratios of sweet sorghum seeds showed great differences between varieties; While the highest crude ash and crude protein ratios were obtained in Erdurmuş and Gülşeker cultivars, respectively, the lowest crude ash and crude protein rates were found in Cowley and Sorge cultivars. In the researches on the raw ash and crude protein contents of sorghum; the crude ash and crude protein ratios were obtained as 1.4-1.6% and 12.1-14.1%, respectively, in sorghum hybrid grains (Isa 2009), as 5.9-9.1% and 18.6-25.2%, respectively, in different sorghum varieties forage (Zhapayev et al. 2015), as 4.8% and 5.1%, respectively, in Ceres-81 sweet sorghum variety (Macedo et al., 2017), as 1.03-2.94% and 10.90-

14.97%, respectively, in sorghum grain (Ebadi et al. 2019), as 1.57% and 11.58%, respectively, in Kisra sorghum cultivar (Mustafa et al. 2021).

ADF, NDF and ADL ratios of the seeds of sweet sorghum cultivars varied between 10.0-24.6%, 24.3-38.0% and 1.07-9.13%, respectively. ADF, NDF and ADL ratios of sweet sorghum seeds differed between cultivars. While the highest ADF, NDF and ADL ratios were obtained from Smith variety; the lowest ADF rate was found in Sorge and Cowley cultivars in the same group, the lowest NDF rate was found in Gülşeker cultivars, and the lowest ADL rate was found in Sorge, USDA-Taiwan and Erdurmuş cultivars, which are in the same group statistically. In the study examining the effects of plant growth regulators on the nutritional values of sweet sorghum, it was reported that the NDF ratios of Cerse-81 sweet sorghum variety ranged from 42.7-73.2 g/kg DM (Macedo 2017). On the other hand, crude fiber ratios were obtained as 2.0-2.7% in sorghum hybrid grain (Issa 2009), as 2.0-2.7% in sorghum grain (Vila-Real et al., 2017), as 1.98-8.99% in different varieties of sorghum kernels (Ebadi et al. 2019), and as 1.68% in Kisra sorghum variety (Mustafa et al. 2021).

DMD and DMI ratios and RFV values of sweet sorghum seeds differed statistically by 1% between cultivars and varied between 69.69-81.09%, 3.16-4.94% and 170.5-309.5%, respectively. While the highest DMD rates were obtained from Sorge, Cowley, Gülşeker and Ulusoy cultivars, which are statistically in the same group, the highest DMI rates were obtained from Ulusoy and Sorge cultivars, and the highest RFV values were obtained from Gülşeker and Sorge cultivars. The lowest DMD rate and RFV value were determined from Smith cultivar, and the lowest DMI rate was determined from USDA-Taiwan cultivar. In the study examining the effects of plant growth regulators on the nutritional values of sweet sorghum, it was reported that the IVD value ranged between 554.0-689.9 g/kg DM (Macedo 2017). On the other hand, it has been reported that the apparent dry matter digestibility and the true dry matter digestibility values in different varieties of sorghum kernels vary between 44.62-76.96% and 64.74-97.52%, respectively (Ebadi et al. 2019).

TDN ratio, DE and ME values of sweet sorghum seeds differed statistically at the level of 1% between cultivars. TDN ratios and DE and ME values of seeds of sweet sorghum cultivars varied between 63.85-74.84%, 3.25-3.74 MJ/kg and 9.85-11.78 Mcal/kg, respectively. While the highest TDN ratios and DE values were obtained from Sorge, Cowley, Gülşeker and Ulusoy cultivars, which are in the same group, the highest ME values were obtained from Ulusoy and Sorge cultivars. The lowest TDN rate and DE value were determined from Smith cultivar, and the lowest ME value from Erdurmuş cultivar. While it has been reported that the available energy content of sorghum grain varies between 9.73-16.08 MJ/kg DM (Black 2001), the apparent metabolisable energy value of different varieties of sorghum kernels has been reported to vary between 2616-3680 g/100 g (Ebadi et al. 2019). On the other hand, it has been reported that ME and apparent metabolisable energy values in sorghum hybrid grain ranged between 3.29-3.59 Mcal/kg and 13.88-14.46 MJ/kg, respectively (Issa 2009).

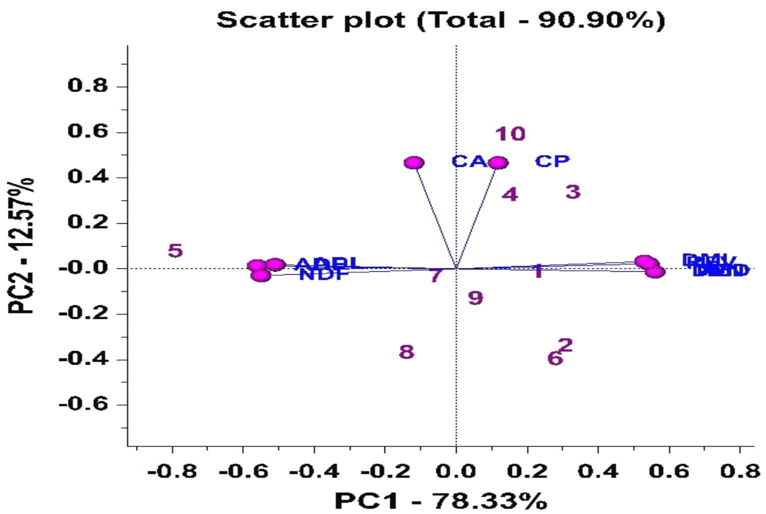

Fig. 1. Vector representation of the relationship between the features examined in terms of average data

With the aid of scatterplot biplot graphics, the relationship between the variations and the characters investigated in the research was visually portrayed. In these graphs, PC-1 represents the productivity of the varieties and PC-2 represents the stability of the varieties (Yan et al., 2000). For this reason, it is desired that an ideal variety should have a high PC-1 value in terms of these characters and a PC-2 value close to zero (Farshadfar et al. 2013). In the research, the total variation between varieties and features was 90.90%, and 78.33% of this rate was PC-1, while 12.57% was PC-2. In this graphic obtained visually with vectors, the narrowing of the angle view between the vectors (DMD, TDN, DMI, RFV, DE and ME, ADL, ADF and NDF) indicates that the relationship between the features is positive and high, and the widening of the angle (ADF and CP, ADF and CA, ADL and CP, ADL and CA, NDF and CP, NDF and CA, CP and NDF) indicates that the relationship is weak, and the angle is greater than 90 0C (as in DMI and CP) indicating a negative relationship. The weak relationship between these characteristics is the cause of the distances of the vectors expressing the features and these vectors from the coordinate plane's centre point (Abate et al. 2015). The ordinate plane can be used to display the inverse relationship between the CA, ADF, NDF, and ADL ratios and other features.

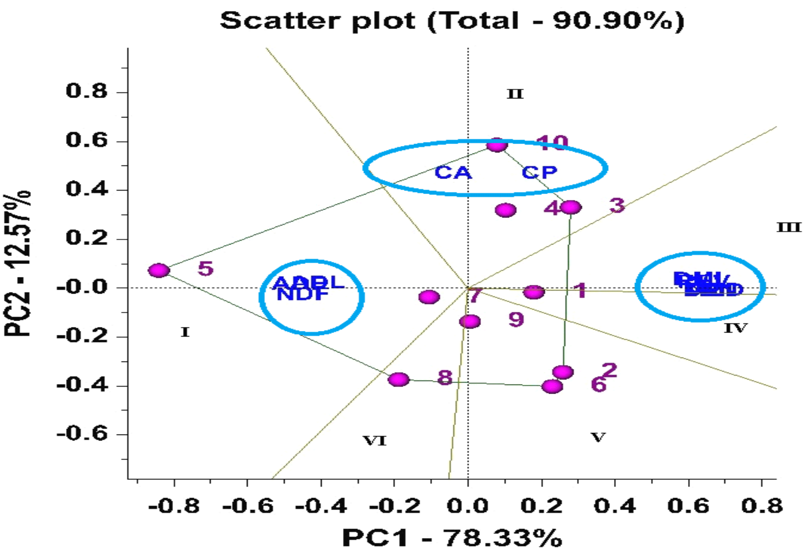

Fig. 2. Illustration of the relationship between the examined features in terms of average data by sectors, polygons and mega environments

The most optimal variety in terms of the characters in each sector are those found at its centre, as seen in Figure 2's sector chart, which uses straight lines to split the graph into parts from the centre to the graphic corners on the x-axis of the coordinate plane. In our research, there are 6 sectors, and in the first sector, variety number 5 (Smith) stands out with its ADF, NDF and ADL characteristics, and in the second sector, varieties number 3 (Gülşeker), 4 (USDA-Taiwan) and 10 (Erdurmuş) were significant with CA and in terms of CP, variety 1 (Ulusoy) in the fourth sector came to the fore in terms of DMI, TDN, RFV, DE and ME. Other varieties did not stand out in terms of any feature. There is a positive relationship between features within the same sector. In addition, it can be said that the varieties in the same sector are close to each other in terms of the characteristics in question, while the varieties in different sectors are far from each other. In the graph, it can be seen that in addition to 6 different sectors, there are 3 separate mega environments. The first mega environment consists of ADF, NDF and ADL, the second mega environment is CA and CP, and the third mega environment is DMI, RFV, TDN, DE and ME. Varieties numbered 2 (Sorge), 6 (Cowley) and 8 (Uzun), located at the corners of the polygon, do not stand out in terms of any feature. In the same sector, however, varieties 3 (Gülşeker), 10 (Erdurmuş), and 5 (Smith) made up the most ideal variety in terms of traits. While Kökten et al. (2017) reported a total variation of 80%, our analysis observed a total variation of 83.75%.

Table 2. Pairwise correlation analysis result of the relationship between features

	CA	CP	ADF	NDF	ADL	DMD	DMI	RFV	TDN	DE
CP	0.3745	1.0000								
ADF	0.2107	-0.1777	1.0000							
NDF	0.1585	-0.2285	0.9496**	1.0000						
ADL	0.2028	-0.1757	0.9211**	0.8124**	1.0000					
DMD	-0.2107	0.1777	-1.0000	-0.9496**	-0.9211**	1.0000				
DMI	-0.1491	0.2185	-0.9067**	-0.9905**	-0.7357*	0.9067	1.0000			
RFV	-0.1633	0.2083	-0.9346**	-0.9957**	-0.7738**	0.9346**	0.9973**	1.0000		
TDN	-0.2107	0.1777	-1.0000**	-0.9496**	-0.9211**	1.0000**	0.9067**	0.9346**	1.0000	
DE	-0.2107	0.1777	-1.0000**	-0.9496**	-0.9211**	1.0000**	0.9067**	0.9346**	1.0000**	1.0000
ME	-0.2107	0.1777	-1.0000**	-0.9496**	-0.9211**	1.0000**	0.9067**	0.9346**	1.0000**	1.0000**

*: Significant at P≤0.05 level, **: Significant at P≤0.01 level.

The scatterplot matrix shows how the characteristics used in the study relate to one another. If the scatter plot matrix representing the relationship between any two features appears as a cloud of dust on the regression curve, it can be said that the relationship between the two features is weak or there is no relationship between them. However, if the distribution is regular and clustered on the regression curve, it can be said that the relationship between the two features is strong (Karaman 2022; Ipekesen et al. 2023). In our research, it was determined that there was a linear and strong relationship between DMD and TDN, DE and ME, between TDN and DE and ME, and between DE and ME, and that the distribution was regular on the regression line and the correlation coefficients (r) were equal to 1. In addition, it was determined that the distribution on the regression line expressing the relationship between CA and CP and other features was in the form of a dust cloud and not regular, and the correlation coefficients were low and mostly negative (Table 2; Figure 3).

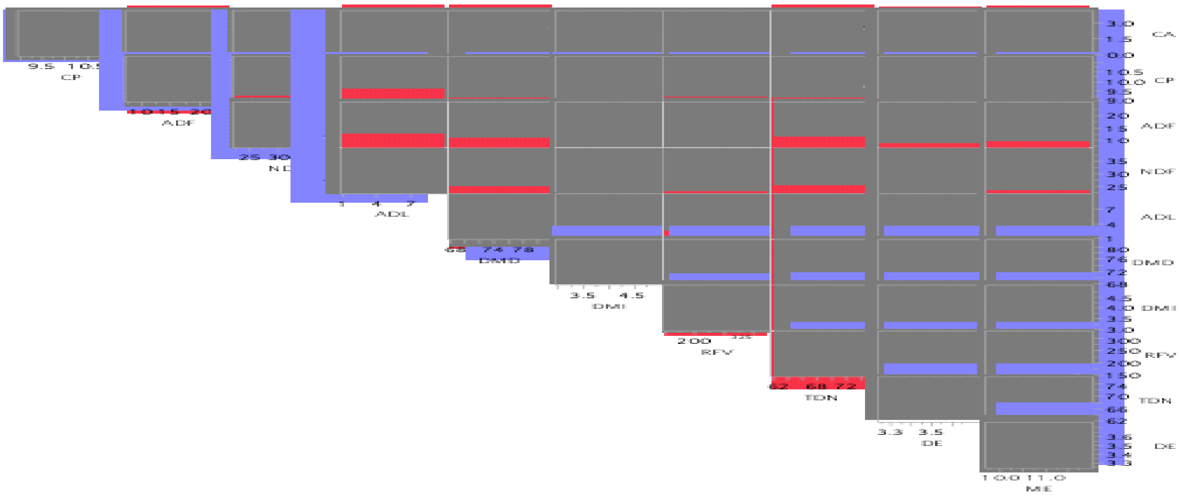

Figure 3. Representation of the correlation between features with scatterplot matrix

4. Conclusions

This Crude ash, CP, ADF, NDF, ADL, DMD, DMI, RFV, TDN, DE and ME values were determined as 0.19-3.74%; 9.03-11.05%; 10.0-24.6%; 24.3-38.0%; 1.07-9.13%; 69.69-81.09%; 3.16-4.94%; 170.5-309.5%; 63.85-74.84%; 3.25-3.74 MJ/kg and 9.85-11.78 Mcal/kg, respectively. Variety Smith stands out with its ADF, NDF and ADL characteristics. Gülşeker, USDA-Taiwan and Erdurmuş were significant in terms of CP. Ulusoy stands out with DMI, TDN, RFV, DE and ME.

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A Study on Determination of Energy Productivity and Greenhouse Gas Emissions in Wheat Production

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HIGHLIGHTS

- In this study, energy productivity and greenhouse gas emissions in wheat production were defined.
- The amount of direct and indirect energy use in wheat production and their shares in total energy consumption were defined.
- The energy use efficiency indicators and total greenhouse gas emissions were defined.
- In order to increase the ratio of renewable energy, non-renewable energy inputs should be reduced and the use of farm manure should be included in wheat production.

Abstract

In this study, energy use efficiency (EUE) and greenhouse gas emissions (GHG) in wheat production were defined, and the energy equivalents (EE) of the inputs in production per unit production area, EUE and GHG values of the product were computed. The data used in the study were obtained from 175 different wheat producing farms in 2021 by conducting face-to-face surveys according to the proportional sampling method. In the study, the amount of direct (DE) and indirect energy (IE) use in wheat production and their shares in total energy consumption were defined. According to the results of the study, total energy input (EI) in wheat production was computed as 19 024.21 MJ/ha and energy output (EO) as 80 585.40 MJ/ha. It was defined that the input with the highest energy consumption was fertilization with a value of 8748.38 MJ/ha. This was followed by seed energy input 4626.79 MJ/ha (24.32%), fuel energy 2697.25 MJ/ha (14.18%), irrigation energy 2362.50 MJ/ha (12.42%), machinery energy 309.52 MJ/ha (1.63%), chemicals energy 269.19 MJ/ha (1.41%), human labor energy 10.58 MJ/ha (0.06%). EUE, energy productivity (EP), specific energy (SE) and net energy (NE) yield values were 4.24, 0.29 kg/MJ, 3.47 MJ/kg and 61561.19 MJ/ha, respectively. Total GHG emission for wheat production was computed as 3784.60 kgCO_{2-eq}/ha. The highest share of total GHG emissions belonged to seed (59.41%). Seed was followed by irrigation (16.84%), nitrogen fertilizer use (14.60%), phosphate fertilizer use (3.99%), fuel use (3.49%), chemicals use (0.98%), machinery use (0.58%) and human labor (0.10%). In addition, the GHG ratio in wheat production was computed as 0.69 kgCO_{2-eq}/ha.

Keywords: Wheat; Energy use efficiency; GHG emission; GHG ratio; Diyarbakır

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1. Introduction

In order for agriculture to contribute to economic growth in a country, it needs to develop rapidly and continuously in accordance with the updated conditions. The desired development in agriculture depends not only on the total capital investments and agricultural supports to be made in the sector, but also on the protection and development of the resources that constitute agricultural production. Due to its strategic importance in all countries, agriculture is a sector that is supported by agricultural policies appropriate to the economic structures of the countries. The main objectives of these supports are to create an organized, highly competitive and sustainable agricultural sector that deals with the economic, social, environmental and international developments entirely for the efficient use of resources (Abay et al. 2005).

Agricultural mechanization is of great importance for increasing productivity in agriculture, reducing production costs, and solving the labor problem that arises due to the aging population. Approximately 35% of production inputs are mechanization inputs (20% mechanization + 15% fuel). Despite this high cost share, mechanization is considered less important than seeds, fertilizers, pesticides and diesel. However, considering that diesel is also a mechanization input, the importantance of the subject emerges (Günindi 2019).

Energy use, GHG emissions and their potential impacts on global climate change are among the most discussed subjects. In this context, more energy use causes significant environmental problems such as GHG emissions that affect human health, so more economical use of inputs becomes important in terms of sustainable agricultural production (Şanlı et al. 2017). In order to increase energy efficiency, it is necessary to either increase yield or reduce inputs. Fuel, chemical fertilizers, pesticides, machinery and tractor inputs, which have a large share in energy inputs, should be reduced (Çelen 2017).

According to researches and other indicators, the origin of wheat is the area between the Euphrates and Tigris rivers, which is called as the southeastern part of Turkey. It is a half-moon shaped geography starting from the southeast of Turkey (from the Taurus Mountains in the southeast), covering Syria, Lebanon and Jordan in the southwest, and the mountainous areas of Iraq and the Zagros Mountains of Iran in the southeast, and extending from these parts to the south (Anonymous 2021a).

World wheat production was around 766 million tons according to the International Grains Council (IGC) 2020-2021 season data. Approximately 66% of the total wheat was produced by the first 10 wheat producing countries. China, which ranked second in wheat production for many years, is the world's largest wheat producer with 136 million tons of wheat production in 2020/21 as a result of increased production and the decrease in production in the European Union. The European Union ranks second with 135.5 million tons, India ranks third with 107 million tons, and Turkey ranks 10th with 18.5 million tons of wheat production (Anonymous 2021b).

Diyarbakir province is one of the few provinces in Turkey in terms of cereals. It ranks third in Turkey in terms of wheat production. Diyarbakir province ranks fourth in Turkey in terms of wheat cultivation area (264000 ha, with 3.3% share), and ranks third in terms of production (845000 tons, with 4.2% share) (Pala et al. 2018).

Studies on EUE and GHG emissions were conducted and continue to be done in the world and in Turkey. Mohammadi et al. (2008), Tipi et al. (2009), Barut et al. (2011), Azarpour (2012), Alipour et al. (2012), Baran and Karaağaç (2014), Baran et al. (2015), Baran (2016), Bayhan (2016), Aydın et al. (2017), Baran et al. (2021), Çelen et al. (2017), Çıtıl et al. (2020), Aydın (2020), Demir et al. (2022), Gökduman et al. (2022), Demir and Gökdoğan (2023), Seydoşoğlu et al. (2023), Turan et al. (2023), Hacıoğlu et al. (2024) conducted studies regarding the subject.

It is important to compare the amount of energy used up for the cultivation of wheat, which is a very important crop in the world and in Turkey, with the energy content of the product obtained at the end of production in terms of EUE of the production system. For this purpose, the processes and inputs used in winter wheat production in Diyarbakır province were examined in detail. The EI used in wheat production were defined by the surveys conducted with the producers in 2021.

2. Materials and Methods

A harsh continental climate prevails in Diyarbakır. Summers are very hot, but winters are not as cold as in Eastern Anatolia Region. The main reason for this is that the Southeastern Taurus arc cuts the cold winds coming from the north. The average of the hottest month is 31 degrees and the average of the coldest month is 1.8 degrees. The average annual precipitation in the city is 496 millimeters and 2% of this precipitation falls in the summer months (Anonymous 2020).

The main material of the study consisted of primary data obtained from face-to-face surveys with wheat producers in Diyarbakır province and its districts. Since it is difficult to conduct a study in all enterprises in the region, the number of surveys was defined by using the proportional sampling method formula to define the number of producers among the farms with the characteristics that we can reach our purpose.

All calculations were performed with the data obtained from wheat producing farms. The data were collected by face-to-face survey technique and all of the surveys were conducted in the farms in Diyarbakır province and its districts. Besides, study, examinations and existing statistical data were also utilized. The collected data were first classified in an appropriate computer software and calculations were completed. Excel tabulation, graphing and analysis software were used to obtain the results from the database and the necessary formulas in the calculations. The following formula was used to define the number of farms to be studied.

In the formula, the P value can be obtained from previous researches or can be estimated intuitively. P = 0.5 should be taken to reach the maximum sample size. Values of P lower or higher than 0.5 reduce the sample size. Therefore, in cases where P is not known, P = 0.5 should be taken since studying with the maxi-mum sample volume will reduce the possible error (Miran 2003; Aksoy and Yavuz 2012). In the formula; n: Sample size, p: Ratio of the producers cultivating wheat (0.50 was taken to reach the maximum sample size), N: Number of the farms in the population, σ2 p: Variance of ratio, r : Deviation from the mean (%5), (According to 95% confidence interval and 0.05 margin of error).

$$n = \frac{(N*p*(1-p))}{(N-1)*\alpha 2p+p*(1-p)} \quad (1)$$

As a result of the calculations, the number of sample farms to be studied was found as 175. The obtained data were collected and grouped in databases to form the calculation parameters to be used in appropriate equations and calculations were performed.

In the study, the EE of the inputs and outputs given in Table 1 were used to calculate the EUE in production, and the GHG emission equivalents given in Table 2 were used to calculate the GHG emission ratio. Energy balance (EB) is given in Table 3, EUE indicators are given in Table 4, energy input types are given in Table 5, and total GHG emissions calculations are given in Table 6. EUE, SE, EP and NE were calculated using the formulas given below (Mandal et al. 2002; Mohammadi et al. 2008; Mohammadi et al. 2010).

$$\text{Energy use efficiency} = \frac{\text{Energy output } (\frac{MJ}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \quad (2)$$

$$\text{Specific energy} = \frac{\text{Energy input } (\frac{MJ}{ha})}{\text{Product output } (\frac{kg}{ha})} \quad (3)$$

$$\text{Energy productivity} = \frac{\text{Product output } (\frac{kg}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \quad (4)$$

Net energy = Energy output (MJ/ha) - Energy input (MJ/ha) (5)

The following equation was used to define the GHG emission (Hughes et al. 2011).

$$GHG_{ha} = \sum_{i=1}^{n} R(i) \; x \; EF(i) \quad (6)$$

GHG_{ha}: GHG emission ($kgCO_{2-eq}$/ha), R(i): Amount of i input ($unit_{input}$/ha), EF(i): GHG emission equivalent of i input ($kgCO_{2-eq}$/unit input).

The GHG ratio is an index defined as the amount of GHG emissions per unit kg of yield. GHG ratio was computed using the equation below (Khoshnevisan et al., 2014; Houshyar et al., 2015).

$$I_{GHG} = \frac{GHG_{ha}}{Y} \quad (7)$$

I_{GHG}: GHG ratio ($kgCO_{2-eq}$/kg) and Y: Yield (kg/ha).

Energy inputs can be classified as direct, indirect, renewable (RE) and non-renewable (N-RE) energies (Mandal et al. 2002; Singh et al. 2003; Koctürk and Engindeniz 2009). Depending on the findings and efficiency indicators defined as a result of the study, solution suggestions were given for the improvement of the current production.

Table 1. EE of inputs and outputs in agricultural production

Inputs	Unit	EE (MJ/unit)	Sources
Human labour	h	1.96	Mani et al. 2007; Karaağaç et al. 2011
Machinery	h	64.80	Singh 2002; Kizilaslan 2009
Combine harvester	h	87.63	Hetz 1992; Çanakcı et al. 2005; Tipi et al. 2009
Chemical fertilizers			
Nitrogen	kg	60.60	Singh 2002; Demircan et al. 2006
Phosphorus	kg	11.10	Singh 2002; Demircan et al. 2006
Chemicals	kg	101.20	Yaldiz et al. 1993; Demircan et al. 2006
Diesel fuel	L	56.31	Singh 2002; Demircan et al. 2006
Irrigation water	m^3	0.63	Yaldiz et al. 1993; Ozalp et al. 2018
Seed	kg	15.07	Singh 2002; Çiçek et al. 2011
Output			
Yield	kg	14.07	Singh 2002; Çiçek et al. 2011

Table 2. GHG emissions equivalents of inputs in agricultural production

Inputs	Unit	GHG emission equivalents ($kgCO_{2-eq}$/unit)	Sources*
Human labour	h	0.700	Nguyen and Hermansen 2012
Machinery	MJ	0.071	Pishgar-Komleh et al. 2012
Nitrogen	kg	4.570	BioGrace-II 2015
Phosphorus	kg	1.180	BioGrace-II 2015
Chemicals	kg	13.900	BioGrace-II 2015
Diesel fuel	L	2.760	Clark et al. 2016
Irrigation water	m^3	0.170	Lal 2004
Seed	kg	7.630	Clark et al. 2016

*Eren et al. (2019)

3. Results and Discussion

3.1. Energy balance

The data collected from the surveyed farms were evaluated and the data used in the calculations were computed by using the equations given in the method section. As a result of the evaluation of the data, the amount of the inputs used in wheat production in Diyarbakır and the yield values are given in Table 3.

