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Phone: +90 362 408 25 15

Fax: +90 362 408 25 15

Email: [bsjagri@blackseapublishers.com](mailto:bsjagri@blackseapublishers.com)

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## SOME ENVIRONMENTAL FACTORS CAUSING FIRST CALVING DIFFICULTIES IN HOLSTEIN FRIESIAN CATTLE

Arzu ÖZDEMİR<sup>1\*</sup>


<sup>1</sup>International Center for Livestock Research and Training, 06850, Ankara, Türkiye

**Abstract:** Calving efficiency, an important target in cattle breeding, has been negatively affected by some environmental factors. Therefore, calving difficulty creates negative economic consequences. In this study, it was aimed to investigate the relationship between the first calving difficulty in Holstein Friesian (HF) cows in terms of management and origin factors. The material of the research consists of 1475 calving difficulty records from 5 different enterprises engaged in HF breeding, located in the Central Anatolia Region of Türkiye, covering the years from 2013 to 2019. The scoring system used to determine calving difficulty: normal without intervention (NB), normal with intervention (NBI), difficult/intervention with equipment (DB), and abnormal birth (AB). In the calving difficulty analysis, the management factor is classified as 1-2-3-4-5 and the origin of the cow is classified as 1 (foreign origin) and 2 (native origin). No findings were observed for NBI scores. Total NB, DB, and AB scores were 1250 (84.74%), 192 (13.01), and 33 (2.25%), respectively. *Chi-square* test was performed to test the differences among farms. Among the enterprises, the highest NB rate was observed in the 5th enterprise with 90.07%, the minimum DB rate was observed in the 5th enterprise with 8.45%, and the AB rate was at least 0.66% in the 1st enterprise. The difference between farms was significant for calving difficulty ( $P < 0.01$ ). Cow origin was not significant on the calving difficulty score. While the NB rates in foreign-origin and native-origin animals were 86.36% and 84.64%, and the DB rates were 13.64% and 12.97%, respectively. The AB score was not seen in foreign-originated cows, but the AB rate was 2.39% in native-origin cows. To reduce the calving difficulties in enterprises, it may be recommended to determine management procedures appropriate to the region and enterprise and to determine semen suitable for the breed, age, and size of the heifer.

**Keywords:** Calving difficulty, First calving, Animal origin, Holstein Friesian

\*Corresponding author: International Center for Livestock Research and Training, 06850, Ankara, Türkiye

E mail: arzuozdemir22@gmail.com (A. ÖZDEMİR)

Arzu ÖZDEMİR  <https://orcid.org/0000-0002-6765-7029>

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### 1. Introduction

In cattle breeding, one of the important targets for dairy cattle farms' business profit is calf productivity, a feature determined by the hereditary structure of the cow and aimed to provide maximum benefit (Kaymakçı, 1987). However, calving difficulty (dystocia birth) is the main reason among the many factors that negatively affect calf productivity. Difficulty in calving is a condition that is frequently encountered in cows, especially at the birth of the first calf, and causes economically important reproductive problems. While losses due to calving difficulties bring about effects such as the death of the calf, birth defects, and anatomical deformations as a result of it remaining in the birth canal, it also causes decreases in productivity characteristics of economic value for the cow, such as maternal deaths, birth canal deformations, reproductive problems, increase in the service period, delay in estrus. Although calving difficulties in cows cause a great economic loss to the producer due to losses due to injury or death, treatment costs, and permanent damage to the mother's fertility, calving difficulties cannot be eliminated from a herd; However, incidents can be greatly reduced with correct management decisions taken before the breeding season

and during pregnancy (Erdoğan, 2023).

Reasons for calving difficulties include factors such as the birth weight and gender of the calf, the live weight and age of the mother at birth, the structure of the birth anatomy, and the prolongation of the gestation period (Meijering, 1984). These factors can prevent the normal progress of the cow's birth process. The incidence of difficult births and the proportion of stillborn calves was higher in primiparous cows than in multiparous cows (Strapáková et al., 2023). Calving difficulty can be a serious financial burden for cattle producers, and this is important for both the health of the animal and the profitability of the business. Additionally, Sakar et al. (2022) suggested that the first calving time of cows should be determined according to the climate and breeding needs of the country and that the first calving age of cows should occur at approximately 23-24 months of age to reduce costs. The economic costs of calving difficulties include factors such as calf loss, veterinary fees, farmer labour costs, and increased risk of health and fertility problems (Meijering, 1984). In addition to the environmental factors of calving difficulty, researchers have examined the effect of direct and maternal genetic components on the ease of calving and have obtained



important findings on this subject (Meijering, 1984; Dekkers, 1994). Therefore, appropriate management procedures and selection strategies are important in reducing calving difficulty. The main purpose of this study is to investigate the relationship between calving difficulty with farm management and cows' origin.

## 2. Materials and Methods

### 2.1. Materials

The study consisted of records covering the years 2013-2019 in 5 different Holstein dairy cattle enterprises in the Central Anatolia Region of Türkiye. The records were obtained from enterprises with automatic milking and herd management systems. Some of the animal material in the enterprises consists of cows that were born abroad and then imported to Türkiye as pregnant heifers, while the other part consists of cows that were born and raised in Türkiye.

### 2.2. Phenotype Data

In the study, calving records of a total of 1475 cows from 5 farms were collected from herds' software programs available in enterprises. All records were then transformed into an Excel program and made ready for analysis. The scoring system used to determine the calving difficulty (Gevrekçi and Akbaş, 2014) is as follows:

1. Normal without intervention; (NB)
2. Normal with intervention; (NBI)
3. Difficult/equipment-involved; (DB)
4. It is an abnormal birth (AB).

In the study, calving difficulty records numbered 1 (n=1250), 3 (n=192), and 4 (n=33) were observed, but calving difficulty records numbered 2 were not observed.

### 2.3. Statistical Analysis

Descriptive statistics and the *Chi-square* test used in the analysis of the calving difficulty of the first parity cows in the study were carried out in the SPSS 10.0 package program (SPSS Inc., Chicago, USA). Farm (1, 2, 3, 4, and 5) and origin (1 = imported, 2 = domestic) were included in the analysis as independent variables.

## 3. Results

In general, the frequency of NB, DB, and AB were 84.74%, 13.01% and 2.25%, respectively. The distribution of birth difficulties by enterprises and the *Chi-square* test results are given in Table 1. Differences in birth difficulty scores according to enterprises were found to be statistically significant ( $P < 0.01$ ). When the distribution of calf difficulties within the enterprises was examined, it was found that NB was at the highest rate in all enterprises, while AB was at the lowest rate. Among the enterprises, the highest NB rate was seen in the 5th enterprise with 90.07%. While the minimum DB rate was observed in the 5th enterprise with 8.45%, the highest rate was observed in the 4th enterprise with 17.64%. The AB rate was at least 0.66% in the 1st enterprise, and the highest was 2.95% in the 4th enterprise.

Data regarding calving difficulties in terms of origin are given in Table 2. When we examined Table 2, it was observed that there was no statistical difference in the frequency rates of NB, DB, and AB in terms of origin. NB rates in imported and domestic animals were 86.36% and 84.64%, respectively. No cases of AB have been observed in animals of imported origin. It was observed that the DB frequency in imported animals was proportionally higher than in domestic animals.

**Table 1.** Distribution of calving difficulties according to farms and Chi-square test results

Score	1. farm n (%)	2. farm n (%)	3. farm n (%)	4. farm n (%)	5. farm n (%)	Total n
NB <sup>1</sup>	253 (%83.77) <sup>ab</sup>	234 (%86.35) <sup>bc</sup>	329 (%83.92) <sup>ab</sup>	189 (%79.41) <sup>a</sup>	245 (%90.07) <sup>c</sup>	1250
DB <sup>2</sup>	44 (%15.57) <sup>ab</sup>	30 (%11.07) <sup>bc</sup>	53 (%13.52) <sup>ab</sup>	42 (%17.64) <sup>a</sup>	23 (%8.45) <sup>c</sup>	192
AB <sup>3</sup>	5 (%0.66) <sup>a</sup>	7 (%2.58) <sup>a</sup>	10 (%2.56) <sup>a</sup>	7 (%2.95) <sup>a</sup>	4 (%1.48) <sup>a</sup>	33
Total	302	271	392	238	272	1475

<sup>1</sup>Normal without intervention, <sup>2</sup>Difficult/equipment-involved, <sup>3</sup>Abnormal birth. The differences between farms in terms of calving difficulty were found to be significant ( $P < 0.01$ ). <sup>a, b, c</sup>Horizontal differences between groups are indicated by the letters.

**Table 2.** Distribution of calving difficulties according to origins and Chi-square test results

Score	1 (Import) n (%)	2 (Türkiye) n (%)	Total n
NB <sup>1</sup>	76 (%86.36)	1174 (%84.64)	1250
DB <sup>2</sup>	12 (%13.64)	180 (%12.97)	192
AB <sup>3</sup>	-	33 (%2.39)	33
Total	88	1387	1475

<sup>1</sup>Normal without intervention, <sup>2</sup>Difficult/equipment-involved, <sup>3</sup>Abnormal birth. The difference between origin in terms of calving difficulty was found to be insignificant.



#### 4. Discussion

In this study, inter-enterprise calving difficulty scores were examined and it was determined that the rate of NB without intervention was 84.74%, and the proportional value of the sum of DB and AB was 15.26%. While some studies in the literature report that the rate of difficult birth in first parity Holstein breed in the USA is up to 28.7% (Meyer et al., 2001; Lombard et al., 2007), this study results was lower. Similarly, Bayram et al. (2015) reported that the total rate of DB and AB cases in the HF was 9.1%. In this study, the differences observed in the calving scores between enterprises in terms of calving difficulty can be attributed to management differences between enterprises. In terms of origin, it is noteworthy that no cases of AB have been found in animals of imported origin, and this result indicates that there is no significant difference in the difficulty of calf birth between farms.

Calving difficulty is an increasingly important problem in dairy cattle, especially in cows in first parity of the HF breed (Meyer et al., 2001; Lombard et al., 2007). Difficult birth negatively affects the health and viability of the born calf, as well as causing a decrease in the cow's milk and fertility in the following lactation, and even cause mortality risk (Dematawewa and Berger, 1997). Difficulty in calving is a very complex traits and is under the influence of many factors. When the studies in the literature on the subject are examined, it has been stated that factors such as parity, body weight, breed, birth anatomy, calf's birth weight and gender are important as the main effect of birth difficulty (Mee, 2008; Kräusslich, 1981). Calving difficulties can be reduced with appropriate management procedures and selection strategies, and the results of studies on this subject show a positive trend in this direction (Meijering, 1984; Philipsson et al., 1979).

#### 5. Conclusion

This study presents the findings obtained by examining the calving records of the first of 1475 cows of the Holstein Friesian breed raised in 5 different enterprises in the Central Anatolia Region. In general, it was observed that birth difficulty scores showed significant differences between enterprises, while these differences were found to be statistically insignificant in terms of origin. In this study, the effect of environmental factors such as farm and origin, as well as maternal factors, on birth difficulties is emphasized, and further research on this subject may be recommended.

**Conflict of Interest:** The author declared that there is no conflict of interest.

**Ethical Consideration:** Ethics committee approval was not required for this study because there was no study on animals or humans.

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We thank the farm owners for their assistance in collecting this data.

#### Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	A.Ö.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### Conflict of Interest

The author declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. Livestock enterprises' own data was taken and there was no experiment conducted at the farm. Also, a signed consent form was also obtained from the owners of the farms.

#### Acknowledgments

We thank the farm owners for their assistance in collecting this data.

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## EFFECT OF DIFFERENT SPRING PLANTING PERIODS ON POMOLOGICAL AND PHYTOCHEMICAL CHARACTERISTICS OF SOME STRAWBERRY VARIETIES

Sinem ÖZTÜRK ERDEM<sup>1\*</sup>


<sup>1</sup>Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Science, Department of Horticulture, 11230, Bilecik, Türkiye

**Abstract:** The study was carried out at Bilecik Seyh Edebali University Agricultural Application and Research Center during the period of 2020-2021. Its objective was to assess the influence of different planting dates (27 March, 10 April, 27 April, 15 May) on the pomological and phytochemical attributes of diverse strawberry cultivars (Albion, Pineberry, Monterey, and Portola). The goal was to determine the optimal spring planting periods and varieties that are well-suited for the region. The Monterey and Albion varieties achieved the highest average fruit weight during the third period, with weights of 14.76 g and 15.92 g, respectively. The Monterey variety exhibited the greatest pH level at 3.88, whilst the Pineberry had the lowest pH level at 3.67. The concentration of soluble solids ranged from 4.73% to 8.56% among the different varieties, and from 5.82% to 6.15% throughout the different planting periods. The Monterey variety exhibited the highest anthocyanin concentration (117.73 µg Plg-3-glu g<sup>-1</sup> dw) among the various strawberry varieties, while the fourth planting period showed the highest content (84.65 µg Plg-3-glu g<sup>-1</sup> dw) among the different times. The concentration of soluble solids ranged from 4.73% to 8.56% among the different varieties, and from 5.82% to 6.15% throughout the different planting periods. The Monterey variety exhibited the highest anthocyanin concentration (117.73 µg Plg-3-glu g<sup>-1</sup> dw) among the various strawberry varieties, while the fourth planting period showed the highest content (84.65 µg Plg-3-glu g<sup>-1</sup> dw) among the different periods.

**Keywords:** Anthocyanin, Colour, *Fragaria ananassa* L., Phenolic content, Planting time

\*Corresponding author: Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Science, Department of Horticulture, 11230, Bilecik, Türkiye

E mail: sinem.erdem@bilecik.edu.tr (S. ÖZTÜRK ERDEM)

Sinem ÖZTÜRK ERDEM  <https://orcid.org/0000-0002-8978-0837>

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### 1. Introduction

The strawberry is a highly significant berry fruit, notable for its distinctive flavor, appearance, and abundant nutritional value (Dzhanfezova et al., 2020). Due to its extensive adaptability and the development of several breeds through selective breeding, this crop can be cultivated in every agricultural location worldwide, ranging from Ecuador to Siberia (Ağaoğlu, 1986). The significance of temperate climate zones in terms of crop productivity and quality is widely acknowledged (Rubinstein, 2015).

In the 2022, world strawberry production was 95 million tons, and Türkiye ranked third with approximately 730000 tons of strawberry production (FAO, 2022). Upon regional analysis, the Mediterranean region emerges as the foremost, followed by the Aegean and Marmara regions while cultivation is mostly done under cover in the Mediterranean, open cultivation is known to be common in the Aegean and Marmara regions (Oguz and Pirlak, 2019).

The growing popularity of strawberry agriculture worldwide is accompanied by increased breeding endeavors aimed at creating novel varieties. The primary

objectives of strawberry breeding include enhancing productivity, size, fragrance, durability, firmness of the fruit, resistance to diseases and pests, adaptability to day-neutral or short-day environments, timing of ripening, and compatibility with different soil types. Recent breeding research has incorporated its health benefits, as indicated (Kafkas, 2004; Ozturk Erdem and Cekic, 2017). Before profitably cultivating these produced cultivars in a different region or ecologically, it is necessary to conduct adaptation studies. The importance of variety adaptation in profitable strawberry cultivation in the recognition that varieties change in response to evolving cultivation systems and ecological conditions in strawberry production.

Many researchers have continued out to determine suitable varieties and planting periods in almost every region for this purpose (Atasay et al., 2006; Balci and Demirsoy, 2006; Ozguven and Yilmaz, 2009; Sezer, 2010; Serce et al., 2012; Alan, 2013; Saracoglu, 2013; Aksu, 2015; Ergun, 2015; Gunduz and Bayazit, 2017; Gecer et al., 2018; Oguz and Pirlak, 2019; Soysal et al., 2019; Ceran, 2023). Several strawberry production research has focused on the Marmara region, which holds



significant economic importance for our country. Gunay (2004), Sarac (2009), Gul (2011), and Ozok (2021) conducted studies that yielded useful insights on this agricultural technique in the region.

The province of Bilecik is located in the southeast of the Marmara region, with agriculture area accounting for about 29.4% of the total land area. Furthermore, within this agricultural land, approximately 11.5% is specifically allocated for fruit cultivation. Due to the presence of three distinct climate types and soil structures in Bilecik province, the range of items cultivated in this limited region is highly diverse. Despite the favorable climate of Bilecik for strawberry cultivation, there is a lack of scientific study, resulting in growers only growing a single type in a limited region. A crucial factor for achieving success in strawberry growing is the first assessment of the appropriate types for the specific region and the optimal timing for planting. The goal of this research was to enhance strawberry production in the Bilecik province and surrounding areas. This was achieved by identifying the suitable type and optimal planting period, as well as evaluating their pomological properties and phytochemical contents, which have significant implications for human health.

## 2. Materials and Methods

The study was carried out at the Agricultural Application and Research Center of Bilecik Seyh Edebali University throughout the years 2020-2021. Ciltar Agricultural Enterprise Ltd.  $\text{\u015f}$ ti obtained chilled seedlings of Albion, Pineberry, Monterey, and Portola varieties to be used as plant material.

Strawberry seedlings were planted in a triangular arrangement on 70-cm-wide tubes with drip irrigation pipes. The tubes were covered with black plastic mulch and the seedlings were placed 30 cm apart in both rows and columns. The plantings in 2020 occurred on four specific dates: March 27 (1st term), April 10 (2nd term), April 27 (3rd term), and May 15 (4th term).

The Gecit Kusagi Agricultural Research Institute conducted a soil study in the research area. As a result, the soil in the study area is somewhat alkaline (pH: 8.02), calcareous (4.68%), loamy (55%), salt-free ( $\text{dS m}^{-1} = 0.162$ ), low in organic matter (1.51%), and high in phosphorus ( $\text{kg da}^{-1} = 14.4$ ). The potassium level was found to be adequate ( $51.72 \text{ kg da}^{-1}$ ). Since planting, an equal amount of fertilizer has been applied to each plant (Sarıdas, 2018).

Temperature, precipitation, and humidity values for Bilecik province, where the study was done, were obtained from the Bilecik Meteorology Directorate for 2020 and 2021. The average temperature in 2020 was determined to be  $13.7 \text{ }^{\circ}\text{C}$  and  $13.3 \text{ }^{\circ}\text{C}$  in 2021.

In the first growing season (in 2020), flowers and stolons that occurred during that year were removed throughout the vegetation period to promote stronger development of the plants (Ağaoğlu, 1986). Harvest began on May 10, 2021, and while average fruit weight ( $\text{g/fruit}$ ), fruit

diameter/length (mm), pH, titratable acidity (%), and water-soluble dry matter amount (%) were analyzed (Cemeroğlu, 2007; Oguz and Pirlak, 2019). The total antioxidant amount was calculated using the TEAC (Trolox equivalent antioxidant capacity) method (Ozgen et al., 2006), the total anthocyanin amount was calculated using Giusti and Wrolstad (2005), and the total phenolic substance amount was calculated using Saracoglu and Ozgen (2015). The Minolta brand color measuring device (CR-300 model) and the Hunter color measurement system were used to measure  $L^*$  (brightness),  $a^*$  (red/green), and  $b^*$  (yellow/blue) on the outside of the fruit (Sacks and Shaw 1994; Gunduz and Ozdemir 2003). The experiment was conducted with three replications using a randomized block design, where each replication consisted of 20 plants. After conducting variance analysis, the application averages were compared using the LSD multiple comparison test. The statistical investigations utilized the MSTAT-C package application.

## 3. Results and Discussion

Adaption studies have been conducted in our country for many years to establish the region's best variety and planting season. Research on the Marmara region began in the late 1900s and has since progressed with the development of new varieties (Erenoglu and Seniz, 1999; Erenoglu et al., 2000; Gunay, 2004; Kaleci and Gunay, 2006; Sarac, 2009; Gul, 2011; Ozok, 2021).

This study examined the quality and phytochemical characteristics of four strawberry varieties cultivated in Bilecik during different spring planting periods in 2020 and 2021. The objective was to identify the most suitable strawberry variety for each planting period.

The trial's first harvest occurred on May 10, 2021, in all four planting periods, with the Portola variety harvesting in the first (March 27) and second (April 10) planting periods, and the Monterey variety harvesting in the third (April 27) and fourth (May 15) planting periods.

The Pineberry cultivar had its first harvest on May 17, later than other varieties. Kaleci and Gunay (2006) determined in their two-year study using seven strawberry varieties under Çanakkale conditions that the first harvest date was in mid-May in the first productive year, and the harvest date was one week later due to ecological conditions in the second year. Gul (2011) observed that the first harvest occurred on June 3 in the low tunnel and on June 6 in open cultivation in his study assessing the yield and development parameters utilizing several day-neutral strawberry cultivars in open and low tunnel circumstances in Tekirdağ ecosystem. In 2002-2003, Günay (2004) conducted a study to determine acceptable strawberry cultivars in open and greenhouse environments in Çanakkale climate conditions. According to the study, the first harvest under open cultivation conditions occurred on the Tuda variety on May 9, 2002, and on the Elsentia variety on May 12, 2003. In a study on the morphological and pomological properties of neutral and short-day strawberry types in Bursa, Ozok (2021)

reported the first harvest date as 11-20 May. The study revealed that the first harvest date was comparable to other studies conducted in the region.

It is known that fruit size in strawberries is a characteristic of the variety. However, it is known that ecological, genetic factors, planting, and care conditions affect fruit size (Hancock, 1999). According to Table 1, the Albion variety had the highest fruit weight with 13.32 g, followed by Monterey with 12.88 g, Portola with 10.38 g, and Pineberry varieties with 6.96 g. There was no significant statistical disparity observed when comparing the second, third, and fourth quarters. The fourth period, occurring on May 15, exhibited the highest mean fruit weight of 11.98 g. It was closely followed by the third period on April 27, which had an average fruit weight of 11.94 g. The second period on April 10 had a slightly lower average fruit weight of 11.22 g. The first period on March 27 had the lowest average fruit weight of 8.42 g. The information is presented in Table 1. The third period (27 April) yielded the highest average fruit weight in the Monterey (14.76 g) and Albion (15.92 g) varieties, while the fourth period (15 May) produced the highest average fruit weight in the Pineberry (8.22 g) and Portola (12.14 g) varieties. Across all four categories, the initial period (March 27) has the smallest fruit weight, as indicated in Table 2. Our study, as well as other studies conducted by Cekic and Aksu (2012), Ruan et al. (2013), and Wan et al. (2014), have found that the Albion variety is considered huge due to its high demand in the Bilecik region.

Mısır (2016) observed that fruit weights varied between 10.0-12.7 g in an adaptation study utilizing three strawberry varieties in Samsun ecological conditions, 12.6 g in the Albion strawberry variety and 12.4 g in the Monterey variety. Fruit weights ranged from 4.80 to 17.81 g, with the Monterey 17.81 g, the Portola 15.96 g, the Albion 13.04 g, and the Pineberry variety 6.19 g, according to Ozok (2021).

A study conducted in the USA utilized refrigerated seedlings to investigate the effects of different planting seasons (March, April, May, June, July, August, and September) on yield. The findings indicated that the crop output decreased when the planting period prolonged beyond the month of May. Based on their research, Moore and Bowden (1968) determined that the months of March, April, and May had the most favorable conditions for planting, resulting in the largest crop yields. Conversely, September was shown to have the least favorable conditions, leading to the lowest yields. Saracoglu (2013) conducted a study to assess the quality and yield of neutral and short-day strawberry cultivars in the Tokat-Kazova region. The study aimed to determine the optimal planting time for these varieties. He discovered that planting periods had no effect on yield in biennial cultivation, but they did have an impact on productivity in annual cultivation. In their study conducted in Eskisehir, Oguz and Pirlak (2019) employed the Albion, Kabarla, San Andreas, Sweet Ann, and Redlans Hope cultivars for planting throughout seven distinct

time intervals. The objective of their research was to determine the optimal variety and planting schedule. The study's results revealed a decline in fruit weight and yield following the third planting session on May 25. Additionally, it was determined that the optimal time for planting was between April 25 and May 10.

The results of the variance analysis indicated that there were statistically significant differences in the length and diameter of the fruits, depending on their variety and time periods. There was no statistically significant distinction observed among the Monterey, Albion, and Portola varieties when analyzing Table 1. The Monterey variety has the largest fruit, measuring 26.41 mm, followed by the Albion (25.85 mm), Portola (25.76 mm), and Pineberry (20.60 mm) variety. The Albion variety had the longest fruit length, measuring 37.52 mm. The Monterey variety had a fruit length of 31.68 mm, the Portola variety had a fruit length of 28.83 mm, and the Pineberry variety had the shortest fruit length at 18.60 mm. Table 2 demonstrates that the Monterey variety yielded fruits with the largest diameter (29.10 mm) and length (34.10 mm) on April 27, during the third planting period, while the lowest measurements were observed during the first planting period. The Pineberry variety exhibited its maximum fruit diameter of 21.16 mm during the fourth planting period. Conversely, its minimum fruit diameter of 20.50 mm was observed during the second planting period, and its minimum fruit length of 17.80 mm was recorded during the third planting period.

According to Ozok (2021), fruit diameter values in the Bursa ecosystem ranged from 19.93 mm (Bursa Derekzık) to 31.83 mm (Yalova-416), while fruit length values varied from 23.98 mm (Pineberry) to 48.75 mm (Mindoir). In this investigation of the performance of several strawberry cultivars during varying planting times, Saracoglu (2013) found that the planting periods' average fruit length and diameter were not statistically significant. The study's findings showed that in the first yield year, the average fruit diameter was 34.07 mm and its length was 41.14 mm, whereas in the second yield year, the average fruit diameter was 28.91 mm and its length was 30.03 mm. The research indicates that strawberry fruit sizes vary depending on the ecological factors, varieties, and cultural processes.

Table 1 displays the variance analysis results, which indicate that there are statistically significant differences in pH level depending on the planting seasons and variety. The Monterey variety exhibits the highest pH level of 3.88, whereas the Pineberry variety demonstrates the lowest pH level of 3.67. The pH levels for the planting periods were recorded to be highest at 3.85 on April 27, 2020, during the third planting period, and lowest at 3.77 on April 10, during the second planting period.

There was no significant change observed in planting seasons, even though there was a substantial difference in the titratable acid ratio across types (Table 1).

**Table 1.** Average values of the examined features

		FW	FD	FL	pH	TTA	TSS	TEAC	TA	TF
Various	Monterey	12.88 <sup>a</sup>	26.41 <sup>a</sup>	31.68 <sup>b</sup>	3.88 <sup>a</sup>	0.59 <sup>c</sup>	5.17 <sup>b</sup>	20.03 <sup>a</sup>	117.73 <sup>a</sup>	2511.62 <sup>c*</sup>
	Pineberry	6.96 <sup>c</sup>	20.60 <sup>b</sup>	18.60 <sup>c</sup>	3.67 <sup>c</sup>	1.02 <sup>a</sup>	8.56 <sup>a</sup>	8.54 <sup>d</sup>	15.26 <sup>d</sup>	2307.18 <sup>d</sup>
	Albion	13.32 <sup>a</sup>	25.85 <sup>a</sup>	37.52 <sup>a</sup>	3.86 <sup>a</sup>	0.74 <sup>b</sup>	5.47 <sup>b</sup>	13.19 <sup>b</sup>	62.83 <sup>c</sup>	2760.37 <sup>b</sup>
	Portola	10.38 <sup>b</sup>	25.76 <sup>a</sup>	28.83 <sup>b</sup>	3.79 <sup>b</sup>	0.59 <sup>c</sup>	4.73 <sup>b</sup>	9.58 <sup>c</sup>	77.28 <sup>b</sup>	2987.31 <sup>a</sup>
	LSD	2.07	2.09	3.68	0.06	0.10	0.73	0.37	5.53	127.80
Period	1	8.42 <sup>b</sup>	22.38 <sup>b</sup>	25.37 <sup>b</sup>	3.81 <sup>ab</sup>	0.73	5.93	9.76 <sup>d</sup>	60.66 <sup>c</sup>	3022.38 <sup>a</sup>
	2	11.22 <sup>a</sup>	24.43 <sup>ab</sup>	29.79 <sup>a</sup>	3.77 <sup>b</sup>	0.76	6.15	14.66 <sup>a</sup>	58.73 <sup>c</sup>	2683.08 <sup>b</sup>
	3	11.94 <sup>a</sup>	25.96 <sup>a</sup>	30.74 <sup>a</sup>	3.85 <sup>a</sup>	0.72	5.82	14.12 <sup>b</sup>	69.07 <sup>b</sup>	2710.09 <sup>b</sup>
	4	11.98 <sup>a</sup>	25.85 <sup>a</sup>	30.73 <sup>a</sup>	3.78 <sup>b</sup>	0.73	6.04	12.82 <sup>c</sup>	84.65 <sup>a</sup>	2150.93 <sup>c</sup>
	LSD	2.06	2.08	3.68	0.06	NS	NS	0.38	5.52	127.80

\*: Letters are statistically significant at the 5% level. FW= fruit weight (g), FD= fruit diameter (mm), FL= fruit length (mm), TA (%)= total titratable acid, TSS (%)= total soluble solids, TEAC= total antioxidant capacity (μmol TE/g ta); TA= total anthocyanin (μg Plg-3-glu/g ta); TF= total phenolic amount (μg GAE/g ta), NS= non-significant.

**Table 2.** Average values of variety and planting period interactions of the examined traits

Various	Period	FW	FD	FL	pH	TTA	TSS	TEAC	TA	TF
Monterey	1	9.70	24.06	28.93	3.91	0.53	5.10	11.46 <sup>e</sup>	100.50 <sup>c</sup>	3593.06 <sup>a*</sup>
	2	13.56	27.33	33.43	3.84	0.63	4.90	23.20 <sup>a</sup>	120.20 <sup>b</sup>	2371.96 <sup>de</sup>
	3	14.76	29.10	34.10	3.87	0.58	5.06	23.60 <sup>a</sup>	112.43 <sup>b</sup>	2176.96 <sup>ef</sup>
	4	13.5	25.13	30.23	3.88	0.61	5.60	21.86 <sup>b</sup>	137.76 <sup>a</sup>	1904.46 <sup>fg</sup>
Pineberry	1	6.06	21.10	19.36	3.63	0.98	7.75	8.43 <sup>gh</sup>	18.30 <sup>h</sup>	3451.96 <sup>a</sup>
	2	6.84	20.50	18.86	3.63	1.03	9.30	7.73 <sup>h</sup>	9.36 <sup>h</sup>	1684.46 <sup>g</sup>
	3	6.68	20.63	17.80	3.75	0.98	8.56	9.06 <sup>fg</sup>	16.00 <sup>h</sup>	1936.13 <sup>fg</sup>
	4	8.22	21.16	18.36	3.68	1.06	8.63	8.93 <sup>fg</sup>	17.36 <sup>h</sup>	2156.13 <sup>ef</sup>
Albion	1	9.88	20.73	28.03	3.92	0.80	6.55	9.50 <sup>f</sup>	42.26 <sup>g</sup>	2634.73 <sup>cd</sup>
	2	13.50	25.13	38.06	3.82	0.77	5.86	18.80 <sup>c</sup>	54.36 <sup>f</sup>	3170.30 <sup>b</sup>
	3	15.92	29.46	42.73	3.87	0.75	4.96	12.93 <sup>d</sup>	60.10 <sup>f</sup>	3400.30 <sup>ab</sup>
	4	14.00	28.06	41.23	3.82	0.63	4.50	11.53 <sup>e</sup>	94.60 <sup>cd</sup>	1836.13 <sup>g</sup>
Portola	1	8.04	23.63	25.13	3.76	0.59	4.30	9.63 <sup>f</sup>	81.56 <sup>e</sup>	2409.73 <sup>de</sup>
	2	10.90	24.73	28.80	3.75	0.59	4.53	8.90 <sup>fg</sup>	50.96 <sup>fg</sup>	3505.56 <sup>a</sup>
	3	10.40	24.63	28.26	3.89	0.57	4.66	10.86 <sup>e</sup>	87.73 <sup>de</sup>	3326.96 <sup>ab</sup>
	4	12.14	30.03	33.10	3.73	0.59	5.43	8.93 <sup>fg</sup>	88.86 <sup>de</sup>	2706.96 <sup>c</sup>
	LSD	NS	NS	NS	NS	NS	NS	0.74	11.04	255.7

\*: Letters are statistically significant at the 5% level. FW= fruit weight (g), FD= fruit diameter (mm), FL= fruit length (mm), TA (%)= total titratable acid, TSS (%)= total soluble solids, TEAC= total antioxidant capacity (μmol TE/g ta); TA= total anthocyanin (μg Plg-3-glu/g ta); TF= total phenolic amount (μg GAE/g ta), NS= non-significant.