Table 3. EB in wheat production

Inputs	EI per hectare (unit/ha)	Energy value (MJ/ha)	Ratio (%)
Human labour	5.40	10.58	0.06
Machinery (Total)	4.47	309.52	1.63
Machinery	3.60	233.28	1.23
Combine harvester	0.87	76.24	0.40
Chemical fertilizers (Total)	248.86	8748.38	45.98
Nitrogen	120.93	7328.36	38.52
Phosphorus	127.93	1420.02	7.46
Chemicals	2.66	269.19	1.41
Diesel fuel	47.90	2697.25	14.18
Irrigation water	3750	2362.50	12.42
Seed	294.70	4626.79	24.32
Total	-	19 024.21	100
Output/Yield	5482	80 585.40	100

According to Table 3, 10.58 MJ/ha of human energy was consumed per unit area for 1 ha area in wheat production, and the ratio of this value to total energy input constituted the lowest input with 0.06%. Among all inputs, fertilizer energy input was computed as the highest with a rate of 45.98%, consuming 8748.38 MJ/ha. This was followed by seed energy input 4626.79 MJ/ha (24.32%), fuel energy 2697.25 MJ/ha (14.18%), irrigation energy 2362.50 MJ/ha (12.42%), chemical energy input 269.19 MJ/ha (1.41%), 309.52 MJ/ha energy was consumed for machinery energy and this value corresponded to 1.63% of total energy input.

When the energy shares in the EE of wheat inputs were ranked, fertilizer energy was the first, diesel energy second, seed energy was the third, irrigation energy was the fourth, machinery energy was the fifth, chemicals (pesticide) energy was the sixth, and human labor energy was the seventh. In the study area, the use of pesticides in wheat production was not intense. EUE indicators in wheat production are given in Table 4.

Table 4. EUE indicators in wheat production

Calculations	Unit	Values
Yield	kg/ha	5482
EI	MJ/ha	19 024.21
EO	MJ/ha	80 585.40
EUE	-	4.24
EP	kg/MJ	0.29
SE	MJ/kg	3.47
NE	MJ/ha	61 561.19

According to Table 4, the total EI value per unit wheat production area was computed as 19 024.21 MJ/ha. The total EO of wheat production was computed as 80 585.40 MJ/ha. The energy use efficiency value was found as 4.24. It EP productivity and NE values were computed as 0.29 kg/MJ and 61 561.19 MJ/ha, respectively.

In EUE studies on wheat, the energy use efficiency values were computed as 3.13 by Shahin et al. (2008) in Ardabil province of Iran, 3.09 by Tipi et al. (2009) in Marmara Region of Turkey, 3.09 by Karaağaç et al. (2011)

in Hacıali district of Adana province in Turkey, 1.76 by Kardoni et al. (2013) in Kuzistan province of Iran, 2.97 by Gökdoğan and Sevim (2016) in Turkey.

In this study, wheat yield per hectare was computed as 5482 kg and in other studies on wheat, the yield was computed as 6357 kg/ha by Shahin et al. (2008), 4346 kg/ha by Tipi et al. (2009), 2587.20 kg/ha by Karaağaç et al. (2011), 4285 kg/ha by Kardoni et al. (2013) and 5237.48 kg/ha by Gökdoğan and Sevim (2016).

In other studies on wheat, Shahin et al. (2008) computed EI as 38,356.39 MJ/ha and EO as 120 097.90 MJ/ha; Tipi et al. (2009) computed EI as 20,653.54 MJ/ha and EO as 63 886.20 MJ/ha; Karaağaç et al. (2011) computed EI as 16 553.94 MJ/ha and EO as 57 985.62 MJ/ha; Kardoni et al. (2013) computed EI as 35605 MJ/ha and EO as 62 989.50 MJ/ha; Gökdoğan and Sevim (2016) computed EI as 25 876.29 MJ/ha and EO as 76 990.96 MJ/ha.

With the net energy value, it is possible to compare the energy values of the farms with a rough approach. The NE yield value was computed as 61,561.19 MJ/ha. In other studies on wheat, net energy values were computed as 81 741.51 MJ/ha by Shahin et al. (2008), 43 232.66 MJ/ha by Tipi et al. (2009), 47 332.26 MJ/ha by Karaağaç et al. (2011), 27 384.50 MJ/ha by Kardoni et al. (2013) and 51 114.67 MJ/ha by Gökdoğan and Sevim (2016).

When DE and IE sources in wheat production were examined, DE sources were computed as 5070.33 MJ/ha and IE sources as 13 953.88 MJ/ha. When evaluated proportionally, DE sources were defined as 26.65% and IE sources were defined as 73.35% (Table 5). RE input was computed as 6999.87 MJ/ha and N-RE energy as 12 024.34 MJ/ha. When evaluated proportionally, RE sources were defined as 36.79% and N-RE sources were defined as 63.21%.

Table 5. Types of EI in wheat production

Energy types	EI (MJ/ha)	Ratio (%)
DE[a]	5070.33	26.65
IE[b]	13 953.88	73.35
Total	19 024.21	100
RE[c]	6999.87	36.79
N-RE[d]	12 024.34	63.21
Total	19 024.21	100

Similarly, in other studies on wheat, Shahin et al. (2008), Tipi et al. (2009), Karaağaç et al. (2011) Kardoni et al. (2013), Gökdoğan and Sevim (2016) defined that DE was more than IE and N-RE energy was more than RE in energy inputs.

3.1. Greenhouse Gas Emissions

Calculations of GHG emissions of wheat production are given in Table 6. According to Table 6, total GHG emissions were computed as 3784.60 $kgCO_{2-eq}$/ha. The highest share in total GHG emissions inputs was seed inputs with a share of 59.41%. This was followed by irrigation inputs (16.84%) and chemical fertilizer inputs (18.59%). The GHG ratio (per kg yield) was defined as 0.69 $kgCO_{2-eq}$/kg. In similar studies, Khoshnevisan et al. (2014) computed total GHG emission as 2711.58 $kgCO_{2-eq}$/ha, Nabavi Pelesaraei et al. (2016) computed GHG emission in kiwifruit production as 1310 $kgCO_{2-eq}$/ha, Mohammadi Barsari et al. (2016) computed GHG emission in watermelon production as 460.41 $kgCO_{2-e}$q/ha, Ozalp et al. (2018) computed GHG emission in pomegranate production as 1730 $kgCO_{2-eq}$/ha.

Table 6. Total GHG emissions in wheat production

Inputs	Unit	Amount per hectare (unit/ha)	Energy value (MJ/ha)	Ratio (%)
Human labour	h	5.40	3.78	0.10
Machinery	MJ	309.52	21.98	0.58
Nitrogen	kg	120.93	552.65	14.60
Phosphorus	kg	127.93	150.96	3.99
Chemicals	kg	2.66	36.97	0.98
Diesel fuel	L	47.90	132.20	3.49
Irrigation water	m^3	3750	637.50	16.84
Seed	kg	294.70	2248.56	59.41
Total	-	-	3784.60	100
GHG ratio (per kg)	-	-	0.69	-

4. Conclusions

As a result of the study, EUE and GHG in wheat production were defined and the EE of the inputs in production per unit production area, the EUE and GHG values of the obtained product were computed. In the study, the amount of direct and indirect energy use in wheat production and their shares in total energy consumption were defined.

According to the results of the study, the total energy consumption in wheat production was computed as 19,024.21 MJ/ha and the energy input as 80 585.40 MJ/ha. It was defined that the input with the highest energy consumption belonged to fertilization with a value of 8748.38 MJ/ha. This was followed by seed energy input 4626.79 MJ/ha (24.32%), fuel energy 2697.25 MJ/ha (14.18%), irrigation energy 2362.50 MJ/ha (12.42%), chemical energy 269.19 MJ/ha (1.41%), machinery energy 309.52 MJ/ha (1.63%), human energy 10.58 MJ/ha (0.06%), respectively.

The EUE, EP, SE and NE values of wheat production were defined as 4.24, 0.29 kg/MJ, 3.47 MJ/kg and 61 561.19 MJ/ha, respectively. Total GHG emission for wheat production was computed as 3784.60 kgCO_{2-eq}/ha. The highest share in total GHG emissions belonged to seeds (59.41%). Seed was followed by irrigation (16.84%), nitrogen fertilizer use (14.60%), phosphate fertilizer use (3.99%), fuel use (3.49%), chemical pesticide use (0.98%), machinery use (0.58%), and human labor (0.10%), respectively. In addition, the GHG ratio in wheat production was computed as 0.69 kgCO_{2-eq}/ha.

The highest energy consumption in fuel-oil input was observed in tillage. Besides, it was seen that fertilizer energy took the second highest place in energy consumption. Therefore, it is thought that different and alternative tillage methods and fertilization methods should be investigated to reduce fuel and fertilizer energy in wheat production.

The use and management of land, which is today's problem, is important for the sustainability of the system. In the early 21st century, greenhouse gases and CO_2 emissions arising from agricultural applications and food safety due to soil environmental degradation caused a rapid increase in the impact of greenhouse gases. There are several carbon emission intensive agricultural applications that stand out. These include ploughing, fertilization, pesticides and irrigation. Careful evaluation is required in order to increase the EUE or decrease the usage of these applications. The conversion of ploughing to non-tillage agriculture, integrated nitrogen management and pest control applications, the improvement of water use by the adaptation of drip irrigation and subsoil irrigation methods will enable to control carbon emissions. Management of water and soil resources, such as improving the control of carbon input from a unit area, increasing its efficiency and reducing losses, is an important strategy (Çelen 2016).

In order to increase the EUE in agriculture, technologies with high EP should be used for the mechanization infrastructure of the farms, tools / machinery with a capacity suitable for the power source should be used, and the necessary power optimization for the enterprise should be provided (Öztürk et al. 2015). In order to increase the ratio of RE, N-RE inputs should be reduced and the use of farm manure should be included in wheat production.

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Farmers' Perceptions of Climate Change Issues in Tetritskaro Municipality, Georgia

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HIGHLIGHTS

- Farmers have more or less similar perceptions of extreme weather events in Tetritskaro, Georgia
- Male farmers mostly have information on climate change issues through their fellow farmers
- Female farmers rely on indigenous knowledge of the local environment
- There is a necessity to develop climate change adaptation-intervention policies in Tetritskaro

Abstract

Agriculture is the traditional and leading field of economy of Tetritskaro Municipality, but it faces the challenge of changing climate. The study examines the perceptions of climate change among male and female farmers in Tetritskaro, including their primary sources of information, chosen adaptation measures, and their respective needs. Climate change data that are available in Tetritskaro focused on characteristic extreme weather events coupled with face-to-face interviews from 254 farmers (male - 53%, female - 47%) was analyzed. The study revealed that men and women have more or less similar perceptions of climate change issues. Male farmers primarily rely on conversations with fellow farmers for information on climate, seasonal prediction, and weather forecasts, while female farmers depend on indigenous knowledge of the local environment. Male and female farmers have adapted to the changes in climate similarly by applying some measures, while the exchange of information between fellow farmers, use of various hail protection products, and crop diversification techniques are more frequent among male farmers. Farmers expressed the need for low-interest loans to purchase agricultural products and equipment and restore/create windbreak zones. Most of the male farmers indicate the need for the introduction of new technologies, while female farmers are more in need of training in agricultural activities. The reliance on the experience of other farmers can be seen as a form of social learning and knowledge sharing. Understanding and respecting these local communication channels and sources of knowledge is important for designing effective extension programs and information campaigns. Addressing the traditional men-women roles and cultural and social norms is critical to increasing the adaptation opportunities of female farmers. The study

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highlights the necessity of developing climate change adaptation policies and interventions in Tetritskaro. The obtained results can be used in other agricultural regions with the same problems.

Keywords: Agriculture, Farmers' needs, Adaptation, Climate change, Development, Tetritskaro Municipality

1. Introduction

Climate change refers to long-term changes in the Earth's climate, including changes in temperature, precipitation, and weather patterns. These changes are predominantly fueled by human activities, notably industrial processes, fossil fuel combustion, and deforestation, which release greenhouse gases like carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) into the atmosphere. These gases trap heat, creating a warming effect known as the greenhouse effect. This warming has various impacts, including rising global temperatures, melting ice caps and glaciers, sea level rise, and a rise in the frequency and intensity of extreme weather events (Easterling 2000; Ossó et al. 2022). Climate change, characterized by an increase in the frequency and intensity of extreme weather events, (Harvey et al.2018; Camila et al. 2019; Balasha and Nkulu 2021; Rasul 2021; Karume et al. 2022; Balasha et al. 2023), is believed to directly impact agricultural production and the well-being of populations worldwide (Pangapanga et al. 2012; Chisale 2013; Ofoegbu et al. 2016; Harvey et al. 2018; Missanjo et al. 2019; Camila et al. 2019; Balasha and Nkulu 2021; Rasul 2021; Karume et al. 2022; Balasha et al. 2023). Anticipated future climate change and the impact of extreme weather events on agriculture escalates the need for socially just timely responses, the development and implementation of measures for adaptation, considering the characteristics of different countries, regions, and communities (Monirul Alam et al. 2017; Adger 2001), where the local farmers can make the greatest contribution. Studies have shown that, when farmers perceive climate change and extreme weather impacts on agriculture, they are more involved in the creation and execution of adaptation plans, as well as the support for policies and programs that aim to solve these problems (Niles et al. 2013; Arbuckle et al. 2013; Bollettino et al. 2020; Nnko at al. 2021). Conversely, when farmers do not perceive these impacts, they may develop or implement inappropriate measures that hinder the adaptation process (Taylor et al. 1988). Therefore, knowing how male and female farmers perceive these impacts and what determines their adaptation strategy (Slegers 2008; Bryan et al. 2009; Mertz et al. 2009; Weber 2010; Zampaligré et al. 2014; Chakraborty et al. 2019; Lee et al. 2019; Buylova et al. 2020;.) can allow us to provide farmers with new opportunities for more targeted all farmers-responsive adaptation policies (Amani et al. 2022; Karume et al. 2022) and to design interventions that are more appropriate for the local context (Balasha et al. 2021; Chuma et al. 2022; Karume et al. 2022).

The purpose of this study is to investigate how farmers in Tetritskaro Municipality in the region of Kvemo Kartli, Georgia, perceive climate change and various extreme weather events. More specifically, we seek to know the most significant extreme events (e.g., strong winds, hail, drought, heavy rainfall, and snowstorms) for Tetritskaro and the most damaging for agriculture. In response to the changing climate, we investigated adaptation measures such as the use of various hail protection products, improvement of irrigation systems, sharing information with fellow farmers, changes in crop types and rotations, use of pesticides, use of fertilizers, changes in crop irrigation, and shifts in the growing season (sowing and harvest dates). As part of the analysis, we examined the differences in perceptions and needs between male and female farmers.

The findings from this study will enhance the comprehension of farmers' knowledge, challenges, and practices. This understanding will, in turn, contribute to enhancing adaptation processes and outcomes, leading to improved responses to climate change and weather extremes in Tetritskaro Municipality, where agriculture is the predominant sector.

2. Materials and Methods

2.1 Study area

The research for this study was conducted in Tetritskaro Municipality, which is located in southeastern Georgia in the Kvemo Kartli region, has only 22500 inhabitants, and occupies 520 km^2 (National 2023) (Figure 1). The municipality is a significant agricultural area with farming, playing a crucial role in the local economy and community livelihoods (Agricultural 2014). Farmers primarily sell their agricultural products both on the local market, in the country's capital, and in nearby Armenia and Azerbaijan (however, the land border with Azerbaijan has been closed in recent years). The municipality of Tetritskaro was selected for this study since agricultural activity is a traditional, leading economic branch of the municipality, on the other hand, the physical-geographical location of the municipality and the difficult terrain determine the peculiarity of temperature and precipitation distribution and the variety of extreme weather events.

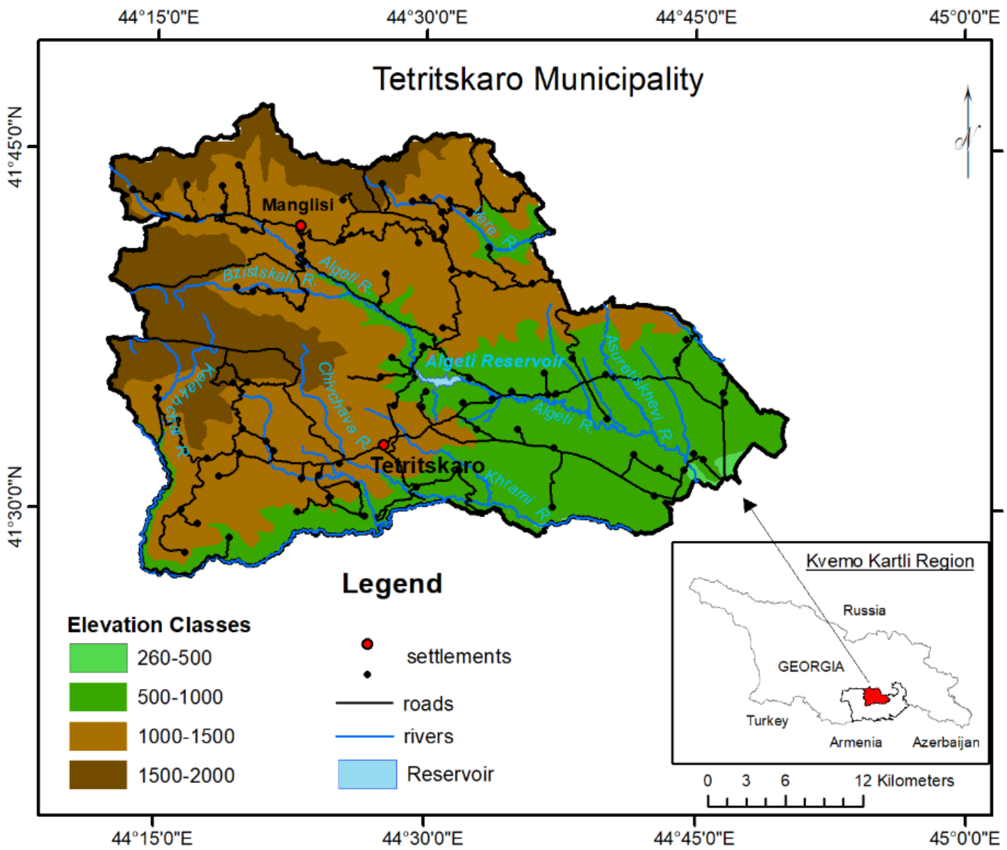

Figure 1. Location of the study area.

The relief of Tetritskaro is characterized by mountainous terrain. A moderately humid climate prevails here with moderately cold winters and long warm summers. Average annual air temperatures in the municipality range from 2 °C in the mountains to 12 °C in the plains (Elizbarashvili 2017). The temperature of the coldest month of the year, January, varies from 0 °C in the lowlands to -11 °C in the mountains, while the temperature of July, the warmest month of the year is 15 °C in the mountains and 25 °C in the lowlands. Average annual precipitations vary from 500 mm in the plains to about 900 mm in the mountains. The maximum precipitations are observed in spring and summer (May 119 mm), and the lowest precipitations – in winter (December 30 mm) (Elizbarashvili 2017; Elizbarashvili et al. 2017).

Air temperatures in Tetritskaro have been increasing by approximately 0.17 °C per decade, precipitation has been decreasing by 5 % per decade over the past several decades (Elizbarashvili et al. 2017).

Strong winds (average > 15 m s-1) and gale-force winds (> 32 m s-1) are the most frequent, long-lasting, and intense events (Elizbarashvili 2017), which can last from several hours to several days. The average number of days with strong winds in the Tetritskaro area is 10. The maximum number of days with strong winds during winter can reach 20. During a strong wind, its highest speed is 28 m s-1in Tetritskaro. However, gale-force winds are also observed in the municipality. Strong winds most often are spread over an area of 500-1000 km^2, its recurrence is 40 %. The recurrence of the occurrence of strong winds in an area of less than 500 km^2 is 25 %, it can also be spread over an area of more than 1000 km^2 (Elizbarashvili 2017). Wind extremes are one of the major processes and problems in the area affecting soil fertility; they also directly damage and destroy crops, causing significant material losses, according to the Agriculture and Rural Development Strategy of Georgia 2021-2027 and Action Plan 2021-2023 (Agriculture 2022). This study also shows similar results according to farmers; the most characteristic extreme weather events in Tetritskaro Municipality are strong winds, and strong winds have the most negative impact on agricultural activities.

Hail events, associated with convective clouds, typically occur in spring and the first half of summer (Elizbarashvili 2017; Elizbarashvili et al. 2014). In Tetritskaro, the largest number of hailstorm days is 14 days a year. In 38 % of cases hail damages the territory with an area of 1 to 3 square kilometers; in 33 % of cases, it damages an area of less than 1 sq. km. An area of more than 3 sq. km is damaged in about 30 % of cases of hailstorms. Rarely, hailstones damage much larger areas, for example, more than 50 square kilometers are damaged in 3 % of cases (Elizbarashvili et al. 2014).

The duration of hailfall is 9-10 minutes. In 60% of instances, hailfall lasts for less than 5 minutes, while in 80% of cases, it lasts less than 10 minutes, and in 3 % of cases, hailfall can last for an hour and a half (Elizbarashvili et al. 2014).

Fog is observed in all seasons, although it is relatively rare in summer. The average number of foggy days in Tetritskaro is 29 days during the winter, spring, and autumn seasons and can in some years exceed 60 days per season. The number of dangerous fog days, when the visibility area is less than 50 m, varies between 7-25 during the year in Tetritskaro (Elizbarashvili 2017).

The average fog duration is 11 hours, and the duration of strong fog (visibility < 50 m) is also 11 hours. The total duration of fog in the Tetritskaro municipality is greatest in winter with an average duration of 315 hours, 295 hours in fall, 260 hours in spring, and only 56 hours in summer. The areas of fog distribution can be several thousands of square kilometers (Elizbarashvili 2017).

According to some previous assessments (Agriculture 2022), fog events affect yield and cause delays in transporting the crops to the market in the Tetritskaro municipality.

Snowstorms are typical of the mountainous regions of Tetritskaro Municipality. The average annual number of snowstorm days is 4, although the largest number of snowstorm days is 12 days. According to the Tetritskaro weather station, during a blizzard, the wind speed may reach 20 m s-1, but speeds of 6-9 m s-1 are most often observed (Elizbarashvili 2017).

Abundant atmospheric precipitations (when their amount is not less than 30 mm per day) occur in the municipality during the warm period of the year, mostly in May and June. The average amount of abundant precipitations is 35-45 mm, and the average duration is 7-12 hours. Abundant precipitations are not frequent and occur on average 4-5 days during the year, nevertheless, extreme precipitations of significant intensity are possible in individual years (Elizbarashvili 2017). For example, the maximum daily precipitations are recorded in Tetritskaro - more than 100 mm.

Dry days are considered the days when the average daily air temperature is higher than 25 °C, atmospheric precipitations are less than 5 mm and the relative humidity is less than 30 %. According to the Atlas of Natural Hazards and Risks of Georgia (Atlas 2012), a severe drought is observed in Georgia once every 15-20 years, and the trend of increasing the frequency of this event is observed both in the territory of Georgia as a whole and in Kvemo Kartli, although according to the same atlas, the drought danger in Tetritskaro Municipality is not high during the vegetation period.

The simultaneous occurrence of the discussed events in the territory of Tetritskaro Municipality is very rare, although some complexes, containing fog, strong wind, and hail, are relatively frequent (Elizbarashvili 2017).

The agricultural insurance project started in Georgia in 2014. The insurance packages available within the project cover losses caused by extreme weather events (for example, natural disasters: floods, storms, and (in the case of citrus) autumn frosts). However, there is a lack of willingness to participate in insurance programs.

There is no tradition of insurance in the agricultural sector of Georgia since farmers do not have proper knowledge and experience regarding these issues. Consequently, this leads them to refrain from insuring their products. In addition, farmers do not trust insurance companies because they are not entirely sure how the insurance company will react in case of the development of negative events. This ambiguity and lack of trust significantly reduce their willingness to participate in the insurance program.

2.2 Research methods

The Law of Georgia on Employment defines *self-employed: "Self-employed - a person working in his enterprise or farm to earn a profit or income (in money or in-kind)"*, according to the Legislative Herald of Georgia. Law of Georgia about employment, Consolidated version (final) (Legislative 2006). According to the Municipal Assessment Report, Tetritskaro (Municipal 2020), there are 263 self-employed farmers, who grow vegetables, fruits, cereals, and other farm crops. We obtained information about these farmers at the municipality's city hall. During the autumn of 2022, the self-employed farmers in Tetritskaro Municipality were interviewed with questionnaires. A total of 254 self-employed farmers from 20 villages were interviewed; 9 farmers refused to participate in the survey or could not be interviewed.

Only self-employed farmers were selected for the research since they are directly responsible for managing agricultural activities and making decisions about the measures required to adjust to climate change and extreme weather events. A face-to-face interview method was used to survey the farmers. Each interview typically lasted between 30 and 40 minutes. We started the survey by introducing ourselves to farmers, explaining the purpose of our study, and offering them to participate in the survey. The questionnaire consisted of closed-ended questions with text-based and yes/no answers, which made it easier for us to collect quantitative data, as well as to categorize and analyze the answers. The face-to-face interviews allowed us to build trust and rapport with farmers, leading to honest and authentic responses, asking follow-up questions, having conversations and discussing their needs, and interests, and clarifying any confusion or misunderstandings.

Information was collected about the age and gender of farmers including the number of years of farming experience they have; how they perceive climate change; extreme weather events; which extreme events are typical for the territory; which extreme weather events have the most negative impact on agricultural activities (together with extreme weather events, we also considered fog, which is characteristic of the territory of the municipality and represents a dangerous weather event); their main sources of information for climate change and extremes as well as for seasonal predictions and weather forecasts; the adaptation measures chosen and implemented in response to climate change and extremes.