The Pineberry variety had the highest titratable acidity rate (1.02%) among all the varieties. The Pineberry variety had the highest titratable acidity rate (1.02%) among all the varieties, but the planting period had the second-highest rate (0.76%) among all the planting periods (Table 2). When investigating the correlation between variety and planting period, it was found that the Pineberry variety exhibited the highest titratable acidity rate (1.06%) during the fourth planting period. Multiple studies have reported that the titratable acidity range was 0.34–1.43% (Gündüz 2003, Gündüz and Özdemir 2012, Gündüz and Gökçek 2019).

Significant changes were seen across different types when analyzing the variance analysis result for the total soluble solids (TSS) content. However, no distinction was discovered between planting seasons, as shown in Table 1. The total soluble solids range from 5.82% to 6.15% across different planting seasons and varieties, with a range of 4.73% to 8.56% (Table 1). According to Table 2, the Pineberry variety had the highest TSS content of

9.30% during the second planting period on April 10th. On the other hand, the Portola variety had the lowest TSS content of 4.30% during the first planting period on March 27th.

In a study done in Bursa ecological conditions, the strawberry cultivars exhibited a range of total soluble solids content, from 6.4% to 9.9%. The titratable acidity content ranged from 0.53% to 0.91%, while the pH levels varied between 3.60 and 4.02. The total soluble solids, titratable acid, and pH values were measured to be 9.9%, 0.91%, and 3.67 in the Pineberry variety, 8.8%, 0.79%, and 3.82 in the Albion variety, 8.6%, 0.74%, and 3.82 in the Portola variety, and 8.0%, 0.53%, and 4.02 in the Monterey variety, respectively. In the study investigating the production and quality features of strawberry varieties (Albion, Camarosa, Festival, Rubygem, Fortuna, Kabarla) in Malatya (end of March), the average weight of fruit during the spring planting season ranged from 7.63 to 11.7 g. The soluble solid content ranges from 7.58% to 10.00%, the titratable acidity is between 0.21% and

0.35%, and the pH ranges between 3.10 and 3.79 (Ozok, 2021).

Our study has been determined to be consistent with other research conducted in different areas.

The total antioxidant content, as measured by the TEAC method, exhibited statistically significant differences among different cultivars and time periods. The Monterey cultivar exhibited the most elevated antioxidant concentration, measuring at 20.03 mol TE/g. It was succeeded by Albion with a concentration of 13.19 mol TE/g, Portola with 9.58 mol TE/g, and Pineberry with 8.54 mol TE/g. When analyzing the variety period interaction table, there was no significant statistical difference observed between the second and third periods in the Monterey variety. The highest TEAC amount was achieved during the second phase, measuring 14.66 mol TE/g. This was followed by the third period with 14.12 mol TE/g, the fourth period with 12.82 mol TE/g, and the first period with 9.76 mol TE/g.

The Monterey variety exhibited the highest concentration of anthocyanins, measuring 117.73 g Plg-3-glu g<sup>-1</sup>. Conversely, the fourth period had the lowest concentration, measuring 84.65 g Plg-3-glu g<sup>-1</sup>. According to the variety period interaction table, the Monterey variety had the highest Plg-3-glu g<sup>-1</sup> concentration of 137.76 g in the fourth period, while the Pineberry variety had the lowest concentration of 9.36 g in the second period. Although there was no significant variation observed among the different planting times, the Pineberry variety exhibited a relatively low level of anthocyanin compared to other varieties.

The total phenolic quantity in the Portola variety was found to be the greatest among varieties at 2987.31 g GAE g<sup>-1</sup>, and the highest among planting rotations at 3022.38 g GAE g<sup>-1</sup> in the first period. When the variety period interaction table was investigated, the second period in the Portola variety was judged to be the highest, and the second period in the Pineberry variety was determined to be the lowest. In their investigation using 20 different strawberry varieties, Capocasa et al. (2009) discovered that total antioxidant and phenolic component concentrations were connected to genotype

rather than growing conditions. Studies show that genetic structure, rather than environmental factors, influences antioxidant and phenol content (Singh et al., 2011; Saridas, 2018).

The color of the strawberries' surface is an important criterion for determining their quality. The vibrant and deep red hue that enhances the market worth of strawberries is attributed to the presence of anthocyanidins, specifically pelargonidin-3-glycoside and cyanidin-3-glycoside (Kosar et al., 2004; Lopez-da Silva et al., 2007). The L, a, and b values were utilized to ascertain the external (side and tip) and internal hue of the fruit. The statistical analysis in Table 3 did not find any significant relationship between color values and planting season. Although the color of the fruit's outer side was not found to have a significant impact on the different varieties, the highest L value for the outer side surface was observed in Albion (43.57), followed by Monterey (20.83) and Portola (19.10). Similarly, the highest L value for the outer end surface was found in Pineberry (41.68), followed by Portola (27.74) and Albion (20.13). Lastly, the highest L value for the inner surface was obtained from Pineberry (43.73), followed by Monterey (23.95) and Albion (21.27).

In a study conducted in the Tokat, Saracoglu (2013) found that the impact of planting times on the L and b values of fruit exterior color was statistically insignificant in both years. The fruit's external color intensity was seen to be greater during the initial year's planting season in August, as compared to other time periods. The L value was determined by research conducted in various ecological conditions to range from 33.7 to 39.8 (Gunduz and Ozdemir, 2003), 30.5 to 35.8 (Ozdemir et al., 2006), and 52.7 to 75.1 (Misir, 2016).

In their study, Oguz and Pirlak (2019) found that the L value in the ecological conditions of Eskisehir was determined to be 31.33 when planted in the sixth period (10 July), 25.40 when planted in the fourth period (10 June), and the average L value of the Albion variety was 25.82. The variation in color values, regarded as a crucial quality parameter, is presumed to be attributable to environmental factors.

**Table 3.** Average fruit color (L, a, b) values

		Exterior /Side			Exterior / Bottom			İnternal		
		L	a	b	L	a	b	L	a	b
Various	Monterey	43.13	20.83	15.53	31.26 <sup>b</sup>	24.25 <sup>a</sup>	19.43	35.09 <sup>b</sup>	23.95	20.08 <sup>a*</sup>
	Pineberry	34.95	20.57	17.76	41.68 <sup>a</sup>	16.22 <sup>b</sup>	18.45	43.73 <sup>a</sup>	17.23	13.83 <sup>b</sup>
	Albion	43.57	17.59	14.89	33.38 <sup>b</sup>	26.01 <sup>a</sup>	20.13	33.21 <sup>b</sup>	22.67	21.27 <sup>a</sup>
	Portola	40.85	22.23	19.10	30.43 <sup>b</sup>	27.74 <sup>a</sup>	20.12	29.88 <sup>b</sup>	22.54	19.27 <sup>a</sup>
	LSD	5.82	4.72	2.66	1.86	4.97	NS	2.93	NS	1.33
Period	1	42.09	19.85	16.18	33.93	25.02	20.50	35.21	22.64	20.18
	2	39.48	21.10	16.63	32.25	23.41	19.77	37.52	22.58	19.29
	3	37.72	21.36	17.71	34.10	21.28	17.73	36.42	17.89	16.39
	4	43.21	18.91	16.76	36.48	24.52	20.13	32.78	23.27	18.58
	LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*= Letters are statistically significant at the 5% level, NS= non-significant.

Studies have shown that fruit color is influenced by factors such as genotype, temperature, and light source (Proctor and Creasy, 1971; Creasy, 1966; Batu et al., 1997). Specifically, when day and night temperatures are high (30/22°C), fruit color tends to be darkened. Research has found an association between lower temperatures (18/12 °C) and a lighter color (Shiow and Camp, 2000).

#### 4. Conclusion

In the study conducted with four different strawberry varieties across four various planting periods under the ecological conditions of Bilecik. It aimed to determine the pomological and phytochemical features of these varieties. Upon analyzing the interaction between variety and time, it was concluded that the planting period did not possess any statistically significant significance. In this region, the cultivation of the Albion variety has been found to excel in terms of fruit weight and quality parameters, as demonstrated in our study. Although the Albion variety is widely utilized, the Monterey and Portola varieties can be recommended as suitable alternatives. As a result of the study, it has been found that the optimal time for spring planting is during the third phase, namely on April 27th. The recently introduced Pineberry variety in our country has been characterized by its small fruit weight and soft fruit flesh, prompting the exploration of various evaluation methods.

#### Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	S.Ö.E.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### Conflict of Interest

The author declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## EFFECT OF BIOCHARS PRODUCED AT DIFFERENT PYROLYSIS TEMPERATURES ON AMMONIUM (NH<sub>4</sub><sup>+</sup>) AND NITRATE (NO<sub>3</sub><sup>-</sup>) LEACHING: COLUMN EXPERIMENT

Cabir Çağrı GENÇE<sup>1</sup>, Halil ERDEM<sup>2\*</sup>

<sup>1</sup>Aksaray University, Aksaray Technical Sciences Vocational School, Department of Plant and Animal Production, 68100, Aksaray, Türkiye


<sup>2</sup>Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 60250, Tokat, Türkiye


**Abstract:** Nitrogen (N) leaching from agricultural soils is a global problem with negative effects on both human health and the environment. Efforts should be made to increase the efficiency of use of plant nutrients and minimize N losses from terrestrial ecosystems to aquatic ecosystems. In this study, the effects of different doses (%0, %1 and %2) of biochar obtained from corn cob and rice husk biomass, which are agricultural production residues, at three different temperatures, on ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) leaching in a coarse-textured soil were investigated. Polyethylene (PE) columns with a diameter of 70 mm and a height of 20 cm were used in the study, which was carried out in three replications according to the randomized plots experimental design. Total nitrogen (27 kg N da<sup>-1</sup>) and water amounts (969 mm) used for 6 tons da<sup>-1</sup> yield of sugar beet were applied. Total water was given to each column in equal volume using drip adjustment sets at one-week intervals, simulating 6 irrigation periods, and the leaked water was collected in each irrigation period and NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were determined. Applications of 1 and 2 doses of corn and rice biochars obtained at three different pyrolysis temperatures caused a significant decrease in NH<sub>4</sub><sup>+</sup> concentrations leaching from the column. Similarly, biochar applications (especially 2% dose) caused a significant decrease in NO<sub>3</sub><sup>-</sup> concentrations leaching from the column. While the total NO<sub>3</sub><sup>-</sup> concentration leaching from the control columns was 149.23 mg kg<sup>-1</sup>, 2% dose of rice husk biochars at 300, 400 and 500 °C temperature applications caused a decrease in the total NO<sub>3</sub><sup>-</sup> concentrations washed from the column by 51%, 55% and 51%, respectively. The results revealed that biochar applications significantly reduced nitrogen leaching from the soil.

**Keywords:** Ammonium, Biochar, Fertilizer, Leaching, Nitrate, Soil

\*Corresponding author: Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 60250, Tokat, Türkiye

E mail: erdemh@hotmail.com (H. ERDEM)

Cabir Çağrı GENÇE  <https://orcid.org/0000-0001-9748-1303>

Halil ERDEM  <https://orcid.org/0000-0002-3296-1549>

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### 1. Introduction

The leaching of nutrients from agricultural lands, due to its adverse effects on ecosystems, is recognized globally as a significant environmental issue (Rashmi et al., 2017). Nitrogen, one of the most commonly used plant nutrients in agricultural production, occupies a central position in this issue (Yahaya et al., 2023). Farmers tend to apply excessive nitrogen in order to achieve higher yields when cultivating high-income crops. This situation exacerbates the issue of nitrogen leaching, particularly in the form of nitrate, further threatening soil fertility. The amount of leaching nitrogen increases proportionally with the quantity of nitrogen-based fertilizers applied (Abascal et al., 2022). Nitrate, known as a mobile ion, particularly enhances the leaching potential in low clay content soils (Forde and Zhang, 1998; Köhler et al., 2006). Sustainable agricultural practices and proper fertilization techniques are of critical importance in overcoming this issue, increasing crop productivity, and reducing environmental adverse effects. As a potential solution, biochar has the capacity to reduce nitrogen leaching from

agricultural production areas (Borchard et al., 2019).

Biochar, a carbon-rich material obtained through the pyrolysis of organic matter, has garnered significant attention due to its potential for carbon (C) sequestration (Teutscheroova et al., 2018; Tepecik et al., 2024) and mitigating climate change through the reduction of greenhouse gas emissions.

Biochar can enhance the cation exchange capacity of soil, thereby preventing leaching of nutrients and harmful chemicals (Elkhilfi et al., 2023). Additionally, biochar improves the physical and chemical structure of soil, increasing its water retention capacity and promoting microbial activity (Banik et al., 2023). Due to its porous structure and surface charge, biochar emerges as a promising material for reducing N leaching (Laird et al., 2010). An increasing number of studies demonstrate that biochar's extensive surface area and surface charge can reduce N leaching (Ding et al., 2010). Indeed, the cation exchange capacity (CEC) is likely responsible for the retention of ammonium (NH<sub>4</sub><sup>+</sup>-N) by biochar (Jellali et al., 2022). However, the sorption properties of biochar



depend on the feedstock and pyrolysis temperature. For instance, Yao et al. (2012) reported that biochars produced at temperatures of 600 °C or higher exhibited the highest nitrate (NO<sub>3</sub>-N) adsorption. Consequently, nitrogen management and biochar applications are significant strategic practices to enhance the sustainability of agricultural lands and leave healthier soils for future generations. This approach and its implementations not only reduce environmental impacts but also increase productivity and profitability in agricultural production. In this study, the effect of biochar applications derived from agricultural residues, such as corn cob and rice husk biomass, obtained at three different temperatures, applied at control, 1%, and 2% doses, on ammonium and nitrate leaching in a coarse-textured soil, was investigated.

## 2. Materials and Methods

### 2.1. Soil and Biochar Materials

Corn cob and rice husk, agricultural residues abundantly available as waste, were used as raw materials for biochar in the experiment. Biomasses collected from the field were brought to the laboratory and initially dried at 70 °C until reaching a constant weight. While corn cobs were ground using a fodder grinder due to their large particle size, rice husks were left untreated without any grinding process. Biochar was produced from corn cob and rice husk biomass through slow pyrolysis in a specially made biochar production chamber (40x25x20 cm<sup>3</sup>) located in the Department of Soil Science and Plant Nutrition at Tokat Gaziosmanpaşa University, Türkiye. The biomass was subjected to pyrolysis at temperatures of 300 °C, 400 °C, and 500 °C individually in a muffle furnace, resulting in the production of biochar. During production, syngas and tar generated were not stored. Some physical and chemical analysis results of the obtained biochars are provided in Table 1.

The soil material used in the study was collected from the Agricultural Research and Application Field of Tokat Gaziosmanpaşa University, Tokat, at a depth of 0-30 cm, and air-dried after passing through a 4 mm sieve. The soil used in the study is slightly alkaline (pH: 7.83), non-saline (EC: 0.74 µS/cm), low in organic matter content (%1.46), and characterized by a sandy loam texture

(%88.40 sand, %7.91 clay, %3.69 silt).

### 2.2. Column Leaching Experiment and Laboratory Analyses

For the leaching experiment, PE columns with a diameter of 70 mm and a height of 20 cm were utilized. Coarse filter papers were placed at the bottom of the columns to prevent the mixture of soil+biochar from spilling, and to protect the filter paper from damage, a fine porous fiber mesh was used to cover the top of the column, secured with a PE clamp (Figure 1).

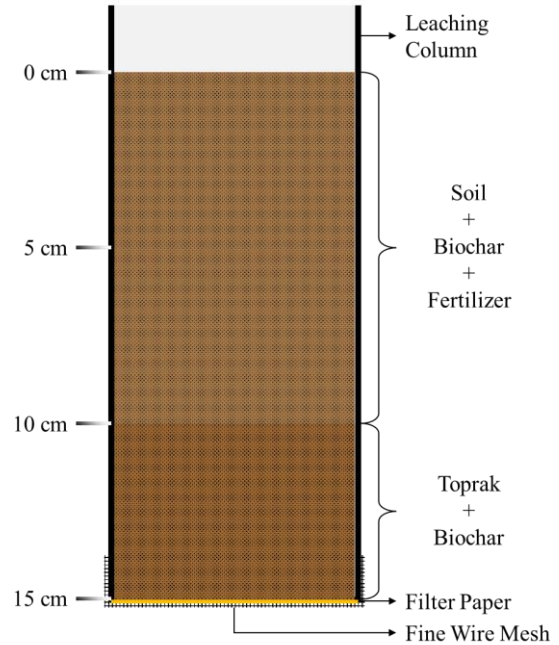


Figure 1. General structure of the column model.

The amount of fertilizer used in the leaching experiments was determined based on the nitrogen requirement of sugar beet plants. According to a study, it was determined that 4-5 kg/ha of pure nitrogen is removed from the soil for the production of 1 ton of sugar beet (İlbaş et al., 2016). The column experiment, conducted in accordance with a randomized complete block design with three replications, involved biochar applications derived from rice husk and corn cob produced at different pyrolysis temperatures (300, 400, and 500 °C) at three different doses (control, 1%, and 2% BC).

Table 1. Some physical and chemical properties of the biochars used in the study

Parameter	Corn Cob			Rice Husk		
	300 °C	400 °C	500 °C	300 °C	400 °C	500 °C
C (%)	43.8	58.6	62.7	48.5	51.7	54.7
N (%)	0.54	0.38	0.29	0.71	0.76	0.58
C/N	81.2	154.2	216.3	68.2	68.1	94.3
P (%)	1.94	2.69	4.12	0.09	0.13	0.18
K (mg kg <sup>-1</sup> )	60.1	96.3	172.7	76.0	82.6	98.6
Mg (mg kg <sup>-1</sup> )	16.3	20.6	26.4	13.6	20.0	22.2
Specific Surface Area (m <sup>2</sup> g <sup>-1</sup> )	156	221	320	68	79	127
pH (1/20)	8.40	8.60	9.72	7.94	8.43	10.4
EC (1/20) (µS cm <sup>-1</sup> )	214	685	1251	176	274	615

Before adding soil (650 g soil) to each column, biochar doses were thoroughly mixed with soil to achieve homogeneity. Subsequently, the soil+biochar mixture was added to the 10-15 cm interval of each column, while a mixture of soil+biochar+fertilizer was added to the 0-10 cm interval. Accordingly, considering a sugar beet yield of 6 tons/ha, a fertilizer rate of 27 kg da<sup>-1</sup> was applied in the form of NH<sub>4</sub>NO<sub>3</sub>. In the experiment, to simulate the irrigation during the sugar beet plant's growing season, the total rainfall received by Tokat province during the sugar beet production season, reported as 969 mm (TAGEM, 2017), was divided into 6 irrigation periods, and a total of 3.73 L of water was applied to each column using drip irrigation sets with one-week intervals (Figure 2). Water applications were conducted weekly, equally to each column, and the experiment was terminated when no further water leakage was observed from the column at the end of the 6<sup>th</sup> week. At the end of each week, the concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in the leachate obtained from each column were determined. Nitrate was measured by spectrophotometry based on the yellow color complex formed by nitrate with sodium salicylate (Fabig et al., 1978), while ammonium was determined based on the green color complex formed by ammonium with nitroprusside salicylate (DEZWAS, 1983).

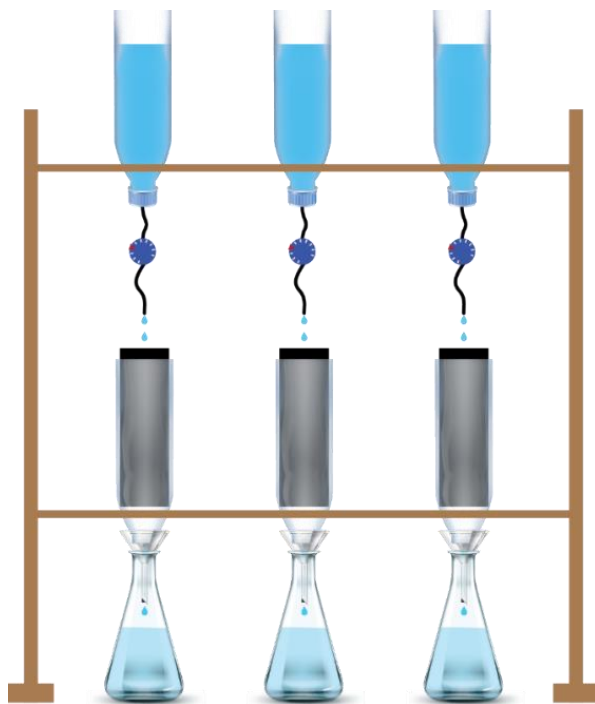


Figure 2. Schematic of the column assembly.

### 2.3. Statistical Analysis

Descriptive statistical data for the concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in the leachates obtained from different doses of biochar applications were determined, and the differences in the effects of different biochar applications on ammonium and nitrate leaching were assessed using one-way analysis of variance (ANOVA) to determine if

they were statistically significant or not. Homogeneity tests for the applications were determined by Duncan grouping. These statistical analyses were conducted using the SPSS 27.0 software package (Genc and Soysal, 2018).

### 3. Results and Discussion

The values of ammonium (NH<sub>4</sub><sup>+</sup>) concentrations in the solutions leached from columns with applications of corn and rice biochars obtained at different pyrolysis temperatures at doses of 1% (BC1) and 2% (BC2) are presented in Figure 3. For all pyrolysis temperatures of corn and rice biochars (except BC2 for CC500), the variation in NH<sub>4</sub><sup>+</sup> concentrations leached from the columns in both 1% and 2% dose applications was found to be statistically significant (P<0.05) (Figure 3 a,b,c; Table 2). Comparing with the control columns, it was observed that the NH<sub>4</sub><sup>+</sup> concentrations leached from the columns with biochar applications were lower (Figure 3abc; Table 2).

Table 2. Total NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations leaking from the columns

Application	NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )
Control	53.27 <sup>m</sup>	149.23 <sup>l</sup>
RH300-%1	29.92 <sup>k</sup>	91.83 <sup>i</sup>
RH300-%2	27.86 <sup>j</sup>	72.49 <sup>e</sup>
RH400-%1	18.39 <sup>g</sup>	86.91 <sup>h</sup>
RH400-%2	13.12 <sup>e</sup>	67.37 <sup>c</sup>
RH500-%1	4.01 <sup>c</sup>	108.84 <sup>k</sup>
RH500-%2	3.70 <sup>b</sup>	72.72 <sup>e</sup>
CC300-%1	6.13 <sup>d</sup>	79.18 <sup>f</sup>
CC300-%2	0.66 <sup>a</sup>	83.00 <sup>g</sup>
CC400-%1	19.89 <sup>h</sup>	69.24 <sup>d</sup>
CC400-%2	16.04 <sup>f</sup>	59.22 <sup>a</sup>
CC500-%1	23.73 <sup>i</sup>	89.02 <sup>i</sup>
CC500-%2	32.28 <sup>j</sup>	60.86 <sup>b</sup>

\*There is no statistical difference between the averages shown with the same letter (P<0.05) (Each column is lettered within itself).

When evaluating the columns under rice husk biochar applications, it was observed that the total NH<sub>4</sub><sup>+</sup> concentration leached from the control application was 53.27 mg L<sup>-1</sup>. However, under RH500 BC1 dose, it decreased by 92.5% to 4.01 mg L<sup>-1</sup>, and under RH500 BC2 dose, the NH<sub>4</sub><sup>+</sup> concentration decreased by 93% to 3.70 mg L<sup>-1</sup> (Table 2). When evaluating the weekly leached NH<sub>4</sub><sup>+</sup> concentrations, it was found that both the control application and the applications of rice husk and corn cob at all doses and temperatures had higher NH<sub>4</sub><sup>+</sup> concentrations leached in the 3rd week compared to other weeks (Figure 3 a,b,c). In contrast, NH<sub>4</sub><sup>+</sup> concentrations leached from the columns were lower in the 1st and 6th weeks (Figure 3 a,b,c). Similar to our findings, Ding et al. (2010) reported that bamboo biochar adsorbed NH<sub>4</sub><sup>+</sup> ions through cation exchange, protecting

against leaching and significantly delaying the vertical movement of  $\text{NH}_4^+$  to deeper soil layers during a 70-day experimental period. Laird et al. (2010) reported that biochar produced from a mixture of oak and walnut sawdust reduced total N and P leaching by 11% and 69%, respectively, with applications ranging from 0%, 0.5%, 1%, and 2%.

Among biochars produced from two different feedstocks, the leaching of  $\text{NH}_4^+$  was observed to be the lowest in the RH500 BC2 dose of rice husk biochar and the CC300 BC2 dose of corn biochar (Table 2). In previous studies, it has been reported that biochar reduces N leaching due to its extensive surface area and surface charge (Ding et al., 2010; Yao et al., 2012). Researchers have reported that biochars protect against leaching by increasing the retention of ammonium ( $\text{NH}_4^+\text{-N}$ ) due to their high cation exchange capacity (CEC) (Alkharabsheh et al., 2021). In a study where pine biochar was mixed with soil at rates of 0.5%, 2.5%, and 10%, and ammonium nitrate was mixed with the soil to achieve a rate of 10 kg/ha, weekly leachate was conducted for 6 weeks. In the study, the application of 10% biochar reduced ammonium leaching by 86% and nitrate by 96% (Sika & Hardie, 2014).

According to the biochar application dose, the total nitrogen (N) content of the soil increased according to the application doses (Şenay and Tepecik, 2024).

Due to nitrate being a highly mobile anion, it tends to leach more in soils with low clay content and coarse to medium texture (Ferretti et al., 2023). Consistent with the literature, it was found that the leached  $\text{NO}_3^-$  concentrations from columns without biochar applications were higher than those from columns with biochar applications (Figure 4 a,b,c). The applications of both corn and rice biochars at doses of 1% and 2% for all pyrolysis temperatures significantly reduced the  $\text{NO}_3^-$  concentrations leached from the columns ( $P < 0.05$ ) (Figure 4 a,b,c; Table 2). Compared to the control applications, especially the rice husk biochar applications at 2% doses led to a significant decrease in  $\text{NO}_3^-$  concentrations leached from the columns. While the total  $\text{NO}_3^-$  concentration leached from the control columns was  $149.23 \text{ mg L}^{-1}$ , this value decreased to  $72.49 \text{ mg L}^{-1}$  (51% reduction) in the RH300 BC2 dose,  $67.37 \text{ mg L}^{-1}$  (55% reduction) in the RH400 BC2 application, and  $72.72 \text{ mg L}^{-1}$  (51% reduction) in the RH500 BC2 dose (Table 2).

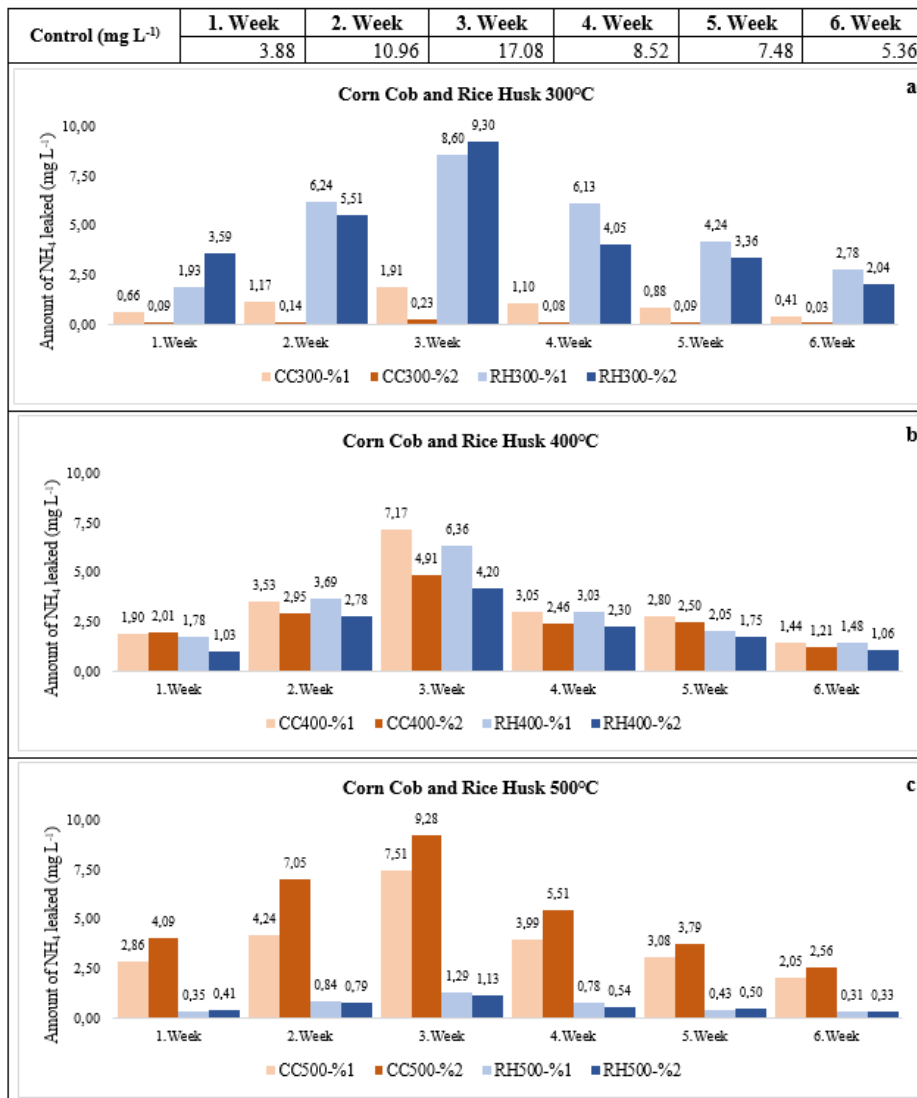


Figure 3. Weekly  $\text{NH}_4^+$  change in leaking water after RH and CC biochar application.

A similar situation was observed for the corn cob biochar at 2% dose for pyrolysis temperatures of 400 (60% reduction) and 500 (59% reduction) (Figure 4 a,b,c; Table 2). The results showed that both corn and rice biochars at 2% dose (except for CC300) significantly reduced NO<sub>3</sub><sup>-</sup> leaching at all temperatures. It has been reported that applications of acacia tree biochar obtained at different pyrolysis temperatures (300, 400, and 500°C) significantly reduced the leached NO<sub>3</sub><sup>-</sup> concentration from the soil, with lower concentrations from biochar applications obtained at pyrolysis temperatures of 400 and 500°C compared to those obtained at 300°C (Uzoma et al., 2011). It has been stated that the properties of biochar change significantly depending on the pyrolysis temperature (Tepecik et al., 2022). Tomczyk et al. (2020) reported that with increasing pyrolysis temperature of the biomass, the amount of volatile organic compounds volatilized increased parallelly with the increase in the surface area of biochar. It has been reported that the application of biochar to sandy soils increases the cation exchange capacity of the soil and therefore the sorption capacity, thus reducing the leaching of nutrient elements such as NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> (Lv et al., 2021). When evaluating the results obtained from the study, it can be concluded

that rice husk and corn cob biochars produced at pyrolysis temperatures of 400 and 500°C are more effective against NO<sub>3</sub><sup>-</sup> leaching. This effectiveness can be attributed to the higher specific surface areas of both materials at 400 and 500 degrees compared to 300°C (Table 1).

When evaluating the results in terms of weekly leached NO<sub>3</sub><sup>-</sup> concentrations, similar to NH<sub>4</sub><sup>+</sup> concentrations, it was observed that the NO<sub>3</sub><sup>-</sup> concentrations leached in the 3<sup>rd</sup> week were higher than other weeks for the control, rice husk, and corn cob biochar applications at all doses and temperatures (Figure 4 a,b,c). However, in the 1<sup>st</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks, the NO<sub>3</sub><sup>-</sup> concentrations leached from the columns were lower (Figure 4 a,b,c).

Günal et al. (2017) investigated the effects of tomato harvest residues-derived biochar produced at 500°C and applied at different doses (Control, 1%, 3%, and 6%) on leached NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations from a loamy soil. The researchers reported that more NO<sub>3</sub><sup>-</sup> was leached in control applications compared to biochar applications, and the amount of leached nitrate increased rapidly after the second leaching (especially in the 2<sup>nd</sup> and 3<sup>rd</sup> leaching), while the NO<sub>3</sub><sup>-</sup> concentration remained constant in subsequent leachings (4<sup>th</sup> and 5<sup>th</sup>).

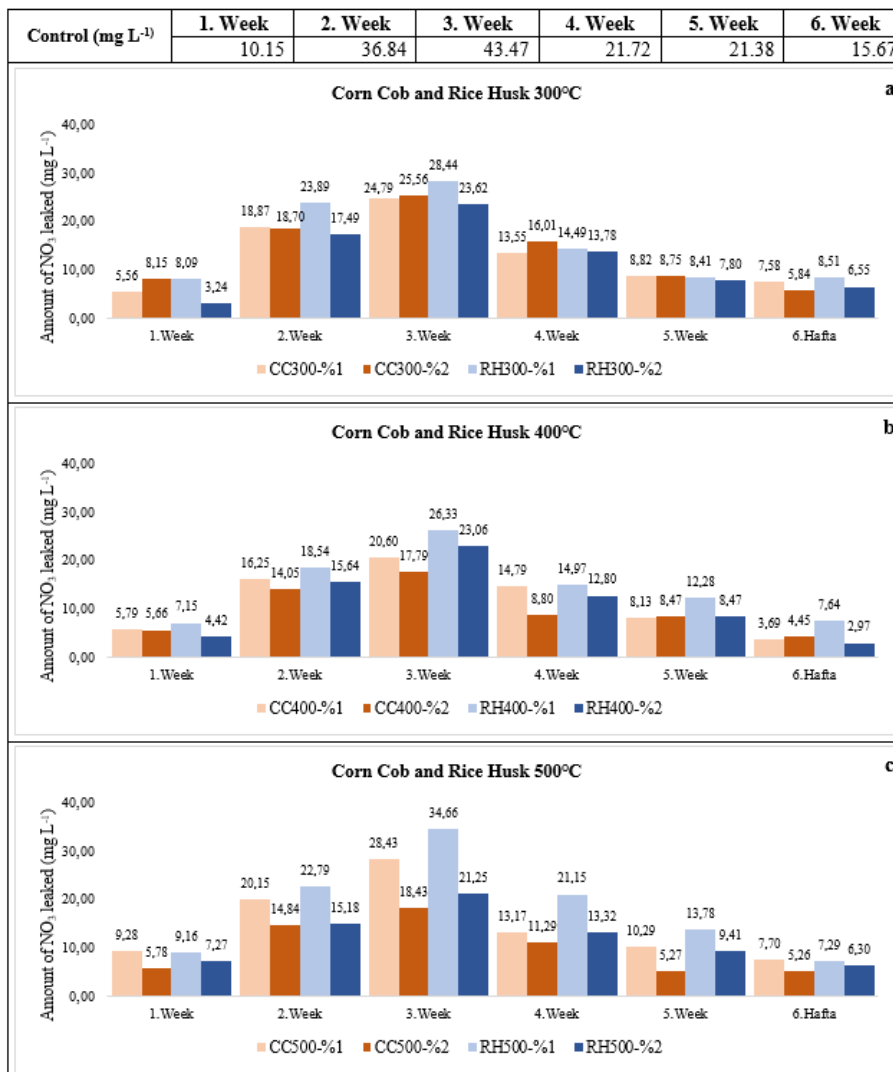


Figure 4. Weekly NH<sub>3</sub><sup>-</sup> change in leaking water after RH and CC biochar application.

Bodur (2016) reported a significant reduction in leached  $\text{NO}_3^-$  concentrations from columns with the application of rice husk biochar at different doses (2.5%, 5%, and 10%) compared to the control application. The researcher detected  $\text{NO}_3^-$  concentrations of  $11.3 \text{ mg L}^{-1}$  and  $3.7 \text{ mg L}^{-1}$  in leachate from the 1<sup>st</sup> week with 2.5% and 5% biochar doses, respectively; however, no nitrate was detected in leachates from other weeks and with a 10% biochar dose.

#### 4. Conclusion

The conversion of corn and rice crop residues, which are extensively produced in our country every year, into biochar and their application to a soil with loamy sand texture has been shown to retain a significant portion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the soil. The variations in leached  $\text{NH}_4^+$  concentrations from columns with the application of corn and rice biochars (except for BC2) at all pyrolysis temperatures and doses (1% and 2%) were statistically significant ( $P < 0.05$ ). The application of rice husk biochar resulted in a reduction of  $\text{NH}_4^+$  concentrations by 92.5% in RH500 BC1 dose and 93% in RH500 BC2 dose.

Similarly, the application of corn and rice biochars at all pyrolysis temperatures and doses (1% and 2%) resulted in a statistically significant ( $P < 0.05$ ) reduction in leached  $\text{NO}_3^-$  concentrations from the columns. Particularly, the effectiveness of rice husk and corn cob biochars at 2% application level in reducing  $\text{NO}_3^-$  leaching was notable. Another significant finding from the study is that biochars with higher pyrolysis temperatures were more effective in reducing  $\text{NO}_3^-$  leaching. These results suggest that biochar applications can significantly reduce the leaching of nitrogen in both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  forms in agricultural soils. The substantial reduction in nitrogen leaching through biochar applications is important for preventing groundwater contamination and enhancing plant uptake of applied nitrogen fertilizers.

#### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	C.Ç.G.	H.E.
C	50	50
D	70	30
S		100
DCP	80	20
DAI	40	60
L	50	50
W	30	70
CR	20	80
SR	20	80
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### Conflict of Interest

The authors declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## EVALUATION OF THE IMPACT OF CLIMATE CHANGE ON LIVESTOCK PRODUCTION FROM THE PERSPECTIVE OF FARMERS: YOZGAT CASE

Hacer TÜFEKÇİ<sup>1\*</sup>, Mehmet AKİF BOZ<sup>1</sup>, Selman YILDIRIM<sup>2</sup>

<sup>1</sup>Yozgat Bozok University, Faculty of Agriculture, Department of Animal Science, 66100, Yozgat, Türkiye


<sup>2</sup>Yozgat Provincial Directorate of Agriculture and Forestry, 66100, Yozgat, Türkiye


**Abstract:** The study aimed to assess the effects of climate change on livestock in the Yozgat province by assessing its influence on farmers. The main material of the study consisted of the data obtained from face-to-face questionnaires with farmers engaged in animal and crop production together in the villages connected to the center of Yozgat province. The study revealed that the majority of farmers fell within the 31-40 age bracket, accounting for 44.8% of the participants. Additionally, 40.0% and 36.0% of the farmers had completed high school and secondary school, respectively. Furthermore, 59.2% of the farmers reported having 5-7 family members. All surveyed farmers confirmed their familiarity with the notion of climate change. According to farmers, drought is the primary concern associated with climate change, followed by global warming and changes in seasons. According to the survey, 90.4% of the farmers reported that climate change has an impact on their region. The observed alterations were identified as a decrease in precipitation, unpredictable and fluctuating precipitation patterns, a reduction in the duration of precipitation, and an increase in water scarcity. All farmers who participated to study stated that climate change negatively affected animal and crop production. As a result, the farmers participating in the survey have a high awareness of climate change, they are affected by these changes in animal production, there are certain practices that they pay attention to in adapting to these processes and reducing their effects, but the need for information, training, legal practices and inspections to be carried out by relevant institutions on this issue has been particularly emphasized.


**Keywords:** Yozgat, Climate change, Global warming, Animal production

\*Corresponding author: Yozgat Bozok University, Faculty of Agriculture, Department of Animal Science, 66100, Yozgat, Türkiye

E mail: [hacer.tufekci@bozok.edu.tr](mailto:hacer.tufekci@bozok.edu.tr) (H. TÜFEKÇİ)

Hacer TÜFEKÇİ  <https://orcid.org/0000-0003-2272-4088>

Mehmet AKİF BOZ  <https://orcid.org/0000-0002-7452-6895>

Selman YILDIRIM  <https://orcid.org/0000-0002-7712-2263>

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### 1. Introduction

Agricultural production and climate change are intricately linked and significant worldwide concerns. Many recent studies have emphasised the significance of interrelationships, particularly those associated with a substantial rise in the average world temperature. The sustainability and productivity of agricultural production systems are mostly determined by climate, which is the most crucial environmental component (Barati et al., 2024). The agricultural sector is a prominent economic sector in Türkiye and is critical for rural sustainability. However, inadequate policies and factors such as climate change and wrong practices are causing rural-urban migration and a declining agricultural sector. In Türkiye, the interaction of livestock systems with the environment is becoming increasingly important in national and local policy agendas in line with climate change mitigation strategies and rural development. In addition to increasing consumption concerns and public interest in climate change, integrating alternative agriculture and food systems into the livestock sector can provide strong benefits (Geß and Hazar Kalonya, 2023).

Climate change threatens the welfare of current and future generations by changing the ecosystem of the planet. Climate changes caused or to be caused by global warming will be seen in different ways according to different regions of the world. Türkiye is among the risk group countries in terms of the potential effects of global warming due to the rise in extreme values in the Eastern Mediterranean region. Our country may be adversely affected by the weakening of water resources, forest fires, drought, desertification and related ecological deterioration due to global warming. For example, arid and semi-arid regions such as South East and Central Anatolia, which are under the threat of desertification, and semi-humid Aegean and Mediterranean regions, which do not have sufficient water, will be more affected by the temperature increase. Climate changes will lead to changes in the natural habitats of animals and plants in agricultural activities and will cause significant problems (Öztürk, 2002; Atalık, 2005; Şen, 2014; Marino et al., 2016). Agriculture is one of the most effective sectors that can ensure human survival. Animal husbandry has an important place in this sector. In the agriculture



sector, livestock farming is considered to be highly resilient to climate change and is thought to play an important role in ensuring food security to meet the demands of the increasing human population by 2050 (Thornton et al., 2007; Meena and Lal, 2018; Reshma Nair et al., 2021).

In the fight against the impacts of climate change on agriculture, two interrelated paths are followed. The first is mitigation policies, that is, reducing greenhouse gas emissions, which means mitigating the negative consequences of climate change. The second is climate change adaptation policies (IPCC, 2001; Akalın, 2014). The relationship between the livestock sector and climate change greatly affects adaptation approaches in the livestock sector (Havlik et al., 2014). In the fight against the impacts of climate change on agriculture, two interrelated paths are followed. The first is mitigation policies, that is, reducing greenhouse gas emissions, which means mitigating the negative consequences of climate change. The second is climate change adaptation policies (IPCC, 2001; Akalın, 2014). The relationship between the livestock sector and climate change greatly affects adaptation approaches in the livestock sector (Havlik et al., 2014).

To enhance animal production in evolving climate conditions, it is imperative to undertake multidisciplinary studies. Additionally, it is crucial to reinforce current agricultural extension systems and formulate sustainable plans encompassing adaptation, mitigation, and recovery approaches. Farmers can utilise climate change assessment at the farm level as a consultation tool, an information source for management, and a component of quality assurance programmes for customers. The study sought to assess the effects of climate change on livestock in the Yozgat province by assessing its influence on farmers.

## 2. Materials and Methods

Study is based on the data obtained from in-person surveys done with farmers involved in animal and crop production in the villages linked to the central area of Yozgat province. For the survey study in Yozgat center, according to the data obtained from the Yozgat Provincial Directorate of Agriculture and Forestry, the number of farmers in 2023 was accepted as the main mass and the sample volume was calculated accordingly. The sample size was calculated with the following proportional sample volume formula given in Equation 1 (Newbold, 1995).

$$n = \frac{Np(1 - p)}{(N - 1)\sigma_{px}^2 + p(1 - p)} \quad (1)$$

In the formula;  $n$  = sample size,  $N$  = Total number of farmers,  $\sigma_{px}^2$  is the variance of the ratio.

The  $p$  value in the proportional sample volume formula expresses the proportion of parts with a certain feature in the main population. To reach the maximum sample volume,  $p=0.50$  should be taken (Akyüz, 2019). In this

study, since it is desired to reach the maximum sample volume,  $p=0.50$  was taken during the calculation, representing the proportion of farmers affected by climate change. As a result of the calculation, the number of farmers to be interviewed was determined to be 135 farmers with 95% confidence interval and 0.05% margin of error. SPSS software was used to analyze the research data (SPSS, 2016). For analysis of the data, firstly, the socio-demographic characteristics of the farmers were revealed, then the information status of the farmers about climate change, the status of being informed about climate change, climate change and evaluations in Yozgat, local impact and factors and effects of climate change observed in Yozgat in the last 10 years, crop loss and compensation situation in the last 5 years due to various reasons, what has been done to adapt to / reduce the effects of climate change and what are the suggestions of farmers to reduce the effects of climate change in their region were analyzed. Simple arithmetic mean and percentage calculations were used in the research.

## 3. Results

The socioeconomic attributes of agricultural households are thought to exert varying influences on farmers' perspectives on climate change and their capacity to adapt (Reddy et al., 2022). The study revealed that 44.8% of the farmers surveyed fell into the age bracket of 31-40, while 24.8% were aged 41-50, 20.8% were aged 51-60, 5.6% were 61 years and beyond, and 5% were aged 26-30 (Table 1). The analysis revealed that the majority of farmers possessed a high school or secondary school education, with 40.0% and 36.0% respectively. Additionally, it was found that the average number of family members in farming households was between 5 and 7, accounting for 59.2% of cases. Furthermore, it was established that the majority of farmers employed the technique of dry farming (84.8%) and focused on producing crops for commercial purposes (89.6%), while also satisfying their own agricultural requirements. Upon examined the farmers' experience in this industry, it was found that the first group (29.6%) consisted of individuals aged 31-40 years, the second group (26.4%) consisted of those aged 41-50 years, and the third group (23.2%) consisted of individuals aged 51-60 years.

All surveyed farmers reported that they do both animal and crop production. They yield once a year and employ both chemical and organic fertilisers, as well as machinery and labour, in their production processes. Furthermore, every farmer stated that they allow their livestock to graze in pastures and house them in barns during the winter season.

Farmers' knowledge about climate change is given in Table 2. All of the farmers participating in the survey stated that they had heard of the concept of climate change before. When climate change is mentioned, farmers report that drought is the first, global warming is the second and changes in seasons as the third. Farmers stated that the causes of climate change are mainly the

increase in air pollution, increase in industrialization, widespread use of chemical pesticides, increase in urbanization, and destruction of forests. Regarding the repercussions of climate change, farmers have indicated that the primary impact will be a rise in temperature and the occurrence of drought. Additionally, there will be an increase in natural catastrophes as a secondary consequence, and the duration and characteristics of the seasons will undergo alterations as a tertiary effect. According to the farmers surveyed, in order to mitigate climate change, it is essential to raise awareness in society, establish legal regulations, implement effective monitoring and oversight, safeguard water resources,

promote the use of renewable energy sources, and restrict the use of chemical fertilisers and pesticides.

Table 3 shows the information status of farmers about climate change. 92.8% of the farmers who participated in the survey reported that they had not participated in any training on climate change before, and at the same time, no information activities were carried out by any institution on climate change or they had no knowledge and information. Farmers reported that they mostly (48.0%) obtained information and news about climate change from TV-radio-newspaper-family-friends-neighbours-public institutions-internet-social media.

**Table 1.** Socio-demographic and production information of farmers

Age	n	%	Education status	n	%
26-30	5	4.0	Primary school	45	36.0
31-40	56	44.8	Middle school	19	15.2
41-50	31	24.8	High school	50	40.0
51-60	26	20.8	Associate degree	3	2.4
61 and over	7	5.6	Master's degree	8	6.4
Gender			Marital status		
Famale	8	6.4	Married	96	76.8
Male	117	93.6	Single	29	23.2
Professional experience (year)			Number of household		
10-20	13	10.4	1-2	13	10.4
21-30	40	32.0	3-4	31	24.8
31-40	34	27.2	5-7	74	59.2
41-50	10	8.0	7 and over	7	5.6
51-60	21	16.8	Your crop production method		
61 and over	7	5.6	Dry agriculture	106	84.8
Property ownership status			Dry-irrigated agriculture	19	15.2
Owner	48	38.4	Your priority in agricultural production		
Tenant	13	10.4	Own need	7	5.6
Owner-tenant	27	51.2	Market orientated	6	4.8
Residence status in Yozgat			Both in one	112	89.6
21-30	22	17.6	Membership status in the agricultural structure		
31-40	37	29.6	Yes-Agricultural Credit Cooperative/Chamber of Agriculture	87	69.6
41-50	33	26.4	Yes-Cooperatives	19	15.2
51-60	29	23.2	Yes-Irrigation Association	9	7.2
61 and over	7	5.6	Yes-Breeders' Association	10	8.0

**Table 2.** Farmers' knowledge about climate change

What comes to mind when you think of climate change?	Agree		Disagree	
	n	%	n	%
Global warming	97	77.6	28	22.4
Changes in seasons	73	58.4	52	41.6
Occurrence of excessive rainfall	31	24.8	94	75.2
Drought	112	89.6	13	10.4
Environmental pollution	54	43.2	71	56.8
Air pollution	34	27.2	91	72.8
More frequent weather events such as floods, storms, tornadoes, etc.	44	35.2	81	64.8
Depletion of the ozone layer	18	14.4	107	85.6
Increasing greenhouse gas effects	3	2.4	122	97.6
Increased CO <sub>2</sub> emissions	3	2.4	122	97.6
The global economic system	19	15.2	115	92

**Table 2.** Farmers' knowledge about climate change (continue)

	Agree		Disagree	
	n	%	n	%
What are the main causes of climate change?				
Increase in air pollution	90	72.0	35	28.0
Increasing use of fossil fuels	61	48.8	63	50.4
Increased industrialization	73	58.4	51	40.8
Rapid population growth	50	40.0	75	60.0
Destruction of forests	63	50.4	62	49.6
Increasing urbanization	65	52.0	60	48.0
Widespread use of chemical drugs	72	57.6	53	42.4
Increased use of motor vehicles	55	44.0	70	56.0
What consequences can climate change have?				
There is an increase in natural disasters	85	68.0	39	31.2
Sudden weather changes occur	50	40.0	75	60.0
The duration and characteristics of the seasons change	80	64.0	45	36.0
Temperature increases, drought occurs	109	87.2	16	12.8
Floods occur as a result of heavy and excessive rainfall	48	38.4	77	61.6
Extreme cold and frost events occur	57	45.6	68	54.4
There is a decrease in water resources	70	56.0	55	44.0
New types of diseases emerge	37	29.6	88	70.4
Some plant and animal species disappear	42	33.6	83	66.4
Crop and animal production decreases	41	32.8	84	67.2
Access to food becomes difficult	35	28.0	90	72.0
Migrations occur	35	28.0	90	72.0
What should be done to prevent climate change?				
It is not possible to prevent climate change	33	26.4	92	73.6
Forests and pastures should be protected and their destruction should be prevented.	70	56.0	55	44.0
Water resources should be protected and renewable energy sources should be used	78	62.4	47	37.6
Water should be saved	63	50.4	62	49.6
The use of chemical fertilizers and pesticides should be limited	76	60.8	49	39.2
Savings should be made in energy use	53	42.4	72	57.6
Harmful waste should be disposed of in a controlled manner	59	47.2	66	52.8
Emission of harmful gases should be prevented and reduced	43	34.4	82	65.6
Strong legal regulations should be made	90	72.0	35	28.0
Society should be made aware of climate change	102	81.6	23	18.4
Effective control and supervision should be carried out	85	68.0	40	32.0

**Table 3.** The status of farmers' being informed about climate change

	n	%
Have you attended any training on climate change?		
Yes-meeting	9	7.2
No	116	92.8
Have any institutions carried out information activities regarding global climate change?		
Yes-Directorate of Agriculture and Forestry-Mukhtar	9	7.2
No-I don't know	116	92.8
Where do you get information and news about climate change?		
TV-radio-newspaper-family-friend-neighbour-public institutions-internet-social media	60	48.0
TV-radio-newspaper-family-friend-neighbour	25	20.0
TV-radio-newspaper-family-friend-neighbour- breeders' association -cooperatives	9	7.2
TV-radio-newspaper-family-friend-neighbour-public institutions-Mukhtar	6	4.8

Upon examined the data on climate change and local effects in Yozgat from Table 4, it is revealed that 9.6% of the farmers were unaware of the influence of climate change on their region, whereas 90.4% acknowledged its impact. Furthermore, a significant majority of 60.0% of farmers expressed a lack of sufficient information

regarding climate change, while 54.4% reported that adequate steps were not being implemented to address this issue. 69.6% of the farmers who participated in the survey stated that the level of being affected by climate change in Yozgat is medium, 20.8% stated that it is high and 5.6% stated that it is low. 49.6% of the farmers

stated that the human impact on climate change in Yozgat is at medium level, 28% at high level and 10.4% at low level.

The factors and impacts of climate change observed in Yozgat during the past decade are presented in Table 5. The poll revealed that most farmers experienced a decline in precipitation, unpredictable and fluctuating precipitation patterns, a shorter duration of precipitation, and instances of water scarcity. Every farmer asserted that climate change had an adverse impact on both agricultural and animal productivity. The primary adverse consequences include inadequate grazing spaces, reduced productivity, heightened production expenses, economic instability, and heat-induced stress. Furthermore, farmers have said that the primary factors contributing to climate change in their region are the inadvertent exploitation of pasture and water resources, excessive grazing, the conversion of pasturelands into other forms of land, and the thoughtless consumption and depletion of existing natural resources.

Table 6 shows the crop loss and compensation status of the farmers participating in the survey in the last 5 years. 94.4% of the farmers stated that they experienced crop losses due to drought, 18.4% due to flood, 45.6% due to hail, 35.2% due to frost and 24% due to storm. When farmers were asked to evaluate the impact of the losses on their income, 16.0% of them stated that it was low, 32.0% stated that it was moderate and 52.0% stated that it was extremely effective. 63.2% of the farmers stated that they were able to compensate for the losses and 36.8% stated that they could not compensate for the losses. 74.4 percent of the farmers surveyed stated that they received any support for losses caused by climate change and 25.6 percent stated that they did not receive any support. According to farmers, agricultural insurance and government help were the primary means of compensation. However, it was claimed that fertiliser, fuel, funds, and machinery support were the most often used kinds of compensation. Furthermore, every farmer surveyed reported no instances of livestock or barn loss resulting from any disaster within the past 5 years.

**Table 4.** Climate change and evaluations in Yozgat, local effect

	Definitely Yes		Yes		No Opinion		No		Absolutely Not	
	n	%	n	%	n	%	n	%	n	%
Climate change affects your region	64	51.2	49	39.2	12	9.6	-	-	-	-
Adequate information on climate change is provided in my region	-	-	9	7.2	42	33.6	67	53.6	8	6.4
Necessary measures are taken in my region regarding climate change	6	4.8	6	4.8	45	36.0	56	44.8	12	9.6
	Unaffected		Low level		Medium level		High level		No Opinion	
	n	%	n	%	n	%	n	%	n	%
What do you think is Yozgat's level of impact from climate change?	-	-	7	5.6	87	69.6	26	20.8	5	4.0
In your opinion, to what extent is the human impact on climate change in Yozgat?	3	2.4	13	10.4	62	49.6	35	28.0	12	9.6

**Table 5.** Factors and effects of climate change observed in Yozgat in the last 10 years

Which effects of climate change do you observe in Yozgat?	Agree		Disagree	
	n	%	n	%
Increased rainfall	14	11.2	111	88.8
Decreased rainfall (drought/desertification)	111	88.8	14	11.2
Irregular rainfall	103	82.4	22	17.6
Delayed rainfall	103	82.4	22	17.6
Shortening of the precipitation period	91	72.8	34	27.2
Increase in frost events	46	36.8	79	63.2
Increase in temperature	93	74.4	32	25.6
Increase in flood events	45	36.0	80	64.0
Increase in hail events	48	38.4	77	61.6
Increase in wind-storms	67	53.6	58	46.4
Increase in day-night temperature difference	71	56.8	54	43.2
Water scarcity	90	72.0	35	28.0
Water pollution	52	41.6	73	58.4
Soil pollution	72	57.6	53	42.4

**Table 5.** Factors and effects of climate change observed in Yozgat in the last 10 years (continue)

	Agree		Disagree	
	n	%	n	%
Do you think that climate change negatively affects crop and animal production?				
Reduction in product quantity	107	85.6	18	14.4
Post-harvest losses occurred	32	25.6	90	72
Heat stress	77	61.6	48	38.4
Increase in weeds and insects	60	48.0	65	52.0
Erosion severity increased	34	27.2	91	72.8
Product variety decreased	27	21.6	98	78.4
Diseases and deaths in farm animals increased	55	44.0	70	56.0
Production cost increased	95	76.0	30	24.0
Pasture areas are insufficient / have low capacity	119	95.2	6	4.8
Negativities increased in farm animals during growth-development and fertility periods	51	40.8	74	59.2
Economic instability	91	72.8	34	27.2
Increase in animal diseases (epidemic diseases)	25	20.0	100	80.0
What are the practices that cause climate change in your region?				
Crop and animal production does not cause climate change	14	11.2	111	88.8
Excessive use of fertiliser	40	32.0	85	68.0
Excessive drug use	89	71.2	36	28.8
Over-irrigation	40	32.0	85	68.0
Burning stubble	95	76.0	30	24.0
Agricultural waste	58	46.4	67	53.6
Establishment of large farms	26	20.8	99	79.2
Gases resulting from animal husbandry activities	20	16.0	105	84.0
Unconscious use of pasture and water resources by people	115	92.0	10	8.0
Overgrazing in pasture areas, conversion to land, etc.	115	92.0	10	8.0
Migration of people out of the country	19	15.2	106	84.8
Population growth	36	28.8	89	71.2
Unconscious consumption and destruction of natural resources (forests, pastures, lakes, streams, etc.)	106	84.8	19	15.2
Breeders who are producers become consumers and cannot continue production	81	64.8	44	35.2

**Table 6.** Product loss and compensation situation in the last 5 years

	n	%
Have you experienced crop loss due to drought?		
Yes	118	94.4
No	7	5.6
Have you suffered crop loss due to flooding?		
Yes	23	18.4
No	102	81.6
Have you experienced crop loss due to hail?		
Yes	57	45.6
No	68	54.4
Have you experienced crop loss due to frost?		
Yes	44	35.2
No	81	64.8
Did you experience any crop loss due to the storm?		
Yes	30	24.0
No	95	76.0
Evaluate the impact of your losses on your income		
Low level	20	16.0
Medium level	40	32.0
Extremely effective	65	52.0
Were you able to compensate for the losses?		
Yes	79	63.2
No	46	36.8

**Table 6.** Product loss and compensation situation in the last 5 years (continue)

	n	%
Have you received any support for losses caused by climate change?		
Yes	93	74.4
No	32	25.6
What are the compensation methods (more than one option can be selected)		
Utilisation of savings	20	16.0
Agricultural insurance	90	72.0
State support	82	65.6
Animal support	12	9.6
Seed support	3	2.4
Machine support	27	21.6
Fertiliser support	85	68.0
Diesel support	82	65.6
Feed support	25	20.0
Credit support	16	12.8
Cash support	47	37.6

**Table 7.** Practices carried out in the last 5 years to adapt to climate change / mitigate its impacts

	Agree		Disagree	
	n	%	n	%
The effects of climate change cannot be stopped/mitigated	44	35.2	81	64.8
Changing the crops planted	65	52.0	60	48.0
Change planting time	89	71.2	36	28.8
Change in time to prepare the field for planting	49	39.2	76	60.8
Change in harvest time	49	39.2	76	60.8
Switching to rotational farming	83	66.4	42	33.6
Insuring products	89	71.2	36	28.8
Conservation tillage	39	31.2	86	68.8
I started growing crops that require less water	37	29.6	88	70.4
Starting to plant multiple crops	40	32.0	85	68.0
I changed my water source	46	36.8	79	63.2
I don't have enough information	9	7.2	116	92.8
Changing irrigation system management	26	20.8	99	79.2
Drip irrigation/sprinkler irrigation preference	30	24.0	95	76.0
Limitation on the use of chemical fertilizers	39	31.2	86	68.8
Using animal manure	35	28.0	90	72.0
Insuring animals	23	18.4	102	81.6
Preferring extensive breeding systems	29	23.2	96	76.8
Get information from experts	37	29.6	88	70.4

**Table 8.** Practices / suggestions that can be done to minimise the effects of climate change

	Agree		Disagree	
	n	%	n	%
The effects of climate change cannot be stopped	20	16.0	105	84.0
Checks and inspections should be increased	73	58.4	52	41.6
Increasing inter-institutional cooperation and presenting region-specific solution suggestions	43	34.4	82	65.6
Conducting training and information activities	92	73.6	33	26.4
Development of good agricultural practices	31	24.8	94	75.2
Increasing organic farming practices	10	8.0	105	84.0
Development of modern irrigation systems	71	56.8	54	43.2
Increasing product diversity	55	44.0	70	56.0
Promoting environmentally friendly products	43	34.4	82	65.6
Conducting soil analysis	56	44.8	69	55.2
Preventing/reducing stubble burning	44	35.2	81	64.8

**Table 8.** Practices / suggestions that can be done to minimise the effects of climate change (continue)

	Agree		Disagree	
	n	%	n	%
Determining the appropriate product pattern	56	44.8	69	55.2
Protecting water resources and providing efficient use opportunities	32	25.6	93	74.4
Improving disaster risk management for rural areas	50	40	75	60
Employment should be increased	23	18.4	102	81.6
Production power should be increased (Supports should be increased / young people should be encouraged / market conditions should be improved)	56	44.8	69	55.2
Harmful waste generation should be reduced	19	15.2	106	84.8
Pasture areas should be protected	71	56.8	54	43.2
Land consolidation	47	37.6	78	62.4

Practices carried out by farmers in the last 5 years to adapt to climate change / reduce its impacts are given in Table 7. When the activities of the farmers participating in the survey are analyzed, it is stated that they insured their products and changed the planting time of the products, switched to rotational agriculture, changed the crops planted, changed the time of preparation for planting and harvesting, and changed the water resources. In addition, 35% of the farmers reported that the impacts of climate change are unstoppable and unmitigable.

In Table 8, practices / suggestions that can be done to minimise the effects of climate change. Farmers mostly stated that training and information activities should be carried out, controls and inspections should be increased, pasture areas should be protected, modern irrigation systems should be developed, soil analyses should be carried out, appropriate crop patterns should be determined and production power should be increased. In addition, farmers reported that practices such as increasing product diversity, developing disaster risk management for rural areas, land consolidation, preventing/reducing stubble burning, promoting environmentally friendly products, increasing cooperation between institutions and presenting region-specific solutions should be implemented.

#### 4. Discussion

Climate change is seen as the biggest obstacle to agricultural development in developing countries. The high dependence on agriculture and related sectors makes many countries vulnerable to climate change phenomena. There is a gap in understanding climate change at macro and micro levels. Farmers' perceptions and opinions on the impacts of climate change on agriculture are the basis for the development of various mitigation and adaptation strategies (Reddy et al., 2022). In one study, it was reported that the same crop yield was affected differently in different regions due to climatic variations (Kumar et al., 2014). In another study, changing temperature and precipitation trends were observed and their effects on different crops in different regions were analyzed (Aggarwal and Swaroop Rani, 2009). In this context, adaptation to changing climates

with climate-resilient technologies and their sensitivity seems to be an effective method for farmers to reduce the negative impacts of climate change (Füssel and Klein, 2006). Nizam (2013) conducted an analysis of the fluctuation in rainfall and temperature, as well as the perception of climate change among farmers in the Anuradhapura region from 1941 to 2010. The study found that most farmers' perceptions closely aligned with a statistical analysis of meteorological data. In their research conducted in several regions, Sarkar and Padaria (2010) and Sarkar and Padaria (2016) found that approximately 38% of the participants were aware of climate change. The researchers noted that the majority of individuals attributed climate change to the rapid process of industrialization. The investigations revealed that the most prominent awareness observed among individuals was a decline in agricultural output.