For statistical analysis of the survey, the collected information was coded as SPSS data (Landau and Everitt 2004) and then cleaned and processed during February 2023. Descriptive statistics − frequencies, means, and percentages were employed for the data analysis. A chi-square test was used to assess whether any observed differences in perception between male and female farmers are statistically significant, or if they could have occurred by chance. The test involves calculating the chi-square statistics, which measures the difference between the observed and expected frequencies, standardized by the expected frequencies. The calculated chi-square value is then compared to a critical value from the chi-square distribution, based on the degrees of freedom and the selected significance level (usually 0.05). If the chi-square value calculated exceeds the critical value, the null hypothesis (no association between gender and perception) is rejected, indicating a significant association between the two variables.

In the article, tables and graphs are constructed according to the percentage of male farmers and female farmers who answer the following in the survey: 1) **average annual air temperature in the territory of Tetritskaro Municipality:** 1. Increasing; 2. Decreasing; 3. Unchanged; 4. Do not know; 2) **Annual amount of precipitation in the territory of Tetritskaro Municipality:** 1. Increasing; 2. Decreasing 3. Unchanged, 4. Do not know; **3) Which of the following extreme weather events is the most typical for the territory of Tetritskaro Municipality?** 1. Hail, 2. Heavy rainfall, 3. Snowstorm, 4. Strong wind, 5. Drought, 6. Fog; 4) **Climate change and extreme weather events have had a negative impact on agricultural activities:** 1. Yes, 2.

No, 3. Do not know; **5) Which of the following extreme weather events has the most significant negative impact on agricultural activities in the territory of Tetritskaro Municipality?** 1. Hail; 2. Heavy rainfall; 3. Snowstorm; 4. Strong wind; 5. Drought; 6. Fog; **6) What is the main source of information about climate change, and extreme weather events?** 1. Television; 2. Internet; 3. Own observation (indigenous knowledge of the local environment); 4. Conversations/information exchange with other farmers **7) Have you carried out any of the adaptation measures listed below?** 1 - Yes 2 – No

1. Crop diversification,
2. Improvement of irrigation systems,
3. Use of pesticides,
4. Use of fertilizers,
5. Watering crops,
6. Early harvest,
7. sharing information,
8. Early sowing,
9. Use of hail protection products.

This study was approved by the Ethics Committee at the Faculty of Exact and Natural Sciences (TSU) № FR1914993-09-03-20. Informed consent was obtained from all participants and the study complies with all ethical regulations.

3. Results and Discussion

53 % of the farmers participating in the study identified as male and 47 % as female. The average age of all participants was 54 with an average of 20 years of farming experience; the average age of male and female farmers was 53 and 55 years, respectively, each with an average of 22 and 18 years of farming experience.

3.1 Sex disaggregated dimensions of farmers' perceptions of climate change and extreme weather events

Most of the male and female farmers believe that the average annual temperature is increasing, and the average annual rainfall is decreasing in Tetritskaro Municipality, Georgia (Table 1). A chi-square test (> 0.05) showed that male and female farmers perceive the trend of changes in air temperature and atmospheric precipitation in the same way.

Table 1. Perceptions of climate change parameters in Tetritskaro Municipality.

№	Farmers		Average annual precipitation	Average annual temperature
1	Male %	Increasing	0.0	82.2
	Female %		0.0	85.7
2	Male %	Decreasing	91.9	0.0
	Female %		89.9	0.0
3	Male %	Unchanged	5.2	9.6
	Female %		6.7	9.2
4	Male %	Do not know	3.0	8.1
	Female %		3.4	5.0

87% of farmers surveyed in Tetritskaro Municipality (88.9% of male farmers and 84.9% of female farmers) believe that climate change and extreme weather events have negatively affected agricultural activities. A lower percentage of both male and female farmers think that these events do not impact agricultural activities or are uncertain (Table 2). No statistically significant difference exists between the opinions of male and female farmers at the 5% level.

The impacts of climate change and extreme weather events on agriculture are complex and influenced by factors such as the region, type of crop, and farming practices. Severe weather events like strong winds, hail, and storms can destroy crops or significantly reduce their yields, leading to income loss for farmers and food insecurity for the population. Climate change can also diminish soil fertility, as higher temperatures, altered rainfall patterns, and other factors change soil composition and quality. This can make crop cultivation more challenging, requiring farmers to invest more resources to maintain soil health. Furthermore, extreme weather can disrupt transportation networks, hindering farmers' ability to deliver their crops to market on time. The presence of dense fog on roads, for example, can cause significant delays in crop transportation, leading to financial losses for farmers and shortages of food for consumers (Harvey et al. 2018; Camila et al. 2019; Balasha and Nkulu 2021; Rasul 2021; Karume et al. 2022; Balasha et al. 2023).

Table 2. Farmers' perceptions of the negative impact of climate change and extreme weather events on agricultural activities in Tetritskaro Municipality.

№		Farmers	Agricultural activities have been adversely affected by climate change and extreme weather events.
1	Agree	Male %	88.9
		Female %	84.9
2	Disagree	Male %	5.2
		Female %	6.7
3	Do not Know	Male %	5.9
		Female %	8.4

When evaluating the effects of climate change and extreme weather events in Tetritskaro Municipality, farmers considered crop destruction, decreased soil fertility, and delays in delivering crops to the market caused by frequent and dense fog on the roads.

According to farmers, the most characteristic extreme weather events in Tetritskaro Municipality are strong winds, hail, and such dangerous events as fog, and they also believe that strong winds, hail, and fog have the most negative impact on agricultural activities. Indeed, 31.9% of male farmers and 34.5% of female farmers consider strong winds to be the most typical extreme weather event, while the majority of male (37.8%) and female (38.7%) farmers believe that strong winds have the most significant negative impact on agricultural activities. Additionally, 31.1% of male and 30.3% of female farmers see hail as the most typical extreme weather event, with 31.9% of male and 35.3% of female farmers attributing the most significant damage to agricultural activities to hail. Furthermore, 28.9% of male and 27.7% of female farmers view fog as the most typical extreme weather event in Tetritskaro Municipality, while 19.3% of male and 10.1% of female farmers believe that fog has the most significant negative impact on agricultural activity (Figure 2).

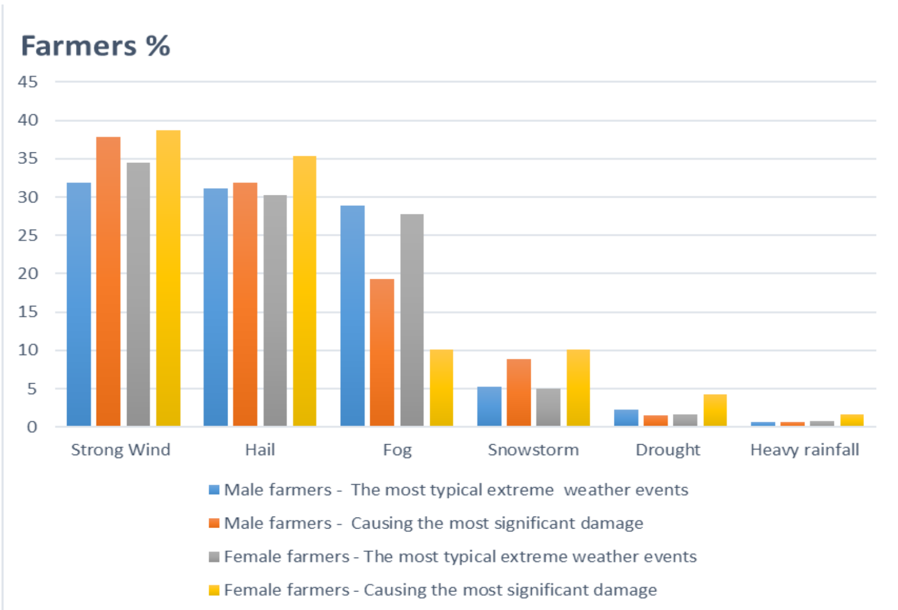

Figure 2. The most typical extreme weather events and extreme weather events cause the most significant damage to agricultural activities in Tetritskaro Municipality.

Far fewer farmers named snowstorms, drought, and heavy rainfall as the most characteristic extreme weather events, and accordingly, far fewer farmers believe that snowstorms, drought, and heavy rainfall have the most negative impact on agricultural activity in Tetritskaro Municipality (Figure 3).

There is no statistically significant difference between the opinions of male and female farmers, except regarding the most significant negative impact on agricultural activity, specifically in the case of fog. This difference may be attributed to local peculiarities and the impact of fog on roads, which will be discussed below.

3.2 Sources of information about climate change, seasonal predictions, and weather forecasts

Farmers relied on various sources of information for updates on climate change, seasonal predictions, and weather forecasts.

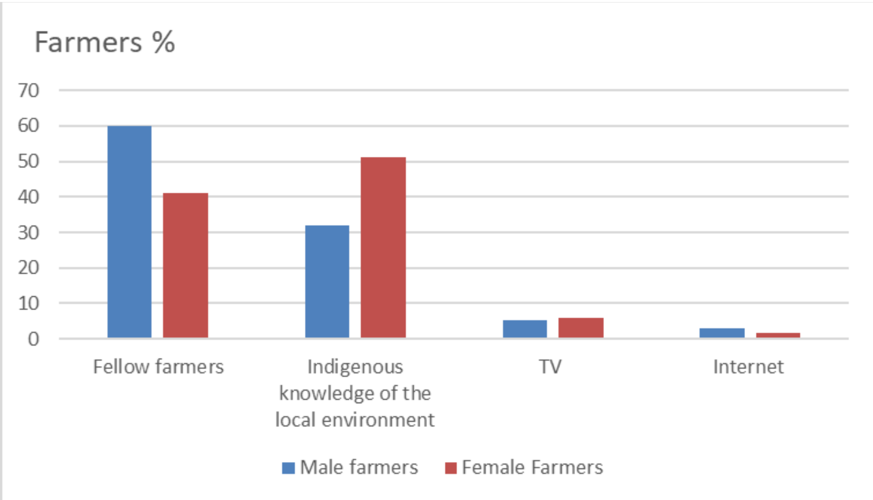

Figure 3. The main sources of information about climate change, seasonal predictions, and weather forecasts among male and female farmers in Tetritskaro Municipality.

Most male farmers (60%) cited conversations with other farmers as their primary source of information on climate change, seasonal predictions, and weather forecasts (Figure 3). On the other hand, the majority of female farmers (51.3%) mentioned indigenous knowledge of the local environment as their main source, such as personal observations of the sky, clouds, and birds, which provide insights into expected weather patterns.

It is important to state while conversations can include the sharing of indigenous knowledge, the two concepts are not interchangeable. Indigenous knowledge is a specific body of knowledge developed by people, often passed down orally from generation to generation. Whereas conversations can involve any type of communication between individuals or groups, indigenous knowledge is not solely based on farmer-to-farmer sharing. Indigenous knowledge is also shaped by a wide range of cultural and environmental factors, including spiritual beliefs, social customs, and historical experiences. It reflects a deep understanding of the relationships between humans and the natural world and is often rooted in local ecosystems and landscapes (Magni 2017).

Only a few male and female farmers rely on the Internet and television for information about climate change, extreme events, seasonal predictions, and weather forecasts. A chi-square test (<0.05) indicated that the primary source of information differs between male and female farmers.

The difference in the main source of information between male and female farmers can be caused by cultural norms, social networks, and communication patterns. Men have a wider circle of friends and get information from them, while women hang around in a narrower circle and therefore, prefer their own observations and indigenous knowledge.

3.3 Male and Female farmers and adaptation practices

We examined the adaptation measures employed by male and female farmers in response to climate change and extreme weather events in Tetritskaro Municipality (Table 3). A notable difference was found in the adoption of several adaptation strategies. Crop diversification is more prevalent among male farmers than female farmers. Male farmers also engage more in information sharing, discussing topics like soil fertility, weather conditions, and agricultural product prices. They are more active in improving irrigation systems and using various hail protection products. However, there were no substantial differences between female and male farmers in the use of some adaptation strategies, such as shifting cropping seasons (sowing and harvest dates), pesticide and fertilizer application, and crop irrigation.

Table 3. Adaptation practices of male and female farmers to climate change and extreme weather events in Tetritskaro Municipality

№	Practices	Male (%)		Female (%)		Pearson Chi-Square
		Yes	No	Yes	No	
1	Crop diversification	88.1	11.9	69.7	30.3	0
2	Improvement of irrigation Systems	14.8	85.2	5	95	0.010
3	Use of pesticides	72.6	27.4	74.8	25.2	0.692
4	Use of fertilizer	77.8	22.2	79.8	20.2	0.690
5	Watering crops	88.9	11.1	91.6	8.4	0.470
6	Early harvest	51.1	48.9	47.1	52.9	0.519
7	Sharing information	62.2	37.8	47.9	52.1	0.022
8	Early sowing	48.1	51.9	54.6	45.4	0.303
9	Use of various hail protection products	53.3	46.7	10.1	89.9	0

3.4 Discussion

3.4.1 Sex disaggregated dimensions of farmers' perception of climate change and extreme weather events in Tetritskaro Municipality

According to the study (Elizbarashvili 2017), average annual air temperatures have been increasing and average annual precipitations have been decreasing in Tetritskaro over the last few decades. Both male and female farmers adequately perceive these changes in climatic parameters, in particular, the trend of decreasing average annual precipitation and increasing average annual air temperatures. The finding that none of the respondents mentioned an increase in precipitation and a decrease in temperature suggests a high level of awareness and observation among the farming community in Tetritskaro.

Several factors could explain why farmers in Tetritskaro municipality can adequately perceive these changes: farmers are often highly attuned to changes in weather patterns and climatic conditions due to their daily interaction with the environment; they may notice changes in rainfall patterns, temperature fluctuations, and other climatic indicators through their farming activities and observations of the natural world; changes in precipitation and temperature can directly affect agricultural productivity and livelihoods. Farmers may be more likely to notice these changes because they have a direct impact on their crops, water availability, and farming practices.

According to Figure 3, in Tetritskaro Municipality, the majority of male farmers name fellow farmers as the main source of information about climate change, seasonal predictions, and weather forecasts, while the majority of female farmers name indigenous knowledge of the local environment as the main source of information. Local and traditional knowledge systems often include observations and practices related to weather and climate. Farmers draw on this knowledge, which has been passed down through generations, to understand and interpret changes in climatic parameters; farmers often rely on information and observations shared within their community networks. Discussions with other farmers, local leaders, and community members can help reinforce awareness of climate change and its impacts.

Farmers perceive and state that strong wind, hail, and fog are the most typical for the municipality, which also corresponds to the data in the previously published literature (Elizbarashvili 2017). Farmers' belief that climate change and extreme weather events adversely affect agricultural activities aligns with current research on global climate change, highlighting its widespread impact on agriculture (Camila et al. 2019; Balasha and Nkulu 2021; Rasul 2021; Karume et al. 2022). Male and female farmers perceive that strong winds, hail, and fog have the most significant negative impact on agricultural activities. Farmers talk about crop destruction, reduced soil fertility, delays in the delivery of crops to the market, and the occurrence of pests. According to historical data, wind erosion is one of the biggest problems affecting soil fertility in Tetritskaro Municipality, according to the Agriculture and Rural Development Strategy of Georgia 2021-2027 and Action Plan 2021-2023 (Agriculture 2022). Strong winds and hail negatively impact agricultural production and yields in the municipality, leading to crop destruction and substantial material losses. (Wheatley 2005; Elizbarashvili 2017).

To address wind extremes and their impacts on soil fertility and crop damage, several precautions can be taken: first, planting trees, shrubs, or other vegetation as windbreaks can help reduce wind speed and protect crops from damage; planting cover crops can aid in shielding the soil from erosion and improve soil fertility, which can be particularly important in areas prone to wind extremes. Increasing soil organic matter through practices such as composting and mulching can help improve soil structure and fertility, making it more resilient to wind erosion; implementing erosion control measures, such as terracing or contour plowing, can help reduce soil erosion caused by wind; planting a variety of crops can aid in mitigating the risk of complete crop failure due to wind damage, as different crops may be more or less susceptible to damage; keeping track of weather forecasts and conditions can help farmers take preventive measures, such as harvesting crops early or securing loose items that could be damaged by the wind; building infrastructure, such as wind-resistant storage facilities or greenhouses, can help protect crops and other assets from wind damage.

Implementing agricultural insurance schemes and risk management strategies can help farmers recover from losses due to extreme weather events.

Farmers should also be aware of practices they should not do, such as overexploiting natural resources, which can further exacerbate environmental degradation and climate change. Additionally, farmers should avoid practices that contribute to greenhouse gas emissions, such as burning crop residues, as this can worsen climate change conditions.

The notable disparity between male and female farmers on fog could be due to local peculiarities. Farmers use the crop both for self-consumption and for sale; during the delivery to the market, fog causes visibility problems on roads and delays traffic. In the region, men primarily drive cars and farm vehicles; therefore, delays caused by fog on the roads are more important for them. In addition, it is important that despite the roadwork carried out in recent years, many highways are in unsatisfactory condition, which, together with extreme events, is an added obstacle to the delivery of agricultural products to the market. Therefore, from this point of view, it is necessary to thoroughly maintain the existing roads in the region.

3.4.2 Sources of information about climate change, seasonal predictions, and weather forecasts

In Tetritskaro Municipality, most male farmers cite other farmers as their primary source of information, whereas most female farmers cite indigenous knowledge of the local environment. Indigenous knowledge is holistic, encompassing not just facts about the environment but also spiritual and cultural aspects. It often includes practices and techniques for sustainable resource management, such as farming, hunting, and medicine-making, which have been developed and refined over centuries of living closely with nature. In the context of climate research, indigenous knowledge of the local environment can be valuable for understanding historical climate patterns, changes in the environment over time, and the impacts of climate change on local ecosystems and communities. Like in Tetritskaro Municipality, farmers in various regions of the world rely on indigenous knowledge of the local environment for weather forecasts and seasonal predictions. However, this valuable knowledge is diminishing globally and is gradually disappearing everywhere (Kolawole et al. 2014; Tume et al. 2019; Radeny et al. 2019; Balasha et al. 2023). Recognizing the importance of indigenous knowledge, it is crucial to support its preservation, transmission, and integration into science and policy-making to enhance climate change adaptation efforts (Magni 2017).

The destruction of crops and income loss caused by climate change and extreme weather events compel farmers to seek new information about weather, climate, and their effects on agricultural practices (Belay et al. 2017). Like other regions of the world, in Tetritskaro Municipality, it is important to share this experience and knowledge among farmers (Balasha et al. 2023). At this time, young are benefiting from the expertise of seasoned farmers, who provide them with valuable information on new crop varieties and practices that are more suited to changing weather patterns. It should be noted that very few farmers receive information through television and the Internet, and they trust the experience of other farmers in the area more than the information they find elsewhere.

Research conducted in other regions of the world has shown that low levels of education and a lack of language skills (Tume et al. 2019; Balasha et al. 2023) are the main factors preventing people from using television and the internet as their primary sources of information. In these countries, weather and climate information on the internet and media is mostly provided in foreign languages such as English and French, rather than in the local language (Balasha et al. 2023), this is not the case in Tetritskaro Municipality. Information about the weather in Georgia is available in the Georgian language on the internet and television. In Tetritskaro Municipality, like many rural areas, there is a strong sense of community and mutual support, which is why very few farmers receive information through television and the Internet. Farmers perceive information from other farmers as more relevant and reliable than information from television or the internet, which may not always be tailored to their specific needs and circumstances. Farmers value the opinions and experiences of their peers over information from external sources. Farmers often trust the knowledge and experience of other farmers in their community, because local farmers understand the area's specific challenges and conditions and can provide practical and context-specific advice. Face-to-face interactions with other farmers provide an opportunity for more personalized and interactive communication, where farmers can ask questions, share experiences, and receive immediate feedback.

The joint use of local and scientific knowledge by farmers is important for adaptation to environmental challenges, and the need to take it into account has been noted in many international studies (Kolawole et al. 2014; Tume et al. 2019; Hosen et al. 2020). Thus, conducting workshops that involve farmers in Tetritskaro Municipality could enable them to integrate local knowledge with scientific information, thereby enhancing their agricultural practices.

3.4.3 Male and female farmers and adaptation strategies in Tetritskaro Municipality

Some studies focused on regions outside of Georgia suggest that the main pesticide users are male farmers (Kishor 2007; Kawarazuka et al. 2020; Ali et al. 2020), while others suggest the contrary (Balasha et al. 2023). In Tetritskaro Municipality, female and male farmers similarly use adaptation measures, such as pesticides, fertilizer, watering crops, early sowing, and early harvest. A notable difference exists among male and female farmers in several adaptation strategies in Tetritskaro Municipality, Georgia. There is a more frequent exchange of information among male farmers, and a higher use of crop diversification techniques, hail protection products, and improved irrigation systems among male farmers. Improved irrigation systems and hail protection products require financial resources and physical effort. We got the result that corresponds to several studies in different regions of the world indicating that female farmers are less adaptable due to limited resources and financial capabilities (Fisher et al. 2015; Jost et al. 2016; Doss et al. 2018; Shahla et al. 2019). The reason for the low adaptation rate of female farmers may be due to the social norms and roles in rural communities (Jost et al. 2016), which is similar to our findings. For example, in Tetritskaro Municipality, Georgia, like in many rural communities worldwide, traditional men-women roles, as well as cultural and social norms, dictate that women should primarily be responsible for domestic duties, while men take care of farming activities, women also often have limited mobility. These men-women roles and societal norms may restrict women's access to essential resources, education, and training needed for agricultural adaptation. This can also make it challenging for them to access markets to sell their products (Udry 1996).

The discussion with farmers in Tetritskaro Municipality showed that women and men farmers have a common interest, specifically, the restoration and creation of windbreaks, which cannot be done by individual farmers, and organized action is necessary to attract and unite resources. Also, both male and female farmers need relatively low-interest loans to purchase agricultural products and equipment, such as pesticides, fertilizer, seeds, various anti-hail equipment, tractors, etc. However, it was revealed that most of the male farmers are interested in introducing new technologies, while female farmers are more in need of training and information in agricultural activities, such as, how to increase soil fertility.

4. Conclusions

In this study, we focused solely on the climatic characteristics of extreme events specific to the region, without considering changes and trends over time in their frequency and intensity. For future research, in addition to the farmer surveys, it is essential to analyze the dynamics of current and future changes in extreme events using large-scale meteorological observations and downscaling techniques, such as regional climate models. This approach will provide detailed information on local climate change trends and extreme weather events. Many studies have used farmer interviews and field observations to assess climate change impacts, primarily focusing on changes in mean annual or seasonal air temperatures and precipitation, as well as adaptation strategies in various landscapes worldwide (Harvey et al. 2014; Belay et al. 2017; Asrat and Simane 2018). However, these studies often highlight a lack of sufficient knowledge regarding the effectiveness and economic aspects of adaptation strategies implemented by farmers. Therefore, future research should aim to conduct more specific studies on each adaptation strategy to enhance our understanding and inform effective decision-making (Balasha et al. 2023).

We consider it interesting to investigate the effectiveness of adaptation strategies in different landscape types not only regarding changes in mean annual or seasonal air temperatures and precipitation but also regarding various extreme weather events since they can have the most negative impact on agriculture. Therefore, for different farm crops, it is necessary to understand these impacts, how they affect yield and profit, and strategies to adapt to them.

The results of this study show that both male and female farmers' perceptions of climate change and extreme weather events align with prior research, which indicates a rise in average annual air temperature and a decline in annual precipitation in Tetritskaro Municipality (Elizbarashvili 2017). They consider strong wind, fog, and hail as the most relevant extreme weather events and consider strong winds and hail to have the most negative impact on agricultural activities. The difference among the male and female farmers was observed only in the case of fog; a greater percentage of male farmers believe that fog has the most negative impact, which can be explained by the fact that more men in the region drive cars and are therefore more likely to be subjected to visibility issues when they deliver their agricultural products to the market.

The primary source of information on climate change, seasonal predictions, and weather forecasts for male farmers is conversations with fellow farmers and local knowledge of the local environment for female farmers. Very few farmers use the internet and television as their main source of information, which can be explained by the fact that farmers more trust their fellow farmers than the information they get from television or the internet. The reliance on the experience of other farmers in the area can be seen as a form of social learning and knowledge sharing that is deeply embedded in rural communities. Understanding and respecting these local communication channels and sources of knowledge is important for designing effective extension programs and information campaigns in rural areas.

It can be concluded that there are significant opportunities for adaptation and enhancing the resilience of the farming community to climate change and extreme weather events in Tetritskaro Municipality. The conditions for further development and modernization of agriculture are very favorable in terms of farmer readiness. Indeed, the farmers are already taking part in adaptation measures to reduce climate-related exposure including extreme weather events.

Male farmers are more inclined to employ various adaptation strategies compared to their female counterparts. This difference can be explained by social norms and roles, resources and financial opportunities in rural communities, as well as adaptation strategy specifics. Thus, addressing these social norms and roles is critical to promoting equity between males and females in agriculture and increasing the adaptation rate of female farmers in other rural communities of Georgia as well.

Discussions with farmers also showed that most male farmers are interested in introducing new technologies, while female farmers are more in need of training in agricultural activities in connection with climate change; both male and female farmers share an interest in restoring and establishing windbreaks., which cannot be done by individual farmers and require organized action and scientific knowledge. Relatively low-interest loans for purchasing agricultural products and equipment are important for both male and female farmers. By taking into account the needs and interests of male and female farmers, interventions can be designed to build the resilience of the community more effectively and sustainably. Therefore, the role of the local, regional, and national governments may be important by promoting the introduction and implementation of various projects or measures, e.g., such as providing affordable loans, distribution of informative agricultural booklets, training, education activities, etc.

Overall, in rural communities, it is critical to address the traditional men-women roles, and cultural and social norms that are typical not only in Tetritskaro Municipality but also in other regions in Georgia to promote equity between males and females in agriculture and increase the adaptation opportunities of female farmers.

The research highlights the importance of developing climate change adaptation policies and empowering female farmers by granting them access to information, resources, training, and involvement in decision-making processes. This need extends beyond Tetritskaro Municipality to other regions of Georgia.

The findings could inform the development of climate change adaptation policies and interventions that consider the distinct needs and interests of male and female farmers. The results can contribute to enhancing the resilience of the farming community.