Adaptation strategies are shaped by multiple factors, including education level, farming family size, gender of the family head, crop-livestock component, access to extension services, and credit from various institutions (Deressa et al., 2011; Elum et al., 2016; Nhemachena and Hassan 2007). In their study, Manjunath et al. (2017) found that crop production in the region is affected by multiple factors including climate, soil, topography, and the institutional and socioeconomic status of farmers. They discovered that 80% of small and marginal farmers believe that regional agriculture is highly susceptible to climate change. Climate change has a greater impact on marginal and smallholders who have less climate-resilient management practices and rely on capital-intensive technologies (Rehmani et al., 2021; Gbetiboubo and Ringler, 2009).

All the farmers involved in the study affirmed their prior knowledge of the idea of climate change. The obtained data exhibited greater values compared to the findings reported by Sarkar and Padaria in 2010 and 2016. According to the survey, farmers ranked drought as the primary concern when discussing climate change, followed by global warming as the secondary concern, and changes in seasons as the tertiary concern. According to the study, 90.4% of the farmers reported that climate change has an impact on their region. Farmers primarily note a decline in precipitation, fluctuations and



variability in rainfall patterns, a reduction in the duration of rainfall, and a consequent lack of water. Every farmer reported that climate change had an adverse impact on both crop and animal production. These findings are comparable to the results of investigations conducted in various geographical areas (Sarkar and Padaria, 2010; Shashidhra and Reddy 2012; Varadan and Kumar, 2014; Sarkar and Padaria, 2016; Reddy et al., 2022).

Despite being aware of the existence of climate change, farmers and policymakers frequently neglect to address its consequences due to socioeconomic and institutional limitations, including a lack of willingness, insufficient capital/resources, and limited knowledge (Tripathi and Mishra 2017). Despite being cognizant of the adverse consequences of excessive utilisation of natural resources, farmers persist in over-exploiting them in the majority of cases. Farmers prioritise maintaining their productivity and income over environmental conservation. Hence, it is imperative to comprehend farmers' perspectives on climate change, their level of sensitivity towards climate change, and the efficacy of agricultural adaptation to climate change. Moreover, the task of developing and implementing climate resilient methods poses a substantial difficulty due to the predominant involvement of small and marginalised farmers in farming systems. The majority of these farmers have limited literacy or education and lack resources, resulting in a low ability to adapt (Gbetibouo and Ringler, 2009; Saroar et al., 2015). Consequently, large-scale adoption of climate-resilient practices is not possible, as most practices are site-specific (McCarthy et al., 2001; Reddy et al., 2022).

## 5. Conclusion

Since the impacts of climate change, adaptation strategies and farmers' knowledge are largely site-specific, location-specific studies are needed. This study sought to ascertain the perspectives of farmers in Yozgat province, situated in the Central Anatolia Region of Türkiye, on the effects of climate change on livestock and the observed alterations. The obtained results are believed to aid to the development of regional plans aimed at mitigating the impacts of climate change. Furthermore, the absence of any comparable field research undertaken in the region, along with the scarcity of such studies in our country, underscores the significance of the present work as a potential catalyst for future investigations. The poll reveals that the farmers involved have a strong understanding of climate change and are impacted by its implications on animal production. Additionally, they employ certain strategies to adapt to these changes and minimise their consequences. Nevertheless, it is crucial to underscore the necessity for key institutions to conduct information dissemination, training programmes, legal enforcement, and inspections pertaining to this matter.

## Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.T.	M.A.B.	S.Y.
C	50	50	
D	50	50	
S	50	50	
DCP			100
DAI	50	50	
L	50	20	30
W	50	30	20
CR	50	50	
SR	50	30	20
PM	20	50	30
FA		50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Permission to conduct the study was obtained with the decision of the Yozgat Bozok University Social Sciences and Humanities Research Ethics Committee dated October 18, 2023 (protocol code: 07/10).

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## HETEROISIS AND DIALLEL ANALYSIS OF YIELD AND YIELD COMPONENTS OF BREAD WHEAT F<sub>1</sub> GENERATION

Harun OCAKTAN<sup>1</sup>, Hüseyin GÜNGÖR<sup>2\*</sup>, Ziya DUMLUPINAR<sup>1</sup>

<sup>1</sup>Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Agricultural Biotechnology 46040, Kahramanmaraş, Türkiye


<sup>2</sup>Düzce University, Faculty of Agriculture, Department of Field Crops, 81620, Düzce, Türkiye


**Abstract:** In this study, half-diallel crosses were performed using six wheat genotypes (Adana-99, Flamura-85, Masaccio, Lucilla, 1635 and 2115). This research was conducted in Kahramanmaraş ecological conditions during the 2019-2020 growing season in a randomized complete block design with three replications. Heading date (HD), grain filling period (GFP), days to maturity (DM), plant height (PH), spike length (SL), grain number per spike (GNS), grain weight per spike (GWS), thousand kernel weight (TKW), grain yield per plant (GY), and chlorophyll content of flag leaf (SPAD value) traits were investigated on F<sub>1</sub> plants and parents. When the mean values of parents and F<sub>1</sub> generations were examined, F<sub>1</sub> mean values were higher than the mean values of parents in heading date (134.06 days), days to maturity (164.04 days), spike length (13.31 cm), grain number per spike (51.64 units), grain weight per spike (2.11 g), thousand-grain weight (36.58 g), grain yield per plant (35.99 g), and chlorophyll content of flag leaf (49.60 SPAD), while lower in grain filling period (41.77 days) and plant height (88.80 cm). According to diallel analyses, it was found that HD, DM, GFP, PH, GWS, and SPAD traits had additive and dominant gene effects, while SL, GNS, TKW, and GY traits had significant dominant gene effects. Partial dominance was observed for HD, DM, PH, GWS, GY, and SPAD traits, while superior dominance was observed for GFP, SL, GNS, and TKW traits. The effects of general combining ability (GCA) and specific combining ability (SCA) were significant for all the traits studied. Positive average heterosis and heterobeltiosis values were determined for GNS, GWS, TKW, and GY traits. In terms of grain yield per plant, the genotypes Adana-99, Lucilla, and Masaccio were identified as potential parents for breeding programs. Combinations of 1635 × 2115, Masaccio × Lucilla, Adana-99 × Lucilla, Adana-99 × Masaccio, Adana-99 × 1635, and Flamura-85 × Lucilla were identified as promising hybrids for grain yield.


**Keywords:** Bread wheat, Diallel analysis, Combining ability, Heterosis, Heterobeltiosis, Inheritance

\*Corresponding author: Düzce University, Faculty of Agriculture, Department of Field Crops, 81620, Düzce, Türkiye

E mail: hgungor78@hotmail.com (H. GÜNGÖR)

Harun OCAKTAN  <https://orcid.org/0000-0003-1711-5712>

Hüseyin GÜNGÖR  <https://orcid.org/0000-0001-6708-6337>

Ziya DUMLUPINAR  <https://orcid.org/0000-0003-3119-6926>

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### 1. Introduction

Wheat plays a fundamental role in human nutrition and is strategically vital for food security; therefore, with the global population increase, improving the yield per unit area is crucial to ensure sufficient and balanced nutrition. In Türkiye, various studies are conducted to improve the quality and productivity of bread wheat, and new varieties and technologies are being developed to enhance both quantity and quality of production. However, breeding programs also need to develop commercial varieties that adapt to changing climate conditions; hence, lines derived from high-performance genotypes (Bayhan et al., 2023). Plant breeders create variations in genetic material by making crosses to develop varieties suitable for their objectives. Parents and hybrid offsprings in recently developed hybrid populations are evaluated for agronomic traits at early stages, and those with superior characteristics are selected. The average values obtained for the parents' features are essential in predicting hybrid performance

and selecting superior parents (Poehlman and Sleeper, 1995). Various methods such as diallel, partial diallel, and line x tester are used for parent selection in hybrid breeding, however the diallel analysis is the most commonly preferred method. Diallel analysis is used to examine the genetic structures of hybrid populations using data obtained from the F<sub>1</sub> generation, determining promising hybrid combinations and parents' general and specific combining abilities (Sing and Chaudhary, 1985). The heterosis concept is utilized to determine the hybrid performance of parents. A high heterosis value is preferred in identifying high-yielding and high-quality hybrid genotypes (Knott, 1965). Heterosis (Ht) refers to the superiority of the F<sub>1</sub> hybrid over the average of parents when two pure lines are crossed. At the same time, heterobeltiosis (Hb) indicates the superiority of the F<sub>1</sub> hybrid over the superior parent (Dumlupınar et al., 2015). The performance of a genotype in the hybridization sequence is defined as a general combining ability.



In contrast, the superiority of hybrid performance between specific genotype pairs is expressed as specific combining ability (Yildirim and Cakir, 1986). General combining ability reflects additive gene effects, while specific combining ability reflects non-additive, dominant and epistatic gene effects (Falconer, 1980). While aiming to develop any trait, the most helpful information for the breeder is to detect the ability of the considered varieties to be parents and the genetic variance that the hybrid population generated from them may have in early generations (Sener et al., 2000). Knowing the inheritance degrees of the selected traits of parents according to the purpose, eliminates the unnecessary combinations and provides insight into which generation to start selection (Toklu and Yagbasanlar, 2005).

This study was aimed to investigate the inheritance of yield and yield components on F<sub>1</sub> combinations obtained from half-diallel crosses among six bread wheat genotypes to determine inheritance degrees and heterosis values to identify general and specific combining abilities, and to select promising hybrid combinations and suitable parents.

## 2. Materials and Methods

This research was conducted during the 2019-2020 growing season in the Kahramanmaraş ecological conditions. The total precipitation during the 2019-2020 growing season was recorded as 492.10 mm, with an average temperature of 13.66 °C (Table 1). The experiment was conducted in a clay-loam, slightly

alkaline, high lime content, salt-free soil with low organic matter, P<sub>2</sub>O<sub>5</sub>, and Mn content, high K<sub>2</sub>O<sub>5</sub> content, and medium Ca, Mg, and Fe content, while Cu and Zn content were at sufficient levels (Table 2).

The study utilized six bread wheat genotypes (Adana-99, Flamura-85, Masaccio, Lucilla, 1635, and 2115) and 15 F<sub>1</sub> combinations obtained using the half diallel analysis method. F<sub>1</sub> seeds and parents were sown on December 22, 2019, in a randomized complete block design with three replications. Plant rows spaced 20 cm apart, 10 cm between two plants and 1 meter long of two rows, as a total plot size of 2 m<sup>2</sup>. At the sowing, 80 kg ha<sup>-1</sup> of phosphorus (P<sub>2</sub>O<sub>5</sub>) and 80 kg ha<sup>-1</sup> of nitrogen (N) in the form of 20-20-0, and 100 kg ha<sup>-1</sup> of nitrogen from 33% ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) as top dressing were applied as fertilizer. Weed control was performed chemically on March 10, 2020, during tillering, and the trial was conducted on rainfed conditions. Harvest was done manually on May 13, 2020 with sickle.

In the study, heading date, grain filling period, days to maturity, plant height, spike length, grain number per spike, grain weight per spike, thousand kernel weight, and chlorophyll content of flag leaf were calculated for the selected ten plants. The grain yield was calculated as per plant.

**Table 1.** Means of climate data of trial year and long years

Months	Precipitation (mm)		Temperature (°C)	
	2019-20	Long Years	2019-20	Long Years
November	39.10	72.28	13.50	12.06
December	198.50	131.88	8.40	7.31
January	88.00	112.15	6.30	5.42
February	72.70	106.58	6.10	7.62
March	173.40	97.74	12.50	12.00
April	61.80	58.56	15.90	16.25
May	18.50	39.66	21.60	20.41
Jun	4.10	6.71	25.00	25.99
Total	492.10	625.56		
Mean			13.66	13.38

**Table 2.** Physical and chemical properties of the trial area

Features	2019-2020	
Saturation	% 58	Clay-Loam
pH	7.75	Slightly Alkalinity
EC dS.m <sup>-1</sup>	0.42	No salinity
CaCO <sub>3</sub> %	20.3	High Lime
Organic matter %	0.57	Slightly
P <sub>2</sub> O <sub>5</sub> kg da <sup>-1</sup>	3.38	Low
K <sub>2</sub> O kg da <sup>-1</sup>	47.96	High
Ca (ppm)	10671	Medium
Mg (ppm)	574	Medium
Cu (ppm)	2.16	Sufficient
Fe (ppm)	5.93	Medium
Mn (ppm)	7.52	Low
Zn (ppm)	0.92	Sufficient

Heterosis (Ht) and heterobeltiosis (Hb) were computed utilizing the formula provided in Equations 1 and 2 (Chang and Smith, 1967; Fonseca and Patterson, 1968):

$$Ht(\%) = \frac{F_1 - MP}{MP} 100 \quad (1)$$

$$Hb(\%) = \frac{F_1 - BP}{BP} 100 \quad (2)$$

where F<sub>1</sub>= value of F<sub>1</sub>; MP= mean value of parents and BP= value of better-parent.

Following preliminary variance analysis using the JMP (Kalayci, 2005), diallel tables were created for each block for traits showing statistically significant variance between F<sub>1</sub> hybrids generations and parents, and analyzed (Hayman, 1954a; Aksel and Johnson, 1963).

Variance analyses of diallel tables were performed by writing necessary formulas into the EXCEL computer program based on the diallel variance analysis method suggested by Jones (1965). The estimation of genetic

variance components through diallel hybrid analysis and the analysis of combining abilities were conducted using the statistical package program TARPOGEN developed by Ozcan (1999) based on the method proposed by Jinks-Hayman (1953), Jinks (1954), and Hayman (1954b, 1958); while the analysis of combination abilities was performed according to Griffing's (1956) Method II and Model I, which include parents.

### 3. Results and Discussion

The average phenotypic values for the examined traits are presented in Table 3, while the heterosis (Ht) and heterobeltiosis (Hb) values (%) are shown in Table 4. The values for genetic parameters calculated for each trait are provided in Table 5, and the general and specific combining ability effects of parents and hybrids are presented in Tables 6 and 7.

**Table 3.** Mean values of agronomic traits from parents and F<sub>1</sub> crosses

Parental Genotypes / Crosses	HD	GFP	DM	PH	SL	GNS	GWS	TKW	GY	SPAD
Adana 99 (1)	121 <sup>K</sup>	54.66 <sup>B</sup>	159.66 <sup>I</sup>	106.4 <sup>B</sup>	14.76 <sup>B</sup>	59.33 <sup>D</sup>	1.95 <sup>F</sup>	31.13 <sup>JK</sup>	28.49 <sup>FGH</sup>	44.64 <sup>KL</sup>
Flamura-85 (2)	124 <sup>I</sup>	53.33 <sup>C</sup>	167 <sup>B</sup>	92.5 <sup>E</sup>	13.3 <sup>C</sup>	47.9 <sup>G</sup>	2.15 <sup>DE</sup>	40.23 <sup>C</sup>	19.24 <sup>LM</sup>	51.2 <sup>CDE</sup>
Masaccio (3)	121 <sup>K</sup>	53 <sup>C</sup>	161.66 <sup>I</sup>	88.46 <sup>FG</sup>	9.4 <sup>G</sup>	32.5 <sup>I</sup>	1.29 <sup>IJ</sup>	33.86 <sup>H</sup>	23.39 <sup>IJK</sup>	49.93 <sup>EFG</sup>
Lucilla (4)	131 <sup>GH</sup>	44.66 <sup>FG</sup>	163.33 <sup>FGH</sup>	90.63 <sup>EF</sup>	11.46 <sup>E</sup>	43.53 <sup>HI</sup>	1.43 <sup>HI</sup>	30.83 <sup>K</sup>	24.56 <sup>IJ</sup>	52.58 <sup>BC</sup>
1635 (5)	139 <sup>C</sup>	38.33 <sup>I</sup>	164.33 <sup>CDEF</sup>	121.56 <sup>A</sup>	13.33 <sup>C</sup>	31.73 <sup>J</sup>	1.12 <sup>JK</sup>	35.23 <sup>FG</sup>	22.49 <sup>JKL</sup>	40.8 <sup>M</sup>
2115 (6)	141 <sup>B</sup>	34.66 <sup>K</sup>	165.33 <sup>C</sup>	102.96 <sup>C</sup>	11.53 <sup>DE</sup>	33.56 <sup>I</sup>	1.04 <sup>K</sup>	28.93 <sup>L</sup>	15.75 <sup>N</sup>	43.15 <sup>L</sup>
1x2	128.66 <sup>I</sup>	61 <sup>A</sup>	177 <sup>A</sup>	81.16 <sup>HI</sup>	15.76 <sup>A</sup>	89.06 <sup>A</sup>	4.11 <sup>A</sup>	46.8 <sup>A</sup>	29.68 <sup>FGH</sup>	51.46 <sup>CDE</sup>
1x3	133.66 <sup>F</sup>	40.66 <sup>I</sup>	159.66 <sup>I</sup>	82.3 <sup>H</sup>	16.03 <sup>A</sup>	60.13 <sup>D</sup>	2.45 <sup>BC</sup>	41.36 <sup>B</sup>	45.7 <sup>D</sup>	49.2 <sup>FG</sup>
1x4	130 <sup>H</sup>	44.33 <sup>G</sup>	160 <sup>J</sup>	81.3 <sup>HI</sup>	16.26 <sup>A</sup>	69.76 <sup>B</sup>	2.58 <sup>B</sup>	38.73 <sup>D</sup>	55.98 <sup>B</sup>	47.37 <sup>HI</sup>
1x5	133.33 <sup>F</sup>	41 <sup>I</sup>	164 <sup>DEFG</sup>	90.23 <sup>EF</sup>	10.46 <sup>F</sup>	54.83 <sup>E</sup>	2.05 <sup>EF</sup>	35.66 <sup>F</sup>	37.74 <sup>E</sup>	46.39 <sup>IJ</sup>
1x6	131.66 <sup>G</sup>	42.66 <sup>HI</sup>	162.66 <sup>HI</sup>	107.13 <sup>B</sup>	12 <sup>DE</sup>	56.16 <sup>E</sup>	2.2 <sup>DE</sup>	38.63 <sup>D</sup>	29.99 <sup>F</sup>	46.64 <sup>IJ</sup>
2x3	130.33 <sup>H</sup>	45.66 <sup>F</sup>	165.33 <sup>C</sup>	90.93 <sup>EF</sup>	15.76 <sup>A</sup>	64 <sup>C</sup>	2.55 <sup>B</sup>	41.43 <sup>B</sup>	30.88 <sup>F</sup>	50.88 <sup>DE</sup>
2x4	127.66 <sup>I</sup>	48.66 <sup>D</sup>	165 <sup>CD</sup>	89.23 <sup>F</sup>	13.43 <sup>C</sup>	53.23 <sup>EF</sup>	2.18 <sup>DE</sup>	38.66 <sup>D</sup>	49.53 <sup>C</sup>	53.88 <sup>B</sup>
2x5	137.66 <sup>D</sup>	38 <sup>I</sup>	166 <sup>B</sup>	102.73 <sup>C</sup>	10.43 <sup>F</sup>	45.3 <sup>GH</sup>	1.65 <sup>G</sup>	32.8 <sup>I</sup>	25.65 <sup>HIJ</sup>	48.38 <sup>GH</sup>
2x6	136.33 <sup>E</sup>	38.66 <sup>I</sup>	164.66 <sup>CDE</sup>	97.53 <sup>D</sup>	11.96 <sup>DE</sup>	41.96 <sup>I</sup>	1.42 <sup>HI</sup>	37 <sup>E</sup>	16.68 <sup>MN</sup>	45.56 <sup>JK</sup>
3x4	128 <sup>I</sup>	47.33 <sup>E</sup>	159.66 <sup>I</sup>	86.66 <sup>G</sup>	14.63 <sup>B</sup>	64.16 <sup>C</sup>	2.26 <sup>CD</sup>	34.63 <sup>GH</sup>	64.14 <sup>A</sup>	52.08 <sup>CD</sup>
3x5	127.66 <sup>I</sup>	44.66 <sup>FG</sup>	160 <sup>J</sup>	107.43 <sup>B</sup>	14.43 <sup>B</sup>	51.33 <sup>F</sup>	1.71 <sup>G</sup>	30.93 <sup>K</sup>	26.13 <sup>HI</sup>	48.54 <sup>GH</sup>
3x6	139 <sup>C</sup>	35.33 <sup>K</sup>	163.66 <sup>EFGH</sup>	79.7 <sup>I</sup>	12.26 <sup>D</sup>	45.83 <sup>GH</sup>	1.44 <sup>HI</sup>	31.96 <sup>IJ</sup>	26.4 <sup>GHI</sup>	51.4 <sup>CDE</sup>
4x5	140.66 <sup>B</sup>	35 <sup>K</sup>	164.66 <sup>CDE</sup>	82.03 <sup>HI</sup>	11.83 <sup>DE</sup>	51.43 <sup>F</sup>	1.54 <sup>GH</sup>	30.13 <sup>K</sup>	20.57 <sup>KL</sup>	45.45 <sup>JK</sup>
4x6	150 <sup>A</sup>	25 <sup>L</sup>	163 <sup>GH</sup>	47.43 <sup>I</sup>	10.16 <sup>F</sup>	31.6 <sup>I</sup>	1.21 <sup>JK</sup>	31 <sup>JK</sup>	15.3 <sup>N</sup>	56.47 <sup>A</sup>
5x6	136.33 <sup>E</sup>	38.66 <sup>I</sup>	165.33 <sup>C</sup>	106.23 <sup>B</sup>	14.2 <sup>B</sup>	55.83 <sup>E</sup>	2.25 <sup>D</sup>	39 <sup>D</sup>	65.5 <sup>A</sup>	50.23 <sup>EF</sup>
F <sub>1</sub> mean values	134.06	41.77	164.04	88.80	13.31	55.64	2.11	36.58	35.99	49.60
Parental mean values	129.50	46.44	163.55	100.42	12.30	41.43	1.50	33.37	22.32	47.05
General mean values	126.14	41.42	156.11	88.33	12.43	49.40	1.86	34.14	30.83	46.42

HD= heading date, GFP= grain filling period, DM= days to maturity, PH= plant height, SL= spike length, GNS= grain number per spike, GWS= grain weight per spike, TKW= thousand kernel weight, GY= grain yield per plant, and SPAD= chlorophyll content of flag leaf.

**Table 4.** Heterosis (Ht) and heterobeltiosis (Hb) values (%) for all studied traits

Crosses		HD	GFP	DM	PH	SL	GNS	GWS	TKW	GY	SPAD
1x2	Hb	5.02	12.62	8.25	-18.41	12.33	66.12	100.48	31.16	24.30	7.38
	Ht	3.75	11.59	5.77	-23.72	6.77	50.10	91.16	16.33	4.17	0.50
1x3	Hb	10.16	-24.46	-0.62	-15.52	32.70	30.97	51.23	27.29	76.10	4.06
	Ht	9.86	-25.61	-1.23	-22.65	8.60	1.34	25.64	22.14	60.42	-1.46
1x4	Hb	-4.05	-10.73	-0.92	-17.47	24.02	35.66	52.66	25.01	128.30	-2.55
	Ht	-13.33	-18.89	-2.03	-23.59	10.16	17.60	32.30	24.41	96.55	-9.90
1x5	Hb	2.56	-11.82	1.23	-20.83	-25.49	20.42	33.98	7.50	48.11	8.60
	Ht	-4.07	-24.99	0.20	-25.77	-29.13	-7.58	5.12	1.25	32.51	3.92
1x6	Hb	0.50	-4.47	0.09	2.34	-8.67	20.90	47.65	28.63	35.63	6.26
	Ht	-6.62	-21.95	-1.61	0.68	-18.70	-5.34	12.82	24.09	5.30	4.48
2x3	Hb	5.83	-14.38	0.50	0.46	38.85	59.20	48.25	11.82	44.86	0.63
	Ht	5.10	-14.90	-1.19	-1.76	18.22	33.61	18.60	2.98	31.97	-0.62
2x4	Hb	-6.81	-1.01	-0.20	-2.58	8.30	16.45	21.78	8.83	148.84	3.83
	Ht	-14.89	-10.17	-1.40	-3.60	0.75	11.12	1.40	-3.87	140.85	2.47
2x5	Hb	4.68	-17.39	0.50	-4.04	-21.75	13.80	1.23	-13.06	22.96	5.17
	Ht	-0.96	-29.18	-0.40	-15.50	-21.75	-5.42	-23.25	-18.46	14.05	-5.50
2x6	Hb	2.89	-12.45	-1.00	-0.23	-3.78	3.01	-11.25	6.99	-4.68	-3.41
	Ht	-3.31	-27.95	-1.59	-5.27	-10.27	-12.40	-33.95	-8.02	-0.54	-11.01
3x4	Hb	-5.76	-3.07	-1.74	-3.2	40.26	68.8	66.17	7.04	191.89	1.61
	Ht	-14.66	-10.69	-2.24	-4.36	27.66	47.4	58.04	2.24	174.17	-0.95
3x5	Hb	-2.04	-2.19	-1.84	2.3	27.02	59.85	42.5	-10.47	14.00	7.01
	Ht	-8.15	-15.73	-2.63	-11.62	8.25	57.93	32.55	-12.2	11.71	-2.78
3x6	Hb	5.84	-19.39	0.09	-16.72	17.3	38.75	25	1.81	34.9	10.44
	Ht	-1.41	-33.33	-1.01	-22.6	6.41	36.52	12.4	-5.6	12.86	2.94
4x5	Hb	-2.65	-15.66	0.5	-22.68	-4.6	36.67	22.04	-8.78	-4.46	-2.65
	Ht	-6.22	-21.63	0.2	-32.51	-11.25	18.14	8.4	-14.47	-8.58	-13.56
4x6	Hb	3.09	-36.96	-0.8	-51	-11.65	-18.02	-1.62	3.74	-15.75	17.99
	Ht	0	-44.02	-1.4	-53.93	-11.88	-27.4	-15.38	0.55	-25.63	7.4
5x6	Hb	-2.62	5.91	0.3	-5.37	14.23	70.99	108.33	20.54	242.57	19.68
	Ht	-3.31	0.86	0	-12.61	6.52	66.3	100.9	9.76	191.24	16.4
Mean	Ht	1.11	-10.36	0.29	-11.53	9.27	34.90	40.56	9.87	65.84	5.60
	Hb	-3.88	-19.11	-0.70	-17.25	-0.64	18.79	21.78	2.74	49.40	-0.51

Ht= heterosis value (%), Hb= heterobeltiosis value (%). P(1)= Adana-99, P(2) = Flamura-85, P(3)= Masaccio, P(4)= Lucilla, P(5)= 1635, P(6)= 2115. HD= heading date, GFP= grain filling period, DM= days to maturity, PH= plant height, SL= spike length, GNS= grain number per spike, GWS= grain weight per spike, TKW= thousand kernel weight, GY= grain yield per plant and SPAD= chlorophyll content of flag leaf.

**Table 5.** Genetic parameters for investigated traits

Genetic Parameters	HD	GFP	DM	PH	SL	GNS	GWS	TKW	GY	SPAD
a	123.46**	167.51**	23.87**	350.43**	350.43**	350.40**	0.88**	37.11**	120.54**	29.32**
b	26.75**	33.62**	11.13**	192.16**	192.16**	147.11**	0.34**	17.03**	271.70**	9.20**
b <sub>1</sub>	89.21**	93.30**	1.02	578.37**	578.37**	866.07**	1.59**	44.24**	801.02**	27.76**
b <sub>2</sub>	6.05**	6.18**	9.53**	89.71**	89.71**	50.14**	0.14**	22.32**	31.54**	4.34**
b <sub>3</sub>	31.31**	42.24**	13.14**	206.15**	206.15**	121.10**	0.32**	11.07**	346.30**	9.84**
E	0.175	0.18	0.18	0.81	0.07	1.26	0.01	0.13	1.45	0.32
D	77.837*	73.31*	7.39	159.34	3.651	121.60	0.21	16.66	19.62	23.31
F	26.552	-9.966	-0.83	38.26	5.921	-39.07	-0.21	12.53	-35.30	14.05
H <sub>1</sub>	99.605	127.53*	48.90	748.14	20.277	519.81	1.32	74.08*	1018.37	37.12
H <sub>2</sub>	136.54*	188.39*	52.71	817.83	17.216	627.53*	1.59	71.98*	1050.78	42.64
(D-H <sub>1</sub> )	-21.77	-54.21	-41.50	-588.84	-16.626	-398.22	-1.11	-57.42	-998.74	-13.81
(H <sub>1</sub> /D) <sup>1/2</sup>	1.131	1.319	2.57	2.17	2.357	2.07	2.51	2.109	7.20	1.26
(H <sub>2</sub> /4H <sub>1</sub> )	0.343	0.369	0.27	0.27	0.212	0.30	0.30	0.24	0.26	0.29
KD/KR	1.355	0.902	0.96	1.12	2.049	0.86	0.66	1.43	0.78	1.63
h <sup>2</sup>	55.047	60.40	0.54	375.19	2.801	560.73*	1.04	28.59	518.21	17.79
K=h <sup>2</sup> /H <sub>2</sub>	0.403	0.321	0.01	0.46	0.163	0.89	0.65	0.40	0.49	0.42
Hg	0.976	0.985	0.927	0.97	0.849	0.96	0.94	0.96	0.89	0.85
Hd	0.513	0.347	0.13	0.18	0.200	0.18	0.12	0.21	0.02	0.49
GCA	121.58**	167.50**	25.58**	350.46**	3.932**	350.42**	0.89**	37.12**	120.52**	29.32**
SCA	26.37**	33.64**	10.92**	192.17**	4.51**	147.15**	0.35**	17.03**	271.68**	9.20**
GCA/ SCA	4.61	4.97	2.34	1.82	0.87	2.38	2.55	2.17	0.44	3.18

\*, \*\* significant at 5% and 1% probability levels, respectively; "D"- "a"- "GCA"= measures additive effect, "H<sub>1</sub>"-"H<sub>2</sub>"-"(b-b<sub>1</sub>-b<sub>2</sub>-b<sub>3</sub>)"- "SCA"= measures dominance effect, F= determines frequencies of dominant to recessive alleles in parents, E= shows environment effect, H<sub>2</sub>/4H<sub>1</sub>= determines proportion of genes with positive and negative effects in the parents,  $\sqrt{(H_1/D)}$ = measures average degree of dominance, (KD/KR)= ratio of the total number of dominant against recessive alleles, GCA= general combining ability, SCA= specific combining ability.

**Table 6.** GCA and SCA values of HD, GFP, DM, PH and SL for crosses and their parents

Parental Genotypes / Crosses	HD		GFP		DM		PH		SL	
	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA
Adana 99 (1)	-3.77**		4.65**		-0.63**		1.26**		1.11**	
Flamura-85 (2)	-2.61**		4.61**		3.21**		0.23		0.35**	
Masaccio (3)	-3.44**		2.24**		-2.00**		-2.62**		0.10	
Lucilla (4)	1.09**		-1.51**		-1.08**		-9.62**		-0.24	
1635 (5)	3.01**		-3.47**		0.21		10.86**		-0.39**	
2115 (6)	5.72**		-6.51**		0.29*		-0.12		-0.94**	
1x2		2.26**		8.63**		10.46**		-12.45**		1.28**
1x3		8.09**		-9.33**		-1.66**		-8.47**		1.80**
1x4		-0.11		-1.92**		-2.24**		-2.46**		2.37**
1x5		1.30**		-3.29**		0.46		-14.01**		-3.28**
1x6		-3.07**		1.42**		-0.95**		13.87**		-1.20**
2x3		3.59**		-4.29**		0.17		1.19**		2.30**
2x4		-3.6**		2.46**		-1.08**		6.50**		0.30
2x5		4.47**		-6.25**		-0.70*		-0.49		-2.55**
2x6		0.42		-2.54**		-2.79**		5.29**		-0.47
3x4		-2.4**		3.50**		-1.20**		6.77**		1.75**
3x5		-4.65**		2.79**		-2.16**		7.06**		1.70**
3x6		3.92**		-3.50**		1.42**		-9.70**		0.08
4x5		3.76**		-3.13**		1.59**		-11.34**		-0.56
4x6		10.38**		-10.08**		-0.16		-34.95**		-1.68**
5x6		-5.19**		5.54**		0.88**		3.36**		2.50**

\*, \*\* significant at 5% and 1% probability levels, respectively; HD= heading date, GFP= grain filling period, DM= days to maturity, PH= plant height, SL= spike length.