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Research Article

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In Vitro Vitamin C Equivalent Antioxidant Capacity, Cytotoxicity and Anti-Cancer Activity of Methanolic *Urtica dioica* L. Leaf Extract as a Food Supplement

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HIGHLIGHTS

- This in vitro study was conducted on some cell cultures originating from vital mammalian tissues.
- The safest concentrations of *Urtica dioica* L. leaf extract were determined as 15.71 mg/ml and 5.14 mg/ml of CC_{50}.
- The extract showed anti-cancer activity on human hepatocellular carcinoma cells with a 2.46 mg/ml of IC_{50}.

Abstract

Antioxidant capacity, cytotoxicity on two vital cell lines and the anti-cancer activity of methanolic *Urtica dioica* L. leaf extract (UDE) collected from Duzkoy, Giresun, Turkey were studied by determining safe concentration. The antioxidant capacity of the extract was expressed as vitamin C equivalency by spectrophotometric MTT assay. The cytotoxic concentration 50% was measured by the linearity between UDE concentrations (CC_{50}) and the cell viability of non-cancer kidney cell lines (BHK-21, MDBK). The anti-cancer activity was conducted on human hepatocellular carcinoma cells (HepG2) by determining inhibition concentration (IC_{50}) on cell proliferation. The vitamin C equivalence of UDE increased linearly by increasing the concentration. The cytotoxic and non-toxic concentrations of UDE were determined on BHK-21 and MDBK with 15.71 mg/ml and 5.14 mg/ml of CC_{50} respectively. The extract inhibited the proliferation of human hepatocellular carcinoma cells with a 2.46 mg/ml of IC_{50}. In conclusion, the present study tried to explain in detail the dose-dependent activity of *Urtica dioica* L. leaf extract. The dose-response results showed that *Urtica dioica* L. leaf extract could have low cytotoxicity, but potential anti-cancer activity at safe concentrations.

Keywords: anti-cancer; cytotoxicity; nettle; Urtica dioica L.

1. Introduction

Urtica dioica L. belongs to the Urticaceae family. It has been identified and widely distributed worldwide and is considered native to Europe, North Africa, Asia, and North America (Upton 2013; Dhouibi et al. 2020; European Commission 2023). It and its extracts are used in both pharmaceutical and food industries as a

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supplement and a food additive for extending shelf-life, ensuring microbial safety of foods and higher consumer acceptability (Alp and Aksu 2010). Phytochemical studies have mainly focused on its bioactive compounds and activities related to antioxidant contents (Körpe et al. 2013; Dhouibi et al. 2020; Veiga et al. 2020). Therefore, a wide range of plants and their extracts were applied as food additives and preservatives during food production and service to improve safety and quality by presenting antioxidant activities at their safe concentrations (Alp and Aksu, 2010; Körpe et al. 2013; Dhouibi et al. 2020).

In addition to antioxidant capacity, several in vitro and in vivo studies of plants aimed to determine the non-toxic doses for preventing adverse effects before using them including *Urtica dioica* L. as a potential therapeutic agent in modern and traditional medicine (Özkol et al. 2012; Sayhan et al. 2012; Dhouibi et al. 2020; Veiga et al. 2020). Recently, the aerial and subsoil parts of *Urtica dioica* L. were experimentally used to treat many cancer types by inhibiting cell proliferation (Konrad et al. 2000; Gözüm et al. 2003). Also, the cancer treatment has a risk of toxicity in different tissues including the kidney and liver by inducing oxidative stress and free radical generation (Özkol et al. 2012; Dhouibi et al. 2020). Therefore, the present study aimed to assess dose-dependently the antioxidant capacity, cytotoxicity on non-cancer kidney cell culture lines and anti-cancer activity on liver cancer cells of methanolic *U. dioica* L. leaf extract (UDE).

2. Materials and Methods

2.1. Extraction of U. dioica L.

The sampling and extraction methods used in this study were adapted from Aydın et al. (2023). Briefly, *U. dioica* L. leaf samples were collected from Duzkoy province (40°56'59.1"N. 38°36'06.5"E), the city of Giresun on the Black Sea coast of Turkey in May 2021. They were identified and authenticated as "*Urtica dioica* L." at the Department of Pharmaceutical Botany, Faculty of Pharmacy in Marmara University, and a herbarium record was created with the code "MARE 23334". The samples were dried in cool room conditions avoiding direct sunlight.

Solid/liquid extraction and evaporation methods were used for the extraction of dried samples. Dried samples were ground by a water-cooled miller. Fifty grams of ground leaves were macerated in 500 ml of methanol (reagent grade. ≥ 99.7%) on a magnetic stirrer for 24 hours. The alcoholic suspension was filtered twice through filter paper (FilterLab-50 g/m2). The solvent in the remaining filtrate was evaporated in a rotary evaporator (Heidolph. Germany) at 40-45°C. 150 mbar, and 135 rpm. The extract was completely concentrated in a vacuum oven (Nüve EV 018. Turkey) at 45°C and -1 bar pressure. A UDE stock solution of 40 mg/ml was prepared in ultra-distilled water and stored at +4 °C for further analysis.

2.2. Handling of Cell Lines

Baby hamster kidney fibroblast (BHK-21, CCL-10), bovine kidney epithelial (MDBK. CCL-22) and human hepatocellular carcinoma (HepG2. HB-8065) cell lines were from ATCC, USA. All cell lines were cultured with Eagle's Minimum Essential Medium (EMEM) in the incubator with the standard condition (SC) of 37 °C and 5% CO2. The medium contained foetal bovine serum (10%). L-alanyl-L-glutamine (200 mM) and 1% penicillin (10.000 unit/ml)-streptomycin (10 mg/ml)-amphotericin B (0.025 mg/ml). For the anti-cancer and cytotoxicity assay, the stock viable cell suspension ($3x10^5$ cell/ml) (100 µl) was transferred in each well ($3x10^4$ cell/well) and kept in SC for 24 h to achieve the confluence of at least 90% in 96-well microplates.

2.3. Vitamin C equivalent antioxidant capacity

Vitamin C (l-ascorbic acid. HPLC grade) was two-fold diluted as 600. 300. 150. 75 and 37.5 µmol with ultra-distilled water. UDE was two-fold diluted as 0.62. 1.25. 2.5. 5. 10 and 20 mg/ml with ultra-distilled water. MTT (2.5-diphenyl-2H-tetrazolium bromide, 1 mg/ml) (380 µl) and each Vitamin C or extract dilution (20 µl) were mixed in an Eppendorf tube and incubated at 37 °C for 4 h. After incubation, DMSO (400 µl) was added to all tubes and mixed well to solve the blue formazan salt formed during the incubation. A 100 µl of each mixture was added quadruplicated to the U-bottom 96-well microplate. The microplate was read at 570 nm.

2.4. Anti-cancer and cytotoxicity assays by MTT

The extract was two-fold serially diluted with the maintaining medium at the concentrations of 0.62, 1.25, 2.5, 5, 10 and 20 mg/ml. Then, each dilution (100 µl) was added to the microplates with monolayer cell culture at six-replicated wells. The cell control wells contained only fresh medium and cells. The blank wells contained the medium without cells. The microplates were incubated in SC for 24 h. 10 µl MTT in PBS (5 mg/ml) was added to each well. After a 4 h incubation in SC. The supernatant was discarded and DMSO (100 µl) was added to wells. The microplate was gently shaken to solubilize the formazan crystals and read at a wavelength of 570 nm (Absorbance 96. Byonoy. Germany).

2.5. Data analysis

The percentage of cell inhibition was calculated using the equation (1) as follows,

Cell viability (%) = (OD sample – OD blank) / (OD control – OD blank) x 100" *(1)*

The 50% cytotoxic concentration (CC_{50}) was calculated from concentration-based-curves after non-linear regression analysis ($y = me^{bx}$). The vitamin C standard curve was generated with optic density (OD, nm) values of five vitamin C dilutions by linear regression analysis (vitamin C equivalence = m x OD_{570} + b, R^2). The equivalence to vitamin C of extract dilutions was calculated concerning the standard curve (Figure 1). Statistical analyses were conducted by using SPSS version 15 software (IBM. USA). The graphs were generated by using Office Excel 2016 (Microsoft. USA).

3. Results

3.1. Vitamin C equivalent antioxidant capacity of UDE

The natural antioxidants in herbal additives such as carotenoids, tocopherol (vitamin E), some phenolic compounds, and ascorbic acid inhibit oxidative damage by free-radical scavenging (Dini 2019; El-Saber Batiha et al. 2021). Previous research suggested that fresh *U. dioica* L. leaves contained various amounts of total ascorbic acid content from 16 to 112.8 mg/100 g fresh weight at different harvest times (Skalozubova and Reshetova 2013; Shonte et al. 2020). The highest vitamin C was measured in August while the lowest was in September (Paulauskienė et al. 2021). As the vegetation period and drying methods influenced the vitamin C content and antioxidant capacity of *U. dioica* L. leaves, it was not varied by meteorological conditions. The highest vitamin C content was determined with low-temperature drying compared to owen drying (Shonte et al. 2020; Garcìa et al. 2021; Paulauskienė et al. 2021). In this study, *U. dioica* L. leaves were harvested in May, dried under cool-dry room conditions and extracted by methanol. The vitamin C equivalence of UDE was calculated concerning the standard curve by linear regression analysis (vitamin C equivalence. µmol = 1191.6 x OD of UDE - 1.4723. $R^2 = 0.998$). The vitamin C equivalence of UDE increased by increasing the concentrations of UDE with a linearity of $R^2 = 0.690$ (vitamin C equivalence, µmol = 29.578 x UDE mg/ml + 501.51) (Figure 1). In harmony with previous studies, the average vitamin C content was calculated as approximately 42.3 µg/mg in methanolic extract of *U. dioica* L. leaves collected from Giresun region in Turkiye.

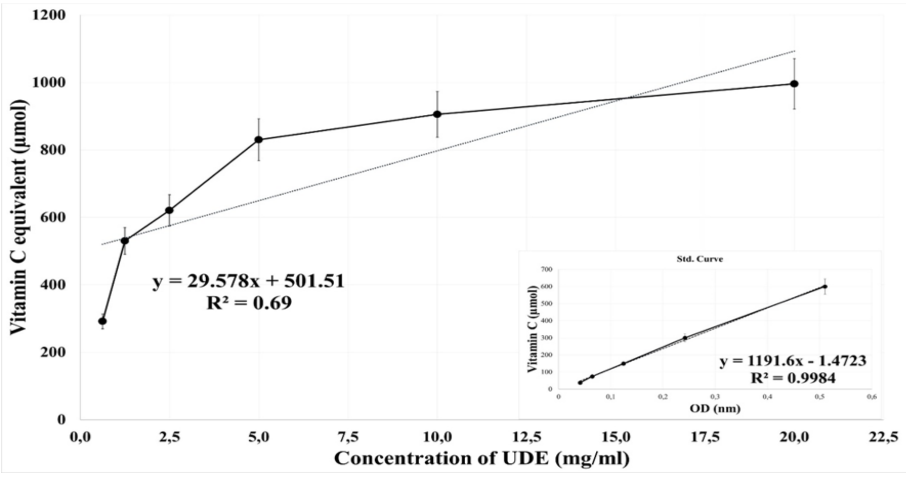

Figure 1. The vitamin C equivalency antioxidant capacity of UDE based on concentration.

3.2. Cytotoxicity of UDE

The cytotoxicity and safety levels of plant extracts vary related to the extraction method and are generally tested with cell lines of mammalian kidney and liver. A previous study suggested that methanolic extract (CC_{50}: 0.702-0.803 mg/ml) was safer than aqueous extract (CC_{50}: 0.37-0.49 mg/ml) of *U. dioica* L. on BHK-21 (Flores-Ocelotl et al. 2018). Also, the ethanolic extracts of *U. dioica* L. showed higher cytotoxicity than the aqueous extracts (Mannila et al. 2022). In this study, the cytotoxic effects of methanolic *U. dioica* L. leaf extract experimented on the viabilities of non-cancer (BHK-21 and MDBK) cell lines with concentration-response curves (Table 1). The relation between the cell viability and the concentration was the cell viability % = $99.474e^{-0.044 \times UDE\ (mg/ml)}$ (R^2 = 0.936) for BHK-21 and the cell proliferation %=$163.76e^{-0.23 \times UDE\ (mg/ml)}$ (R^2 = 0.913) for MDBK (Figure 2). The CC_{50} was calculated as 15.71 mg/ml and 5.14 mg/ml for BHK-21 and MDBK respectively (Table 1). The Higher CC_{50} than the previous study indicated that the methanolic extract of *U. dioica* L. leaves might be a safer and potential food supplement and additive.

Table 1. The dose-response effects of UDE on % cell viability.

UDE Conc. (mg/ml)	Non-Cancer Cells		Cancer Cell
	BHK-21	MDBK	HepG2
	Mean±SD (%)	Mean±SD (%)	Mean±SD (%)
20	44.66±5.00c	1.13±4.44e	2.37±3.38d
10	56.36±6.50bc	43.21±4.30d	22.65±2.30c
5	73.59±9.15b	61.73±8.35c	34.49±5.25bc
2.5	93.45±4.25ab	79.42±4.01b	44.05±2.30b
1.25	99.82±8.35ab	101.54±2.02a	60.05±6.61a
0.62	99.95±5.51a	102.82±1.51a	60.13±5.45a
P value	<0.01	<0.01	<0.01
CC_{50} (mg/ml)	15.71	5.14	2.46

3.3. Anti-cancer activity of UDE

Various studies have recently demonstrated the cytotoxic and anti-cancer properties of *U. dioica* L. in particular against colon, gastric, lung, prostate and breast cancers (Esposito et al. 2019). Aqueous extract of *U. dioica* L. leaf cultured in Iran inhibited the proliferation of human breast cancer (MCF-7) at 2 mg/ml after 72h treatment (Fattahi et al. 2013, 2018). Its aqueous extract (from 5 to 30 μg/mL) harvested in Turkey has shown a dose-dependent inhibition effect on three breast cancer cell lines (MCF-7, MDA-MB-468, and MDA-MB-231) with IC_{50}s of 14-18 μg/ml (Karakol et al. 2022). For Its dichloromethane extract against both mouse and human breast cancer cells, IC_{50} was determined between 31.37 and 38.14 mg/ml. Similarly, its anti-cancer activity was measured by inhibiting the metastasis of breast cancer with 20 mg/kg daily injection treatment in vivo mouse models (Mansoori et al. 2017; Mohammadi et al. 2017). For its anti-cancer activities on human prostate cancer, the dichloromethane and aqueous extracts inhibited the proliferation of PC3 and LNCaP cells with 15.54 μg/ml and 42-50 μg/ml of IC_{50}, respectively (Durak et al. 2004; Levy et al. 2013; Mohammadi et al. 2016). Meanwhile, no cytotoxicity was determined in non-cancer human prostate stromal cells (Konrad et al. 2000). *U. dioica* L. hydroalcoholic (50:50 v/v) extract inhibited 25.4% of human hepatocellular carcinoma (HepG2) cell viability at the concentration of 0.35 mg/ml (Carvalho et al. 2017). In this study. The anti-cancer effect of UDE on the proliferation of HepG2 cells was determined by spectrophotometric MTT assay with the concentration-response curve (cell proliferation % = $74.941e^{-0.163 \times UDE\ (mg/ml)}$., R^2 = 0.965) (Figure 2). The IC_{50} was calculated as 2.46 mg/ml for human hepatocellular carcinoma (Table 1). These IC_{50} values present the potential anti-cancer activity through hepatoprotective and reducing tumorigenesis activities of methanolic extract of *Urtica dioica* L. leaf used at safe concentrations.

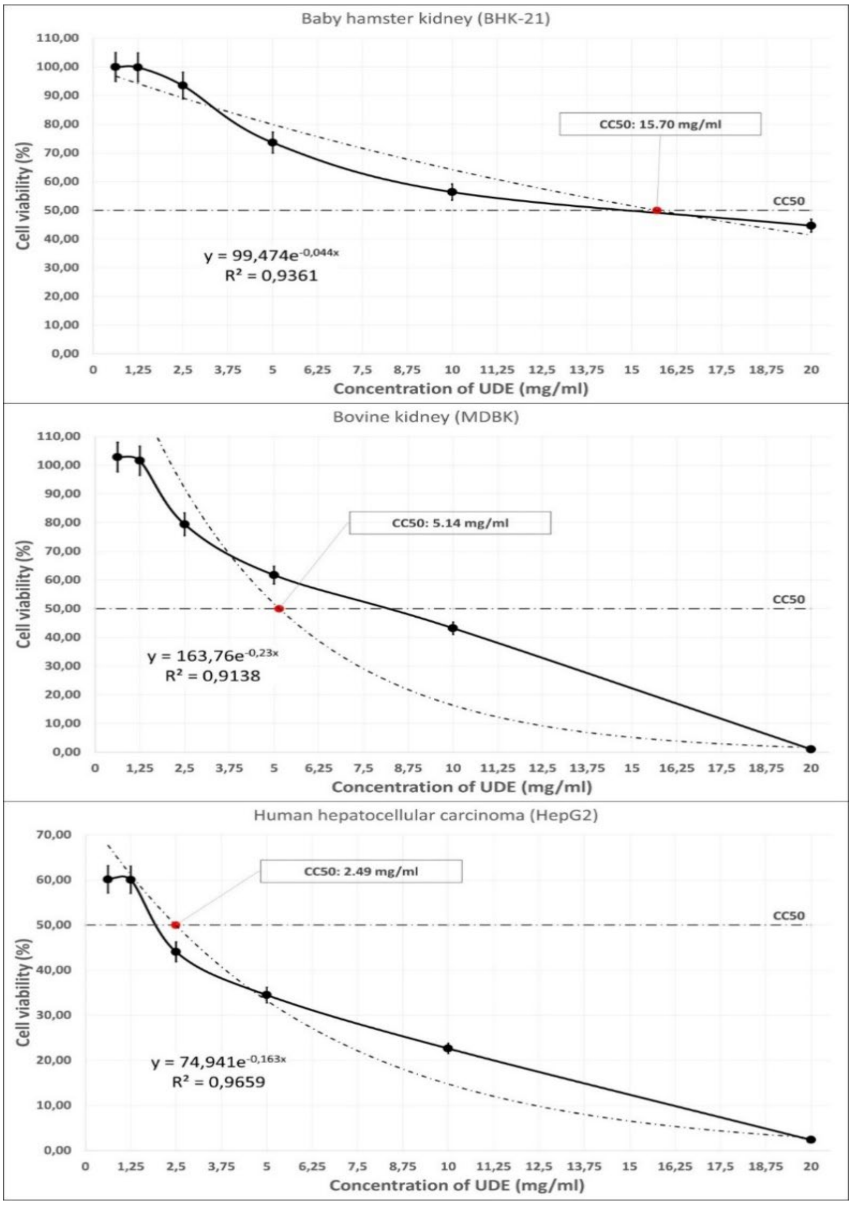

Figure 2. The cell viability and inhibition with CC_{50} (mg/ml) of UDE

4. Conclusions

In conclusion, the present study gives information about the bioactivity of *U. dioica* L. leaf harvested during the growing season in Giresun City, Turkey. The extract had dose-dependently antioxidant capacity expressed as vitamin C equivalency. Non-toxic and safe concentrations of the extract were determined in detail on vital cell lines originating from kidney tissue, which are generally used in food and drug research for consumer safety. The dose-response results indicated that the extract was less toxic to kidney cells while inhibiting the proliferation of liver cancer cells at a lower concentration.

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Farming Legumes for Food Security and Agricultural Sustainability in Somalia

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HIGHLIGHTS

- Agriculture is an essential input in human life all over the world.
- Legume production – especially for cowpea is mainly affected by insufficient rainfall, high temperature, drought, insects, diseases and weeds.
- It is of great importance for the government to provide agricultural inputs to local farmers, provide loans and grants, disseminate agricultural information, provide training and provide new ways and techniques for better agriculture, determine the adaptation abilities of different legumes, thereby ensuring food security and agricultural sustainability.

Abstract

This research was carried out in 2023 in a total five different regions of Somalia, including Mogadishu the capital city, along with four other towns: Afgooye, Baydoa, Jowhar and Belet-hawa, to reveal the problems faced by legume growers and to investigate ways to solve them, thus contributing to the security of food and agricultural sustainability. A total of 100 farmers, 20 randomly selected from each city/town, participated in 30 survey questions face to face. As a result of the research conducted in the mentioned regions, it was seen that there are problems in pest and disease control, weed control, fertilizer application and irrigation techniques and water use in legume agriculture. These problems are the main factors affecting the production of legumes. To prevent such problems and mistakes, farmers need to pay extra attention to get better quality and higher yields from their legume production, and sustainability-based studies must be increased to solve such problems, especially for food security.

Keywords: Farmer problems, Legume productions, Rural development, Sustainable agriculture

1. Introduction

Agriculture is an essential input in human life all over the world. It provides all the nutrients, vitamins and minerals that both animals and humans need. It provides proteins that both build and repair the body and carbohydrates that provide energy for the body to healthy life. Agriculture has a crucial impact in

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ensuring the security of food besides promoting economic development in both developing and developed nations (Yurtkuran, 2021). Climate changes pose a persistent and increasing challenge to the sustainability of global food security. The changing of temperature and rainfall patterns have a significant impact on crop yields in numerous growing nations where agricultural production plays a crucial role (Aziz & Peksen, 2020; Ali Warsame & Hassan Abdi, 2023; Doruk Kahraman & Kahraman, 2023). In organic farming - not using mineral fertilizers prevents significant greenhouse gas emissions from fertilizer production and field fertilizer application. This system leads to the formation of organic matter in the soil and the retention of atmospheric carbon. A conclusion can be drawn that organic agriculture has a significant potential to contribute towards the mitigation of climate change (Holka et al., 2022). In this respect, legumes are of great importance due to their symbiotic nitrogen fixation mechanism.

Among the various legumes grown in the world, beans, chickpeas, cowpeas, peanuts, lentils, peas and soybeans are the most preferred legumes worldwide. In terms of production, the highest annual growth rate was observed in soybeans (4.5%), followed by cowpeas (4.1%), lentils (3.5%), peanuts (2.2%), peas (1.8%), followed by beans (1.5%) and chickpeas (1%). In terms of productivity, the highest annual growth rate was observed in peanuts (1.5%), followed by soybeans (1.4%), cowpeas (1.4%), lentils (1.4%), beans (1.1%) and chickpea (0.9%), respectively (Nigam et al., 2021).

Legumes and cereals stand out as excellent sources of high-quality protein due to their significant protein content. The development of protein concentrates and isolates further enhances their versatility, allowing for integration into a wide array of consumer products. Beyond their nutritional value, plant proteins offer distinct health advantages over animal protein sources, including reduced cholesterol levels, reduced risk of cardiovascular diseases, increased satiety, and potential applications in the development of new therapeutics, such as utilizing plant proteins for drug delivery mechanisms (Nosworthy et al., 2023).

Food security is a major issue in Somalia, which mostly caused hunger and scarcity in 2011/2012, 2015/2016 and 2017, normally related to rainfall scarcity (Abdi-Soojeede, 2018). According to official data, the area where legumes are grown in Somalia is large, but the total production amount and unit area yield are very low. Like the other plants, legume production is mainly affected by insufficient rainfall, high temperature, drought, insects, diseases and weeds (Kahraman & Gökmen, 2022; 2023). The combination of these factors prevents farmers from achieving higher yields per unit area. A previous study (The Ministry of Planning, 2021) shows that the area planted with cowpeas in Somalia is large, but the production and yield per unit area is very low. Legume production – especially for cowpeas is mainly affected by insufficient rainfall, high temperatures, drought, insects, diseases and weeds. The combination of these factors prevents farmers from achieving higher yields. The surface area of Somalia is 637657 square kilometers. According to estimates from the World Bank/FAO report, Somalia possesses around 3 million hectares of arable land, with approximately 2.3 million hectares suitable for rain-fed crop cultivation, while the remaining 700,000 hectares, primarily located along the Shabelle and Juba rivers, have the potential for crop production through pumped or controlled irrigation. However, at present, only 110,800 hectares are actively being irrigated (FAO, 2020; Worldbank, 2020). The agricultural sector, constituting about 70% of the gross domestic product from 2013 to 2016, stands as the primary employer for rural households and serves as a crucial contributor to export income (The Ministry of Planning, 2021).

This research was conducted in the provinces between and surrounding Somalia's two largest rivers. The reason for choosing these regions is that farmers in this region mostly grow edible legumes. Within the scope of the research, the main problems and current situation of legume producers were determined and a situation assessment was made to ensure food security and determine the principles of sustainability.

2. Materials and Method

It is known that Somalia imports more legume products than some other countries produce or even export. Somalia's legume exports are very low. Somalia's exports are mainly affected by a combination of factors such as recurrent drought and scarcity, unpredictable rainfall patterns, insecurity, as well as lack of support for farmers. Most farmers in Somalia are small-scale farmers who produce agricultural products only for domestic consumption, which may not be sufficient to meet the needs and food security of Somalia's growing population. This situation has forced Somalia to import many products from other countries. Therefore, this study was planned to serve to ensure sustainability, especially in healthy human nutrition and food safety.

This research was conducted in 5 different regions of Somalia including Mogadishu the capital city, along with four other towns: Afgooye, Baydoa, Jowhar and Belet-hawa in 2023, a total of 100 farmers, 20 randomly selected from each city/town, answered 30 survey questions. The results obtained from the survey questions were converted into a percentage (%) rate (Kahraman & Onder, 2015; Karimi et al., 2024).

3. Results and Discussion

In this part of the article, the Questions of the survey are abbreviated as "Que" term.

Que 1) In your opinion, what factors affect legume production?

a) Human factor= 7%

b) Biotic factor= 18%

c) Climate factor= 60%

d) Edaphic factor (Soil structure and composition)= 15%

Survey responses indicate that primary challenges encountered by legume growers revolve around biotic factors, including insects, pests, and diseases, as well as adverse climatic conditions like insufficient rainfall and elevated temperatures. The changing climate and environmental warming contribute to a decline in agricultural production (Mohamed & Nageye, 2021).

Que 2) In your opinion, which climate factors affect legume production the most?

a) Rainfall= 33%

b) Temperature= 50%

c) Relative humidity= 2%

d) Wind= 15%

The results show that the main problems faced by legume growers are adverse climatic conditions such as lack of rainfall and high temperatures. A one-degree Celsius rise in temperature results in a 3% reduction in agricultural production (Mohamed & Nageye, 2021).