**Table 7.** GCA and SCA values of GNS, GWS, TGW, GY and SPAD for crosses and their parents

Parental Genotypes / Crosses	GNS		GWS		TKW		GY		SPAD	
	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA
Adana 99 (1)	10.94**		0.47**		1.73**		3.93**		-1.47**	
Flamura-85 (2)	3.54**		0.34**		3.44**		-4.21**		1.31**	
Masaccio (3)	-1.33**		-0.07		-0.20		1.93**		1.23**	
Lucilla (4)	-0.48**		-0.11		-1.85**		3.76**		2.29**	
1635 (5)	-4.86**		-0.26		-1.33**		-0.50**		-2.69**	
2115 (6)	-7.82**		-0.37**		-1.78**		-4.90**		-0.68**	
1x2		23.00**		1.37**		5.97**		-2.13**		2.75**
1x3		-1.07**		0.11		4.18**		7.75**		0.56
1x4		7.72**		0.30		3.20**		16.21**		-2.32**
1x5		-2.83**		-0.09		-0.39		2.23**		1.67**
1x6		1.46**		0.16		3.02**		-1.12**		-0.08
2x3		10.21**		0.35		2.53**		1.07		-0.53
2x4		-1.41**		0.03		1.42**		17.90**		1.41**
2x5		-4.95**		-0.36		-4.97**		-1.71**		0.89**
2x6		-5.33**		-0.49		-0.33		-6.29**		-3.94**
3x4		14.39**		0.51		1.02**		26.37**		-0.32
3x5		5.94**		0.11		-3.20**		-7.38**		1.12**
3x6		3.40**		-0.06		-1.73**		-2.71**		1.98**
4x5		5.19**		-0.02		-2.35**		-14.70**		-3.02**
4x6		-11.69**		-0.24		-1.04**		-15.64**		5.99**
5x6		16.93**		0.95**		6.44**		38.82**		4.73**

\*, \*\* significant at 5% and 1% probability levels, respectively; GNS= grain number per spike, GWS= grain weight per spike, TKW= thousand kernel weight, GY= grain yield per plant, and SPAD= chlorophyll content of flag leaf.

### 3.1. Heading Date

In the study, the average heading date for parents was 129.50 days, while the average for F<sub>1</sub> hybrid combinations was 134.06 days. Among the parents, the longest heading date was observed in the genotype 2115 (141.0 days), while the shortest heading date was obtained from the genotypes Adana-99 and Masaccio (121.0 days). Among the hybrids, the longest heading date was measured in the hybrid Lucilla × 2115 (150 days), while the shortest heading date was recorded in the combinations Flamura-85 × Lucilla and Masaccio × 1635 (127.66 days) (Table 3). Heterosis values for heading date ranged from -6.81% (Flamura-85 × Lucilla) to 10.16% (Adana-99 × Masaccio), while heterobeltiosis values varied from -14.89% (Flamura-85 × Lucilla) to 9.86% (Adana-99 × Masaccio) (Table 4). In terms of heading date, an average heterosis of 1.11% and an average heterobeltiosis of -3.88% were obtained in the hybrid populations (Table 4). Diallel variance components a, b, b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> were significant for heading date in the study. Among the calculated genetic parameters for heading date, additive gene variance (D) and dominance variance corrected for gene distribution (H<sub>2</sub>) were significant at 0.05. The significance of D and H<sub>2</sub> and the negative value of D-H<sub>1</sub> highlight the importance of dominant gene effects in heading date. The fact that the square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> is greater than 1 (1.131) indicates the presence of

overdominance. The difference in the frequency of dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) from 0.25 (0.343) suggests unequal frequencies. Additionally, the ratio of dominant to recessive alleles (KD/KR) greater than 1 (1.355) supports the predominance of dominant alleles. However, as the value of K = (h<sup>2</sup>/H<sub>2</sub>) is below 1 (K = 0.403), the adequate number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.976 and 0.513, respectively. The ratio of general combining ability to specific combining ability greater than 1 indicates the superiority of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effects were obtained from the parents 2115 (5.72) and 1635 (3.01), while the lowest were from the parents Adana-99 (-3.77) and Masaccio (-3.44). The highest specific combining ability effect was obtained from the hybrid Lucilla × 2115 hybrids (10.38), while the hybrids showing the lowest specific combining ability effects were the combinations 1635 × 2115 (-5.19), Masaccio × 1635 (-4.69), and Flamura-85 × Masaccio (-3.61) (Table 6). For heading date, the significance of both additive and dominant gene variance, epistatic effects, and the inability to determine the adequate gene pair number suggest that selection for this trait should be deferred to later generations. Tulukcu (2004) identified dominant gene effects as dominant in the inheritance of heading date, whereas Nazeer et al.



(2004) and Akram et al. (2008) stated that additive gene effects were dominant. Sharma et al. (2002) found that dominant and additive gene effects control heading date.

### 3.2. Grain Filling Period

In the research, the average grain-filling period for parents was 46.44 days, whereas it was 41.77 days for F<sub>1</sub> hybrid combinations. Among the parents, the highest grain filling period was observed in the genotype Adana-99 (54.66 days), while the lowest was obtained from the genotype 2115 (34.66 days). Among the hybrids, the highest grain-filling period was recorded in the hybrid Adana-99 × Flamura-85 (61 days), whereas the lowest grain-filling period was found in the hybrid Lucilla × 2115 (25 days) (Table 3). Heterosis values for the grain filling period in F<sub>1</sub> hybrid populations ranged from -36.96% (Lucilla × 2115) to 12.62% (Adana-99 × Flamura-85), while heterobeltiosis values varied from -44.02% (Lucilla × 2115) to 11.59% (Adana-99 × Flamura-85) (Table 4). An average heterosis of -10.36% and an average heterobeltiosis of -19.11% was determined for the grain-filling period in the hybrid populations (Table 4). Significant diallel variance components a, b, b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub> were found for the grain filling period in the study. Among the calculated genetic parameters for the grain filling period, additive gene variance (D), dominance gene variance (H<sub>1</sub>), and dominance gene variance corrected for gene distribution (H<sub>2</sub>) were found to be significant at the 0.05 level. According to the diallel hybrid analysis, the significance of additive gene variance (D) and dominance variance (H<sub>1</sub> and H<sub>2</sub>), along with the negative value of D-H<sub>1</sub>, emphasizes the importance of dominant gene effects in the manifestation of the grain-filling period. The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (1.319) indicates the presence of overdominance. The deviation of the frequency of dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) from 0.25 (0.369) suggests unequal frequencies of dominant and recessive alleles in the parents. The negative F value determines the direction of dominant and recessive alleles (-9.966), and the KD/KR ratio is less than 1, which indicates the predominance of recessive alleles. Since the value of K = (h<sup>2</sup>/H<sub>2</sub>) is below 1 (K = 0.321), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.985 and 0.347, respectively. The ratio of general combining ability to specific combining ability (4.97) was calculated to be greater than 1. This ratio above 1 indicates the superiority and importance of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effects were obtained from the parents Adana-99 (4.653) and Flamura-85 (4.611), while the lowest were from the parents 2115 (-6.514) and 1635 (-3.472). The highest specific combining ability effect was obtained from the combination Adana-99 × Flamura-85 (8.625). The hybrid showing the lowest specific combining ability effects was the combination Lucilla × 2115 (-10.08) (Table 6). For the grain-filling period, the significance of both additive and

dominance variance, the inability to determine the effective gene pair number, and the moderately low narrow-sense heritability suggest that selection for this trait should be deferred to later generations. Kutlu (2012) and Celik (2016) have also found similar results. The significance of additive and dominance gene variance, the inability to determine the adequate gene pair number, and the moderately low narrow-sense heritability suggest that selection for this trait should be deferred to later generations.

### 3.3. Days to Maturity

In the study, the average days to maturity were 163.55 for the parents and 164.04 for the F<sub>1</sub> hybrids. Among the parents, the highest DM was observed in the genotype Flamura-85 genotype (167 days), while the lowest DM was obtained from the genotype Adana-99 (159.66 days). Among the hybrids, the highest DM was recorded in the hybrid Adana-99 × Flamura-85 (177 days). In contrast, the lowest DM was 159.66 days in the combinations Adana-99 × Masaccio and Masaccio × Lucilla (Table 3). Heterosis values for DM in F<sub>1</sub> hybrid populations ranged from -1.84% (Masaccio × 1635) to 8.25% (Adana-99 × Flamura-85), while heterobeltiosis values varied from -2.63% (Masaccio × 1635) to 5.77% (Adana-99 × Flamura-85) (Table 4). Regarding the DM, an average heterosis of 0.29% and an average heterobeltiosis of -0.7% were determined for the hybrid populations (Table 4). In the half-diallel variance analysis table for the studied population, additive variance (a), dominance variance (b), and its components (b<sub>2</sub>, b<sub>3</sub>) were found to be significant. In the half-diallel hybrid analysis, all genetic parameters were found to be insignificant, and it was observed that D-H<sub>1</sub> was negative. The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (2.572) indicates the presence of overdominance. The negative value of the difference between additive and dominance variance (D-H<sub>1</sub>) indicates that dominance gene variance is more significant than additive gene variance. The deviation of the frequency of dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) from 0.25 (0.269) is consistent with the significance of the b<sub>2</sub> sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The negative F value determining the direction of dominant and recessive alleles (-0.825) and the KD/KR ratio less than 1 (0.958) suggest that recessive alleles are predominant. Since the value of K = (h<sup>2</sup>/H<sub>2</sub>) is below 1 (K = 0.01), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.927 and 0.128, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (2.34) was calculated to be greater than 1. This ratio above 1 indicates the superiority and importance of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effect was obtained

from the parent Flamura-85 (3.208), while the lowest was from the parent Masaccio (-2.0). The highest specific combining ability effect was obtained from the combination Adana-99 × Flamura-85 (10.464), and the phenotypic value obtained from this combination was the highest (177 days). The hybrids showing the lowest specific combining ability effects were the combinations Flamura-85 × 2115 (-2.786) (Table 6). For DM, the significance of both additive and dominance variance, the inability to determine the effective gene pair number, and the fit of the inheritance of the trait to the additive-dominant model can be expressed. Hammad et al. (2013) and Celik (2016) have also found similar results. Our findings align with previous studies, and Hammad et al. (2013) reported that specific combining ability is positive.

### 3.4. Plant Height

In the investigation, the average plant height for parents was 100.42 cm, while it was 88.80 cm for F<sub>1</sub> hybrid combinations. The average plant height for F<sub>1</sub> hybrid combinations was lower than for parents. Among the parents, the highest plant height was observed in the genotype 1635 (121.56 cm), while the lowest plant height was obtained from the genotype Masaccio (88.46 cm). Among the hybrids, the highest plant height was recorded at 107.43 cm in the hybrid Masaccio × 1635, whereas the lowest plant height was 47.43 cm in the hybrid Lucilla × 2115 (Table 3). Heterosis values for plant height in F<sub>1</sub> hybrid populations ranged from -51% (Lucilla × 2115) to 2.34% (Adana-99 × 2115), while heterobeltiosis values varied from -53.93% (Lucilla × 2115) to 0.68% (Adana-99 × 2115). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Flamura-85 as the parent (Ht: -4.96%, Hb: -9.97%), while the lowest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Lucilla as the parent (Ht: -19.39%, Hb: -23.60%). Average heterosis of -11.53% and heterobeltiosis of -17.25% were determined for plant height in hybrid populations (Table 4). Components a, b, b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> were significant in the diallel variance analysis for plant height. Additive gene variance (D), dominant gene variance (H<sub>1</sub>), genes' distribution corrected dominant gene variance (H<sub>2</sub>), and environmental variance (E) were found to be insignificant for plant height. The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (2.167) indicates the presence of overdominance. The negative difference between additive and dominance variance (D-H<sub>1</sub>) suggests that dominant gene variance is more remarkable than additive gene variance. The deviation of the frequency of dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) from 0.25 (0.273) is consistent with the significance of the b<sub>2</sub> sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The positive F value determining the direction of dominant and recessive alleles (38.258) and the KD/KR ratio greater than 1 (1.117) suggest that dominant alleles are

predominant. Since the value of K = (h<sup>2</sup>/H<sub>2</sub>) is below 1 (K = 0.459), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.969 and 0.183, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (1.82) was calculated to be greater than 1. This ratio greater than 1 indicates the superiority and importance of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effect was obtained from the parent 1635 (10.864), while the lowest was from the parent Lucilla (-9.619). The highest specific combining ability effect was obtained from the combination Adana-99 × 2115 (13.866) and the combination Masaccio × 1635 (7.058), while the hybrids showing the lowest specific combining ability effects were the combination Lucilla × 2115 (-34.95) (Table 6). There is a complete similarity in the significance of the additive (a, GCA) and dominant (b, b<sub>2</sub>, b<sub>3</sub>, SCA) gene effect components obtained by the two evaluation methods. This contradictory situation arises from non-allelic gene interactions. For plant height in the population, both additive and dominance variance were found to be effective, indicating that the inheritance of the trait fits the additive-dominant model. Similar results have also been found by Akgun and Topal (2002). The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (2.167) indicates the presence of overdominance. In this study, the significance of both additive and dominance variance for plant height, epistatic effects, the inability to determine the effective gene pair number, and the low narrow-sense heritability suggest that the selection planned for this trait should be postponed to future generations.

### 3.5. Spike Length

In the study, the average spike length for parents was 12.30 cm, while it was 13.31 cm for F<sub>1</sub> hybrid combinations. Among the parents, the highest spike length was observed in the genotype Adana-99 (14.76 cm), while the lowest spike length was obtained from the genotype Masaccio (9.4 cm). Among the hybrids, the highest spike length was recorded at 16.26 cm in the hybrid Adana-99 × Lucilla, whereas the lowest spike length was 10.16 cm in the combination Lucilla × 2115 (Table 3). Heterosis values for spike length in F<sub>1</sub> hybrid populations ranged from -25.49% (Adana-99 × 1635) to 40.26% (Masaccio × Lucilla), while heterobeltiosis values varied from -29.13% (Adana-99 × 1635) to 27.66% (Masaccio × Lucilla). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Masaccio as the parent (Ht: 31.23%, Hb: 13.83%), while the lowest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype 1635 as the parent (Ht: -2.12%, Hb: -9.47%). Average heterosis of 9.27% and heterobeltiosis of 0.64% were determined for spike

length in hybrid populations (Table 4). Components a, b,  $b_1$ ,  $b_2$ , and  $b_3$  were significant in the diallel variance analysis for spike length. Additive gene variance (D), dominant gene variance ( $H_1$ ), genes' distribution corrected dominant gene variance ( $H_2$ ), and environmental variance (E) were found to be insignificant for spike length. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.357) indicates the presence of overdominance. The negative difference between additive and dominance variance ( $D-H_1$ ) suggests that dominant gene variance is more significant than additive gene variance. The deviation of the frequency of dominant and recessive alleles ( $H_2/4H_1$ ) from 0.25 (0.212) is consistent with the significance of the  $b_2$  sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The positive F value determining the direction of dominant and recessive alleles (5.921) and the KD/KR ratio greater than 1 (2.049) suggest that dominant alleles are predominant. Since the value of  $K = (h^2/H_2)$  is below 1 ( $K = 0.163$ ), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.849 and 0.2, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (0.87) was calculated to be less than 1. This ratio of less than 1 indicates the superiority and importance of specific combining ability and, consequently, non-additive gene variance (Table 5). The highest general combining ability effect was obtained from the parent Adana-99 (1.114), while the lowest was from the parent 2115 (-0.936). The highest specific combining ability effect was obtained from the combination 1635 × 2115 (2.504) and the combination Adana-99 × Lucilla (2.367), while the hybrids showing the lowest specific combining ability effects were the hybrid Adana-99 × 1635 (-3.279) (Table 6). According to the results of three evaluation methods, the negative value of D- $H_1$ , the low value of narrow-sense heritability (0.2), and the GCA/SCA ratio less than 1 (0.87) indicate the dominance of dominant variance for this trait. For this trait, Balci and Turgut (2002), Sharma et al. (2002), Bao et al. (2009) reported the dominance of additive gene variance; Yagdi and Ekingen (1995) reported the dominance effect; Khan et al. (2010), Nazeer et al. (2011) reported the superiority and importance of both additive and non-additive gene variance. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.357) indicates the presence of overdominance. In this study, the significance of dominant gene variance for spike length, epistatic effects, the inability to determine the effective gene pair number, and the low narrow-sense heritability suggest that the selection planned for this trait should be postponed to future generations.

### 3.6. Grain Number per Spike

In the research, the average grain number per spike for parents was 41.43, while 55.64 for F<sub>1</sub> hybrid combinations. Among the parents, the highest grain number per spike was observed in the genotype Adana-99 (59.33), while the lowest grain number per spike was obtained from the genotype 1635 (31.73). Among the hybrids, the highest grain number per spike was recorded at 89.06 in the hybrid Adana-99 × Flamura-85, whereas the lowest grain number per spike was 31.6 in the hybrid Lucilla × 2115 (Table 3). Heterosis values for grain number per spike in F<sub>1</sub> hybrid populations ranged from -18.02% (Lucilla × 2115) to 70.99% (1635 × 2115), while heterobeltiosis values varied from -27.4% (Lucilla × 2115) to 66.3% (1635 × 2115). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Masaccio as the parent (H<sub>t</sub>: 51.51%, H<sub>b</sub>: 35.36%), while the lowest average heterosis value was obtained from the hybrid series with the genotype 1635 as the parent (H<sub>t</sub>: 23.13%), and the heterobeltiosis value was obtained from the hybrid series with the genotype Adana-99 as the parent (H<sub>b</sub>: 11.22%). Average heterosis of 34.9% and heterobeltiosis of 18.79% were determined for grain number per spike in hybrid populations (Table 4). In the diallel variance analysis for grain number per spike, components a, b,  $b_1$ ,  $b_2$ , and  $b_3$  were significant. Genes' distribution corrected dominant gene variance ( $H_2$ ) and heterozygote locus dominance effect ( $h^2$ ) were significant at the 0.05 level. In contrast, additive gene variance (D), dominant gene variance ( $H_1$ ), and environmental variance (E) were found to be insignificant. The insignificance of environmental variance (E) suggests that genetic factors play a more significant role than environmental factors for this trait. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.068) indicates the presence of overdominance. The negative difference between additive and dominance variance ( $D-H_1$ ) suggests that dominant gene variance is more incredible than additive gene variance. The deviation of the frequency of dominant and recessive alleles ( $H_2/4H_1$ ) from 0.25 (0.302) is consistent with the significance of the  $b_2$  sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The negative F value determining the direction of dominant and recessive alleles (-39.074) and the KD/KR ratio less than 1 (0.856) indicate that recessive alleles are predominant. Since the value of  $K = (h^2/H_2)$  is below 1 ( $K = 0.894$ ), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were 0.955 and 0.177, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (2.38) was calculated to be greater than 1. This ratio greater than 1 indicates the superiority and importance of general combining ability and,

consequently, additive gene variance (Table 5). The highest general combining ability effect was obtained from the parent Adana-99 (10.944), while the lowest was from the parent 2115 (-7.818). The highest specific combining ability effect was obtained from the Adana-99 × Flamura-85 hybrids (23.004), and the phenotypic values obtained from this combination were also the highest (89.06). The hybrids showing the lowest specific combining ability effects were the Lucilla × 2115 combinations (-11.688), and the phenotypic values for these combinations were phenotypically low (31.6) (Table 7). According to the variance analysis method Hayman (1954) used, both the GCA variance corresponding to additive variance and the SCA variance corresponding to dominance variance were significant. Thus, we can infer that dominance gene variance is dominant for grain number per spike. For this trait, Balci and Turgut (2002), Akram et al. (2011), Yildirim et al. (2014) reported the dominance of additive gene variance; Yagdi and Ekingen (1995) reported the dominance effect; Akgun and Topal (2002) reported the superiority and importance of both additive and non-additive gene variance. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.068) indicates the presence of overdominance for the grain number per spike. In this study, the significance of dominant gene variance for grain number per spike, epistatic effects, the inability to determine the effective gene pair number, and the low narrow-sense heritability suggest that the selection planned for this trait should be postponed to future generations.

### 3.7. Grain Weight per Spike

In the investigation, the average grain weight per spike for parents was 1.50 g, while it was 2.11 g for F<sub>1</sub> hybrid combinations. The average grain weight per spike of F<sub>1</sub> hybrid combinations was higher than that of the parents. Among the parents, the highest grain weight per spike was observed in the genotype Flamura-85 (2.15 g), while the lowest grain weight per spike was obtained from the genotype 2115 (1.04 g). Among the hybrids, the highest grain weight per spike was recorded at 4.11 g in the hybrid Adana-99 × Flamura-85, whereas the lowest grain weight per spike was 1.21 g in the hybrid Lucilla × 2115 (Table 3). Heterosis values for grain weight per spike in F<sub>1</sub> hybrid populations ranged from -11.25% (Flamura-85 × 2115) to 108.33% (1635 × 2115), while heterobeltiosis values varied from -33.95% (Flamura-85 × 2115) to 100.9% (1635 × 2115). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Adana-99 as the parent (Ht: 57.2%, Hb: 33.41%), while the lowest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Flamura-85 as the parent (Ht: 32.10%, Hb: 10.79%). Average heterosis of 40.56% and heterobeltiosis of 21.78% were determined for grain weight per spike in F<sub>1</sub> hybrid populations (Table 4). Components a, b, b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> were significant in the diallel variance analysis for grain weight per spike. Additive gene variance (D), dominant gene variance (H<sub>1</sub>),

genes' distribution corrected dominant gene variance (H<sub>2</sub>), heterozygote locus dominance effect (h<sup>2</sup>), and environmental variance (E) were found to be insignificant. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.51) indicates the presence of overdominance. The negative difference between additive and dominance variance (D-H<sub>1</sub>) suggests that dominant gene variance is more significant than additive gene variance. The deviation of the frequency of dominant and recessive alleles  $(H_2/4H_1)$  from 0.25 (0.303) is consistent with the significance of the b<sub>2</sub> sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The negative F value determining the direction of dominant and recessive alleles (-0.213) and the KD/KR ratio less than 1 (0.662) indicate that recessive alleles are predominant. Since the value of K =  $(h^2/H_2)$  is below 1 (K = 0.651), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were found to be 0.936 and 0.119, respectively. Both general combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (2.55) was calculated to be greater than 1. This ratio greater than 1 indicates the superiority and importance of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effect was obtained from the parents Adana-99 (0.473) and Flamura-85 (0.336), while the lowest GCA effect was from the parent 2115 (-0.367). The highest SCA effect was 1.369 from the hybrid Adana-99 × Flamura-85, while the lowest SCA effect was -0.485 from the combination Flamura-85 × 2115 (Table 7). Considering the three evaluation methods, both additive and dominant variances were found to be effective for grain weight per spike in the population, indicating that the inheritance of the trait conforms to the additive-dominant model. For this trait, Borghi and Perenzin (1994), Balci and Turgut (2002), Hassan et al. (2007) reported the significance of additive gene variance, Mann and Sharma (1995), Akgun et al. (2002) reported the significance of non-additive gene effects, Nazeer et al. (2011) reported the significance of epistatic gene effects, and Sener (1997) reported the presence of non-allelic interactions. The square root of the mean dominance degree  $(H_1/D)^{1/2}$  greater than 1 (2.51) indicates the presence of overdominance for grain weight per spike. In this study, the significance of both additive and dominant gene variance for grain weight per spike, the inability to determine the effective gene pair number, and the low narrow-sense heritability suggest that the selection planned for this trait should be postponed to future generations.

### 3.8. Thousand Kernel Weight

In the study, the average thousand kernel weight for parents and F<sub>1</sub> hybrids was recorded as 34.14 g. While the average thousand kernel weight for parents was 33.37 g,

it was 36.58 g for F<sub>1</sub> hybrid combinations. The average thousand kernel weight of F<sub>1</sub> hybrid combinations was higher than that of the parents. Among the parents, the highest thousand kernel weight was observed in the genotype Flamura-85 (40.23 g), while the lowest thousand kernel weight was obtained from the genotype 2115 (28.93 g). Among the hybrids, the highest thousand kernel weight was recorded at 46.8 g in the hybrid Adana-99 × Flamura-85, whereas the lowest thousand kernel weight was 30.13 g in the hybrid Lucilla × 1635 (Table 3). Heterosis values for thousand kernel weight in F<sub>1</sub> hybrid populations ranged from -13.06% (Flamura-85 × 1635) to 31.16% (Adana-99 × Flamura-85), while heterobeltiosis values varied from -18.46% (Flamura-85 × 1635) to 24.41% (Adana-99 × Lucilla). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype Adana-99 as the parent (Ht: 23.92%, Hb: 17.64%), while the lowest average heterosis and heterobeltiosis values were obtained from the hybrid series with the genotype 1635 as the parent (Ht: -0.85%, Hb: -6.82%). Average heterosis of 9.87% and average heterobeltiosis of 2.74% were determined for thousand kernel weight in F<sub>1</sub> hybrid populations (Table 4). Components a, b, b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> were found to be significant in the diallel variance analysis for thousand kernel weight. Dominant gene variance (H<sub>1</sub>) and genes' distribution corrected dominant gene variance (H<sub>2</sub>) were found to be significant at the 0.05 level. In contrast, additive gene variance (D), heterozygote locus dominance effect (h<sup>2</sup>), and environmental variance (E) were found to be insignificant. Since environmental variance (E) was insignificant, genetic factors contribute more to this trait than environmental factors. The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (2.109) indicates the presence of overdominance. The negative difference between additive and dominance variance (D-H<sub>1</sub>) suggests that dominant gene variance is more significant than additive gene variance. The deviation of the frequency of dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) from 0.25 (0.243) is consistent with the significance of the b<sub>2</sub> sub-parameter, indicating unequal frequencies of dominant and recessive alleles in the parents. The positive F value determining the direction of dominant and recessive alleles (12.532) and the KD/KR ratio greater than 1 (1.434) indicate that dominant alleles are predominant. Since the value of K = (h<sup>2</sup>/H<sub>2</sub>) is below 1 (K = 0.397), the effective number of genes could not be determined for the trait. The trait's broad-sense heritability (H<sub>g</sub>) and narrow-sense heritability (H<sub>d</sub>) were found to be 0.960 and 0.212, respectively. Both general combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level, and the ratio of general combining ability to specific combining ability (2.17) was calculated to be greater than 1. This ratio greater than 1 indicates the superiority and importance of general combining ability and, consequently, additive gene variance (Table 5). The highest general combining ability effect was obtained

from the parent Flamura-85 (3.438), while the lowest GCA effect was from the parent Lucilla (-1.854). The highest SCA effect was obtained from the combinations 1635 × 2115 (6.442) and Adana-99 × Flamura-85 (5.971), while the lowest SCA effect was from the hybrids Flamura-85 × 1635 (-4.971) (Table 7). Based on the analysis by Hayman (1954), it can be said that dominant gene variance is predominant for a thousand kernel weight. For this trait, Mann and Sharma (1995) reported the significance of overdominance, Tosun et al. (1995) reported the significance of non-additive effects, Kutlu et al. (2015) reported the significance of both additive and non-additive effects, Ronga et al. (1995) reported the significance of additive gene effects, and Sener et al. (2000) reported the significance of epistatic gene effects. The square root of the mean dominance degree (H<sub>1</sub>/D)<sup>1/2</sup> greater than 1 (2.109) indicates the presence of overdominance for thousand kernel weight. In this study, the significance of dominant gene variance for thousand kernel weight, the inability to determine the effective gene pair number, epistatic effects, and the low narrow-sense heritability suggest that the selection planned for this trait should be postponed to future generations.

### 3.9. Grain Yield

In the research determined the average grain yield per plant for both parents and F<sub>1</sub> hybrids as 30.83 g. While the average grain yield per plant for parents was 22.32 g, the average for F<sub>1</sub> hybrid combinations was 35.99 g. The average value of grain yield per plant for F<sub>1</sub> hybrid combinations was higher than that of the parents. Among the parents, the highest grain yield per plant was obtained from the genotype Adana-99 (28.49 g), while the lowest was from the genotype 2115 (15.75 g). Among the hybrids, the highest grain yield per plant was recorded at 65.5 g in the 1635 × 2115 combination, while the lowest was 15.30 g in the hybrid Lucilla × 2115. Heterosis values for grain yield per plant in F<sub>1</sub> hybrid populations ranged from -15.75% (Lucilla × 2115) to 242.57% (1635 × 2115), while heterobeltiosis values ranged from -25.63% (Lucilla × 2115) to 191.24% (1635 × 2115). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series where genotype Lucilla was the parent (Ht: 89.76% and Hb: 75.47% respectively), while the lowest average heterosis value was from the genotype Flamura-85 (Ht: 47.26%). Heterobeltiosis value was from the 2115 genotype (Hb: 36.65%). The hybrid populations obtained an average of 65.84% heterosis and 49.40% heterobeltiosis values for grain yield per plant. In the study, diallel variance components a, b, b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> were significant for grain yield per plant. From the calculated genetic parameters for grain yield per plant, additive genetic variance (D), dominant genetic variance (H<sub>1</sub>), and additive × additive interaction variance (b<sub>2</sub>) were found to be significant at the 0.05 level. In contrast, the others were found to be insignificant. The average degree of dominance (H<sub>1</sub>/D)<sup>1/2</sup> being more significant than 1 (7.204) indicates the presence of overdominance.

The negative difference between additive and dominant variances ( $D-H_1$ ) indicates that the dominant genetic variance is greater than the additive genetic variance. The F value determining the direction of dominant and recessive alleles being negative (-35.300) and the KD/KR ratio being less than 1 (0.778) indicate that recessive alleles are predominant. Since the value of  $K = (h^2/H_2)$  for the number of effective genes is less than 1 ( $K = 0.493$ ), the number of effective genes could not be determined for the examined trait. The broad sense heritability ( $H_g$ ) and narrow sense heritability ( $H_d$ ) for the examined trait were 0.886 and 0.018, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level statistically, and the ratio of GCA to SCA was calculated to be less than 1 (0.44), indicating that SCA and hence non-additive genetic variance are superior and essential. The highest GCA effect was obtained from the female parents Adana-99 (3.933) and Lucilla (3.755), while the lowest GCA effects were obtained from the female parents 2115 (-4.903) and Flamura-85 (-4.212). The highest SCA effect was obtained from the hybrid 1635 × 2115 (38.821), while the lowest SCA effects were obtained from the hybrid Lucilla × 2115 (-15.64), which also had the lowest average grain yield per plant phenotypically (15.3 g). In the examined population, in the half-diallel variance analysis table, additive variance (a), dominance variance (b), and all components ( $b_1, b_2, b_3$ ) were found to be significant. In the half-diallel hybrid analysis, genetic parameters were found to be statistically insignificant, but  $D-H_1$  was determined to be negative. The adverse determination of  $D-H_1$  indicates that dominant genetic variance is more important and superior for this trait. Evaluation of the compatibility abilities according to the variance analysis method found both GCA variance corresponding to additive variance and SCA variance corresponding to dominance variance to be significant. However, since the ratio of GCA to SCA (0.44) was calculated to be less than 1, it indicates that dominance genetic variance is more important and superior. Similarly, many researchers such as Sener (1997), Tulukcu (2004), and Kutlu (2012) have expressed the difficulty of selection due to the effectiveness of many genes and the low, narrow sense heritability in the inheritance of yield. Our findings are consistent with previous studies. In this study, the importance of dominant variance for grain yield per plant, epistatic genetic effects, and the low, narrow sense heritability suggest that selection planned for this trait should be postponed to subsequent generations.

### **3.10. Chlorophyll Content of Flag Leaf (SPAD)**

Regarding chlorophyll content of flag leaf, the average for parents was 47.05 SPAD, while the average for  $F_1$  hybrid combinations was 49.60 SPAD. Among the parents, the highest chlorophyll content of flag leaf was obtained from the genotype Lucilla (52.58 SPAD), while the lowest was from the genotype 1635 (40.8 SPAD). Among the hybrids, the highest chlorophyll content of flag leaf was recorded

as 56.47 SPAD for the hybrid Lucilla × 2115, while the lowest was 45.45 SPAD in the hybrid Lucilla × 1635. Heterosis values for chlorophyll content of flag leaf in  $F_1$  hybrid populations ranged from -3.41% (Flamura-85 × 2115) to 19.68% (1635 × 2115), while heterobeltiosis values ranged from -13.56% (Lucilla × 1635) to 16.4% (1635 × 2115). The highest average heterosis and heterobeltiosis values were obtained from the hybrid series where the genotype 2115 was the female parent ( $H_t$ : 10.19%,  $H_b$ : 4.04%), while the lowest average heterosis value was from the genotype Flamura-85 ( $H_t$ : 2.72%), and the heterobeltiosis value was from the hybrid series where the genotype Lucilla was the female parent ( $H_b$ : -2.91%). In the hybrid populations, an average of 5.60% heterosis and -0.51% heterobeltiosis values were obtained for chlorophyll content of flag leaf. From the calculated genetic parameters for chlorophyll content of flag leaf, additive genetic variance (D), dominant genetic variance ( $H_1$ ), and additive × additive interaction variance ( $b_2$ ) were found to be statistically insignificant. The average degree of dominance ( $(H_1/D)^{1/2}$ ) is greater than 1 (1.262), indicating the presence of overdominance. The negative difference between additive and dominant variances ( $D-H_1$ ) indicates that the dominant genetic variance is greater than the additive genetic variance. The F value determining the direction of dominant and recessive alleles is negative (14.054), and the KD/KR ratio is greater than 1 (1.628), indicating that dominant alleles are predominant. Since the value of  $K = (h^2/H_2)$  for the number of effective genes is less than 1 ( $K = 0.417$ ), the number of effective genes could not be determined for the examined trait. The broad sense heritability ( $H_g$ ) and narrow sense heritability ( $H_d$ ) for the examined trait were 0.853 and 0.489, respectively. General combining ability (GCA) and specific combining ability (SCA) were found to be significant at the 0.01 level statistically, and the ratio of GCA to SCA was calculated to be greater than 1 (3.18), indicating that SCA and hence non-additive genetic variance are superior and essential. The highest GCA effect was obtained from the female parent Lucilla (2.293), while the lowest GCA effect was from 1635 (-2.685). The highest SCA effect was obtained from the hybrid Lucilla × 2115 (5.993), while the lowest SCA effects were obtained from the hybrid Flamura-85 × 2115 hybrid (-3.936). In the examined population, in the half-diallel variance analysis table, additive variance (a), dominance variance (b), and all components ( $b_1, b_2, b_3$ ) were found to be significant. In the half-diallel hybrid analysis, genetic parameters were found to be statistically insignificant, but  $D-H_1$  was determined to be negative. Evaluation of the compatibility abilities according to the variance analysis method found both GCA variance corresponding to additive variance and SCA variance corresponding to dominance variance to be significant. Additionally, the ratio of GCA to SCA being more significant than 1 (3.18) indicates that general combining ability and, hence, additive genetic variance

are superior and vital despite the significance of additive x additive interactions. Furthermore, despite the significance of additive genetic variance, non-additive and epistatic genetic effects and low, narrow sense heritability suggest that selection planned for this trait should be postponed to subsequent generations.

#### 4. Conclusion

In conclusion, six bread wheat genotypes (Adana-99, Flamura-85, Masaccio, Lucilla, 1635 and 2115) used as parents and 15 F<sub>1</sub> generations obtained from their half-diallel crosses were investigated using biometric-genetic diallel methods to develop high-yielding and superior-quality new domestic bread wheat genotypes. Heterosis and heterobeltiosis values were calculated. When the heritability degrees of the examined traits were considered, narrow sense heritability values were relatively small for all traits, suggesting that selection should be carried out in subsequent generations. Adequate variation was observed in the traits examined in the study, and the determination of suitable hybrids and parents for the investigated traits suggests that the population under study could be utilized to develop desired varieties. Regarding grain yield per plant, the genotypes Adana-99, Lucilla, and Masaccio exhibited high values, indicating their potential as parental genotypes for breeding programs. Additionally, hybrids such as 1635 x 2115, Masaccio x Lucilla, Adana-99 x Lucilla, Adana-99 x Masaccio, Adana-99 x 1635, and Flamura-85 x Lucilla emerged as promising hybrids.

#### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.O.	H.G.	Z.D.
C	30	35	35
D	40	30	30
S		50	50
DCP	70	15	15
DAI	40	40	20
L	80	10	10
W	20	50	30
CR	10	60	30
SR	10	60	30
PM	40	20	40
FA	20		80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### Conflict of Interest

The authors declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this BSJ Agri / Harun OCAKTAN et al.

study because of there was no study on animals or humans.

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## GREEN SUPPLIER SELECTION USING IMF SWARA AND FUZZY WASPAS TECHNIQUES FOR THE SUPPLY OF AGRICULTURAL PESTICIDES

Hüseyin Fatih ATLI<sup>1\*</sup>, Gül SENİR<sup>2</sup>

<sup>1</sup>Iskenderun Technical University, Vocational Schools, Department of Production Management and Marketing, 31200, Hatay, Türkiye


<sup>2</sup>Niğde Ömer Halisdemir University, Faculty of Economics and Administrative Science, Department of International Trade and Logistics Management, 51100, Niğde, Türkiye


**Abstract:** The concept of sustainability is constantly increasing in importance in all areas of life with its human, social, economic and environmental dimensions. With the impact of global climate change and other environmental factors, concerns about sustainable agriculture and access to sufficient and reliable food are increasing. Reports of the Food and Agriculture Organization of the United Nations (FAO) and other international organizations also confirm this. For this reason, awareness has been created all over the world regarding the United Nations 17 Sustainable Development Goals (SDGs). With the increasing awareness of environmental protection worldwide, green supply chain management (GSCM) has become an important issue for businesses to achieve environmental sustainability. Nowadays, many managers and business owners pay special attention to green supplier selection to gain competitive advantage. Therefore, green supplier selection remains a critical decision for businesses. Businesses need to consider many economic and environmental criteria in the decision process to select the most suitable supplier. The aim of this study is to choose the most suitable green supplier for the supply of agricultural pesticides. Decision makers in selecting the most suitable green supplier for agricultural pesticide supply are business managers and academicians who are experts in the relevant field. In this study, an effective solution based on the combination of IMF SWARA (Improved Fuzzy Stepwise Weight Assessment Ratio Analysis) and fuzzy WASPAS (Weighted Aggregated Sum Product Assessment) methods is proposed to help agricultural enterprises that need to choose the best pesticide supplier. According to the research results, the criteria were determined as cost, quality and green product in order of importance, starting from the most important. In the ranking of the alternatives, alternative 1 ranked first with the highest value. This research proposes a framework to determine the most suitable alternative for green supplier selection through a combined approach of fuzzy multi-criteria decision making involving relevant stakeholders.

**Keywords:** Green supplier selection, Supply chain, Agricultural marketing, Fuzzy logic, Multi criteria decision making

\*Corresponding author: Iskenderun Technical University, Vocational Schools, Department of Production Management and Marketing, 31200, Hatay, Türkiye

E mail: hfatih.atli@iste.edu.tr (H. F. ATLI)

Hüseyin Fatih ATLI  <https://orcid.org/0000-0002-1397-1514>

Gül SENİR  <https://orcid.org/0000-0001-5454-2321>

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### 1. Introduction

In recent years, with increasing awareness of environmental protection worldwide, GSCM has attracted considerable interest from researchers and practitioners alike. This growing interest has been fueled by a worsening environment, increasing pollution levels, overflowing landfills and dwindling raw material resources. In addition, increased government regulation, stronger public awareness and consumer pressures are making businesses more vigilant about the environmental impacts of their operations. Environmental management is becoming increasingly important as organizational stakeholders such as governments, customers, employees, competitors and communities care about environmental protection. Businesses today should not neglect environmental issues if they want to survive in the global market (Van Hoek, 1999; Hashemi et al., 2015).

In order to sell products in certain countries, businesses need to ensure that their products comply with environmental regulations as well as implement strategies to voluntarily reduce their environmental impact. The integration of environmental, economic and social performance to achieve sustainable development has become an important business challenge for the new century (Verghese and Lewis, 2007). The concept of sustainability is an approach that creates a balance between nature and humans and ensures that existing resources are transferred to future generations without being destroyed (Turna and Solmaz, 2022). Legal and regulatory initiatives have emerged in developed countries, particularly in Europe and Japan. Some pioneering businesses joined the green supply chain trend long before the EU environmental orders came into force. To achieve long-term success in the global market, businesses should not only emphasize financial



conditions when evaluating their suppliers, but also consider a variety of criteria, including pro-environmental concerns (Lee et al., 2009).

The green supplier evaluation process is highly complex for a variety of reasons, including the diversity of influencing factors (Kumar et al., 2014), the mix of quantitative and qualitative selection criteria (Sarkis and Talluri, 2002), and the breadth and diversity of suppliers along the supply chain (Bai and Sarkis, 2010). Increased outsourcing, complex and tightening government and regional policies, and conflicting corporate and supply chain objectives have increased the importance and complexity of green supplier selection decisions. Green supplier selection requires the incorporation of environmental criteria into traditional supplier selection practices and approaches (Govindan et al., 2015). While price, quality and service level have been the dominant traditional green supplier selection criteria, carbon footprint and emissions, energy efficiency, water use and recycling initiatives have become more common environmental criteria (Choi, 2013).

Therefore, the objective of this study is to select the most suitable green supplier for the supply of agrochemicals. Since green supplier selection is suitable for the use of methods that can evaluate a large number of criteria

together, the study utilized the methods of MCDM. Firstly, the importance levels of the criteria were found by using IMF SWARA, and then the most suitable green supplier was selected by using fuzzy WASPAS method. There are many different studies on green supplier selection in the literature. In this study, both economic and environmental criteria are used for green supplier selection and a comprehensive green supplier selection model is proposed. In addition, it is the first paper in the literature to integrate IMF SWARA and fuzzy WASPAS methods for green supplier selection for the procurement of pesticides. The study is expected to contribute to the literature as it fills the mentioned gaps in the literature.

In the introduction part of the study, which consists of six sections, information about the literature review is presented. In the second section, materials and methods and in the third section, the findings of the analysis are presented. In the fourth section, the findings and similar studies in the literature are interpreted together and a discussion section is included. The study is completed with the fifth and final section, the conclusion section.

### 1.1. Literature Review

There are many different studies on green supplier selection in the literature.

**Table 1.** Literature review (Turkish studies)

Author(s) and Year	Method	Sector
Şişman (2016)	fuzzy MOORA (multi-objective optimization by ratio analysis)	White Goods
Denizhan et al. (2017)	AHP (analytic hierarchy process), fuzzy AHP	Machinery Manufacturing
Çelik and Ustasüleyman (2018)	fuzzy AHP, fuzzy TOPSIS (technique for order preference by similarity to ideal solution)	Fitted Kitchen
Daldır and Tosun (2018)	fuzzy AHP, fuzzy WASPAS (weighted aggregated sum product assessment)	Manufacturing
Özkır (2018)	TOPSIS	Automotive
Koca and Behdioğlu (2019)	ENTROPY, Heuristic fuzzy TOPSIS	Automotive
Madenoğlu (2019)	TOPSIS-F, VIKOR-F (multi-criteria optimization and compromise solution), GRA-F, ARAS-F, SWARA-F (step-wise weight assessment ratio analysis)	Furniture
Madenoğlu (2020)	SWARA, GIA (gray relational analysis)	Production
Öztürk and Paksoy (2020)	DEMATEL (the decision making trial and evaluation laboratory) -QFD-AT2 fuzzy AHP	Food
Soyer and Türkay (2020)	ANP (analytic hierarchy process)	White Goods
Akın (2021)	Trapezoidal fuzzy flexible cluster	Food
Çalık (2021)	BWM (best-worst method), CRITIC (criteria importance through intercriteria correlation), COPRAS (complex proportional assessment), ENTROPY, MABAC, WASPAS	Food
Erbıyık et al. (2021)	ELECTRE (elemination and choice translating reality english), SWARA	Automotive
Kılınç and Yağmahan (2021)	GIA and AHP	Automotive
Cezlan (2022)	AHP, TOPSIS	Health
Dalay and Sari (2022)	fuzzy DEMATEL	Food
Kara and Yalçın (2022)	SWARA, TOPSIS	Tourism
Karatas and Ozcelik (2022)	EDAS (evaluation based on distance from average solution), VIKOR	Electricity
Uçkun et al. (2023)	fuzzy AHP and fuzzy QFD	Automotive

**Table 2.** Literature review (English studies)

Author(s) and Year	Method	Sector
Lee et al. (2009)	fuzzy AHP	High Technology
Kuo et al. (2010)	DEA (data envelopment analysis), ANP, ANN (artificial neural network), MADA	Electronics Industry
Bali et al. (2013)	IFS, GRA	Automobile
Kannan et al. (2013)	fuzzy AHP, fuzzy TOPSIS, fuzzy MOLP	Automobile
Yazdani (2014)	AHP, fuzzy TOPSIS	Automotive
Freeman and Chen (2015)	AHP, ENTROPY, TOPSIS	Electronics Industry
Hashemi et al. (2015)	ANP, GRA	Automotive
Kuo et al. (2015)	DANP (analytical hierarchy process), VIKOR	Electronics Industry
Wang Chen et al. (2016)	fuzzy AHP, TOPSIS	Manufacturing Sector
Gupta and Barua (2017)	BWM, fuzzy TOPSIS	Automobile
Yazdani et al. (2017)	DEMATEL, COPRAS, MOORA	Food
Banaeian et al. (2018)	TOPSIS, VIKOR, GRA	Agri-Food
Shi et al. (2018)	GRA, TOPSIS	Agri-Food
Zhu and Li (2018)	H2TL, Choquet Integral	Automobile
Duan et al. (2019)	AQM, SWARA	Paper Industry
Gupta et al. (2019)	AHP, TOPSIS, MABAC, WASPAS	Automotive
Mati'c et al. (2019)	FUCOM (full consistency method), COPRAS	Construction
Miranda-Ackerman et al. (2019)	TOPSIS	Agri-Food
Phochanikorn and Tan (2019)	fuzzy DEMATEL, fuzzy ANP	Food
Ramakrishnan and Chakraborty (2020)	TOPSIS	Automobile
Kazemitash et al. (2021)	RBWM (rough best worst method)	Biofuel Companies
Puška et al. (2021)	PIPRECIA, MABAC	Agriculture
Tirkolae et al. (2021)	AHP-fuzzy TOPSIS	Food
Ecer (2022)	fuzzy AHP	Home Appliances Manufacturer
Puška et al. (2022)	fuzzy LMAW, fuzzy CRADIS	Agriculture
Wang and Van Thanh (2022)	SF-AHP, CODAS (combinative distance-based assessment)	Agriculture

These studies are given in two different tables in Turkish and English. Table 1 shows the Turkish studies in the literature.

When the studies in Table 1 are examined, it is observed that the studies were conducted between the years 2016-2023 and many different MCDM methods were used and the studies were mostly concentrated in the automotive and food sectors.

Table 2 presents the English studies conducted in the literature. When the studies in Table 2 are examined, it is observed that the studies were conducted between 2008 and 2022 and many different MCDM methods were used and the studies were mostly concentrated in the automotive, food and agriculture sectors.

## 2. Materials and Methods

In this section, information about the data set used in the study, the analysis methods and the criteria used in the analysis are given.

### 2.1. Data Set Used in the Study

In order to select the most suitable green supplier for the supply of agricultural pesticides, data were collected from the enterprises using agricultural pesticides and academicians working in the relevant field by survey method. The data of the study belongs to the year 2024.

### 2.2. Criteria Used in the Study

In order to select the most suitable green supplier for the supply of pesticides, the criteria accepted in the relevant

literature and determined comprehensively were determined as decision criteria in accordance with the MCDM methods. The ten criteria are given in Table 3 were used for green supplier selection.

**Table 3.** Green supplier selection criteria

Criteria	Criteria/ Codes	Author(s) and Year
Green Product	C1	Hashemi et al. (2015), Kazemitash et al. (2021), Puška et al. (2022)
Green Competence	C2	Freeman and Chen (2015), Hashemi et al. (2015), Puška et al. (2022)
Environmental Management System	C3	Hashemi et al. (2015), Puška et al. (2022), Tirkolae et al. (2021)
Recycling	C4	Puška et al. (2022)
Pollution Control	C5	Gupta et al. (2019), Hashemi et al. (2015), Lee et al. (2009), Puška et al. (2022)
Quality	C6	Freeman and Chen (2015), Gupta et al. (2019), Lee et al. (2009), Puška et al. (2022)
Cost	C7	Freeman and Chen (2015), Gupta et al. (2019), Puška et al. (2022)
Logistics Service	C8	Puška et al. (2022)
Innovativeness	C9	Puška et al. (2022)
Technological Competence	C10	Puška et al. (2022)

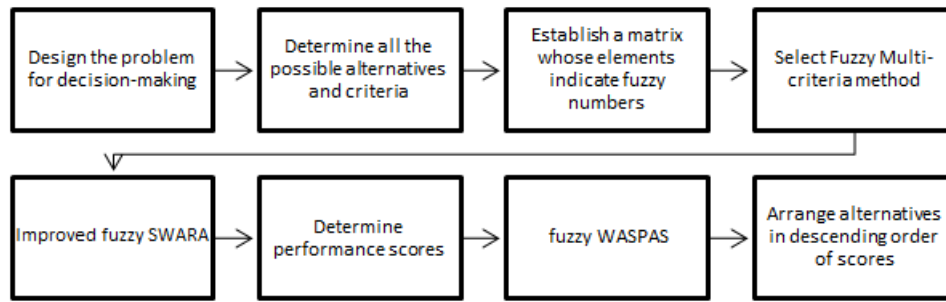


Figure 1. Research flowchart for green supplier selection (Atlı, 2024).

For each of the five alternatives, the decision makers' task is to identify potential criteria that will complete the decision-making process. The flow chart of the MCDM process is shown in (Figure 1).

**2.3. Analysis Methods Used in the Study**

In the study, the MCDM methods were utilized. In order to select the most suitable green supplier for the supply of pesticides, first the importance levels of the criteria were determined with IMF SWARA and then the most suitable one was selected among the alternatives with fuzzy WASPAS. MCDM methods are methods that enable the identification, selection, ranking and classification of multiple alternatives with a large number of criteria (Vassilev et al., 2005).

IMF SWARA and fuzzy WASPAS techniques used in working with fuzzy numbers and application steps are given. Scales used to convert numbers into fuzzy numbers are also presented. The weights of the criteria were calculated with the IMF SWARA method. Then, alternative rankings of green supplier selection in agricultural pesticide supply were obtained by using the fuzzy WASPAS method.

**2.4. Fuzzy Logic and Fuzzy Numbers**

Fuzzy sets, basic operations, concepts and properties are given in this article. According to Zadeh (2015), one of the main contributions of fuzzy logic is to provide a basis for progress from binarization to gradation, from binary to pluralism, from black and white to shades of grey. Fuzzy logic; It is based on the concepts of fuzzy set and subset (Zadeh, 1965). There are membership functions in different forms that define fuzzy sets analytically and represent their membership degrees, and the most commonly used among the various forms of fuzzy membership functions are triangular, trapezoidal, Gaussian and generalized bell curve membership functions (Sergi, 2021). In this study, triangular fuzzy numbers were used. Triangular fuzzy numbers were created to maximize the accuracy of the evaluations in uncertain evaluations when making decisions (Arslankaya and Göraltay, 2019). Equation 1 is given in (Hudec, 2016), and the graph drawn for the function is given in (Figure 2).

$$\mu_{\tilde{a}}(x) = \begin{cases} 0, & \text{if } x \leq l \\ \frac{x-l}{m-l}, & \text{if } l \leq x \leq m \\ \frac{u-x}{u-m}, & \text{if } m \leq x \leq u \\ 0, & \text{if } u \leq x \end{cases} \quad (1)$$

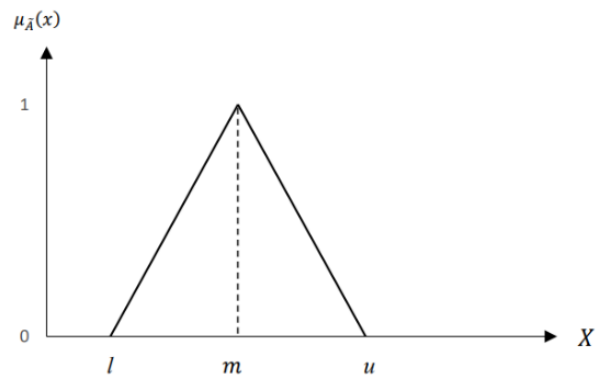


Figure 2. Triangle membership function (Hudec, 2016).

**2.5. Calculation of Criterion Weights with the IMF SWARA Method**

SWARA technique, introduced by Kersuliene et al. (2010), is an MCDM technique used to calculate the weights of selection criteria. The SWARA technique has an algorithm that can be easily followed by decision makers, and the weights of the criteria can be determined by following these application steps (Zolfani et al., 2021). According to Mardani et al. (2017), SWARA method; unlike the classical MCDM method, it tries to predict the preferences of decision makers and includes these predictions to evaluate the process.

To overcome the many uncertainties that exist in an evaluation process, the fuzzy SWARA technique was developed based on fuzzy sets (Mavi et al., 2017). Fuzzy SWARA is a subjective evaluation technique (Zolfani et al., 2021). Vrtagić et al. (2021) suggested using a new scale by developing the IMF SWARA method, which is a new approach to overcome the shortcomings of the technique for making pairwise comparisons between criteria. The current literature shows that researchers used IMF-SWARA method to analyze different topics. Zolfani et al. (2021) used IMF SWARA and F-MABAC methods to solve the logistics village selection problem with very complex and uncertain conditions based on fuzzy approaches.

Vrtagić et al. (2021) developed an integrated fuzzy model to determine the safety degree of observed road sections. The paper's major contribution is the development of the IMF SWARA method. Vrtagić et al. (2021) applied IMF SWARA to determine the values of the weight coefficients of the criteria and used the fuzzy MARCOS method for the final ranking of the sections. It is crucial to provide a consistent and realistic evaluation tool to reflect the subjective evaluations carried out by decision-makers (Vrtagić et al., 2021). For this purpose, the IMF SWARA

(Vrtagić et al., 2021) method will be used to determine the criterion weights and the application steps of the technique as follows.

Step 1. Determine the rank value of the criteria: After determining the criteria, the ranking value of these criteria is determined.

Step 2. Making pairwise comparisons between criteria: Decision makers determine the relative importance of each criterion with the help of the linguistic variables (scale) given in Table 4.

**Table 4.** The linguistic scale for the IMF SWARA technique and TFNs (Vrtagić et al., 2021)

Linguistic Variable	Abbreviation	TFN Scale		
Absolutely less significant	ALS	1	1	1
Dominantly less significant	DLS	1/2	2/3	1
Much less significant	MLS	2/5	1/2	2/3
Really less significant	RLS	1/3	2/5	1/2
Less significant	LS	2/7	1/3	2/5
Moderately less significant	MDLS	1/4	2/7	1/3
Weakly less significant	WLS	2/9	1/4	2/7
Equally significant	ES	0	0	0

**Table 5.** Fuzzy linguistic scale for evaluating alternatives (Liang et al., 2021)

(Linguistic Variables)	(Rating)	(TFNs)		
Very poor (VP) / Very low (VL)	1	0.1	0.2	0.3
Poor (P) / Low (L)	2	0.2	0.3	0.4
Slightly poor (SP) / Slightly low (SL)	3	0.3	0.4	0.5
Fair (F) / Medium (M)	4	0.4	0.5	0.6
Slightly good (SG) / Slightly high (SH)	5	0.5	0.6	0.7
Good (G) / High (H)	6	0.6	0.7	0.8
Very good (VG) / Very high (VH)	7	0.7	0.8	0.9

Step 3. Computing the coefficient value: For each fuzzy number, the following steps are followed and  $k_j$ ,  $q_j$ ,  $w_j$  values are calculated.

$k_j$ : The coefficient value

$q_j$ : Weights values of the criteria

$w_j$ : Fuzzy weight coefficients values of the criteria

The coefficient  $k_j$  value for each fuzzy number is calculated using Equation 2.

$$\tilde{k}_j = \begin{cases} \tilde{1}, & j = 1 \\ \tilde{s}_j, & j > 1 \end{cases} \quad (2)$$

Afterward, weights values of the criteria  $\tilde{q}_j$  are calculated by using Equation 3.

$$\tilde{q}_j = \begin{cases} \tilde{1}, & j = 1 \\ \frac{\tilde{q}_{j-1}}{k_j}, & j > 1 \end{cases} \quad (3)$$

Finally, fuzzy weight coefficients values of the criteria are calculated with the help of Equation 4.

$$\tilde{w}_j = \frac{\tilde{q}_j}{\sum_{j=1}^n \tilde{q}_j} \quad (4)$$

Step 4. Defuzzifying the criteria weights: In the final step of the IMF SWARA technique, fuzzy values are defuzzified by using Equation 5 as follows.

$$w_{Crisp\ value} = \frac{w^{(l)} + 4w^{(m)} + w^{(u)}}{6} \quad (5)$$

## 2.6. Ranking of Alternatives with the Fuzzy WASPAS Method

MCDM methods can be effectively applied to determine the value and degree of utility of various fields and prioritize their implementation (Turskis, 2008). WASPAS method is one of these methods. Zavadskas et al. (2012) was developed as a combination of two approaches known as WSM and WPM, which are frequently used in MCDM. Turskis et al. (2015), the fuzzy logic approach and WASPAS method were integrated and introduced into the literature as the fuzzy WASPAS method. The fuzzy WASPAS method is an effective decision-making tool that is widely used due to its ease in complex calculations, simplicity, and high accuracy and consistency in ranking alternatives. The advantageous features of the WASPAS method include its own sensitivity analysis and the ability to check consistency while listing alternatives (Chakraborty, 2014).

Fuzzy WASPAS method was preferred to obtain alternative rankings in green supplier selection. Linguistic variables given by decision makers according to the performance of supplier alternatives in agricultural pesticide supplier selection will be converted

into triangular fuzzy numbers through Table 5.

In the fuzzy WASPAS method proposed by Turskis et al. (2015), the following fuzzy WASPAS steps were used:

Step 1. Creating a fuzzy decision matrix: In Equation 6,  $m$  indicates the number of alternatives, while  $n$  indicates the number of criteria.

$$\tilde{X} = \begin{bmatrix} \tilde{x}_{11} & \dots & \tilde{x}_{1j} & \dots & \tilde{x}_{1n} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{x}_{i1} & \dots & \tilde{x}_{ij} & \dots & \tilde{x}_{in} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{x}_{m1} & \dots & \tilde{x}_{mj} & \dots & \tilde{x}_{mn} \end{bmatrix}; i = \overline{1, m}, j = \overline{1, n} \quad (6)$$

Step 2. Creating the normalized decision matrix: The values required to create the normalized decision matrix are calculated using Equation 7.

$$\tilde{\tilde{x}}_{ij} = \begin{cases} \frac{\tilde{x}_{ij}}{\max_i(\tilde{x}_{ij})} & \text{if benefit is the criterion} \\ \frac{\min_i(\tilde{x}_{ij})}{\tilde{x}_{ij}} & \text{if cost is the criterion} \end{cases} \quad i = \overline{1, m}, j = \overline{1, n} \quad (7)$$

Step 3. Using Equation 8, the weighted normalized fuzzy decision matrix for WSM is determined. Using Equation 9, the weighted normalized fuzzy decision matrix for WPM is determined.

$$\tilde{\tilde{X}}_q = \begin{bmatrix} \tilde{\tilde{x}}_{11} & \dots & \tilde{\tilde{x}}_{1j} & \dots & \tilde{\tilde{x}}_{1n} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{\tilde{x}}_{i1} & \dots & \tilde{\tilde{x}}_{ij} & \dots & \tilde{\tilde{x}}_{in} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{\tilde{x}}_{m1} & \dots & \tilde{\tilde{x}}_{mj} & \dots & \tilde{\tilde{x}}_{mn} \end{bmatrix}; \tilde{\tilde{x}}_{ij} = \tilde{x}_{ij} \tilde{w}_j \quad i = \overline{1, m}, j = \overline{1, n} \quad (8)$$

$$\tilde{\tilde{X}}_p = \begin{bmatrix} \tilde{\tilde{x}}_{11} & \dots & \tilde{\tilde{x}}_{1j} & \dots & \tilde{\tilde{x}}_{1n} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{\tilde{x}}_{i1} & \dots & \tilde{\tilde{x}}_{ij} & \dots & \tilde{\tilde{x}}_{in} \\ \vdots & \ddots & \vdots & \ddots & \vdots \\ \tilde{\tilde{x}}_{m1} & \dots & \tilde{\tilde{x}}_{mj} & \dots & \tilde{\tilde{x}}_{mn} \end{bmatrix}; \tilde{\tilde{x}}_{ij} = \tilde{x}_{ij}^{\tilde{w}_j} \quad i = \overline{1, m}, j = \overline{1, n} \quad (9)$$

Step 4. Calculate values of the optimality function: Values of the optimality function is calculated separately according to WSM and WPM, respectively, using Equation 10 and Equation 11. The fuzzy performance measurement value for each alternative is calculated using Equation 12 and Equation 13.

$$\tilde{Q}_i = \sum_{j=1}^n \tilde{\tilde{x}}_{ij}, \quad i = \overline{1, m} \quad (10)$$

$$\tilde{P}_i = \prod_{j=1}^n \tilde{\tilde{x}}_{ij}, \quad i = \overline{1, m} \quad (11)$$

$$Q_i = \frac{1}{3}(Q_{i\alpha} + Q_{i\beta} + Q_{i\gamma}) \quad (12)$$

$$P_i = \frac{1}{3}(P_{i\alpha} + P_{i\beta} + P_{i\gamma}) \quad (13)$$

Step 5. The integrated utility function value for an alternative can be determined by Equation 14. In cases where WSM and WPM approaches are considered to have equal impact, the value of  $\lambda$  is taken as 0.5. Otherwise, the  $\lambda$  value is calculated with Equation 15.

$$K_i = \lambda \sum_{j=1}^m Q_i + (1 - \lambda) \sum_{j=1}^m P_i, \lambda = 0, \dots, 1, \quad 0 \leq K_i \leq 1 \quad (14)$$

$$\lambda = \frac{\sum_{i=1}^m P_i}{\sum_{i=1}^m Q_i + \sum_{i=1}^m P_i} \quad (15)$$

Step 6. Rank preference order. Choose an alternative with maximal  $K_i$  value.

### 3. Results

#### 3.1. Calculation of Criterion Weights with the IMF SWARA Method

The criteria for selecting green suppliers for the supply of agricultural pesticides were evaluated by ten experts. The decision-making expert group that evaluates the criteria; It consists of businesses that use agricultural pesticides and academicians in the related field. As a result of the evaluation, the IMF SWARA method was applied to obtain the weights.

Step 1. The first step in the IMF SWARA method, ranking the criteria from most important to least important, was done by each decision maker one by one. The ranking results were obtained as shown in Table 6.

Step 2. Linguistic evaluations of the importance levels between the criteria determined by the decision makers have been converted into fuzzy numbers through Table 7.

Step 3. In this step, firstly, coefficient  $k_j$  values were reached by using Equation 2 with the help of  $s_j$  values. Then, the importance vector  $q_j$  values of each criterion were calculated using Equation 3. Finally, the weights of the criteria  $w_j$  were calculated using Equation 4. The  $k_j, q_j, w_j$  values calculated for each criterion of the decision makers are shown in Table 7.

Step 4. In the final step of the IMF SWARA technique, fuzzy values was defuzzified by using Equation 5. The geometric mean of the criterion weights was calculated and the final weights of the criteria were obtained as shown in Table 8.

According to Table 8, cost (C7) is the most important criterion for decision makers, with a relative importance score of 0.131. This is followed by quality (C6), green product (C1) and pollution control (C5). Innovativeness (C9) was seen to be a less critical criterion.