Que 3) What kind of edible legumes do you grow most?

a) Cowpea= 76%

b) Mung bean= 11%

c) Pea= 7%

d) Soybean= 6%

Based on the research findings, Somalia predominantly cultivates cowpeas and mung beans as the primary legume crops. In the southern regions, rain-fed agriculture is prevalent, particularly in the Juba River and Shabelle basins, with two growth seasons corresponding to the Deyr and Gu rainy seasons. The cultivated crops encompass vegetables, millet, sorghum, corn, peanuts, cowpeas, sesame, mung beans and cassava. These kinds of agricultural products serve dual purposes, catering to both human consumption and animal feed (Boitt et al., 2018).

Que 4) How do you prepare your soil before planting seeds?

a) Shovel by hand = 50%

b) Tractor = 50%

Most farmers in Somalia who do not have a tractor and cannot afford to rent one use the tiring and time-consuming hand hoeing method. Farmers who have the means and power use rental tractors to make their work faster and easier despite constraints such as limited labor force and farm equipment in Somalia, many farmers continue to rely on traditional methods, such as hand hoeing. Nevertheless, there are instances of exceptions where individuals opt for rented older model tractors with more expensive working hours during initial tillage (Abdi-Soojeede, 2018).

Que 5) When do you sow your seeds?

a) Jilaal season (hot and dry season)= From late December to early March = 7%

b) Gu' season (rainy season)= From late March to early June = 71%

c) Hagaa season (dry, windy and rarely rainy season)= End of June - beginning of September = 7%

d) Deyr season (little rainy season) = From the beginning of September to the end of December = 15%

The “main Gu” rains begin in April and end in June. This is normally followed by the Hagaa season, a period of showers and cloudy skies that usually ends in August. Deyr rains begin in mid-October and end at the end of December. The Jilaal dry season occurs between Deyr and Gu, it is the most severe time of the year with the highest temperatures and almost no rainfall (Madany, 1992). The results obtained from the survey show that the main growing seasons in Somalia are Gu and Deyr seasons. Somalia's agricultural calendar is characterized by two main seasons: crop production during the Gu season, spanning from April to June, and crop cultivation in the Deyr season, which occurs from October to December (Boitt et al., 2018). Besides adaptation for seasons, breeding studies against different stress conditions are of great importance (Doruk Kahraman, 2021; Okumuş et al., 2023).

Que 6) What sowing methods do you use?

a) Sowing in row = 72%

b) Spreading sowing= 28%

Row planting of legumes is recommended as it facilitates crop management practices during hoeing, weeding, fertilizer application and harvesting (Bozoglu et al., 2007; Beshir et al., 2019).

Que 7) What is your sowing density of legumes?

a) Between rows 20-25cm, On row 4-8cm= 2%

b) Between rows 40-50cm, On row 5-10cm= 98%

Cowpeas which are the most grown legumes in the region, are grown as the main crop, the planting norm is recommended with a spacing of 40x20 cm (Nderi, 2020).

Que 8) How to irrigate your plants?

a) Surface irrigation= 21%

b) Bucket use = 22%

c) Canal use= 57%

The primary sources of water loss in and around canals include leakage from canal embankments, openings created by rodents along the canals (mouse holes), and surface runoff. In agricultural lands, water loss occurs through surface evaporation, over-irrigation, and percolation (Omar, 2018).

Que 9) What irrigation methods do you use?

a) Drip irrigation= 65%

b) Surface irrigation= 35%

Drip irrigation systems are extensively employed to enhance the efficient utilization of agricultural water. This technique involves the gradual delivery of water directly to the plant's base, minimizing water loss due to evaporation and reducing the overall quantity of water needed (Kumari & Singh, 2016). Drip irrigation offers the benefit of water conservation, as it minimizes water wastage. Presently, numerous companies have emerged, introducing cost-effective and user-friendly drip irrigation systems to the market (Omar et al., 2022).

Que 10) Do you use certified seeds?

a) Yes= 30%

b) No= 70%

While 70% of the farmers participating in the survey stated that they did not use certified seeds, 30% declared that they used certified seeds. The result obtained from this research is like a study conducted by another study that focused on chickpea cultivation in Sarayönü-Konya region (Kahraman & Onder, 2015).

Que 11) Why you do not use certified seeds?

a) Expensive= 62%

b) Insufficient government support= 27%

c) I have available seeds= 11%

Almost 62% of the farmers who participated in the survey stated that certified seeds were expensive for them. Additionally, 27 of them stated that they could not use certified seeds because there was insufficient government support and 11% of farmers said they had existing seeds.

Que 12) From where do you obtain the seeds?

a) Self-production= 42%

b) Purchase from a seed dealer= 30%

c) Obtained from other farmers= 28%

In Somalia, farmers commonly acquire seeds from local seed traders in villages, particularly after periods of drought. During favorable seasons, the majority either rely on saved seeds or purchase them from relatives (Manyasa & Ismail, 2005).

Que 13) How many kg da_{-1} of seeds do you use?

a) 10 kg and low= 40%

b) 10-12 kg= 29%

c) 12-14 kg= 15%

d) 14-16 kg= 9%

e) 16 kg and more= 7%

The area where legumes are grown in Somalia is quite large, but production and yield per hectare are very low. Legume production is mainly affected by insufficient rainfall, high temperature, drought, insects, diseases and weeds.

Que 14) Do you intercrop?

a) Yes= 85%

b) No= 15%

Most of the surveyed farmers indicated that they practice intercropping to optimize land usage, as it involves cultivating different crops together on the same piece of land. Additionally, different crops grown together in the same field do not compete for the same overall resources, reducing competition between crops. Moreover, applying such a method also helps in increasing efficiency Intercropping offers advantages, including enhanced yield stability and increased productivity per unit area. It also contributes to reduced pest issues and minimizes the reliance on agrochemicals, all while fostering biodiversity (Jensen et al., 2020).

Que 15) What crops do you intercrop with legumes?

a) Cereals= 92%

b) Vegetables= 8%

Most legume farmers in Somalia grow legume crops, often in combination with cowpeas and grains such as sorghum or maize. In small-scale agriculture, the practice of intercropping legumes with cereals emerges as a crucial factor in enhancing farm productivity. This approach contributes to the supply of essential plant nutrients, particularly nitrogen, in the soil. Furthermore, it plays a vital role in improving the physical properties of the soil (Mohamed Abdi, 2020). The intercropping of maize with cowpeas has been documented to enhance light interception, decrease water evaporation, and promote better conservation of soil moisture in comparison to sole cultivation of maize (Ghanbari et al., 2010).

Que 16) Do you practice crop rotation?

a) Yes= 100%

According to the survey, all farmers declared that they implemented crop rotation. The result obtained from this research is like a study conducted by Kahraman and Önder (2015) on chickpea cultivation in Sarayönü-Konya region.

Que 17) Which plants do you rotate with?

a) Cereals= 86%

b) Forage crops= 14%

While 86% of the farmers surveyed said that they planted legumes together with cereals, the remaining 14% of farmers declared that they planted legumes together with forage crops.

Que 18) Do you use organic fertilizer?

a) Yes= 40%

b) No= 60%

Most of the farmers who use organic fertilizer in Somalia make their organic fertilizer through manure obtained from farm animals like cows, chickens, and goats. Additionally, they incorporate green manure sourced from young plants, particularly various types of legumes. In sustainable agriculture, organic fertilizers play a vital role and offer a variety of benefits that contribute to both environmental and agricultural well-being. Organic fertilizers contribute to enhancing soil structure and increasing the capacity of water retention. They promote the growth and development of beneficial microorganisms, thereby improving the soil's capability to retain water and resist erosion. Key benefits of organic fertilizers include the enhancement of soil texture, increased water retention, and improved resistance to erosion (Sharma & Chetani, 2017). Organic fertilizers are mainly cost-effective, and readily available from local materials, in contrast to chemical fertilizers (Solomon Wisdom et al., 2012; Ucar et al., 2021).

Que 19) Do you use fertilizer?

a) Yes= 60%

b) No= 40%

Some of the Somali farmers use both organic and inorganic fertilizers to increase crop yields. Previous reports have indicated that the application of both organic and inorganic fertilizers by farmers leads to increased yields and the preservation of soil fertility (Chukwu et al., 2012).

Fertilizers provide the essential nutrients that plants need. Plants need nitrogen, phosphorus, potassium, calcium, magnesium and other microelements for growth and development. Fertilizers contain essential nutrients required for plant growth, and therefore, they are applied to the soil to enhance its physical, biological, and chemical properties. Fertilizers are also used to increase soil fertility (Sharma & Chetani, 2017). However, improper or excessive application of fertilizers may lead to environmental issues and soil degradation. Hence, it is crucial to employ fertilizers responsibly and in a balanced manner. Inorganic fertilizers, characterized by their high costs, can have adverse environmental effects if not managed properly (Morris, 2007).

Que 20) What type of fertilizers do you use?

a) DAP (18-46)= 86%

b) NPK 20-20-20= 12%

c) NPK 15-15-15= 2%

While 86% of the surveyed farmers declared that they use DAP for legumes, 12% of the farmers chose NPK 20-20-20 fertilizer type for legumes. The remaining 2% of farmers stated that they use NPK 15-15-15 fertilizer type in legume cultivation.

Que 21) How do you control weeds?

a) Chemical using= 42%

b) Mechanic control= 30%

c) Cultural control= 28%

Weeds compete with crops or desirable plants for vital resources, including water, nutrients, sunlight, and space. The rivalry between weeds and cultivated plants for essential resources can result in diminished yields and stunted growth in the cultivated plants. Weeds also cause economic harm not only to farmers but to the entire country. Crop losses because of weeds are estimated to vary between 10-20% and exceed US$ 15 billion annually in the USA alone (Bridges, 1994). Nevertheless, a more recent and likely more precise estimate places the annual cost of non-native weeds to U.S. agriculture at $27 billion (Pimentel et al., 2000).

Que 22) Where do you get agricultural information?

a) Friends= 49%

b) From nowhere= 32%

c) Company= 19%

Due to weakness and fragility, agricultural policies struggle to disseminate important agricultural information, provide early warnings and provide necessary training to farmers. He noted that the most cost-effective factor for enhancing rural agricultural development is ensuring sufficient access to knowledge and information in various fields. This includes new agricultural technologies, early warning systems for events like droughts, pests, and diseases, as well as advancements in seedlings, fertilizers, credit options, and market price information (Balit et al., 1996).

Que 23) A) Do you get agricultural information from official institutions?

a) Yes= 55%

b) No= 45%

Some non-governmental organizations and volunteers support the idea of agricultural extension services, including the provision of farm inputs, access to farm credit, and the provision of education and information to farmers. However, according to the survey results, 45% of the farmers participating in the survey do not receive information and agricultural extension services from government institutions, non-governmental organizations or volunteers. The results are like the survey conducted by another study (Maow & Temizel, 2021).

B) If yes, how many times a year?

a) Once= 50%

b) Twice= 50%

Research confirms that the importance of education will result in the enhancement of farmers' skills in agricultural work. Enhancing farmers' capabilities through training proves more advantageous than offering financial assistance in terms of boosting production and income. (Maow & Temizel, 2021).

Que 24) Can you sell your products easily?

a) Yes= 60%

b) No= 40%

Farmers lack advanced knowledge and skills in agribusiness and marketing, persisting in traditional systems for their livelihoods. Hence, the degradation of the environment and the utilization of resources in agriculture pose a threat to the production of food (Doruk Kahraman & Gökmen, 2021; Ali, 2022).

Que 25) Do you have your silo?

a) Yes= 20%

b) No= 80%

Farmers interviewed about seed storage and seed purchasing reported that the quantity of seeds saved after harvest depends on the area of the land to be planted in the next season. Most farmers in Somalia, especially small-scale farmers, still use traditional seed storage by storing their seeds in sacks, plastic jars, and clay pots. The seeds are combined with ash and then put into sacks, 200-litre closed barrels, plastic containers, or earthenware containers, based on the number of seeds and the availability of containers (Longley et al., 2001). Traditional seed storage methods have been associated with a decrease in seed quality. Post-harvest crop losses are high due to poor storage structures as well as less knowledge and skills for all farmers (Abdi-Soojeede, 2018).

Que 26) Do you want to continue legume farming?

a) Yes= 100%

According to the survey, farmers stated that they would never give up and continue legume farming despite the difficulties they faced. It has been found that after facing problems such as drought and climate change, lack of government support, insufficient agricultural inputs, lack of certified seeds, farmers still want to continue growing legumes.

Que 27) How much area do you cultivate legumes?

a) 1 hectare and low= 12%

b) 2 hectare= 37%

c) 5 hectare= 25%

d) 10 hectare= 13%

e) More than 10 hectare= 13%

The results show that most of the legume growers manage small plots of land such as 2 hectares, which means that the majority of the legume growers engage in small-scale farming. Most of the subsistence farmers operate on a small scale, mainly in the Southern regions, participating in crop production, typically managing an average land area ranging from 0.2 to 3 hectares (The Ministry of Planning, 2021).

Que 28) How many years have you been growing legumes?

a) 5 years and less= 25%

b) 5-7 years= 30%

c) 10 years= 40%

d) 15 years and more= 5%

Almost 95 percent of the farmers participating in the survey have been growing legumes for periods ranging from 5 to 10 years. Despite facing several challenges, these agricultural practitioners expressed satisfaction with their current farming methods. They stated that they were satisfied with the farming method they chose.

Que 29) Do you own all agricultural tools and equipment?

a) Yes= 45%

b) No= 55%

The research shows that not all farmers have agricultural tools and equipment, so they miss out on important machines such as tractors that would make their work easier less time-consuming and also less laborious because the machines do the work. In addition, most farmers who can afford it use rental tractors for hours during land preparation at high prices.

In Somalia, limitations like a shortage of labor force and farm equipment persist, leading farmers to rely on traditional technologies such as manual hoeing. However, there are situations where people use rented older model tractors with more expensive working hours during initial tillage (Abdi-Soojeede, 2018).

Que 30) What are the important problems you encounter in legume production?

a) Insect and disease infestation,

b) Weed,

c) Drought and climate change,

d) Water stress,

e) Soil degradation,

f) Insufficient farm inputs,

g) Lack of certified seeds,

h) High taxes,

i) Insecurity,

j) Lack of qualified personnel,

k) Poor infrastructures such as roads,

l) Lack of support from the government,

m) Lack of market access.

Farmers continue traditional practices as they struggle to access sufficient technical and support services, due to the lack of strong agricultural planning in the country and the insecurity of rural areas and the lack of control mechanism, most of the farmlands are controlled by terrorist groups. For this reason, agricultural production is low (Abdullahi & Arısoy, 2022).

4. Conclusions

The results of the study show that the main problems faced by legume growers in Somalia are biotic factors like insects, pests, diseases, and adverse climatic conditions like lack of rainfall and high temperatures. It shows that most of the farmers do not use certified seeds, most of them irrigate their crops through canals which wastes a lot of water, and they also control weeds by using chemicals like herbicides. The research also shows that not all farmers have agricultural tools and equipment, so they do not have important facilities such as tractors that would make their work easier and less time-consuming and also less laborious as machines do the work. It shows that most legume growers manage small plots of land such as 2 hectares, which means that most legume growers are small-scale farmers. Additionally, according to the research, the most grown legumes in Somalia are cowpeas and mung beans. It was also concluded that most legume growers use DAP as fertilizer for legumes. According to the survey questions applied, it shows that farmers want to continue growing legume crops despite facing many difficulties. Most farmers stated that they did not receive support from the government and faced difficulties such as high taxes on products and reduced access to markets due to poor infrastructure such as roads.

Based on the results obtained from the survey questions, farmers can be advised to control weeds, pests and diseases through crop rotation. Additionally, implementing appropriate chemicals such as pesticides and herbicides is essential in this regard. Farmers are required to utilize water usage by using irrigation methods such as drip irrigation and also to preserve water by storing it so that it can be used when water is needed or there is a shortage of water and rainfall. Farmers should stop using old methods such as surface irrigation, which wastes water.

Since it shows that most of the legume producers do not use certified seeds, it may be recommended that they use certified seeds to obtain high-quality and high-yield legume products. Finally, it is of great importance for the government to provide agricultural inputs to local farmers, provide loans and grants, disseminate agricultural information, provide training and new ways and techniques for better agriculture, and determine the adaptation abilities of different legumes, thereby ensuring food security and agricultural sustainability.

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Comprehensive Analysis of Flowering Patterns in One-Year-Old Long Shoots of Apple Cultivars with Contrasted Bearing Habits

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HIGHLIGHTS

- There is a significant difference in the quality of floral buds among apple cultivars in shoot zones.
- "Amasya' shoots exhibited a gradual increase in floral bud quality as they extended towards the distal zone.
- In 'Braeburn' and 'Granny Smith,' floral bud quality variables remained stable along the shoot.
- The properties of spur leaves along the shoot have been found to be crucial for maintaining consistent productivity.

Abstract

Before developing orchard management strategies, it is crucial to conduct architectural analysis at the shoot level for the specific type of fruit to be grown. The objective of this study was to analyze flowering patterns in 1-year-old long shoots of three apple cultivars ('Amasya,' 'Braeburn,' and 'Granny Smith') with different bearing behaviors. This analysis aimed to gain a better understanding of their growth dynamics and reproductive activities. The shoot was divided into three consecutive zones (proximal, median, and distal) based on node number. When comparing the number of floral and vegetative buds in the three zones along the shoot, less contrast was observed between alternate-bearing ('Amasya') and regular-bearing cultivars ('Braeburn' and 'Granny Smith'). However, a notable difference in floral bud quality variables was evident in shoot zones between biennial-bearing and regular-bearing cultivars. In 'Braeburn' and 'Granny Smith' with a regular bearing habit, floral bud quality variables remained stable along the shoot. In contrast, 'Amasya' shoots exhibited a gradual increase in floral bud quality from the proximal zone towards the distal zone. 'Amasya' had the fewest spur leaves per inflorescence, both in the proximal zone (0.89) and the median zone (2.87) of one-year-old long shoots, whereas they were nearly equal for 'Braeburn' (5.67 and 6.09, respectively) and 'Granny Smith' (5.55 and 6.59, respectively). In the proximal zone, 'Amasya' had a spur leaf size of 0.48, while 'Braeburn' and 'Granny Smith' exhibited similar spur leaf sizes of 3.39 and 3.59, respectively. This study underscores the significance of floral bud quality, especially the properties of spur leaves along the shoot, in ensuring optimal and consistent productivity. Finally, with the exception of internode length, morphometric traits did not significantly differ between cultivars when considering the entire shoot. However, these differences became apparent when examining successive zones along the shoot.

Keywords: branching; floral quality; tree architecture; reproductive; bearing behaviors

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1. Introduction

In temperate fruit trees, understanding branching and bearing behaviors is crucial due to their crucial agronomic and economic implications. Tree architectural analysis, which allows for the quantification of form and shape, has the potential to characterize growth and branching patterns, as well as flowering and fruiting habits (Halle et al. 1978; Costes et al. 2006). However, these patterns represent complex processes, the mechanisms of which are not yet fully understood, particularly in perennial species characterized by a succession of growth units (Tromp 2005; Hanke et al. 2007; Lauri and Corelli-Grappadelli 2014). Consequently, such an analysis is not easily comprehensible due to the intricate nature of perennial woody plants.

Several classifications have been applied in the architectural characterization of apple trees based on their growth habits (spur and standards, from upright to weeping) (Dennis et al. 1996; Lane et al. 1996), tree forms (columnar, upright, spreading, drooping, and weeping) (UPOV 1982), and fruiting habits (Types I to IV) (Lespinasse 1977), as well as the position of the scaffold branches along the trunk (Lespinasse 1992). In addition, a more detailed modeling approach to architectural traits has been studied in apples (Costes and Guédon 1997; Costes and Guédon 2002; Renton et al. 2006; Wang et al. 2020). These models mainly focused on branching patterns, which have the potential to affect training, pruning, and orchard management efficiency.

In apple trees, there is a significant variability in flowering patterns among different cultivars (Lauri and Lespinasse 1993; Costes and Guédon 2002). This diversity in flowering patterns has significant implications for orchard management strategies, especially for cultivars that display synchronized or desynchronized flowering rates over the years. Understanding the architecture of 1-year-old long shoots, where flowers are located, can greatly assist in the development of effective orchard management strategies for these cultivars. Comprehensive knowledge of flowering patterns and shoot architecture in apple trees is crucial for devising effective orchard management strategies, especially for cultivars that exhibit biennial bearing over time. It's essential to consider the architectural traits of apple trees, such as branch distribution and fruiting positions, as these factors can impact fruit yield and overall productivity. In this study, we conducted a quantitative and qualitative characterization of flowering patterns on 1-year-old long shoots in three apple cultivars ('Amasya,' 'Braeburn,' and 'Granny Smith') that exhibit distinct bearing behaviors.

The aim was to gain a better understanding of their growth dynamics and reproductive activities. To achieve this, we compared 'Amasya,' which demonstrates alternate bearing patterns, with 'Braeburn' and 'Granny Smith,' both known for regular bearing patterns. We examined variables related to vegetative and reproductive bud development and the quality of floral buds along the annual shoots, which we partitioned into three different successive zones, in addition to analyzing the shoot structures.

2. Materials and Methods

The study orchard was established in 2013 with row spacing set at 4.0 m and tree spacing at 1.0 m. It was located at the Egirdir Fruit Research Institute in Isparta, Türkiye (37° 48' 52.16'' N, 30° 52' 39.66'' E). Following planting, the trees were trained in a trellis spindle system with minimal pruning. Orchard management practices, including irrigation, nutrition, pest and disease control, as well as weed management, were conducted in accordance with local commercial orchards from 2013 to 2019.

For this study, we selected three different apple cultivars, each with unique genetic backgrounds and levels of vigor: 'Amasya,' 'Braeburn,' and 'Granny Smith,' all grafted onto M.9 rootstock. 'Amasya' is characterized by its desirable features such as eating quality and aroma, but it is subject to irregular yield constraints (Atay et al. 2018). 'Granny Smith' and 'Braeburn,' both popular apple cultivars globally, exhibit consistent yields. 'Braeburn' is distinguished by high-density branching and lateral fruiting, while 'Granny Smith' is known for terminal fruiting and a high mortality rate of axillary buds (Lauri et al. 1997; Fumey et al. 2011).

The trees were arranged in a randomized block design with three replications, and each replication consisted of one tree in 2019. We selected twelve shoots (four per tree) per cultivar, which were gathered from four different directions (North, South, East, and West) during the first week of April. The chosen shoots were typically healthy and bearing, with base diameters of the 1-year-old wood exceeding 5 mm, and each shoot's total length exceeded 40 cm (see Figure 1). Nodes were numbered from the proximal to distal end and categorized into three zones (proximal, median, and distal).

Figure 1. One-year-old long shoots - A: 'Amasya', B: 'Braeburn', and C: 'Granny Smith'

We recorded the nature of buds (whether they were vegetative or generative) node by node along the shoots at full bloom. Full bloom occurred on April 24-26 for 'Amasya' and April 30-May 2 for 'Braeburn' and 'Granny Smith.' We quantified the number of flowers per inflorescence on the flowering buds. Furthermore, we also counted the number of spur leaves per inflorescence. The size of the spur leaves was determined based on a scale ranging from 1 to 5, as previously described in Atay and Atay (2022). In summary, this scale consists of five different intervals: 1) 0-10 cm^2, 2) 11-20 cm^2, 3) 21-30 cm^2, 4) 31-40 cm^2, and 5) >41 cm^2 spur leaf area.

As part of the morphometric traits analysis, we measured the shoot length in cm, from the base to the top, using a fabric tape measure. We used a digital caliper to determine the basal diameter of the shoots in mm. The nodes were counted from the base of the shoots towards the top, and then the internode lengths were calculated for each cultivar on each tagged shoot. We also counted the number of flowering buds per shoot for each cultivar.

Statistical analyses were conducted using R statistical software version 4.1.3 (R Core Team, 2022). The 'ggplot2' package was utilized for graph generation. ANOVA procedures were performed to compare zone effects. Violin plots are recommended for summarizing distributions and displaying all data points effectively, making them a preferable choice for clear communication and visual appeal in presenting important results.

3. Results

Buds on one-year-old wood were individually examined for all cultivars, and assessments were conducted along the shoot zones, from the proximal to the distal end. The number of generative buds in the proximal zone of the shoots was higher for 'Braeburn' compared to 'Amasya' and 'Granny Smith'. The proximal zone was primarily characterized by vegetative buds for 'Amasya' and 'Granny Smith'. In the median zone of the shoots, the average number of generative buds for 'Granny Smith' was relatively lower compared to the other two cultivars. Conversely, in the distal zone on one-year-old long shoots, more than 95% of the nodes for all cultivars were characterized by the presence of generative buds (Figure 2).

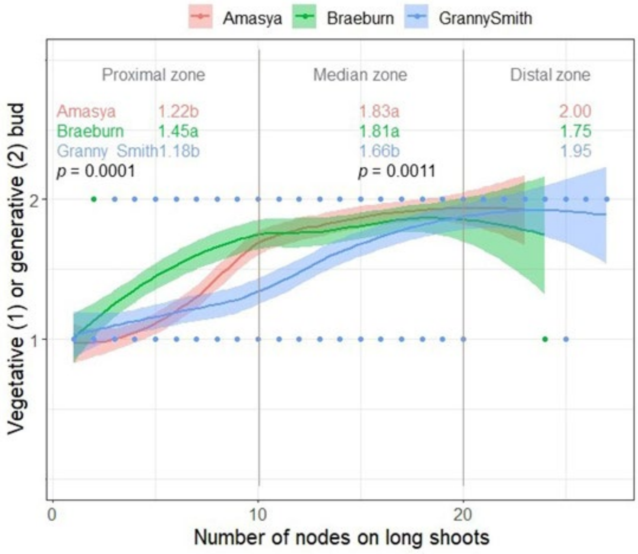

Figure 2. Effect of shoot zone on frequencies of vegetative or generative buds in 'Amasya,' 'Braeburn,' and 'Granny Smith' apples

After the proportion of floral differentiation along 1-year–old long shoots was observed, floral bud quality in the different shoot zones was examined in detail. The number of flowers per inflorescence in 'Amasya' was significantly lower in both the proximal (*p*=0.0001) and median zones (*p*=0.0003) of the shoots compared to the other cultivars (Figure 3). In the distal zone of the shoots, the number of flowers per inflorescence was quite similar for all cultivars, ranging from 4.67 to 5.00 number/inflorescence. This suggests that the number of flowers per inflorescence in 'Amasya' gradually increased towards the distal zone, and the difference diminished in the distal zone of the shoots.

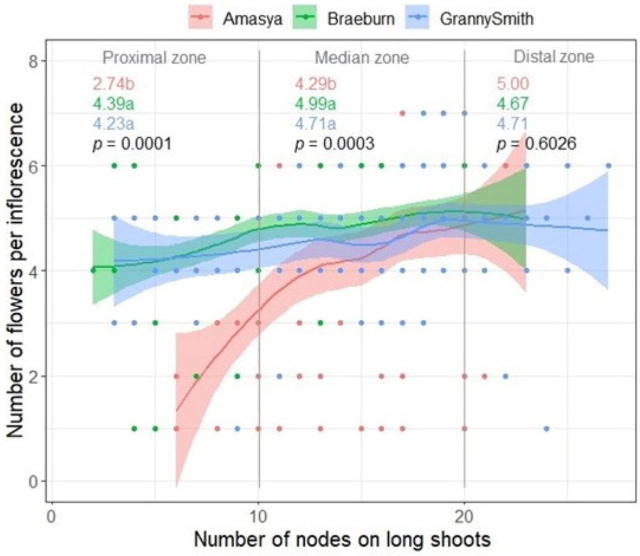

Figure 3. Effect of shoot zone on number of flowers per inflorescence in 'Amasya,' 'Braeburn,' and 'Granny Smith' apples

'Amasya' had the fewest spur leaves per inflorescence, both in the proximal zone (0.89 number/inflorescence) and the median zone (2.87 number/inflorescence) of one-year-old long shoots, whereas they were nearly equal for 'Braeburn' (5.67 and 6.09 number/inflorescence, respectively) and 'Granny Smith' (5.55 and 6.59 number/inflorescence, respectively) (Figure 4). There was no significant difference in the number of spur leaves per inflorescence among all cultivars (6.67, 5.33, and 6.57 number/inflorescence for 'Amasya,' 'Braeburn,' and 'Granny Smith,' respectively) in the distal zone of the shoots.

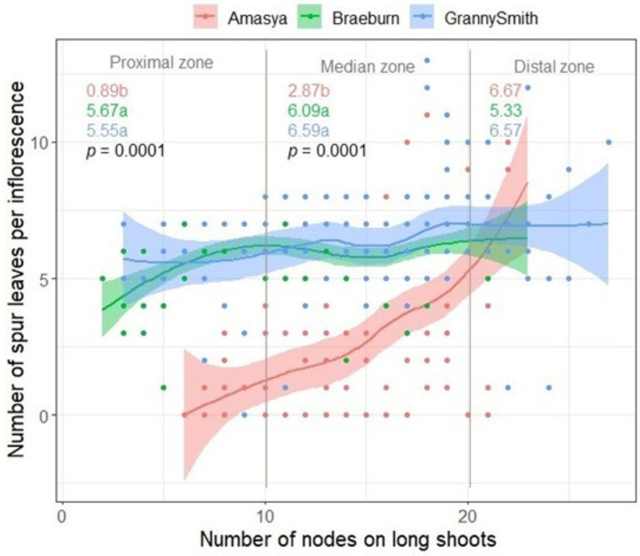

Figure 4. Effect of shoot zone on the number of spur leaves per inflorescence in 'Amasya,' 'Braeburn,' and 'Granny Smith' apples

In addition, the spur leaf size in the proximal and median zones of 'Amasya' shoots was significantly smaller than that of 'Braeburn' and 'Granny Smith' (Figure 5). In the proximal zone, 'Amasya' had a spur leaf size of 0.48, while 'Braeburn' and 'Granny Smith' exhibited similar spur leaf sizes of 3.39 and 3.59, respectively. Among the cultivars, the highest spur leaf size in the median zone was observed in 'Granny Smith' (4.15), followed by 'Braeburn' (3.80). 'Amasya' had relatively smaller spur leaf size in the distal zone of shoots compared to the other two cultivars, but there were no significant differences among these cultivars. The spur leaf size per inflorescence for 'Amasya' gradually increased from the proximal zone to the distal zone, while the sizes for 'Braeburn' and 'Granny Smith' remained more stable along the shoot.

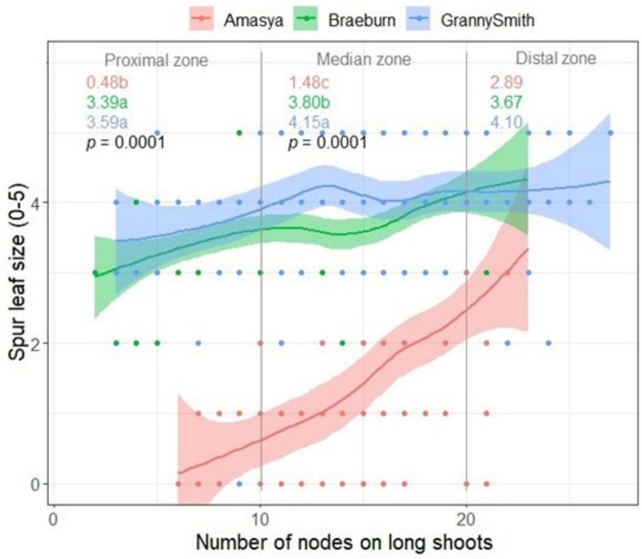

Figure 5. Effect of shoot zone on spur leaf size in 'Amasya,' 'Braeburn,' and 'Granny Smith' apples

The mean shoot length was slightly longer in 'Braeburn' (54.08 cm) and 'Granny Smith' (53.75 cm) than in 'Amasya' (47.83 cm), with no statistically significant differences (Figure 6A). Mean shoot diameter ranged from 7.87 mm ('Amasya') to 8.37 mm ('Granny Smith') (Figure 6B). 'Granny Smith' shoots had a slightly higher node number (mean: 21.25), but this difference was not statistically significant when compared to the other cultivars (Figure 6C). On the other hand, internode length, as leaf separators, varied depending on the cultivar, with the highest mean internode length found in 'Braeburn' (mean: 2.78 cm), while it was 2.48 cm and 2.53 cm for 'Amasya' and 'Granny Smith,' respectively (Figure 6D). Additionally, the number of flowering buds per shoot did not vary significantly by cultivar, with mean values ranging from 9.83 ('Granny Smith') to 12.08 ('Braeburn') among the one-year-old long shoots of these cultivars (Figure 6E).

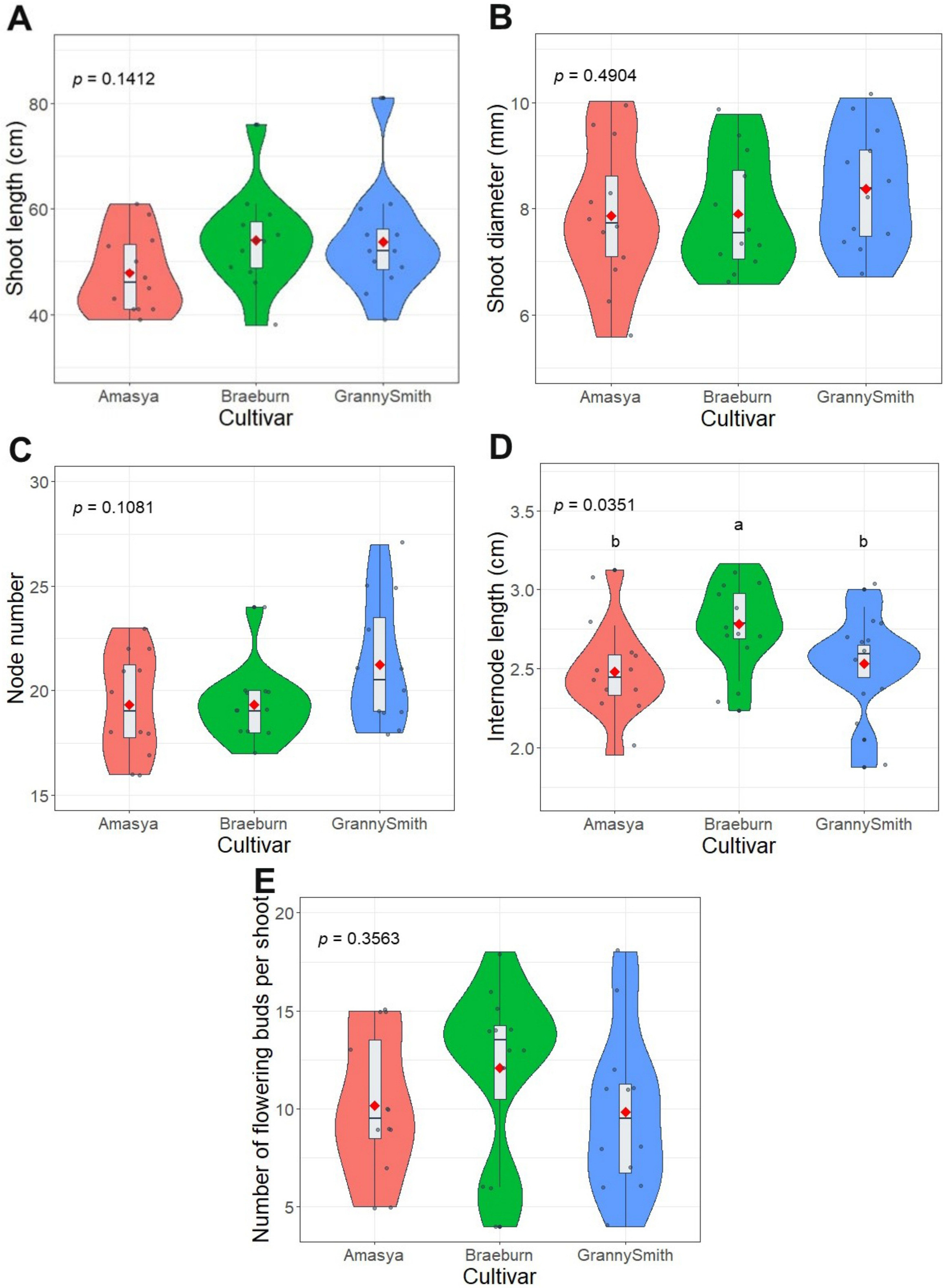

Figure 6. Morphometric traits of one-year-old long shoots in the cultivars 'Amasya,' 'Braeburn,' and 'Granny Smith'. (A) Shoot length (cm), (B) Shoot diameter (mm), (C) Node number, (D) Internode length (cm), and (E) Number of flowering buds per shoot. Different letters indicate statistical significance according to the LSD multiple-range test at $p < 0.05$.

4. Discussion

Three successive zones, comprising the proximal, median, and distal portions, are a common feature in long shoots of fruit trees (Costes and Guedon 2002; Solar et al. 2005; Stanley 2016; Meszaros et al. 2020). In this study, these zones were identified along the 1-year-old shoots of 'Amasya,' 'Braeburn,' and 'Granny Smith,' based on their positions (distance from the base and apex). The number of vegetative and generative buds was estimated for each cultivar in these three zones along the shoots. The distribution of vegetative and generative buds clearly depended on the zone along the shoot. In 'Amasya' and 'Granny Smith,' the proximal zone predominantly contained vegetative buds, forming a pool of buds that enables the tree to respond to damage, pruning, and aging by developing epicormic shoots (Gordon et al. 2006; Atay and Koyuncu 2012). It was observed that 'Braeburn' exhibited bearing behavior in every zone along the shoot, although it was relatively lower in the proximal part, consistent with the findings of Costes et al. (2003) in their description of the architectural development of 'Braeburn'.

The median zone was primarily composed of generative buds for 'Amasya,' while it consisted of vegetative buds for 'Granny Smith'. The third (distal) zone was identified as the floral zone, containing a significant number of associated generative buds for all cultivars. Indeed, the frequency of generative buds varies with terminal bearing behavior in apples, depending on cultivars, shoot type, and length (Lauri and Corelli-Grappadelli 2014; Pallas et al. 2018). The nature of the terminal bud and other buds near the apex on 1-year-old shoots was consistently determined as generative buds in all the cultivars studied. Given the higher light levels, photosynthetic capacity, and hormonal activity in this region, a greater intensity of flowering can be expected in the distal zone (Ferree 1989; Barritt et al. 1991; Tromp 2005; Gottschalk and van Nocker 2013).

The relationship between bud position and fate along the annual shoots determines the flowering patterns, which are associated with bearing habits (e.g., regular or biennial) (Guedon et al. 2001; Costes et al. 2006; Pallas et al. 2018). However, in our study, we observed less contrast between biennial bearing and regular bearing cultivars when comparing these vegetative and generative buds in the three zones along the shoots. 'Amasya,' with irregular bearing patterns, displayed a similar generative bud rate to 'Braeburn' or 'Granny Smith,' characterized as regular cultivars in each zone. This suggests that there is no simple relationship between flowering patterns and regular bearing capacity. Hormonal equilibrium may have a more significant impact than the number of flower buds (Tromp 2005; Bangerth 2006; Hanke et al. 2007).

The number of flower organs, flower size, the number of flowers per inflorescence, as well as the area and number of leaves of bourse shoots, have been proposed for analyzing floral bud quality in apples (Lauri et al. 1996; Ferree et al. 2001; Jackson 2003). The number of flowers per inflorescence remained relatively stable across all zones in 'Braeburn' and 'Granny Smith.' There was a gradual increase in the number of flowers per inflorescence toward the distal zone of shoots in 'Amasya.' This trend was also observed in the number of spur leaves per inflorescence and spur leaf size for 'Amasya.' In fact, both the number and size of spur leaves per inflorescence were limited in the proximal and median zones in ‘Amasya’, whereas they were more similar to those of 'Braeburn' and 'Granny Smith' in the distal zone. Generally, the size of reproductive buds (leaf number) differs along apple shoots in one-year-old shoots (Lauri 2007). However, this trend was only obvious in ‘Amasya’, which has irregular yield constraints, in our study. Floral bud quality variables showed stability along the shoot in 'Braeburn' and 'Granny Smith,' which exhibit a regular bearing habit. In contrast to the number of vegetative or generative buds, there was a clear difference in floral bud quality variables in shoot zones between biennial bearing and regular bearing cultivars studied. These findings confirm that floral bud quality, especially spur leaf properties, is strongly associated with the bearing capacity of apples (Ferre and Schmid 2004; Elsysy and Hirst 2017; Atay and Atay 2022). A sufficient amount of spur leaves in apples is crucial for long-term productivity due to its effect on photosynthetic performance, carbohydrates, phytohormones, and nutritional status (Ferree et al. 2001; Wertheim and Schmidt 2005; Madail et al. 2012; Atay and Atay 2022).

In general, differences in the morphometric traits of 1-year-old long shoots between cultivars are expected, possibly due to different growth habits (Lauri and Lespinasse 1993; Costes and Guédon 2002). However, in the present study, there were no differences in shoot length, shoot diameter, node number, and flowering bud number between cultivars when considering the entire shoot scale. These results confirm that weak genetic control may appear in morphological traits when bud position and growth units along the shoot are not taken into account (Verheij 1996; Costes 2003; Sadok et al. 2015). Therefore, no significant effects could be observed in whole shoot traits between cultivars. One possible cause of these finding could be that the shoot age in perennial plants has a greater impact than branching behavior (Costes 2003). In our study, we used 1-year-old long shoots with a similar crop load, and this stability in the studied shoots may have influenced these findings.

Interestingly, internode length appeared to be significantly impacted by the cultivar effect in the present study. A similar result was found for some horticultural crops by Da Silva et al. (2014) and Sadok et al. (2015), demonstrating that internode lengths were mainly genetically controlled. The internode lengthening process plays a crucial role in maintaining water conductance, light interception, hydraulic efficiency, and mechanical stability as it affects the stiffness of shoots and the number and size of the leaves (Cochard et al. 2005; McCulloh and Sperry 2005; Sadok et al. 2013). Therefore, internode lengthening traits are suggested as one of the most important architectural components for achieving high productivity (Sadok et al. 2015). Since our study was conducted on only three cultivars trained with the same system, further analysis of different cultivars in various growing systems, under different environmental and orchard management conditions, would be relevant.

5. Conclusions

In conclusion, the results demonstrate differences in flowering patterns along the three successive zones (proximal, median, and distal) on the 1-year-old shoots of 'Amasya,' 'Braeburn,' and 'Granny Smith.' There was less contrast observed between alternate bearing ('Amasya') and regular bearing cultivars ('Braeburn' and 'Granny Smith') when comparing the number of vegetative or generative buds in these three zones along the shoot. However, a clear difference in floral bud quality variables was evident in shoot zones between biennial bearing and regular bearing cultivars studied. Floral bud quality variables remained stable along the shoot in 'Braeburn' and 'Granny Smith,' which have a regular bearing habit, while there was a gradual increasing trend from the proximal zone towards the distal zone in 'Amasya' shoots. The results of this study highlight the importance of floral bud quality, especially spur leaf properties along the shoot, in ensuring optimal and consistent productivity.

Author Contributions: The author has read and agreed to the published version of the manuscript.

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Drought Tolerance in Rice (*Oryza Sativa* L.): Impact, Performance and Recent Trends

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HIGHLIGHTS

- Rice is a primary cereal crop for over half the world's population, meeting 35-60% of their calorie needs.
- Drought is a significant environmental factor affecting rice production, particularly in Asia, impacting around 30% of farmers.
- Drought-tolerant rice varieties have been developed to enhance and stabilize rice yields, with Nepal releasing 11 such varieties.
- Understanding the types and impacts of drought is crucial for effective drought management and mitigation strategies.

Abstract

Drought poses a significant challenge to rice cultivation in Asia's rain-fed regions, which is expected to worsen with climate change. This article presents a comprehensive review of the current state of knowledge on drought tolerance in rice, based on a literature review of 52 relevant articles. The articles were chosen based on their relevance to the topic of drought tolerance in rice. The selected articles were then analyzed using a qualitative approach to summarize and synthesize their findings into three main sections: impact, performance, and recent trends. The article highlights several key findings on the development of drought-tolerant rice cultivars, including the identification of genes that control responses to water availability, the use of submergence-tolerant varieties in flood-prone lowlands, and the importance of physiological, biochemical, and molecular adaptation processes in improving rice's tolerance to drought stress. The article emphasizes the importance of marker-assisted breeding and cultivation in semi-arid and rainfed environments to develop more drought-tolerant cultivars. The development of drought-tolerant rice cultivars is crucial to ensure food security and mitigate the effects of climate change in Asia's rain-fed regions. The article also discusses various types of droughts and their effects on different plant species and drought pressures. As the global population increases, the demand for rice as a dependable food crop also rises. To meet this demand, rice cultivation must be expanded to rainfed areas. However, rice's adaptation mechanisms and habitat make it one of the most challenging crops for breeders to develop drought-tolerant varieties. Overall, this article provides important insights and recommendations to improve rice productivity and address the challenges associated with drought in rice cultivation.

Keywords: Rice; *Oryza sativa;* Drought tolerance; Stress response; Impacts; Sukhadhan

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1. Introduction

Rice is a perennial, semi-aquatic, self-pollinated cereal crop that belongs to the Poaceae family (Adhikari et al., 2022). It is the primary cereal crop for more than half the world's population (Dar et al., 2020). Over half of the world's population consumes rice as their primary staple diet, which meets their calorie needs by 35 to 60% (Dhakal et al., 2020). Nearly every part of the world—including the tropics, subtropics, and tropical and temperate regions—now has rice-growing areas (Ji et al., 2012). Developmental nations produce most of the world's rice, with China and India alone accounting for nearly 95% of global production (Dhakal et al., 2020). According to FAO, the rice yield in 2019 was 4661 kg/ha, while the yearly output was 755473800 tons (Adhikari et al., 2022). More than 90% of the world's rice production and consumption occurs in Asia, which is superior to other continents in production and consumption (Panda et al., 2021; Vinod et al., 2019). More than half of the country's population's daily calorie needs are met by rice, a significant food crop in Nepal. It can be grown in Nepal's diverse agro-climate zones, which range from the terai (50 masl) to the mid-hills and high mountains valley (3050 masl), which is Jumla, the highest altitude of any rice-growing region in the world (Adhikari et al., 2022; Dhakal et al., 2020).

Rice contributes significantly to Nepal's agricultural GDP, aids national food security, and improves people's standard of living (Gauchan et al., 2014). Several environmental factors have reduced global rice output, particularly in the lowlands of Asia; roughly 30% of farmers have been impacted (Dar et al., 2020). In order to give nutrients and water, rice is often produced in flooded circumstances; however, due to the low water availability, around half of the rice lands have seen some reduction in production as a result of the drought (Gusain et al., 2015). About 34 million hectares of rainfed lowland rice and 8 million hectares of highland rice in Asia are under drought stress (Dar et al., 2020). Most of Nepal's rice fields are prone to drought, and around two-thirds are rain-fed (Figure 1) (Gauchan et al., 2014). Drought is one of the main abiotic variables inhibiting rice growth, development, output, and productivity and lowering rice areas (Dhakal et al., 2020; Guo et al., 2013). Therefore, disseminating these drought-prone types more widely, creating and promoting various drought-resistant rice varieties has been crucial in enhancing and stabilizing rice yields. Drought tolerance is the multifunctional outcome of many molecular, morphological, and biochemical traits (Sahebi et al., 2018). The creation of rice cultivars resistant to drought was started in the 1960s by the International Rice Research Institute (IRRI). However, hopeful outcomes did not appear until around ten years ago (Dar et al., 2020). Nepal has created and released around 11 drought-tolerant rice varieties to address productivity and food security issues with IRRI's technical assistance (Gauchan et al., 2014). Sukhadhan-1, Sukhadhan-2, and Sukhadhan-3 were published in 2011, while Sukhadhan-4, Sukhadhan-5, and Sukhadhan-6 were released in 2014 as drought-tolerant rice varieties created in Nepal. These varieties can help improve grain output and quality, leading to poverty eradication and improved health and standard of living for farmers (Dar et al., 2020; Vinod et al., 2019).

The objective of this article is to provide an overview of the importance of rice as a primary cereal crop for a significant portion of the world's population, the environmental and agricultural factors that can impact rice production, and the efforts made to develop drought-resistant rice varieties in order to enhance rice yield, productivity, and food security.

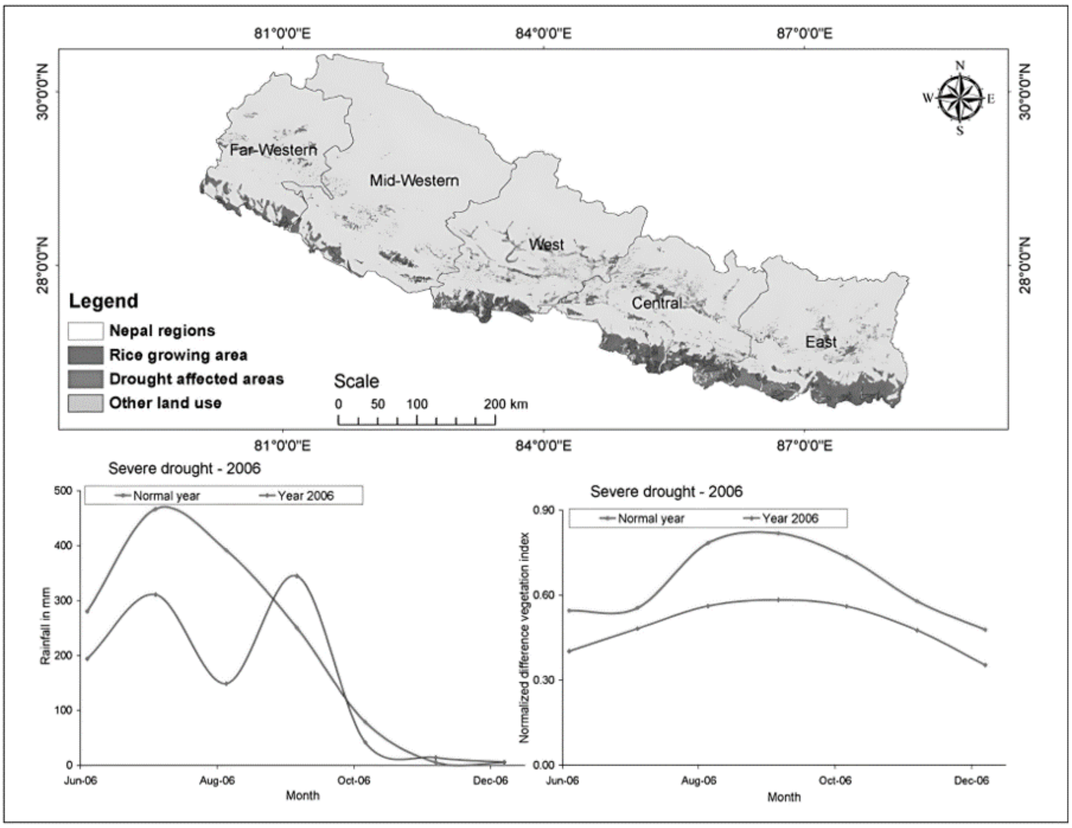

Figure 1. Geospatial mapping of drought-prone rice production locations in Nepal (Source: Gumma et al., 2011).

2. Materials and Methods

This manuscript is based on a literature review of 52 articles selected from various scientific databases. The articles were chosen based on their relevance to the topic of drought tolerance in rice (*Oryza sativa* L.) and their publication dates (from 2010 to 2022). The selection process was conducted by searching for articles using relevant keywords such as "drought tolerance," "rice," "*Oryza sativa*," "stress response," and "Impacts." The selected articles were studied for their impact, performance, and recent trends in drought tolerance in rice. These articles were then summarized in a coherent manner to provide an overview of the current state of knowledge in this field. The analysis of the selected articles was conducted using a qualitative approach, which involved summarizing and synthesizing the findings of each article. The concept and findings from the selected articles were organized into three main sections: impact, performance, and recent trends. Each section provides a detailed description of the key findings of the selected articles and their implications for the development of drought-tolerant rice varieties. Overall, this manuscript presents a comprehensive review of the current state of knowledge on drought tolerance in rice, based on a selection of 52 relevant articles.