**Table 6.** Ranking of criteria according to decision makers

Code	Criteria	DM1	DM2	DM3	DM4	DM5	DM6	DM7	DM8	DM9	DM10
C1	Green Product	1	5	9	10	9	8	1	5	8	3
C2	Green Competence	9	7	8	9	10	6	6	7	7	4
C3	Environmental Management System	7	8	4	3	6	10	4	9	6	2
C4	Recycling	8	4	10	6	8	7	7	4	3	7
C5	Pollution Control	6	6	5	5	7	9	2	1	2	1
C6	Quality	4	3	2	1	1	5	5	2	10	5
C7	Cost	3	1	1	2	2	4	3	3	9	6
C8	Logistics Service	10	2	3	4	3	1	8	6	1	10
C9	Innovativeness	2	9	7	7	4	2	9	8	5	9
C10	Technological Competence	5	10	6	8	5	3	10	10	4	8

**Table 7.** The weights of criteria were calculated by using the IMF SWARA technique

Code	$\tilde{s}_j$			DM1										Crips Value
				$\tilde{k}_j$	$\tilde{q}_j$		$\tilde{w}_j$							
C1				1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.216	0.232	0.251	0.232
C9	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.177	0.185	0.195	0.186	
C7	2/9	1/4	2/7	1.222	1.250	1.286	0.669	0.640	0.605	0.145	0.148	0.152	0.148	
C6	2/9	1/4	2/7	1.222	1.250	1.286	0.548	0.512	0.471	0.118	0.119	0.118	0.118	
C10	1/4	2/7	1/3	1.250	1.286	1.333	0.438	0.398	0.353	0.095	0.092	0.089	0.092	
C5	2/9	1/4	2/7	1.222	1.250	1.286	0.359	0.319	0.274	0.078	0.074	0.069	0.074	
C3	2/7	1/3	2/5	1.286	1.333	1.400	0.279	0.239	0.196	0.060	0.055	0.049	0.055	
C4	2/9	1/4	2/7	1.222	1.250	1.286	0.228	0.191	0.152	0.049	0.044	0.038	0.044	
C2	1/3	2/5	1/2	1.333	1.400	1.500	0.171	0.137	0.102	0.037	0.032	0.026	0.032	
C8	1/2	2/3	1	1.500	1.667	2.000	0.114	0.082	0.051	0.025	0.019	0.013	0.019	
				DM2										
C7				1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.122	0.124	0.128	0.125
C8	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.100	0.100	0.099	0.100	
C6	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.122	0.124	0.128	0.125
C4	2/7	1/3	2/5	1.286	1.333	1.400	0.778	0.750	0.714	0.095	0.093	0.091	0.093	
C1	2/9	1/4	2/7	1.222	1.250	1.286	0.636	0.600	0.556	0.078	0.075	0.071	0.075	
C5	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.122	0.124	0.128	0.125
C2	1/4	2/7	1/3	1.250	1.286	1.333	0.800	0.778	0.750	0.097	0.097	0.096	0.097	
C3	2/9	1/4	2/7	1.222	1.250	1.286	0.655	0.622	0.583	0.080	0.077	0.075	0.077	
C9	1/4	2/7	1/3	1.250	1.286	1.333	0.524	0.484	0.438	0.064	0.060	0.056	0.060	
C10	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.122	0.124	0.128	0.125
				DM3										
C7				1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.242	0.264	0.293	0.265
C6	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.198	0.211	0.228	0.212	
C8	2/7	1/3	2/5	1.286	1.333	1.400	0.636	0.600	0.556	0.154	0.158	0.163	0.158	
C3	2/9	1/4	2/7	1.222	1.250	1.286	0.521	0.480	0.432	0.126	0.127	0.126	0.127	
C5	2/5	1/2	2/3	1.400	1.500	1.667	0.372	0.320	0.259	0.090	0.084	0.076	0.084	
C10	2/5	1/2	2/3	1.400	1.500	1.667	0.266	0.213	0.156	0.064	0.056	0.046	0.056	
C9	2/5	1/2	2/3	1.400	1.500	1.667	0.190	0.142	0.093	0.046	0.038	0.027	0.037	

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C2	2/7	1/3	2/5	1.286	1.333	1.400	0.148	0.107	0.067	0.036	0.028	0.020	0.028
C1	2/9	1/4	2/7	1.222	1.250	1.286	0.121	0.085	0.052	0.029	0.023	0.015	0.022
C4	1	1	1	2.000	2.000	2.000	0.060	0.043	0.026	0.015	0.011	0.008	0.011
DM4													
C6				1.000	1.000	1.000	1.000	1.000	1.000	0.142	0.147	0.155	0.148
C7	2/5	1/2	2/3	1.400	1.500	1.667	0.714	0.667	0.600	0.101	0.098	0.093	0.098
C3	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.142	0.147	0.155	0.148
C8	1/3	2/5	1/2	1.333	1.400	1.500	0.750	0.714	0.667	0.106	0.105	0.103	0.105
C5	1	1	1	2.000	2.000	2.000	0.375	0.357	0.333	0.053	0.053	0.052	0.053
C4	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.142	0.147	0.155	0.148
C9	1/4	2/7	1/3	1.250	1.286	1.333	0.800	0.778	0.750	0.113	0.114	0.116	0.115
C10	1/4	2/7	1/3	1.250	1.286	1.333	0.640	0.605	0.563	0.091	0.089	0.087	0.089
C2	2/5	1/2	2/3	1.400	1.500	1.667	0.457	0.403	0.338	0.065	0.059	0.052	0.059
C1	2/5	1/2	2/3	1.400	1.500	1.667	0.327	0.269	0.203	0.046	0.040	0.031	0.039
DM5													
C6				1.000	1.000	1.000	1.000	1.000	1.000	0.130	0.134	0.137	0.134
C7	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.130	0.134	0.137	0.134
C8	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.130	0.134	0.137	0.134
C9	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.107	0.107	0.107	0.107
C10	2/9	1/4	2/7	1.222	1.250	1.286	0.669	0.640	0.605	0.087	0.085	0.083	0.085
C3	2/9	1/4	2/7	1.222	1.250	1.286	0.548	0.512	0.471	0.071	0.068	0.065	0.068
C5	2/9	1/4	2/7	1.222	1.250	1.286	0.448	0.410	0.366	0.058	0.055	0.050	0.055
C4	2/9	1/4	2/7	1.222	1.250	1.286	0.367	0.328	0.285	0.048	0.044	0.039	0.044
C1	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.130	0.134	0.137	0.134
C2	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.107	0.107	0.107	0.107
DM6													
C3				1.000	1.000	1.000	1.000	1.000	1.000	0.159	0.165	0.173	0.166
C5	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.159	0.165	0.173	0.166
C1	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.159	0.165	0.173	0.166
C4	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.130	0.132	0.135	0.132
C2	2/9	1/4	2/7	1.222	1.250	1.286	0.669	0.640	0.605	0.106	0.106	0.105	0.106
C6	2/9	1/4	2/7	1.222	1.250	1.286	0.548	0.512	0.471	0.087	0.085	0.082	0.085
C7	1/4	2/7	1/3	1.250	1.286	1.333	0.438	0.398	0.353	0.070	0.066	0.061	0.066
C10	1/4	2/7	1/3	1.250	1.286	1.333	0.351	0.310	0.265	0.056	0.051	0.046	0.051
C9	1/4	2/7	1/3	1.250	1.286	1.333	0.280	0.241	0.198	0.045	0.040	0.034	0.040
C8	1/2	2/3	1	1.500	1.667	2.000	0.187	0.145	0.099	0.030	0.024	0.017	0.024
DM7													
C1				1.000	1.000	1.000	1.000	1.000	1.000	0.227	0.246	0.270	0.247
C5	1/4	2/7	1/3	1.250	1.286	1.333	0.800	0.778	0.750	0.182	0.191	0.203	0.191
C7	2/7	1/3	2/5	1.286	1.333	1.400	0.622	0.583	0.536	0.141	0.143	0.145	0.143
C3	1/4	2/7	1/3	1.250	1.286	1.333	0.498	0.454	0.402	0.113	0.111	0.109	0.111
C6	2/9	1/4	2/7	1.222	1.250	1.286	0.407	0.363	0.313	0.092	0.089	0.084	0.089
C2	2/9	1/4	2/7	1.222	1.250	1.286	0.333	0.290	0.243	0.076	0.071	0.066	0.071
C4	1/4	2/7	1/3	1.250	1.286	1.333	0.267	0.226	0.182	0.060	0.055	0.049	0.055
C8	2/7	1/3	2/5	1.286	1.333	1.400	0.207	0.169	0.130	0.047	0.042	0.035	0.041
C9	1/3	2/5	1/2	1.333	1.400	1.500	0.156	0.121	0.087	0.035	0.030	0.023	0.030
C10	1/3	2/5	1/2	1.333	1.400	1.500	0.117	0.086	0.058	0.026	0.021	0.016	0.021



DM8													
C5				1.000	1.000	1.000	1.000	1.000	1.000	0.160	0.167	0.175	0.167
C6	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.160	0.167	0.175	0.167
C7	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.160	0.167	0.175	0.167
C4	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.131	0.133	0.136	0.133
C1	1/4	2/7	1/3	1.250	1.286	1.333	0.655	0.622	0.583	0.105	0.104	0.102	0.104
C8	1/4	2/7	1/3	1.250	1.286	1.333	0.524	0.484	0.438	0.084	0.081	0.077	0.081
C2	1/4	2/7	1/3	1.250	1.286	1.333	0.419	0.376	0.328	0.067	0.063	0.057	0.063
C9	1/4	2/7	1/3	1.250	1.286	1.333	0.335	0.293	0.246	0.054	0.049	0.043	0.049
C3	2/9	1/4	2/7	1.222	1.250	1.286	0.274	0.234	0.191	0.044	0.039	0.034	0.039
C10	2/9	1/4	2/7	1.222	1.250	1.286	0.224	0.187	0.149	0.036	0.031	0.026	0.031
DM9													
C8				1.000	1.000	1.000	1.000	1.000	1.000	0.162	0.173	0.188	0.174
C5	1/2	2/3	1	1.500	1.667	2.000	0.667	0.600	0.500	0.108	0.104	0.094	0.103
C4	2/5	1/2	2/3	1.400	1.500	1.667	0.476	0.400	0.300	0.077	0.069	0.056	0.068
C10	1/3	2/5	1/2	1.333	1.400	1.500	0.357	0.286	0.200	0.058	0.049	0.038	0.049
C9	1/3	2/5	1/2	1.333	1.400	1.500	0.268	0.204	0.133	0.044	0.035	0.025	0.035
C3	1/4	2/7	1/3	1.250	1.286	1.333	0.214	0.159	0.100	0.035	0.027	0.019	0.027
C2	2/9	1/4	2/7	1.222	1.250	1.286	0.175	0.127	0.078	0.028	0.022	0.015	0.022
C1	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.162	0.173	0.188	0.174
C7	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.162	0.173	0.188	0.174
C6	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.162	0.173	0.188	0.174
DM10													
C5				1.000	1.000	1.000	1.000	1.000	1.000	0.158	0.164	0.172	0.164
C3	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.158	0.164	0.172	0.164
C1	0	0	0	1.000	1.000	1.000	1.000	1.000	1.000	0.158	0.164	0.172	0.164
C2	2/9	1/4	2/7	1.222	1.250	1.286	0.818	0.800	0.778	0.129	0.131	0.134	0.131
C6	2/9	1/4	2/7	1.222	1.250	1.286	0.669	0.640	0.605	0.106	0.105	0.104	0.105
C7	2/9	1/4	2/7	1.222	1.250	1.286	0.548	0.512	0.471	0.087	0.084	0.081	0.084
C4	2/9	1/4	2/7	1.222	1.250	1.286	0.448	0.410	0.366	0.071	0.067	0.063	0.067
C10	1/4	2/7	1/3	1.250	1.286	1.333	0.359	0.319	0.274	0.057	0.052	0.047	0.052
C9	2/7	1/3	2/5	1.286	1.333	1.400	0.279	0.239	0.196	0.044	0.039	0.034	0.039
C8	1/3	2/5	1/2	1.333	1.400	1.500	0.209	0.171	0.131	0.033	0.028	0.022	0.028

Table 8. The final criteria weights

Code	Criteria	Final weights
C7	Cost	0.131
C6	Quality	0.130
C1	Green Product	0.109
C5	Pollution Control	0.107
C3	Environmental Management System	0.084
C8	Logistics Service	0.066
C4	Recycling	0.065
C2	Green Competence	0.061
C10	Technological Competence	0.058
C9	Innovativeness	0.058

3.2. Ranking of Alternatives with the fuzzy WASPAS Method

The agricultural production enterprise where the application was carried out was asked to evaluate its five main suppliers of agricultural pesticides (A1, A2, A3, A4 and A5) according to the determined criteria with linguistic variables (Table 5). For this purpose, fuzzy WASPAS method was used to obtain alternative rankings in green supplier selection. A fuzzy decision matrix was created according to Equation 6. Here, *m* indicates the number of alternatives and *n* indicates the number of criteria.

Depending on whether the selected criterion is a benefit or cost criterion, the initial Decision Matrix is normalized and the normalized decision matrix is shown in Table 10. Finally, according to the criterion weight values determined in Table 8, WSM was calculated as shown in

Table 11 and WPM in Table 12.

The  $\lambda$  value calculated according to Equation 15 was found to be 0.539. Accordingly, in the ranking made using Equation 15, Alternative 1 received the highest value and

the highest ranking score with 0.715. Then, the ranking values were determined as Alternative 3 (0.707), Alternative 5 (0.698), Alternative 2 (0.653) and Alternative 4 (0.577).

**Table 9.** Fuzzy initial decision matrix

	A1	A2	A3	A4	A5
C1	0.5	0.4	0.4	0.4	0.4
	0.6	0.5	0.5	0.5	0.5
	0.7	0.6	0.6	0.6	0.6
C2	0.7	0.6	0.6	0.4	0.6
	0.8	0.7	0.7	0.5	0.7
	0.9	0.8	0.8	0.6	0.8
C3	0.7	0.6	0.7	0.5	0.6
	0.8	0.7	0.8	0.6	0.7
	0.9	0.8	0.9	0.7	0.8
C4	0.7	0.6	0.6	0.5	0.6
	0.8	0.7	0.7	0.6	0.7
	0.9	0.8	0.8	0.7	0.8
C5	0.7	0.6	0.6	0.5	0.6
	0.8	0.7	0.7	0.6	0.7
	0.9	0.8	0.8	0.7	0.8
C6	0.5	0.6	0.7	0.4	0.7
	0.6	0.7	0.8	0.5	0.8
	0.7	0.8	0.9	0.6	0.9
C7	0.5	0.5	0.7	0.3	0.7
	0.6	0.6	0.8	0.4	0.8
	0.7	0.7	0.9	0.5	0.9
C8	0.5	0.5	0.7	0.2	0.7
	0.6	0.6	0.8	0.3	0.8
	0.7	0.7	0.9	0.4	0.9
C9	0.6	0.4	0.7	0.3	0.7
	0.7	0.5	0.8	0.4	0.8
	0.8	0.6	0.9	0.5	0.9
C10	0.6	0.4	0.7	0.3	0.7
	0.7	0.5	0.8	0.4	0.8
	0.8	0.6	0.9	0.5	0.9

**Table 10.** Normalized fuzzy decision matrix

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
A1	<i>l</i>	0.714	0.778	0.778	0.778	0.778	0.556	0.600	0.556	0.667	0.667
	<i>m</i>	0.857	0.889	0.889	0.889	0.889	0.667	0.500	0.667	0.778	0.778
	<i>u</i>	1.000	1.000	1.000	1.000	1.000	0.778	0.429	0.778	0.889	0.889
A2	<i>l</i>	0.571	0.667	0.667	0.667	0.667	0.667	0.600	0.556	0.444	0.444
	<i>m</i>	0.714	0.778	0.778	0.778	0.778	0.778	0.500	0.667	0.556	0.556
	<i>u</i>	0.857	0.889	0.889	0.889	0.889	0.889	0.429	0.778	0.667	0.667
A3	<i>l</i>	0.571	0.667	0.778	0.667	0.667	0.778	0.429	0.778	0.778	0.778
	<i>m</i>	0.714	0.778	0.889	0.778	0.778	0.889	0.375	0.889	0.889	0.889
	<i>u</i>	0.857	0.889	1.000	0.889	0.889	1.000	0.333	1.000	1.000	1.000
A4	<i>l</i>	0.571	0.444	0.556	0.556	0.556	0.444	1.000	0.222	0.333	0.333
	<i>m</i>	0.714	0.556	0.667	0.667	0.667	0.556	0.750	0.333	0.444	0.444
	<i>u</i>	0.857	0.667	0.778	0.778	0.778	0.667	0.600	0.444	0.556	0.556
A5	<i>l</i>	0.571	0.667	0.667	0.667	0.667	0.778	0.429	0.778	0.778	0.778
	<i>m</i>	0.714	0.778	0.778	0.778	0.778	0.889	0.375	0.889	0.889	0.889
	<i>u</i>	0.857	0.889	0.889	0.889	0.889	1.000	0.333	1.000	1.000	1.000

**Table 11.** The weighted normalised matrix for WSM

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	Q(i)
A1	L	0.078	0.048	0.065	0.051	0.083	0.072	0.078	0.037	0.038	0.039	0.589
	M	0.094	0.054	0.075	0.058	0.095	0.087	0.065	0.044	0.045	0.045	0.662
	U	0.109	0.061	0.084	0.065	0.107	0.101	0.056	0.051	0.051	0.051	0.738
A2	L	0.063	0.041	0.056	0.043	0.071	0.087	0.078	0.037	0.026	0.026	0.527
	M	0.078	0.048	0.065	0.051	0.083	0.101	0.065	0.044	0.032	0.032	0.600
	U	0.094	0.054	0.075	0.058	0.095	0.116	0.056	0.051	0.038	0.039	0.676
A3	L	0.063	0.041	0.065	0.043	0.071	0.101	0.056	0.051	0.045	0.045	0.582
	M	0.078	0.048	0.075	0.051	0.083	0.116	0.049	0.059	0.051	0.051	0.660
	U	0.094	0.054	0.084	0.058	0.095	0.130	0.044	0.066	0.058	0.058	0.741
A4	L	0.063	0.027	0.047	0.036	0.059	0.058	0.131	0.015	0.019	0.019	0.474
	M	0.078	0.034	0.056	0.043	0.071	0.072	0.098	0.022	0.026	0.026	0.527
	U	0.094	0.041	0.065	0.051	0.083	0.087	0.078	0.029	0.032	0.032	0.593
A5	L	0.063	0.041	0.056	0.043	0.071	0.101	0.056	0.051	0.045	0.045	0.573
	M	0.078	0.048	0.065	0.051	0.083	0.116	0.049	0.059	0.051	0.051	0.651
	U	0.094	0.054	0.075	0.058	0.095	0.130	0.044	0.066	0.058	0.058	0.731
$\Sigma Q_i$												3.108

**Table 12.** The weighted normalised matrix for WPM

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	P(i)
A1	L	0.964	0.985	0.979	0.984	0.973	0.926	0.935	0.962	0.977	0.977	0.708
	M	0.983	0.993	0.990	0.992	0.987	0.949	0.913	0.974	0.986	0.986	0.776
	U	1.000	1.000	1.000	1.000	1.000	0.968	0.895	0.984	0.993	0.993	0.840
A2	L	0.941	0.976	0.967	0.974	0.958	0.949	0.935	0.962	0.954	0.954	0.643
	M	0.964	0.985	0.979	0.984	0.973	0.968	0.913	0.974	0.967	0.967	0.716
	U	0.983	0.993	0.990	0.992	0.987	0.985	0.895	0.984	0.977	0.977	0.784
A3	L	0.941	0.976	0.979	0.974	0.958	0.968	0.895	0.984	0.986	0.986	0.693
	M	0.964	0.985	0.990	0.984	0.973	0.985	0.880	0.992	0.993	0.993	0.763
	U	0.983	0.993	1.000	0.992	0.987	1.000	0.866	1.000	1.000	1.000	0.829
A4	L	0.941	0.952	0.952	0.963	0.939	0.900	1.000	0.905	0.939	0.938	0.553
	M	0.964	0.965	0.967	0.974	0.958	0.926	0.963	0.930	0.954	0.954	0.633
	U	0.983	0.976	0.979	0.984	0.973	0.949	0.935	0.948	0.967	0.967	0.707
A5	L	0.941	0.976	0.967	0.974	0.958	0.968	0.895	0.984	0.986	0.986	0.685
	M	0.964	0.985	0.979	0.984	0.973	0.985	0.880	0.992	0.993	0.993	0.755
	U	0.983	0.993	0.990	0.992	0.987	1.000	0.866	1.000	1.000	1.000	0.820
$\Sigma P_i$												3,634

**Table 13.** Integrated utility function values of the WASPAS-F method

	$Q_i$	$P_i$	$\Lambda$	$K$	Rank
A1	0.663	0.775		0.715	1
A2	0.601	0.714		0.653	4
A3	0.661	0.762	0.539	0.707	2
A4	0.531	0.631		0.577	5
A5	0.652	0.753		0.698	3
	3.108	3.634			

#### 4. Discussion

According to the results of the analysis conducted to select the most suitable green supplier for the supply of pesticides, cost, quality and green product were ranked in the first three places in the importance levels of the criteria. In the ranking of alternatives, alternative 1 was determined as the best supplier among five alternative suppliers.

There are many different studies on green supplier selection in the literature. However, among the studies, the study on green supplier selection especially in the agricultural sector and overlapping with this study belongs to Puska et al. Puška et al. (2022) used Z-Numbers, fuzzy LMAW and fuzzy CRADIS Model for green supplier selection using a hybrid fuzzy MCDM model in an uncertain environment in agriculture. According to the results obtained in this study, cost, quality and recycling criteria ranked in the top three in terms of importance levels. When the importance levels of the criteria are compared, it is seen that the first two rankings of the importance levels of the criteria obtained in this study and the study of Puška et al. (2022) are the same. In this direction, it can be said that the important points in selecting the most suitable green supplier are focused on cost and quality, and that price is important as the main economic indicator in the supply of agrochemicals. Therefore, according to the experts' opinion, it can be interpreted that in the selection of green suppliers, it is necessary to obtain raw materials and production materials of excellent quality and at affordable prices. However, in Puška et al. (2022) study, the third most important criterion was found to be recycling. In this study, the third most important criterion is green products. In both studies, in addition to economic criteria, ecological criteria ranked third in the ranking. In this context, it can be interpreted that ecological criteria should also be taken into consideration when selecting green suppliers, and that the raw materials and production materials supplied should not only be of good quality and affordable, but also at an environmentally acceptable level.

On the other hand, in Puška et al. (2022), the best alternatives among the six alternative suppliers were determined as alternative 2 (A2) and alternative 3 (A3). When the ranking of the alternatives is compared, it is seen that the ranking of the alternatives obtained in the Puška et al. (2022) study and this study differ. It can be interpreted that the different persons and institutions where the data were collected had an effect on this result.

#### 5. Conclusion

In this study, the most suitable green supplier for the supply of agricultural pesticides was selected by using the data collected from pesticide-using enterprises and academicians working in the related field. The importance levels of the criteria were determined with the IMF SWARA method and the most suitable green

supplier was selected with the fuzzy WASPAS method. It is thought that the results of the study will serve as a guide for both decision makers and other stakeholders and will also be an incentive for agricultural supply chain stakeholders.

Like every study, this study has various methodological limitations such as the data set, the methods used, and the criteria used. In fact, these limitations can shed light on future studies. Methodologically, new studies can be conducted in future studies by using different MCDM methods and their integrated forms. The importance levels of the criteria and green supplier selection can be evaluated by using recent methods that are not included in the literature review table (Tables 1 and 2). Data was collected in 2024 for the ten criteria identified in this study. Different indicators can be taken as criteria in future studies. On the other hand, in this study, both economic and environmental criteria are used for green supplier selection and a comprehensive green supplier selection model is proposed. In the studies to be conducted in this context in the literature, the results obtained by considering the criteria in detail can be compared and the studies to be conducted can fill an important gap.

#### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.F.A.	G.S.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### Conflict of Interest

The authors declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## DETERMINING AND MAPPING BIOMASS ENERGY POTENTIAL FROM AGRICULTURAL RESIDUES IN SYRIA

Laith GHANEM<sup>1</sup>, Gürkan Alp Kağan GÜRDİL<sup>1\*</sup>, Mohamwdeltayip Omer Salih EISSA<sup>1,2</sup>, Bahadır DEMİREL<sup>3</sup>

<sup>1</sup>Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, 55200, Samsun, Türkiye

<sup>2</sup>Bahri University, Faculty of Engineering, Department of Agricultural Engineering, Khartoum Bahri, Sudan

<sup>3</sup>Erciyes University, Faculty of Agriculture, Department of Biosystems Engineering, 38030, Kayseri, Türkiye

**Abstract:** Syria faces a problem of restricted access to fossil fuels due to limited resources. In this paper, the potential of biomass and the energy value produced from agricultural residues for 32 agricultural crops has been studied. Data from the Syrian Ministry of Agriculture for the year 2016 were utilized to determine the total annual potential of field and orchard agricultural residues using the residue-to-product ratio. The study also examined the distribution of regions with the highest production of agricultural waste in the country. The research found that approximately 1.93 million tons of agricultural residues were produced, with 0.698 and 1.213 million tons for field and orchard crops, respectively. The most significant agricultural residues came from olive trees, wheat plants, and orange trees, accounting for 35%, 11%, and 10%, respectively. The possible heat value from field and orchard crops was 23972 and 44932 Btu, respectively. This quantity provides 17.6% of Syria's energy consumption. The provinces with the highest production of agricultural residues were Aleppo, Lattakia, and Tartus, with values of 12.35, 11.8, and 8.04 PJ, respectively. According to the study, agricultural residues in Syria have the potential to be a sustainable source for biomass.

**Keywords:** Biomass, Syria, Green energy

\*Corresponding author: Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, 55200, Samsun, Türkiye

E mail: ggurdil@omu.edu.tr (G. A. K. GÜRDİL)

Laith GHANEM



<https://orcid.org/0009-0005-5195-2647>

Gürkan Alp Kağan GÜRDİL



<https://orcid.org/0000-0001-7764-3977>

Mohamwdeltayip Omer Salih EISSA



<https://orcid.org/0000-0003-0186-1112>

Bahadır DEMİREL



<https://orcid.org/0000-0002-2650-1167>

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### 1. Introduction

Syria is a country with a rich agricultural history. Historically, agriculture has been a cornerstone of the Syrian economy, providing livelihoods for a significant portion of the population. However, due to the war, oil wells ceased operations, and traditional agricultural practices were disrupted, leading to a decline in agricultural productivity. With the depletion of fossil fuel resources in Syria, according to 2017 statistics the country's production dropped to only 47.8% of its energy needs (WHO, 2022). Globally and particularly in Syria, the use of biomass from agricultural residues is considered essential to meet energy needs (Hamza, 2007). The biomass residues referred to here are the leftovers after harvesting the main crop in agriculture, including stem cutting, trimming, straw, stalks, leaves, and branches (Karaca, 2023), which are valuable energy resources. Biomass can be converted into energy production, which can be used for electricity generation and heating, serving as an alternative to traditional cooking fuels, especially in rural areas with limited access to conventional energy sources (Tun et al., 2019). By utilizing biomass energy, economic development and

increased investment can be achieved. Encouraging rural communities to engage in the collection and processing of biomass residues creates investment opportunities. Project owners can establish small facilities for biomass processing, providing employment opportunities and stimulating economic growth. This, in turn, reduces reliance on central energy networks and improves living conditions (Ginni et al., 2021). Additionally, biomass residues are directly linked to crop production during agricultural activities. The more crops produced, the more crop residues generated, as residues constitute a certain percentage of the total crop (Karaca, 2022). The energy potential of biomass can be calculated if these parameters are known. Crop production and biomass residues, along with their agricultural development, depend on environmental factors such as climate and soil (Avcıoğlu et al., 2019).

Several studies have been conducted on the potential of biomass resources worldwide. These studies have been published to assess agricultural biomass residues and their potentials. (Shahbeik et al., 2024) it was found that converting agricultural residues into biofuel using the Hydrothermal Liquefaction (HTL) method holds promise



in alleviating financial burdens associated with fossil fuel use, (Wang and Wu, 2023) found that biomass has proven itself as a primary fuel, contributing to reducing carbon emissions in the electricity grids of the United Kingdom. Therefore, it is regarded as highly important for mitigating greenhouse gas emissions.

(Naeimi et al., 2023) studied the possible heat value of agricultural residues available for 10 agricultural products in Azerbaijan, and the value was found to be 19.61 T.J. The most contributing crops were thin maize and tobacco. (Askarova et al., 2022) studied the potential of renewable energy in Kazakhstan and found that the country could annually produce 37.26 million tons of biomass resulting from waste, with the potential to generate 466.47 P.J of energy. This accounts for 61% of the country's total energy production. The study also highlighted that dry agricultural residues could be burned with coal in power plants. (Avcıoğlu et al., 2019) identified the agricultural biomass energy potential in Türkiye. The study utilized characteristics of agricultural residues, moisture levels, and low heat values for dry matter. A mathematical model was developed to calculate the energy potential of agricultural biomass residues. The theoretical biomass quantity and energy potential were determined for field crops and orchard crops in Türkiye, amounting to 59.43 kilotons and 15.882 kilotons, respectively. The total available energy from biomass residues was estimated at 298.955 T. joules for field crops and 65.491 T.J for orchard crops.

Demirel et al. (2019) utilized the waste-to-product ratio to study the energy potentials of crop residues in Sudan. The thermal energy was approximately 154 gigajoules for the 2015 crop, with thin maize being the largest contributor. Karaca (2019) determined the biomass potentials and the possible energy production values for agricultural residues in the Hatay province. The total thermal value of agricultural residues was found to be 13.36 gigajoules. The aim of this study is to identify the biomass potentials and energy produced from agricultural residues in Syria to reduce dependence on imported fuels and maintain energy security.

Tun and Juchelková (2019) studied the importance of using biomass energy in the agricultural and livestock sectors to mitigate the consumption of fossil fuels in the energy sector. They found that biomass energy could cover 50% of the total energy consumption in the country. The energy generated from residues was 15.9 million tons of oil equivalent (Mtone). Karaca et al. (2017) studied the potential of agricultural biomass residues in the Samsun province of Türkiye. The total heating value (THV) was found to be 6.46 GJ, with hazelnuts being the major contributor.

In the first part of the study, agricultural biomass residues in Syria were examined. The structural and physical characteristics of different crop types were determined to obtain the energy potential of biomass residues. These characteristics included the residue-to-product ratio, residue moisture, and residue energy

value. Based on these values, it was possible to identify agricultural crops containing biomass residues with higher energy potentials. In the second part of the study, theoretically, the potential energy values available for Syria were calculated using computed values such as residue moisture and residue product ratio. The study explored crops that produce larger biomass and, consequently, higher energy potentials. It is important to know the regions where crops are intensively cultivated, the types of residues they produce, and the characteristics of these residues, as well as their energy capacities. This information is crucial for making informed decisions about the installation of renewable biomass energy stations and sustaining energy supplies based on biomass residue potentials.

## 2. Materials and Methods

The study was conducted in the Syrian Arab Republic, situated between latitude 32 - 37.5 degrees north and longitude 35.5 - 42 degrees east of Greenwich. Syria is considered part of the Asian continent, covering an area of 185,180 square kilometers, divided into 14 provinces: Damascus, Rural Damascus, Homs, Hama, Aleppo, Lattakia, Tartus, Sweida, Daraa, Quneitra, Idlib, Al-Raqqa, Dair-Ezzor, and Hassakeh. It should be noted that Damascus is solely a residential area and does not contain agricultural lands. The Mediterranean climate predominates in the coastal region, while the climate varies based on geographical location and topography. The coastal areas experience a more moderate climate, with hot and dry summers and mild, rainy winters. The vegetation consists mainly of shrubs influenced by the Mediterranean climate. The central and eastern regions of Syria, on the other hand, have a desert climate.

According to data from the Syrian Ministry of Agriculture, the arable land in Syria amounts to 6.082 million hectares, of which 5.77 million hectares are utilized. Agricultural production for the studied field crops in this research reached 4.215 million tons, and 2.966 million tons for orchard crops. The studied area for field crops encompasses 2.711 million hectares, while orchard agricultural land covers 1.042 million hectares. In total, the areas studied in the research constitute approximately 3.75 million hectares, representing 65% of the total cultivated agricultural land in Syria.