3. Results and Discussion

3.1. Types of droughts

Drought is a complex phenomenon with significant impacts on various sectors of society. It is defined as the absence or insufficiency of precipitation, which may be measured as a decrease in precipitation quantity and can occur for a brief time or be protracted across many years (Carroll et al., 2021). Drought has become a severe global concern, and the severity of disasters like drought has significantly grown since the 1970s, with a projection of continued increase in the twenty-first century, according to the earth system model (Liu et al., 2016). The impact of drought on water supplies, agricultural output, and social activity is significant and has been studied extensively (Caloiero et al., 2021). The strength and severity of a drought may be determined through drought indicators, which are essential in assessing and analyzing the danger of drought (Aghelpour et al., 2020). Numerous research efforts have been made globally to comprehend and efficiently address drought (Bae et al., 2019). Rice plants adapt to drought stress through morphological adjustments in their root system, reducing water loss through transpiration, and accumulating osmoprotectants and antioxidants (Figure 2). They also undergo changes in gene expression to prioritize survival over growth. Understanding these mechanisms can aid in the development of strategies to improve rice plant drought tolerance. Understanding drought is crucial for effective management of water resources and related sectors. It is divided into four categories to facilitate study and research on drought: socioeconomic, agricultural, hydrological, and meteorological/climatological droughts (Wang et al., 2016). Additionally, droughts are classified into three categories based on their severity, length, and geographical distribution (Zargar et al., 2011). Overall, the study of drought is critical for the development of appropriate mitigation and adaptation strategies to address its negative impacts on society and the environment. The utilization of drought indicators can aid in effective management and preparedness to minimize drought impacts.

3.1.1. Meteorological drought

Meteorological drought, which is caused by an imbalance between precipitation and evapotranspiration, is a type of water shortage (Liu et al., 2016). This type of drought occurs due to a lack of precipitation, which may also be accompanied by an increase in potential evapotranspiration (Caloiero et al., 2021). It has been observed that non-meteorological droughts are linked to meteorological droughts, which underscores the importance of studying meteorological drought to comprehend its interplay with other types of droughts (Bae et al., 2019). The primary cause of meteorological drought is a decline in precipitation, which leads to a shortage of soil moisture, also known as agricultural drought (Liu et al., 2016). Additionally, increased evaporation at high frequency can lead to hydrological drought, while a more substantial reduction in precipitation can lead to socioeconomic drought (Liu et al., 2016). The standardized precipitation index (SPI) is a widely used indicator for studying meteorological droughts, which takes into account the variability of precipitation (Caloiero et al., 2021). Understanding meteorological droughts is crucial because they can significantly impact water resources, agriculture, and social activities. By studying meteorological droughts and their interplay with other types of droughts, policymakers and researchers can develop effective strategies to mitigate the negative consequences of droughts on society and the environment.

3.1.2. Agricultural drought

Agri-drought, also known as agricultural drought, is a type of drought that occurs when soil moisture levels are below what is needed for optimal plant growth (Liu et al., 2016). This can be caused by a lack of precipitation, low soil moisture, reduction in reservoirs, and differences between actual and potential evapotranspiration (Bae et al., 2019). The impact of agricultural drought on crops can lead to reduced yields or complete crop failure, which can further impact the environment and society (Van Loon, 2015). The severity of agricultural drought is influenced by various factors, including soil temperature, soil properties, evapotranspiration, precipitation, and the physiological and ecological traits of the crops being grown (Liu et al., 2016). Monitoring agricultural drought is essential for predicting and mitigating its impact. Various drought monitoring indices have been developed, and they can be classified into site-based and remote sensing methods. Remote sensing techniques have made it possible to accurately assess the impact of drought on crops at a larger scale, and simple agricultural drought indices can be created using this information (Liu et al., 2016).

3.1.3. Hydrological drought

A hydrological drought is defined as a water shortage that results in an imbalance in the availability of water resources, a decline in water supplies or aquifers, and reservoir depletion (Liu et al., 2016). It occurs when the amount of water demanded exceeds the amount of water available in the hydrological system (Bae et al., 2019). Hydrological droughts have a significant impact on both land and aquatic ecosystems globally, highlighting the need for drought monitoring (Van Loon, 2015). It is closely related to meteorological drought, as a lack of precipitation leads to reduced water availability in the hydrological system (Wang et al., 2016). Various indicators are used to identify and index hydrological drought, such as streamflow, groundwater levels, and lake and reservoir storage (Van Loon, 2015).

3.1.4. Socioeconomic drought

Socioeconomic drought is often caused by the interplay of climatic factors, resource availability, and the societal response to drought impacts. It can result in economic losses, reduced income, unemployment, increased poverty, and food insecurity, among others (Liu et al., 2016; Kundu et al., 2021). It can also cause social and political unrest, migration, and conflicts over scarce resources (Kundu et al., 2021). Therefore, understanding the socioeconomic impacts of drought is crucial in developing effective policies and strategies to mitigate and adapt to drought risks.

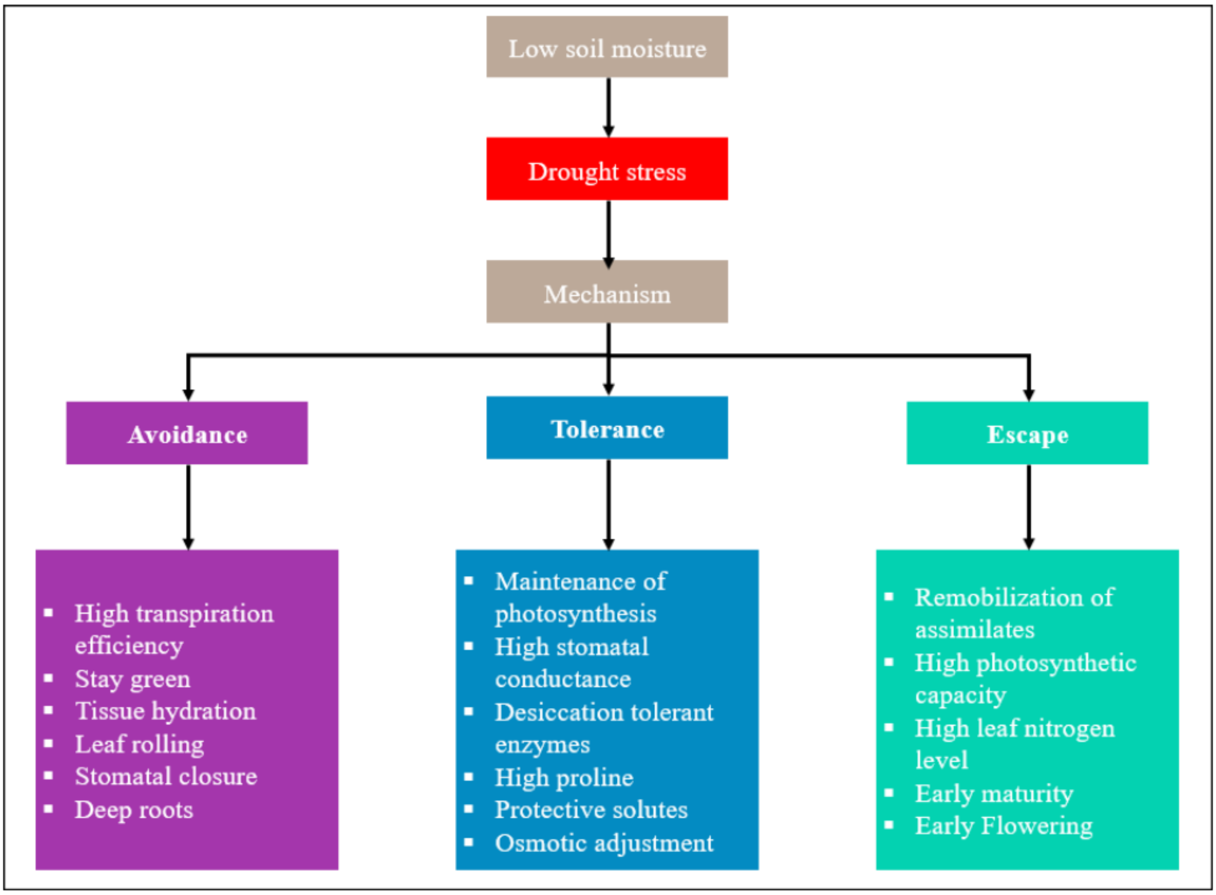

Figure 2: Mechanisms involved in rice plant adaptation to drought stress (Source: Panda et al., 2021).

3.2. Effect of drought stress

Plants are significantly affected by drought under abiotic stress, which is the primary factor limiting agricultural yield and ultimately determining the order of plant species. Stomata closure, decrease in water content, and drought stress are some effects that can be observed by regulating gas flow. Significant water loss can lead to the accumulation of reactive oxygen species (ROS), which disrupts metabolism and enzyme-catalyzed processes, potentially causing plant death (Hà, 2014).

3.2.1 Morphological stress

Plants exhibit a complex response to drought stress, which includes morphological changes. The most common sign of drought stress is reduced plant growth, and most plant species experience changes in leaf architecture and structure due to drought stress (Lum et al., 2014). The immediate impact of drought is impaired germination, which negatively affects standing ability (Ndjiondjop et al., 2018). Drought stress affects the whole green plant surface, and the response of plants to this stress is complex because it involves the interaction of many types of stresses affecting a plant's response at every key developmental stage over time and space (Shekhar et al., 2021). Drought stress is a severe abiotic factor that limits rice output in the rainfed and upland habitat by causing plants to produce fewer leaves per plant and smaller leaves (Ndjiondjop et al., 2018; Shekhar et al., 2021). Drought stress during the tillering phases harms the number of tillers, leaf length, and area (Shekhar et al., 2021). Under full irrigation, rice leaves typically do not roll, but during a drought, they roll in, preserving their positive internal water status. Drought is a factor in leaf rolling and burning of the leaf tips, particularly in vulnerable rice cultivars. Leaf rolling during a drought is reversible, but leaf tip drying is not (Ndjiondjop et al., 2018).

Drought stress lowers metabolic activity as a result of the lack of water. Reduced metabolic activity lowers turgor pressure, impairs the plant's cell division and elongation processes, and lowers plant height (Singh et al., 2018). The most crucial time for controlled watering and drainage may be during the vegetative stage. According to studies, the blossoming period is the most vulnerable to water scarcity (Yang et al., 2019). Grain weight may decrease due to the loss in absorption and nitrogen availability to reproductive plant parts brought on by drought stress. Under drought stress, yield/plant was substantially correlated with plant height, panicle length, grains/panicle, and grain weight/panicle (Bhutta et al., 2019). Drought stress impacts plant height, the number of tillers, and panicles during the vegetative stage. The elongation of the cell is constrained during drought stress, which shortens the internode's length and causes a drop in plant height (Haque et al., 2016). Raising root weight under water stress may be an adaptive process that lowers water intake owing to more root development during dry circumstances (Davatgar et al., 2009). At the vegetative drought stage root, the dry matter often declines, and forcing a drought during blooming resulted in almost the same reaction. Studies show drought stress inhibits root development (Haque et al., 2016). Water stress is often responsible for reductions in the fresh and dry weight of leaves and shoots, chlorophyll a, b, and photosynthetic pigments, and carotenoids that impact photosynthesis. In rice, a reasonable tolerance to shoot dryness was found (Usman et al., 2012). Stunting is the most crucial morphological result of drought stress (Piveta et al., 2021).

3.2.2 Physiological Stress:

Drought is a significant environmental stress that can have a detrimental impact on crop yield and plant development in large agricultural areas (Saha et al., 2019). Plants exhibit various physiological responses to changes in their water status and adapt to their surroundings. One of the first indications of water scarcity in rice plants is a decrease in growth. In response to drought stress, plants may develop additional aerial organs. This is accompanied by changes in the physio-chemical characteristics of cell walls and a decrease in the rate of cell division. When tissue water status decreases, the stomata close to reduce the transpiration rate, thereby conserving water in the plant (Ndjiondjop et al., 2018). Water stress during the blooming stage leads to a higher proportion of empty grains due to reduced carbohydrate synthesis, weakened sink strength, and fertilized ovary abortion. Furthermore, terminal drought stress during grain filling leads to a reduction in kernel mass and size, which results in chalky grains. This occurs due to loosen starch packing in the endosperm, which lowers grain production and mass (Kumar et al., 2020). Stomatal closure, reduced gas exchange, and increased water stress can lead to the accumulation of reactive oxygen species (ROS). Rice seedlings may exhibit severe drought symptoms due to a decrease in chlorophyll content and an increase in H2O2 and malondialdehyde (MDA) in response to drought stress (Hà, 2014).

Drought stress can reduce photosynthetic activity due to stomatal or non-stomatal processes. Stomatal closure, which is the initial response to drought stress, results in a reduction in photosynthesis and a disruption of critical photosynthesis-related processes, including CO2 supply, electron transport in thylakoids, peroxidative degradation of lipids, and water balance (Anjum et al., 2011). Drought stress can also result in an increase in proline buildup, which provides osmotic adjustment to defend cell turgor. Proline can also support enzyme protein stability and cell membrane integrity (Patmi et al., 2020). Drought stress reduces photosynthetic pigments and carotenoids, leading to a decline in leaf photosynthetic rate (Usman et al., 2012). Under restricted drought circumstances, physiological characteristics such as relative water content, leaf water potential, stomata conductance, transpiration, and leaf temperature are altered. Reactive oxygen species (ROS) damage to chloroplasts due to drought stress is associated with a decrease in chlorophyll content (Piveta et al., 2021).

3.3. Drought toleract rice varieties

In rain-fed regions of Southeast Asia, drought is a major environmental stress that reduces rice productivity across more than 24 million hectares of land, as noted by Adhikari et al. (2019). To address this challenge, the International Research Rice Institute (IRRI) has developed drought-tolerant rice varieties that offer better performance and higher yields to help ensure food security in regions that are prone to drought, as reported by Dar et al. (2020). One such genotype developed by IRRI is the breeding line IR74371-70-1-1, which has been distributed under the names Sahbhagi Dhan in India, Sukhadhan in Nepal, and BRRI Dhan in Bangladesh, and has shown promising results in withstanding drought stress, as reported by Anantha et al. (2016).

3.3.1 Sukhadhan Varieties in Nepal

With the assistance of the International Research Rice Institute (IRRI), Nepal has developed and released nine drought-tolerant rice cultivars, including the Sukhadhan series (Sukhadhan-1,2,3,4,5,6) (Gauchan et al., 2022). Sukhadhan-1, 2, 3, and Sukhadhan-4,5,6 were released in 2011 and 2014, respectively (Adhikari et al., 2019). The Sukhadhan series has varying maturation periods, with Sukhadhan-1 taking around 125 days to mature, followed by Sukhadhan-2, Sukhadhan-3, Sukhadhan-4, Sukhadhan-5, and Sukhadhan-6 maturing in about 120–125 days. Among the Sukhadhan series cultivars, Sukhadhan-5 has the highest yield of approximately 3.2–4.2 tons/ha, while Sukhadhan-3 performs well under drought-like conditions (Gauchan et al., 2022). Sukhadhan-2, Sukhadhan-5, Sukhadhan-3,4 (76 grains), Sukhadhan-1 (75 grains), and Sukhadhan-6 (68 grains) have been identified as the cultivars with the most effective grains per panicle under drought conditions (Dhakal et al., 2020). This series of drought-tolerant Sukhadhan rice cultivars shows promising potential for replacing older rice varieties in both irrigated and drought-prone areas, as they produce higher yields under varying water conditions (Adhikari et al., 2019).

3.3.2 Sahbhagi Dhan in India

In 1997, IR55419-04 and "Way Rarem" were crossed to produce the drought-tolerant and high-yielding Sahbhagi Dhan rice variety (Anantha et al., 2016). It was introduced by the Central Rain-fed Upland Rice Research Station of India in 2009 as an early-duration drought-tolerant variety (Dar et al., 2020; Rai et al., 2020). Research conducted in Eastern India demonstrated that Sahbhagi Dhan has the most consistent yields and outperforms other rice varieties (Dar et al., 2020). Sahbhagi Dhan was created to thrive in drought-prone areas of India and has a yield advantage of 0.5 to 1 ton/ha over other rice varieties during droughts (Kumar et al., 2021). Although it is resistant to leaf blasts, it is only moderately resistant to brown spots, sheath rot, stem borer, and leaf folder. Sahbhagi Dhan matures in approximately 105 days and produces an average yield of 4 to 5 tons/ha (Rai et al., 2020). By allowing early access to food, increasing cropping intensity, attracting better markets, reducing input costs, improving grain quality, and enhancing grain resistance to pests and diseases, Sahbhagi Dhan has enabled farmers to increase their yields and free up labour resources (Dar et al., 2020).

3.3.3 BRRI Dhan in Bangladesh:

The Bangladesh International of Nuclear Agriculture (BINA) developed a series of drought-tolerant rice varieties, which include BRRI dhan-55, BRRI dhan-42, BRRI dhan-43, BRRI dhan-48, BRRI dhan-33, BRRI dhan-56, BRRI dhan-57, BRRI dhan-66, BRRI dhan-71, and BRRI dhan-83 (Shelley et al., 2016). These varieties have demonstrated superior yield potential and exhibit reasonable resistance to leaf blight and sheath blight (Rahman et al., 2021). Among these varieties, BRRI dhan-55 has the highest average yield potential of approximately 4 tons/ha (Shelley et al., 2016). Furthermore, under drought conditions, BRRI Dhan-56 and BRRI Dhan-57 have demonstrated a yield potential of around 4-4.5 tons/ha and have been reported to outperform other rice varieties in Bangladesh (Rahman et al., 2021).

3.4. Impacts of Sukhadhan

Sukhadhan, a rice variety preferred by farmers in areas with lower water supply, has been found to be able to thrive without constant irrigation supply, which is a significant benefit in rain-fed environments where drought stress can harm plant function and result in complete crop failure and lower output. Tripathi et al. (2012) reported that Sahbhagi Dhan, a type of Sukhadhan, has an increased average grain production, particularly under drought conditions, and its early maturing characteristics allowed farmers to increase cropping intensity effectively, accommodating supplementary short-duration crops in the present cropping system. Furthermore, Kar et al. (2018) found that Sahbhagi Dhan produces uniform average grain weight in all water systems when used in DSR (direct-seeded rice), unlike other cultivars, whose grain weight significantly decreased at either 10 kPa or 40 kPa. Additionally, Anantha et al. (2016) observed that Sahbhagi Dhan can compete with weeds and sustain more substantial plant populations under stress compared to other recently produced rice varieties under direct dry seeding due to its high seedling emergence leading to early vegetative development, combined with its semi-tall height. Studies have also shown that the semi-dwarf plant type of Sukhadhan-3 responds well to production inputs, including fertilizer and watering (Deo et al., 2019), while Sukhadhan-5, which had the greatest CGR and maximum LAI, significantly increased photosynthetic rate and accumulation (Mahato & Adhikari, 2017). The highest photosynthesis efficiency is found in low-lying sections of rice, indicating that as altitude increases, photosynthesis efficiency tends to decrease (Li & Lin, 1993; Dhakal et al., 2020). Although Sahbhagi Dhan showed several positive traits for responding to drought, it also demonstrated several negative traits. Its vegetative development is comparably weak during dry seasons owing to low temperatures or low solar exposure, as opposed to other genotypes that lack lateral root response to drought (Anantha et al., 2016). Moreover, its performance in experiments under lowland drought was observed to fluctuate for midseason shoot biomass under vegetative stage stress owing to varying climatic circumstances compared to other genotypes (Anantha et al., 2016). Adopting Sahbhagi Dhan may result in lower input and labor costs along with an increase in yield and cropping intensity. However, there is no quantitative data to support these claims, which bring behavioural reactions to bear in managing risk, household habits, and investing (Dar et al., 2020). Sukhadhan also offers a greater byproduct, such as straw used to feed animals, and closer spacing results in a high rate of tillers per unit area, which is the cause of the highest byproduct output (Dhakal et al., 2020).

3.5. Ongoing campaigns to raise awareness of drought-tolerant rice plants

Climate change scenarios are expected to worsen the drought vulnerability situation for rice cultivation in rain-fed regions of Asia, which is already a major challenge for farmers (Serraj et al., 2011). In response, researchers have been exploring genomic-based methods to increase drought tolerance in rice by identifying genes that control molecular, cellular, and developmental responses to water availability (Tuberosa & Salvi, 2006). Additionally, in flood-prone lowland areas with heavy rainfall and locations susceptible to flooding, submergence-tolerant varieties like sub-A have helped to ensure food security (Ismail et al., 2013). Physiological, biochemical, and molecular adaptation processes are also being studied to improve rice's tolerance to drought stress, including morphological responses, seed germination, seedling development, leaf and root characteristics, physiological response, leaf photosynthesis, and osmolyte buildup (Panda et al., 2021). Different novel strategies have been developed to develop drought tolerant rice varieties and to improve drought stresses (Figure 3). In Nepal, researchers commonly use polyethylene glycol for in vitro testing of rice genotypes for drought resistance. In one study, mature embryos of three aromatic Indica rice types and one non-aromatic variety were used to develop callus and test for drought tolerance (Joshi et al., 2011). Overall, these efforts aim to develop rice varieties that can better withstand drought and submergence stress to ensure food security and mitigate the effects of climate change in Asia's rain-fed regions.

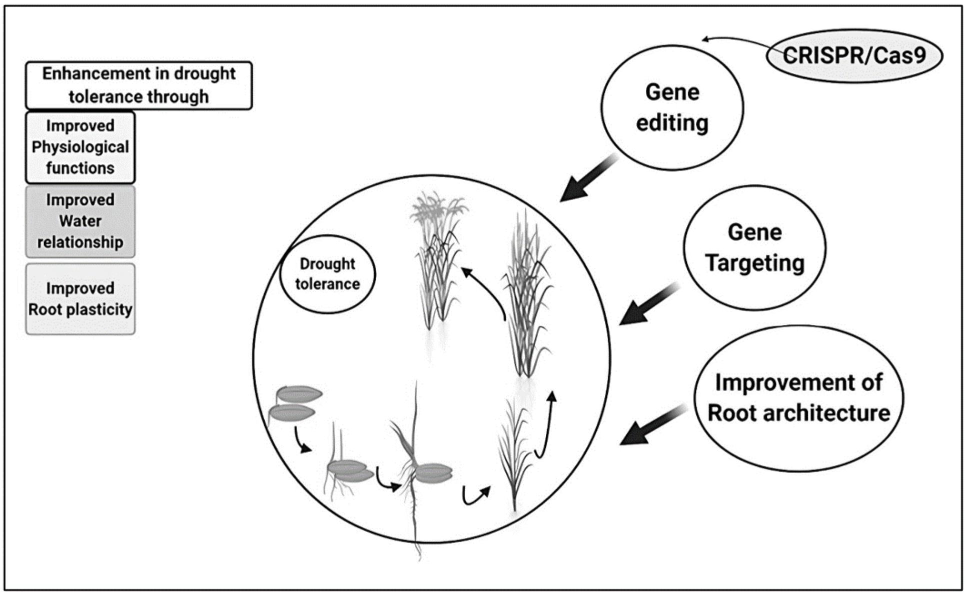

Figure 3: Novel techniques for improving drought resistance in rice plants (Source: Rasheed et al., 2020).

4. Conclusions

In conclusion, the increasing incidence of drought caused by climate change poses a significant challenge to rice cultivation in Asia's rain-fed regions. This article highlights several significant advances in the development of drought-tolerant rice cultivars. These include the identification of genes that control responses to water availability, the use of submergence-tolerant varieties in flood-prone lowlands, and the importance of physiological, biochemical, and molecular adaptation processes in improving rice's tolerance to drought stress. To develop more drought-tolerant cultivars, marker-assisted breeding and cultivation in semi-arid and rainfed environments are essential. Furthermore, to better understand the effects of drought tolerance on rice performance and to identify new trends in rice cultivation, further research is needed. Overall, these efforts are essential to ensure food security and mitigate the effects of climate change in Asia's rain-fed regions. The article's key findings demonstrate that Sukhadhan, a drought-tolerant rice variety, can thrive without constant irrigation supply, and its early maturing characteristics allow for increased cropping intensity. The use of submergence-tolerant rice varieties, such as sub-A, has been critical in ensuring food security in flood-prone lowlands. Developing drought-tolerant rice cultivars requires identifying genes that control responses to water availability, physiological, biochemical, and molecular adaptation processes, and marker-assisted breeding and cultivation in semi-arid and rainfed environments. Additionally, further research is necessary to assess the effects of drought tolerance on rice performance and to identify new trends in rice cultivation.

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Review Article

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Indonesian Perspective: Identification of Stevia Plant (*Stevia Rebaudiana* Bertoni M.) as Medicine Prospects

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HIGHLIGHTS

- Description of Stevia Plant (*Stevia Rebaudiana* Bertoni M.)
- Content of Bioactive Compounds of Stevia Plant (*Stevia Rebaudiana* Bertoni M.)
- Plant Pest Organisms on *Stevia Rebaudiana* Plants
- How to Use The Stevia Plant Folk Recomend by Medicine of Indonesian Indigenous Tribes

Abstract

Stevia plant has the potential for development as a source of natural sweeteners, a plant from South America and indigenous to Paraguay, has shown several advantages including the level of sweetness that reaches 100-200 the sweetness of sugar cane and low calorie also constituting a source of many substances with a nutritional effect on the human beings. Folk medicine of Indonesian indigenous tribes recommends it particularly as a substance strengthening the heart, the circulatory system and regulating blood pressure. It exhibits antibacterial, antifungal and anticaries properties. This stevia study aimed to educate people about the efficacy and safety of the stevia plant as a sweetener to replace sugar and medicines by diabetic patients as a part of Indonesia perspective the aims also to enrich information about the stevia plant which has the potential for development as a source of natural sweeteners, and as a medicine for diabetes with a bright prospects in the future.

Keywords: Identification of Stevia Plant; *Stevia Rebaudiana* Bertoni M; Medicine Prospects

1. Introduction

Sugar is a strategic commodity because it is consumed by all levels of society whose exploitation comes from on-farm to off-farm and is multi-dimensional regarding technical, social, economic, and political interests. Indonesian national sugar demand in 2014 reached 5.7 million tonnes. Sugar consumption for 2022/23 is expected to increase to 7.8 MMT of raw sugar equivalent from 7.6 MMT in 2021/22. In line with population growth and expected recovery of demand from the food and beverage industry, 2023/24 sugar

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consumption is forecast to reach 7.9 MMT of raw sugar equivalent. The Indonesian government is pursuing a National Sugar Self sufficiency Program (Director General of Plantations of Indonesia 2013); to meet the demand for sugar by looking for alternatives to other natural sweeteners that are safer than synthetic sweeteners to meet local needs.

Sugar is a sweetener for food and beverages. Based on the manufacturing process, sugar is divided into two categories, synthetic sugar and natural sugar. Synthetic sugar is artificial sugar, for example saccharin and cyclimate, while natural sugar is processed and obtained from plants that contain sweeteners, like sugarcane, coconut, palm, and stevia.

Annual non-calorie plant Stevia (*Stevia rebaudiana* Bertoni M.) based sugar contains glycoside, glycosides, steviosides, steviolbiosides, rebauldiosides A and B and several other sweetening ingredients in diterpenes as aglucon groups which can be used as an alternative source of sweetening in Indonesia (Directorate of Various Plants 2000). It has approved for use on the European Union (EU) list of alternative sugars. Stevia is mostly cultivated in South America but most of the world's commercial sales and production are made in Asia (IFST 2012).

Chemical analysis has shown that the stevia plant sweetener is a mixture of glycoside compounds. Several studies on stevia sugar have found that stevia sugar contains protein, fiber, carbohydrates, zinc, vitamin A, vitamin C, sodium, and magnesium. Its use in herbal form, replace sugar. Moreover, stevia has anti-fungal and anti-bacterial functions, which reduce flu symptoms, and is good as a skin care ingredient that can treat digestive disorders.

The cultivation and processing of stevia plants have developed rapidly in Indonesia, seen from the increasing area of stevia plantations and the increasing variety of uses of stevia products. Stevia products can be found both in the form of dry leaves and powder. Stevia products can be found both in the form of dry leaves and powder. Seed, stem cuttings, tillers, or tissue culture methods in large quantities in Indonesia can do stevia propagation

Therefore this stevia study aimed to educate people about the efficacy and safety of the stevia plant as a sweetener to replace sugar and medicines by diabetic patients as a part of Indonesia perspective. This review article could enrich information about the stevia plant which has the potential for development as a source of natural sweeteners, and as a medicine for diabetes with a bright prospects in the future.

2. Stevia Plant Description and Propagation

The stevia plant (Stevia rebaudiana Bertoni M.) was first discovered in the border area between Paraguay and Brazil, South America in 1887 by the American taxonomist Antonio Bertoni and named it Eupatorium rebaudianum Bertoni. Allegedly more than 80 types of stevia species grow wild in North America and 200 species naturally in South America, but only Stevia rebaudiana is used as a sweetener. It is commonly known as sweet leaf, sugar leaf, or simply stevia. It is used as sweetner in Japan since 1970 (Raini and Isnawati 2011). Indonesian local residents use this plant as a sweetener in food and drinks. The stevia plant is estimated to have entered Indonesia in 1977 on the cooperation of Japanese and Indonesian entrepreneurs, since then the stevia plant has become a commodity that has opportunities to be cultivated locally (Rukmana 2003).

According to Syamsuhidayat and Hutapea (1991) stevia plants are classified as following:

Kingdom : Plantae

Division : Spermatophyta

Sub-division : Angiospermae

Class : Dicotyledonae

Ordo : Campanulatae

Family : Compositae

Genus : Stevia

Species : Stevia rebaudiana Bertoni M.

Syn Eupatorium rebaudianum

The stevia plant is an annual shrub with a plant height that can reach 90 cm. The stem is round, fluffy, segmented, and forms many branches. Stevia has a single leaf, the leaves sit opposite each other, the leaves are oval and have fine hairs. Leaves 2-4 cm long and 1-5 cm wide. The stevia has a shallow taproot system with hairy roots that grow in the soil as a thicket. Stevia plants have compound flowers, in the form of panicles that grow at the ends of the stems and axils of the leaves. Stevia fruit is box-shaped, brown, and surrounded by hairs. Stevia seeds are dirty white and needle-shaped (Suhendi 1987). The following shows the shape of the stevia plant, which can be seen in Figure 1.

Figure 1. Stevia Plants

Source: (https://plants.ces.ncsu.edu/plants/stevia-rebaudiana/)

The stevia plant has a strong regeneration capacity and is resistant to pruning. Young stevia shoots will emerge from the base of the stem if pruned. Young shoots also emerge during degeneration at the end of the reproductive phase. Harvesting stevia is done by pruning the stems and leaving only the base. Pruning can continue until the plants are 3 to 4 years old.

Stevia plants can adapt well to various growing environments. Mubiyanto (1990) has mentioned that stevia plants can grow in areas with an altitude of 200-1500 meters above sea level, air temperature between 14°C-27°C, precipitation of 1600-1850 mm/year and 2-3 dry months. Infertile soil can be used to grow stevia provided enough available water. In general the soil should be fertile has loose, lots of humus, with good aeration and drainage, pH 4-5, with sufficient soil water contents between 43%-47%. Soil that is too wet will facilitate the spread of root by soil-borne pathogens.

Stevia propagation can be done generatively using seeds or vegetatively using stem cuttings, shoot cuttings, of shoots, and also plant tissue culture. The stem and shoot cuttings originating from the primary branch should have 3-4 nodes. Propagation of stevia by seeds is difficult because stevia seeds have a very low germination rate of 11% (Goettemoeler and Ching 1999).

The first propagation of stevia stem cuttings was carried out in the greenhouse of the Bogor Plantation Research Institute Indonesia in February 1983, the experimental results showed that the top of the stevia stem, both from the scion and its branches, was the best material for propagating stevia by stem cuttings.

Plant propagation by cuttings can be defined as a process of propagation using vegetative parts which then grow to perfection. Based on the part used as propagation material, there are 3 types of cuttings, namely root cuttings, stem cuttings (shoot cuttings are stem cuttings), and leaf cuttings (Hartmann et al. 1997).

In stem cuttings, the part of the plant taken as material for cuttings can determine success in cutting. The apical part has a higher success rate than the basal part of the plant. Good cuttings can be taken from young, woody stems. Woody cuttings are more resistant to unfavorable environmental conditions. Vegetative propagation basically forms adventitious organs that do not yet exist in cuttings. In stem cuttings, the adventitous roots that are formed, while potential shoots (not adventitious) already exist. Stem cuttings must immediately form roots so that the water supply to the canopy occurs immediately (Hartmann et al. 1997).

The process of forming adventitious roots consists of three stages, namely (1) cell differentiation followed by the formation of meristematic cells (root initiation), (2) differentiation of meristematic cells until root primordia are formed, and (3) emergence of new roots (Ashari 1995).

Vegetative propagation through stem cuttings, and root growth is an important factor during plant growth because they promote shoots induction and grow after the roots. Cuttings taken from an ancient material are unsuitable because they are not prone to rooting. Young cuttings are better because the roots can be induced rapidly (Wudianto 1993).

Vegetative propagation has many advantages, including being able to produce perfect plants with roots, stems, and leaves in a relatively short time, are true to type, fast. Factors that influence the success of cutting can be classified into three, namely plant factors, environmental factors, and implementation factors. Plant factors include the type of cutting material, age of cutting material, food content, water content and growth regulators. Environmental factors include growth media, humidity, temperature, and light, while implementation factors include the time of taking cuttings and treatment with growth regulators (Rochiman and Harjadi 1973).

Propagation of stevia using tissue culture technique, explants. 2mm long shoots were, cultured on Linsmainer and Skoog medium amended with 10 mg/l kinetin, for 50 days. The induced shoots were separated into several clusters, consisting of 2-4 shoots and subcultured. After 30 days, the growing shoots were separated to shoot elongation medium containing namely Linsmainer and Skoog media amended with 1 mg/l kinetin. The shoots that reached 3-5 cm in length, were rooted on Linsmainer and Skoog medium added with 0.1 mg/l NAA (Gunawan 1992).

3. Content of Bioactive Compounds

Stevia leaves contain apigenin, austroinulin, avicularin, beta-sitosterol, caffeic acid, campesterol, caryophyllene, sentaureidin, chlorogenic acid, chlorophyll, cosmosiin, synanoside, daukosterol, diterpene glycosides, dulcosides A-B, funikulin, formic acid, gibberellic acid, gibberellins, indole-3-acetonitrile, isoquercithrin, isosteviol, jihanol, kaempferol, kaurene, lupeol, luteolin, polystacoside, quercitrin, rebaudiosid A-F, scopoletin, sterebin A-H, steviol, steviolbioside, steviolmonosida, stevioside, stevioside a-3, stigmasterol, umbelliferon, and santofil (Raini and Isnawati, 2011). The main content of stevia leaves is steviol derivatives, especially stevioside (4-15%), rebaucide A (2-4%) and C (1-2%) and dulcoside A (0.4-0.7%). (Directorate General XXIV Consumer Policy and Consumer on Health Protection, Scientific Committee on Food in Raini and Isnawati, 2011).

4. Similarities with Other Plants

The stevia plant has similar secondary metabolite groups to the gotu kola plant. *Centella asiatica* can also be an alternative to preventing and treating diabetes. *Centella asiatica* contains asiaticoside compounds. Asiaticoside compounds in gotu kola plants can also improve memory, concentration and alertness. This is possible because asiaticoside can help smooth the circulation of oxygen and nutrients and protect brain cells from oxidative damage by free radicals. After all, the fatty acid content is very high and easily oxidized (Bermawi et al. 2005).

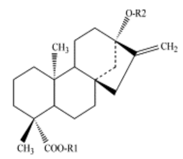
The chemical constituents of *Centella asiatica* leaves include a triterpenoid glycoside compound called asiaticoside (a heteroside compound), which is a secondary metabolite compound belonging to the terpene group which is efficacious for accelerating wound healing, asiaticic acid and madekasat (Haralampidis et al. 2002). Phillips et al. (2006) suggested that these terpenes are fats synthesized from the primary metabolite Acetyl CoA via the Mevalonic Acid (MAP) pathway or the basic intermediates of glycolysis via the Methylerythritol Phosphate (MEP) pathway. Three Acetyl CoA molecules combine to form mevalonic acid. This 6-carbon intermediate compounds undergoes pyrophosphorylation, carboxylation and dehydration to form Isopentenyl pyrophosphate (IPP). IPP is a terpene 5 C block precursor. IPP can also be formed from glycolysis intermediates or carbon reduction cycles in the photosynthetic process (Taiz and Zeiger 2002).

5. Stevioside biosynthetic pathway

Primary metabolite biochemical compounds play a role in the plant bodys nutrition and main metabolic processes. In contrast, secondary metabolites (including the active ingredient in stevioside compounds in the stevia plant) influence the ecological interactions between plants and their environment. In each plant, even between plant organs, biosynthesis of secondary metabolites occurs which varies depending on the environmental factors in which the plant grows. Plant secondary metabolites can be grouped into 3 main groups: terpenes or terpenoids, alkaloids or secondary products containing nitrogen, phenylpropanoids, and other phenolic compounds.

Steviosides are natural compounds belonging to the terpene group. This compound has a very sweet taste, 250-300 times sweeter than sucrose (cane sugar), and is low in calories (Chatsudthipong 2009). Steviosides from the stevia plant have greater potency, function, and sweetener characteristics than other sweeteners. In addition, stevioside also has hypoglycemic properties (Djas 2005), so it can be used as an alternative for preventing and treating *Diabetes mellitus*.

The structure of the stevia sweetener components, especially the steviosides contained in the leaves, can be seen in Figure 2.



	Compound name	R1	R2
1	steviol	H	H
2	steviolbioside	H	β-Glc-β-Glc(2→1)
3	stevioside	β-Glc	β-Glc-β-Glc(2→1)
4	rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	rebaudioside C (dulcoside B)	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Figure 2. Stevioside structure and compound bonds. Rebaudiosides D and ER R1 contain 2-β-Glc- β-Glc-(2→1). Rebaudiosides A, B, C, D, E and F in group R2 are added 3 carbons with the prefix β-Glc. Rebaudioside F one β-Glc replaced with β-Xyl.

The formula of stevioside ent-kaurene and gibberellins (GAs) is formed via the 2-C-Methyl-D-erythritol-4-phosphate(MEP) pathway (Totte et al. 2000). The enzymes catalyzing the first two pathways namely 1-deoxy-D-Xylulose-5-phosphate (DXS) synthase and 1-deoxy-D-Xylulose-5-phosphate reductoisomerase (DXR) were cloned using transcriptase-PCR. DXS and DXR from the stevia plant contained N-base plastid sequences and showed high similarity to other plants. In addition, it was shown through Escherichia coli functional clones of cDNAs coding proteins (Totte et al. 2003). Kim et al. (1996) found that the highest HMG-CoA reductase activity was the main enzyme of the mevalonic acid (MVA) pathway to IPP that occurs in the chloroplasts of the stevia plant. It is suspected that steviol is synthesized via the mevalonic acid (MVA) pathway, but no evidence supports this claim.

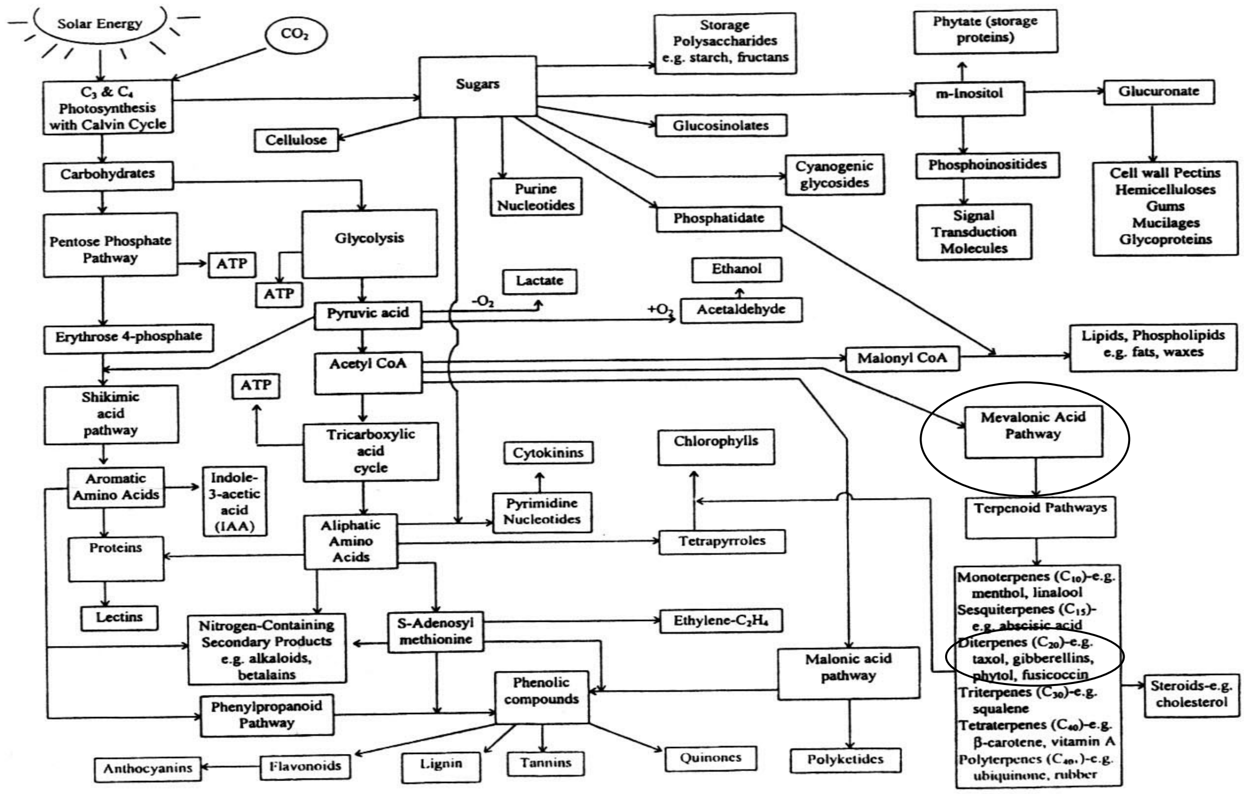


Figure 3. Pathways of stevioside biosynthesis

6. Use of The Stevia Plant

Stevia leaf is used as a sweetner by the Indonesian people. Stevia grown in Indonesia comes from Japan, Korea and China. These plant materials come from seeds so that the growth of stevia plants in the field is very diverse. The quality of stevia leaves is influenced by many environmental factors such as soil type, irrigation, lighting and air circulation. It is also affected by bacterial and fungal disorders. The quality of stevia sweetener is based on its aroma, taste, appearance and level of sweetness. Unlike other sweeteners, stevia doesn't give off a bitter after taste. The secret to stevia's sweetness lies in its complex molecule called stevioside which is a glycoside composed of glucose, sophorose and steviol (Raini and Isnawati 2011).

7. Mechanism of Action of Stevioside Compounds

Steviosides are hydrolyzed to steviol, before being absorbed in the small intestine. The absorption of stevioside and rebaudioside A was deficient, with a permeability coefficient of 0.16. 10-6 and 0.11. At 10-6 cm/sec, steviol at a concentration of 100 mmol/l is absorbed more effectively with a very high permeability coefficient of 44.5. 10-6 cm/sec while in secretory 7.93. 10-6 cm/second (Geuns et al. 2003 in Raini and Isnawati 2011). The absorption of the steviol aglycone in its single form (steviol) is better than in the mixed form of stevia (rebaudioside A, rebaudioside C, stevioside and dulcoside A), more than 93% remains in the mucosal fluid and 76% of steviol is absorbed in both the Duodenum-jejunum and ileum (Koyama et al. 2003 in Raini and Isnawati 2011).

Research conducted by Wang et al. (2004) and Raini and Isnawati 2011 showed that low levels of steviol were found in plasma after 8 hours post oral administration of 0.5 g/kg of steviosida (95% purity). Research to determine the bio-transformation of stevioside was carried out by incubating 50 mg/l stevioside (purity> 96%) in chicken excreta under anaerobic conditions for 24 hours, the result showed that 20% of stevioside hydrolyzed to steviol (Geuns et al. 2003 and Raini and Isnawati 2011).

Another study was carried out by incubating 40 mg of stevioside (85% purity) and 40 mg of rebausid A (90% purity) in faeces derived from 11 volunteers under anaerobic conditions for 72 hours. Steviosides are hydrolyzed to steviol aglycones within 10 hours and rebaudiosides within 12 hours. Steviol remained unchanged for 72 hours, indicating that bacterial enzymes could not break down the steviol structure (Gardana et al. 2003, Raini and Isnawati 2011).

Research on the metabolism of steviol in rats and humans was carried out using human liver microsomal preparations derived from 10 donors and rat liver microsomal preparations. The metabolite profile obtained from human liver microsomes was similar to that of mice, mass spectroscopic analysis showed the presence of 2 dihydroxy metabolites and 4 monohydroxy metabolites. One additional mono hydroxy metabolite was present in the rat preparations. Liver microsomal clearance of steviol in humans is 4 times lower than in mice (Wang et al. 2004 in Raini and Isnawati 2011).

8. Plant Pest Organisms on *Stevia rebaudiana* plants

Pests that commonly attack stevia plants include: aphids (*Aphis* sp.), armyworm (*Heliothis* sp.), grasshoppers, and caterpillars. While the diseases that are often found include: root rot, *Sclerotium rolfsiii wilt disease, Alternaria alternata* leaf spot, and *Fusarium* sp.

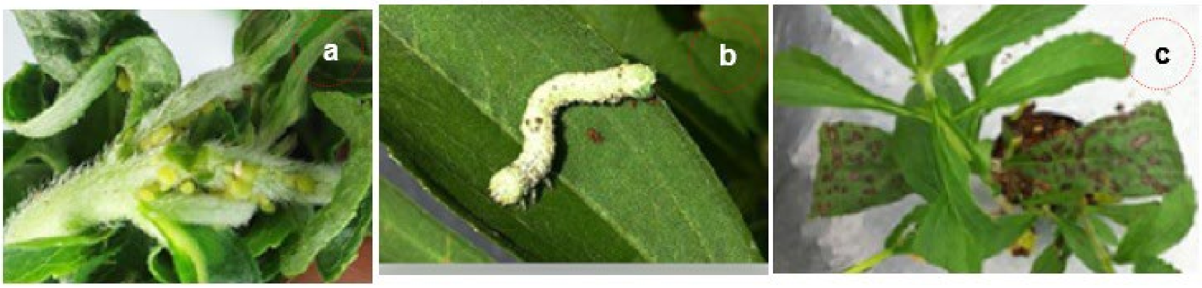


Figure 4. (a) *Aphis* sp., (b) *Heliothis* sp., and (c) leaf spot

Source: https://ditjenbun.pertanian.go.id/mengenal-stevia-pemanis-pengganti-gula-dari-tanaman-stevia-rebaudiana-dan-pengendalian-opt-secara-pht/

8.1 Control by technical culture

Control by technical culture can be done by garden sanitation and fertilizer application. Garden sanitation is carried out through manual weed control or by installing plastic mulch. *Stevia rebaudiana* plants are known to be very weak in competing with weeds, especially at the start of growth. Weed control should be done before weeds flower and seed to reduce weed seed reserves in the soil. Weed growth decreases after the *Stevia rebaudiana* plant canopy covers the soil surface. Garden sanitation is accompanied by applying urea, TSP, and KCL fertilizers at a dose of 1 g each per plant after one week of planting. Fertilization is repeated every time after harvest.

8.2 Mechanical control

Mechanical control can be done by pruning and manual control. Pruning the *Stevia rebaudiana* plant is done when it is about 2 weeks old by pruning each end of the plant so that branches form and produce more leaves. Pruning is also done on the leaves attacked by plant pest oganisms. Control is done manually by taking pests or affected plant parts, then destroying them so they do not spread to other healthy plants.

8.3 Biological control

The type of disease that causes many stevia plants to wilt is the fungus *Poria hypolateria*. Priority control uses biological control agents (APH) or APH secondary metabolites.

8.4 Control with vegetable pesticides

The most common pests that attack stevia plants are aphids (*Aphis* sp.) which damage leaf shoots and caterpillars *Heliothis* sp. eat leaves. Both types of pests can be controlled with vegetable pesticides in the form of neem seed extract.

9. Uses of the Stevia Plant

Stevia has many health benefits that more than 500 studies have proved; It does not affect blood sugar levels, is safe for people with diabetes, prevents tooth decay by inhibiting the growth of bacteria in the mouth, helps improve digestion, and relieves stomach pain. It is good for managing weight, to limit high-calorie sweet foods (Raini and Isnawati 2011). In addition, Kiat (2013) added that the benefits of stevia are that it contains zero calories, loses weight because insulin regulates the body stores less fat, improves digestive function, is good for children with special needs (autism), lowers blood pressure, and reduces inflammation.

Studies in Indonesia have shown that stevia does not break down at high temperatures as saccharin or aspartame. Steviosides are heat resistant up to 200°C (392°Fahrenheit), so they can be used in almost all food recipes (Raini and Isnawati 2011).

Natural sweeteners derived from stevia are safe for health. This is evidenced by reports of no adverse effects on the people of Paraguay who have been using stevia leaves as a sweetener in their drinks and beverages for more than one hundred years. Research in Japan reported that rabbits and rats given stevia leaf extract or pure stevioside did not cause negative effects on growth, behavior, reproduction, and other characteristics (Adinegoro 1986).

10. How to Use The Stevia Plant

According to Kiat (2013), Stevia leaves can be directly used as a sweetener. The way to use it is by drying it. The drying process does not require high heat. It is enough to dry in the sun for about 12 hours on a household scale. Drying it more will reduce the stevioside levels by drying the stevia leaves in the microwave for 2 minutes, then pulverizing them. This powder can be directly consumed as a food sweetener.

Stevia sweetener can also be made in liquid form, namely by soaking it for 24 hours and then storing it in the refrigerator. The ratio of water to stevia is 1:4. Do not use stevia directly if the leaves are exposed to pesticides or other chemicals. It is better to wash it thoroughly before soaking it so that it is not contaminated with substances harmful to health.

11. Stevia leaf drying and storage process:

People in Indonesia cut stevia branches 10-15 cm high from the ground, then pick off the leaves. They do not use Stevia leaves contaminated with pesticides or chemical fertilizers.

- They wash and rinse the stevia leaves under running water until clean.
- They dry the stevia leaves with a clean towel or tissue, or simply drain until the water is gone.
- They place the leaf in direct sunlight.

• They let the stevia leaves dry but remain green in color, crunchy, and crumble to the touch. Approximately it takes 2-3 days for stevia leaves to dry. Do not dry it too long because the leaves turn brown and will yield low.

• They crush the dried stevia leaves using a coffee grinder, or you can also crush them using the back of a spoon by placing the dried leaves in a bowl or crushing them with a mortar.

• They store stevia leaf powder in a glass bottle with a lid in a cool and dry place.

12. Conclusion

Stevia has many health benefits, does not affect blood sugar levels, is safe for people with diabetes, prevents tooth decay by inhibiting the growth of bacteria in the mouth, helps improve digestion and relieves stomach pain. Good for managing weight, to limit high-calorie sweets. Apart from that, other benefits of stevia are that it contains zero calories, loses weight because insulin regulates the body stores less fat, improves digestive function, is good for children with special needs (autism), lowers blood pressure, and reduces inflammation. Stevia plant has the potential for development as a source of natural sweeteners, and as a medicine for diabetes, with bright prospects in the future.

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