### 2.1. Selection of Agricultural Crops for Biomass Residue

An annual production energy of 4.2 million tons of field crops suitable for agriculture and over 2.865 million tons of orchard crops was chosen for evaluation of the biomass potential in Syria. A total of 32 different crops were considered in two categories. These are listed below:

- Field Crops Studied: Wheat, barley, potatoes, corn, cotton, sunflowers, beans, lentils, tomatoes, red watermelon, onions, chickpeas, sugar beets, peanuts, sesame, and tobacco.



- Orchard Crops: Olives, grapes, apples, oranges, mandarins, lemons, apricots, plums, peaches, pomegranates, cherries, pears, figs, pistachios, walnuts, and almonds.

Based on the 2016 statistics from the Ministry of Agriculture and Agrarian Reform, the annual production quantities for the 32 crops in the country were collected. Residue types from crops (straw, stalks, peels, stem leaves, pruning, etc.) were selected, and the amount of waste production, its percentage, and its lower heating value (LHV) were obtained. Data analysis was performed based on the physical characteristics of agricultural and orchard crop residues presented in Table (1). The crop product quantity (AAP), residue product ratio (RPR), lower heating value (LHV), and availability ratios (A) were used in the mathematical model that was introduced. The total heat value from agricultural production were calculated, as shown in the flowchart in Figure (1).

**2.2. Calculation of Available Agricultural Residues (AAR):**

The value of ARR (Available Agricultural Residues) represents the total annual production of biomass

obtained from agricultural residues. The ARR value varies depending on the quantities of agricultural production in tons (AAP), the percentage of residue product ratio (RPR), and the percentage availability of residues (A). ARR is calculated according to the equation 1 (Karaca, 2015)

$$ARR = AAP * RPR * A \tag{1}$$

Residue product ratio (RPR) vary from one region to another depending on agricultural practices and alternative uses of residues. For example, when rice is cut about 5 cm above the ground, the RPR value is 1.75; if it is cut more than 5 cm during harvesting, the RPR will decrease by up to 0.452 (Avcioglu et al., 2019).

**2.3. Calculating the Potential Available Energy**

Equation 2 below was utilized to compute the potential energy available for dry biomass: (Jorjani et al., 2021).

$$THV = AAR * LHV \tag{2}$$

where THV is the total heat value of agricultural residues in GJ, and LHV is the lower heat value of dry crop residues in MJ.kg<sup>-1</sup>. Values for PRP, A, and THV were obtained from previous research, as presented in Table 1.

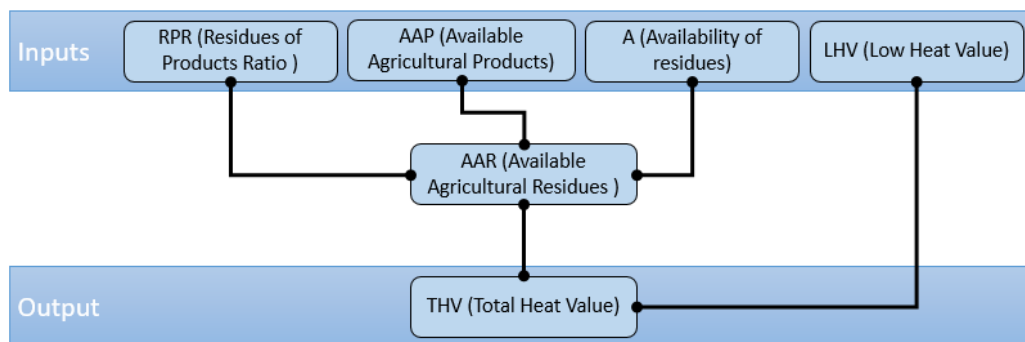


Figure 1. is a flowchart for calculating the total heat value.

Table 1. Residue of products ratio, availability, and THV for residues of major agricultural crops in Syria

Crops	(AAP)	Area	Residues	RPR	A%	LHV	Reference
	1000 Ton	1000 Ha					
Wheat	1726	1179	Straw	0.8	15	17.9	(Karaca et al., 2017)
Barley	954	1244	Straw	0.9	15	17.5	(Karaca et al., 2017)
Potato	507	22	Vines	0.1	50	15.34	(Soucek and Jasinskas, 2020)
Tomato	415	9	Stalks	0.3	60	13.7	(Akinbomi et al., 2014)
Watermelon	213	7	Stalks	0.15	50	20.5	(Ronzon and Piotrowski, 2017)
Lentils	113	123	Stalks	1.74	20	14.7	(Unal and Alibas, 2007)
Maize	79	18	Stalks	1	60	18.5	(Karaca et al., 2017)
	79	18	cob	0.64	60	18.4	(Karaca et al., 2017)
Dry Onion	79	5	Husk	0.1	100	16.51	(Malat'ák and Dlabaja, 2016)
Cotton	41	17	Stalks	2.3	60	18.2	(Karaca, 2019)
Chickpeas	31	56	Stalks	1.3	60	18.5	(Karaca, 2019)
Sugar beet	11	12	Roots	1	40	17.21	(Brachi et al., 2017)
Tobacco	8	0.3	Roots	2.27	60	16.1	(Turker et al., 2022)
Peanut	7	7	Stalks	1.5	60	18	(Gao et al., 2016)
	7	7	Hull	0.28	60	18	(Gao et al., 2016)

**Table 1.** Residue of products ratio, availability, and THV for residues of major agricultural crops in Syria (continue)

Crops	(AAP)	Area	Residues	RPR	A%	LHV Mj	Reference
	1000 Ton	1000 Ha					
Beans	26	7	Root- Leaf	1.45	15	14.7	(Turker et al., 2022)
Sunflower	3	3	Stalks	2.5	60	14.2	(Karaca, 2019)
Sesame	2	2	Stalks	0.5	56	12.4	(Demirel et al., 2019)
Total	4215	2711.3					
<b>Fruits Crops</b>							
Orange	725	26	Pruning	0.35	80	18.1	(Turker et al., 2022)
Olives	668	692	Pomace	0.4	90	19.7	(Karaca, 2019)
	668	692	Pruning	1.2	50	18.5	(Turker et al., 2022)
Apples	452	52	Pruning	0.19	80	17.8	(Turker et al., 2022)
Mandarin	260	11	Pruning	0.28	80	17.6	(Turker et al., 2022)
Grapes	213	47	Pruning	0.42	80	18.0	(Turker et al., 2022)
Lemon	188	7	Pruning	0.3	80	17.6	(Turker et al., 2022)
Cherries	76	29	Pruning	0.19	80	21.7	(Turker et al., 2022)
Pomegranate	3149**	5	Pruning	9	80	17	(Karaca, 2019)
	69.9						
Almond	55	72	Pruning	0.6	80	18.2	(Turker et al., 2022)
Peach	52	7	Pruning	0.4	80	18.2	(Turker et al., 2022)
Apricot	50	14	Pruning	0.19	80	20	(Turker et al., 2022)
Pistachio	50	60	Pruning	0.44	80	18.5	(Turker et al., 2022)
Fig	39	9	Pruning	0.21	80	18.2	(Turker et al., 2022)
Plum	1450**	4	Pruning	7	80	17.3	(Karaca, 2019)
	31.1						
Pear	26	4	Pruning	0.22	80	18.2	(Turker et al., 2022)
Walnuts	11	3	Pruning	0.66	50	19	(Gürdil et al., 2021)
Total	2966	1042					
Total Summation	7181	3753.3					

\*\*1000 trees.

### 3. Results and Discussion

Agricultural residues in the studied crops amounted to 0.698 and 1.213 million tons for field and orchard crops, respectively. The potential heat value from field and orchard crops was 23.972 and 44.932 million gigajoules. For field crops, Table (2) indicates that the per-hectare production of agricultural residues is 14.64 tons. This value reflects a high productivity level for agricultural residues. The per-hectare production in Syria for tomatoes is good, reaching 8.3 tons, while corn and cotton yield 4.318 and 3.328 tons of agricultural residues per hectare, respectively. In orchard crops, the per-hectare productivity of orange agricultural residues is 7.8 tons compared to olives, which amount to 0.92 tons per hectare. The per-hectare productivity of mandarins and lemons is high, reaching 5.294 and 6.445 tons, respectively. On the other hand, barley and wheat have low per-hectare productivity, amounting to 0.103 and 0.175 tons, respectively.

Based on the agricultural land area for each crop and the per-hectare productivity of agricultural residues, Figure (2) illustrates the percentage of agricultural residues for each crop. In Syria, olives constitute 35% of the total weight of agricultural residues, primarily due to the extensive cultivation areas and the utilization of olive

pomace as agricultural residue. Oranges constitute 10% of agricultural residues despite being cultivated on only 26 thousand hectares. The high per-hectare productivity of agricultural residues contributes significantly to this percentage. Apples represent 6% of agricultural residues and are cultivated on 52 thousand hectares. Corn accounts for 4% of the weight percentage of agricultural residues and is grown on an area of 18 thousand hectares. Both cotton, tomatoes, and mandarins each contribute 3% to the total weight of agricultural residues. However, it's worth noting the high per-hectare productivity of tomato residues, cultivated on only 9 thousand hectares but with a yield of 74.7 thousand tons. In contrast, cotton is grown on 17 thousand hectares with a yield of 56.58 thousand tons.

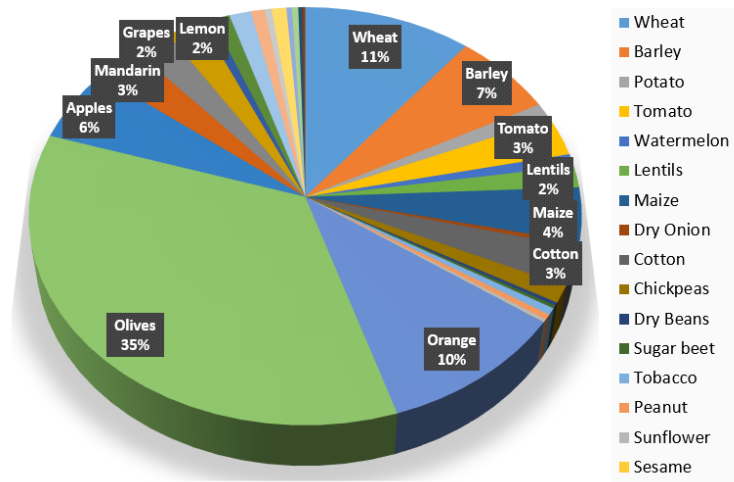


Figure 2. AAR of major crops in Syria (%).

Table 2. Values of AAR and THV variables and hectare productivity

Crops	Area (1000 Ha)	AAR (Ton)	THV (Gj)	AAR/ha
Wheat	1179	207120	7414896	0.175
Barley	1244	128790	4507650	0.103
Potato	22	25350	777738	1.152
Tomato	9	74700	2046780	8.3
Watermelon	7	15975	654975	2.282
Lentils	123	39324	1156125.6	0.319
Maize	18	77736	2870165	4.318
Dry Onion	5	7900	260858	1.58
Cotton	17	56580	2059512	3.328
Chickpeas	56	24180	894660	0.431
Dry Beans	12	10400	357968	0.86
Sugar Beet	0.3	4400	482420.4	14.64
Tobacco	7	7200	259200	1.02
Peanut	7	2698.5	87097.5	0.38
Sunflower	2	4500	127800	2.25
Sesame	3	560	13888	0.186
Total	2711.3	697995.5	23971733.5	
Fruits				
Orange	26	203000	7348600	7.80
Olives	692	641280	24304512	0.92
Apples	52	68704	2445862.4	1.321
Mandarin	11	58240	2050048	5.294
Grapes	47	71568	2576448	1.52
Lemon	7	45120	1588224	6.445
Cherries	29	11552	501356.8	0.398
Pomegranate	5	22672.8	770875.2	4.53
Almond	72	26400	960960	0.366
Peach	7	16640	605696	2.37
Apricot	14	7600	304000	0.542
Pistachio	60	17600	651200	0.293
Fig	9	6552	238492.8	0.728
Plum	4	8120	280952	2.03
Pear	4	4576	166566.4	1.14
Walnuts	3	3630	137940	1.21
Total	1042	1213254.8	44931733.6	
Total summation	3753.3	1911250.3	68903467.1	

**Table 3.** Distribution of agricultural residues and the amount of the annual total calorific value in the Syrian governorates and their percentages

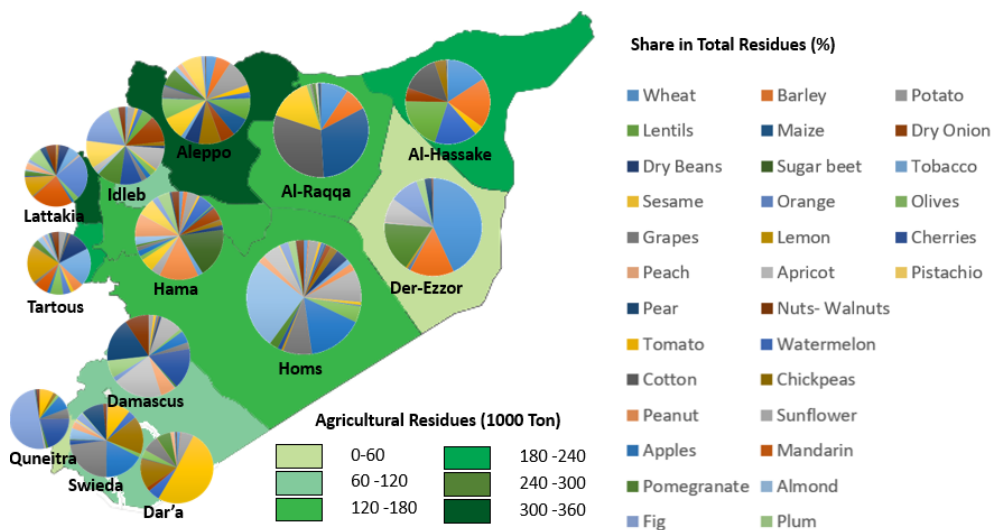
Governorate	Residues-AAR (Ton)	Share in Total Residues (%)	Total Heating Value – THV. (GJ)	Share in Total Heating (%)
Aleppo	345078	18.09	12569097	18.28
Lattakia	330558	17.32	11992486	17.43
Tartous	218946	11.47	8072696	11.74
Al-Hassake	206344	10.82	7198417	10.47
Al-Raqqa	159741	8.37	5808792	8.44
Hama	160847	8.43	5771469	8.39
Homs	134816	7.06	4948290	7.19
Idleb	101155	5.3	3670342	5.33
Damascus Countryside	81495	4.27	3015555	4.38
Dar'a	81022	4.24	2622933	3.81
Sweida	58014	3.04	2047163	2.98
Dair-Ezzor	24137	1.26	860117	1.25
Quneitra	5299	0.27	188403	0.27
Total	1907458	100	68765766	100

Table (3) shows the distribution of agricultural waste in each governorate and the percentage of agricultural waste in each governorate. It can be noted that the city of Aleppo is more productive of agricultural waste, as it produces 345.07 kilotons of agricultural waste, or about 18.09% of the total weight value of agricultural waste, as it accounts for 40.6%. Of potato production, 32.5% of chickpea production, 29.5% of sesame production, and 29.9% of pistachio production, Figure (3), and the resulting agricultural waste can generate thermal energy amounting to 12.57 PJ, Table (3).

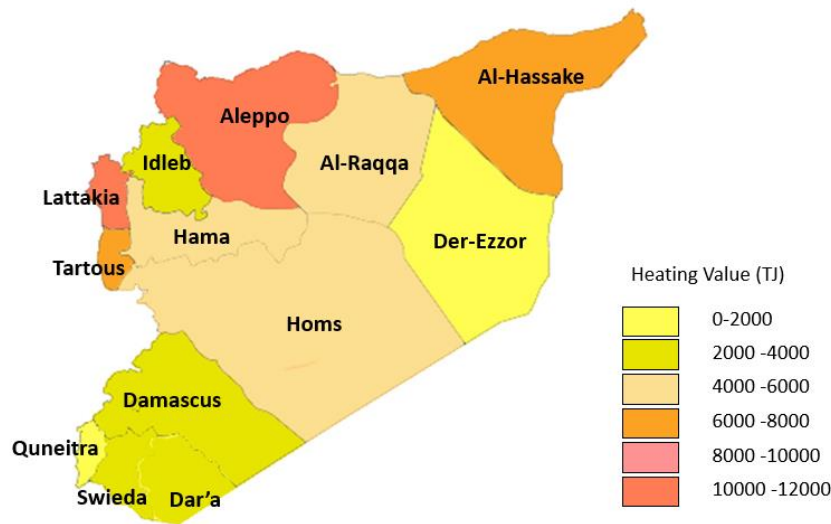
According to the table, Lattakia province produces 330.5 kilotons of agricultural residues, approximately 17.32% of the total quantity. Despite its smaller area compared to eastern cities such as Al-Hassakeh and Deir Ezzor, Lattakia plays a significant role due to cultivating 87.5% of oranges and 78.8% of mandarins, resulting in higher agricultural residue production. The estimated annual thermal energy value obtainable from Lattakia is 11.99

GJ. Tartus province produces 218,946 kilotons of agricultural residues, accounting for 11.47% of the total quantity. Tartus province contributes significantly to the production of 58.2% of lemons, 33.9% of dry beans, 20% of olives, and mandarins.

The city with the least production of agricultural residues is Quneitra due to its small area of 180 square kilometers. As for Dair-Ezzor province, the limited agricultural residue production can be explained by the ongoing war. In 2011, it produced 47,159 tons of corn, 105,029 tons of cotton, 185,258 tons of sugar beets, and 2,119 tons of sesame) (Syrian Ministry of Agriculture, 2011). However, there are no available data for these crops in 2016. Al-Raqqa and Al-Hassakeh, despite dominating wheat and barley production, contribute 10.47% and 8.44% of agricultural residues, respectively, due to the diversity of crops cultivated in Al-Hassakeh, as shown in Table (3).



**Figure 3.** Map of agricultural residues distribution in Syria.



**Figure 4.** The distribution map of heating value based on agricultural residues in Syria.

**Table 4.** The production per hectare from agricultural residues in several countries compared to Syria

Country	Arable land 10 <sup>6</sup> Ha	AAR 10 <sup>6</sup> Ton	Productivity (Ton/Ha)	References
Türkiye	23.95	75.084	3.13	(Avcıoğlu et al., 2019)
Sudan	19.82	11.2	0.56	(Demirel et al., 2019)
Azerbaijan	2.09	1.09	0.52	(Naeimi et al., 2023)
Syria	3.75	1.91	0.51	

From Table (3), we observe that the production per hectare from agricultural residues is acceptable compared to Sudan and Azerbaijan but is low compared to Türkiye. The results can be explained by the soil fertility, crop diversity, and the utilization of modern technology in agriculture (Akkoyunlu, 2013). Additionally, the war in Syria played a significant role in the decline of agricultural production.

#### 4. Conclusion

Syria is an agriculturally rich country with diverse crops, and the agricultural residues can be utilized for energy generation. This paper identified the distribution of agricultural residues in the Syrian Arab Republic and the total heat value that can be obtained annually from each province. The importance of this paper lies in Syria being an energy-importing country in need of sustainable energy. The total quantity of unused agricultural residues in Syrian lands for 2016 was 1.907 million tons, 698 and 1.213 million tons for field and orchard crops, respectively. The total calorific value obtained was 68.76 gigajoules. Olive, wheat, oranges, and barley accounted for 35%, 11%, 10%, and 7% of agricultural residues, respectively. Aleppo, Lattakia, and Tartous were the top provinces in terms of calorific value production, with percentages of 18.09%, 17.34%, and 11.47%, respectively.

The sustainability of biomass residues, especially in regions cultivating olives, citrus fruits, wheat, and barley, is crucial for choosing and establishing biomass energy stations. Energy can be obtained through pellet or

briquette technology from wheat, barley straw, and olive pomace. Corn residues, with their high moisture content, and the pulp resulting from olive processing, are valuable biomass residues for biogas production. In addition to biomass energy conversion methods, utilizing biomass with techniques for biomass use, fertilizer production, construction materials, chipboard production, and the production of bio-based products like bio-plastics are feasible. Obtaining higher value-added biological products and energy with minimal residues in the bio-refinery system is possible. Agricultural residues can be used as inputs for bio-based products in Türkiye. However, regulatory and financial challenges in collecting and transporting agricultural residues, coupled with a lack of public awareness about their use, pose challenges for ensuring economic sustainability and energy security for Syria, which imports most of its energy needs.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	L.G.	G.A.K.G.	M.O.S.E.	B.D.
C	60	20	10	10
D	50	30	10	10
S		80		20
DCP	70	10	10	10
DAI	50	20	10	20
L	25	25	25	25
W	25	25	25	25
CR	20	30	20	30
SR	25	25	25	25
PM	25	25	25	25
FA	25	25	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## AGAROSE-RESOLVABLE SSR MARKERS BASED ON ddRADSeq IN CHICKPEA

Duygu SARI<sup>1\*</sup>


<sup>1</sup>Akdeniz University, Faculty of Agriculture, Department of Field Crops, 07070, Antalya, Türkiye

**Abstract:** Exploitation of genetic diversity is essential for sustainable crop production. Molecular markers have potential to reach these goals in more rapid and efficient manner. Here, we developed genomic SSR markers from chickpea with the use of ddRADSeq data. 1,396 SSR regions with an average of 530 SSR/Mb in the whole genome were successfully identified. Considering different types of repeats, dinucleotides were the most frequent type accounting for 62.03% of the total SSR regions identified, followed by trinucleotides (25.50%) and tetranucleotides (4.58%). The AT/TA motif was greatly characterized among dinucleotide repeats, and it was also the most common type in the chickpea genome accounting for 36.5% of the total SSR regions identified, followed by AG/GA (139) and TC/CT (135) among dinucleotide motifs. Considering their genomic distribution and simple visualization on agarose gels, we examined SSR regions of 10 bp and longer for identification of SSR markers. A total of 10 SSR markers were successfully designed and resulted in successful polymorphic bands among chickpea genotypes. Consequently, the results show that ddRADSeq is effective for marker development and these markers might be valuable for biodiversity studies, marker-assisted selection (MAS) and linkage map construction in chickpea.

**Keywords:** Chickpea, ddRADSeq, Marker, SSR, Sequencing

\*Corresponding author: Akdeniz University, Faculty of Agriculture, Department of Field Crops, 07070, Antalya, Türkiye

E mail: duygusari@akdeniz.edu.tr (D. SARI)

Duygu SARI  <https://orcid.org/0000-0002-7909-2627>

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### 1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most significant legume crops in the family Fabaceae, subfamily Faboideae (van der Maesen et al., 2007). *C. arietinum* is the only cultivated species of the genus *Cicer*. The cultivated chickpea is considered to be evolved from its wild ancestor, *C. reticulatum* Ladiz by selection (van der Maesen, 1987). Chickpea is mainly cultivated in India, Australia, Ethiopia, Türkiye and Myanmar (FAOSTAT, 2022). It was globally grown in 14.8 million ha and world chickpea production was about 18.1 million tons in 2022 (FAOSTAT, 2022). Chickpea is a diploid ( $2n = 2x = 16$ ) and self-pollinated plant having a genome size of approximately 740 Mb (Varshney et al., 2013). It is high in carbohydrates (60-65%), plant-based protein (20-22%) and fat (6%), and a good source of vitamins (vitamin A, B, folate, and thiamine) and minerals (iron, potassium and zinc) (Gaur et al., 2016). It also plays significant role in the soil fertility enrichment and crop rotation because of its capacity to fix the atmospheric nitrogen (Herridge et al., 1993).

The chickpea production has been influenced by many environmental factors in the worldwide (Sari et al., 2022). Because genetic diversity in the cultivated chickpea was limited, an attempt to increase production has not been sufficient (Roorkiwal et al., 2014). The prime objective of chickpea breeding is improving high-

yield and high-quality varieties. Molecular-marker assisted breeding have potential to reach these goals in more rapid and efficient manner. Molecular markers have the potential to reveal the genetic diversity among genotypes (Cui et al., 2017) and also efficient tools for biodiversity studies, segregation analysis, construction of genetic physical and genetic maps as well as transcript profiling (Singh et al., 2010). So far, random amplified polymorphic DNA (RAPD) (Iruela et al., 2002; Talebi et al., 2008), amplified fragment length polymorphism (AFLP) (Nguyen et al., 2004; Shan et al., 2007), simple sequence repeat (SSR) (Sethy et al., 2006), inter simple sequence repeat (ISSR) (Sudupak, 2004; Amirmoradi et al., 2012; Aggarwal et al., 2015) and internal transcribed spacer (ITS) (Singh et al., 2008) have been conducted to identify genetic diversity in chickpea. In recent years, the advances of high-throughput sequencing techniques (or next-generation sequencing (NGS)) have prompted the identification of high-quality markers such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers in various natural or mapping populations of chickpea (Gujaria et al., 2011; Gaur et al., 2012; Kujur et al., 2015; Sari et al., 2023). Opposite to SNP markers, SSRs are very simple and practical. They are also greatly informative, abundant in the genome, multiallelic and locus specificity, and have been widely utilized because of the co-dominance and



highly reproducible nature for plant breeding applications (Davey et al., 2011; Sakiyama et al., 2014). Several number of SSR markers have also been applied in genetic diversity studies (Seyedimoradi et al., 2019), linkage mapping (Ahmad et al., 2014) and QTL analysis for the identification of candidate genes (Jha et al., 2019). Nevertheless, the identification of markers might be restricted due to the narrow genetic base in chickpea (Sethy et al., 2006; Gujaria et al., 2011). So, it is crucial to find alternative markers for chickpea genomics-assisted breeding applications.

With an aim of enhancing the marker repository and development of the breeder friendly markers in chickpea, the present study focused on identification and development of SSR markers with the use of double digest restriction site-associated DNA sequencing

(ddRADSeq) data from 20 chickpea accessions compared with a reference genome sequence. The developed markers were also tested on chickpea germplasm with a simple and low-cost agarose gel electrophoresis.

## 2. Materials and Methods

### 2.1. Plant Material

A total of 20 chickpea accessions from nine different regions in Africa, America, Asia, and Europe were evaluated for ddRADSeq analysis in this study (Table 1). The highest number of accessions was from India (8), followed by Spain (3), Türkiye (2), Russian Federation (2), Mexico (2), Iran (1), the United States (1) and Ethiopia (1). The seeds of each accession in the collection were sown in pots for DNA analysis.

**Table 1.** List of the chickpea genotypes used ddRADseq analysis

No	Species	Kabuli/Desi	Gen bank Number/Name	Origin	Continent
1	<i>Cicer arietinum</i>	Kabuli	ILC 200	Russian Fed	Europe
2	<i>Cicer arietinum</i>	Desi	ICC 552	India	Asia
3	<i>Cicer arietinum</i>	Kabuli	ILC 3507	Spain	Europe
4	<i>Cicer arietinum</i>	Desi	ICC 506	India	Asia
5	<i>Cicer arietinum</i>	Desi	ICC 988	Mexico	America
6	<i>Cicer arietinum</i>	Desi	ICC 5912	India	Asia
7	<i>Cicer arietinum</i>	Kabuli	ILC 3500	Spain	Europe
8	<i>Cicer arietinum</i>	Desi	ICC 5714	India	Asia
9	<i>Cicer arietinum</i>	Desi	ICC 8325	India	Asia
10	<i>Cicer arietinum</i>	Desi	ICC 5434	India	Asia
11	<i>Cicer arietinum</i>	Desi	ICC 7509	Iran	Asia
12	<i>Cicer arietinum</i>	Desi	ICC 4929	India	Asia
13	<i>Cicer arietinum</i>	Desi	ICC 12031	Mexico	America
14	<i>Cicer arietinum</i>	Desi	ICC 10301	USA	America
15	<i>Cicer arietinum</i>	Kabuli	Hasanbey	Türkiye	Europe
16	<i>Cicer arietinum</i>	Kabuli	CA 2969	Spain	Europe
17	<i>Cicer arietinum</i>	Desi	ICC 1069	Russian Fed	Europe
18	<i>Cicer arietinum</i>	Desi	ICC 8262	Türkiye	Europe
19	<i>Cicer arietinum</i>	Desi	ICC 533	India	Asia
20	<i>Cicer arietinum</i>	Desi	ICC 8617	Ethiopia	Africa

### 2.2. DNA Extraction

Extraction of genomic DNA was conducted using the cetyltrimethylammonium bromide (CTAB) method given by Doyle and Doyle (1990) with some modifications such as the use of extra chloroform:isoamyl alcohol and 70% ethanol cleaning steps to increase DNA purity. Quality of extracted DNA was checked on a 1% agarose gel.

### 2.3. Library Preparation, Sequencing and SSR Identification

The ddRADseq library involved using a modified version of the protocol from Peterson et al. (2012). Main difference was that we used different restriction enzymes. Briefly, for the library preparation, we digested ~200 ng DNA per sample with two restriction enzymes, *VspI* (Asel, Thermo Fisher) and *EcoRI* (methylation sensitive, Thermo Fisher), and ligated P1 and P2 adapters to the fragments' restriction ends. Before ligation,

Ampure XP beads (Beckman Coulter Genomics) were used to clean digestion products. After ligation, 15 cycles of PCR amplification with genotype specific indexed primers were performed. The PCR products were visualized on an agarose gel and combined and equalized in concentration. The genomic library with insert size of 300–450 bp was run on Illumina HiSeq platform using the 2x150 bp paired-end sequencing protocol. The ddRAD sequencing data of 20 accessions have been deposited in the National Center for Biotechnology Information (NCBI) Sequence-Read Archive (SRA) database with the accession number of PRJNA1064701.

For bioinformatic analysis, the raw data were demultiplexed with *Je* (v1.2) (Girardot et al., 2016). Quality control and preprocessing of FASTQ files were done using *fastp* (Chen et al., 2018), and reads were trimmed by removing bases with quality score with an



average Phred score less than 15. The cleaned data were mapped to kabuli reference genome 1.0 (Varshney et al., 2013) using Bowtie 2 (v2.2.6) (Langmead and Salzberg, 2012). Variant calling was performed in FreeBayes (Galaxy Version 1.1.0.46-0) (Garrison and Marth, 2012) with genotype specific individual 'alignment files in BAM format' by selecting following parameters: simple diploid calling with filtering, and coverage of 20X. SNPs were removed from the variant files using VCFfilter (Galaxy Version 1.0.0). The separate .vcf files containing insertions and deletions were merged into a single data file. The combined variant file was organized in Microsoft Excel in order to remove duplicated regions and arrange the SSRs by sizes. SSR regions were visualized with Integrated Genome Browser (IGB) (Freese et al., 2016) using BAM files of the genotypes and the chickpea reference genome.

**2.4. Primer Design and PCR Amplification**

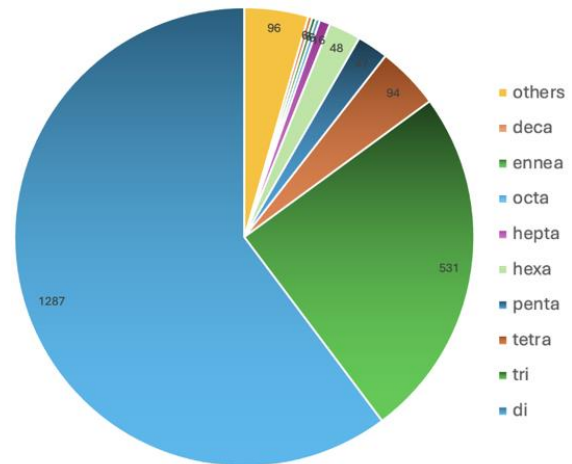
To develop the SSR markers, flanking sequences of the identified SSRs were extracted as the target sequence based on the chickpea reference genome by using IGB software. For designing forward and reverse primers, Primer3Plus (Untergasser et al., 2007; <https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>) was used with the following characteristics: optimal length of primers of 18–27 bp, melting temperature (Tm) between 50 and 60 °C, 30–70% GC, and PCR amplicons of 150–500 bp. The primer pairs were checked for possible duplication using IGB software. The designed primers were later controlled for possible matches of with other loci in the genome. All markers were termed as CA-D(I)-X-XXX format, where "CA" stands for chickpea, "D" and "I" for deletion and insertion, "X" for chromosome number, and "XXX" for start of the chromosomal position.

10 SSR markers which were evenly distributed on each chromosome, were selected from the designed primers pairs to be validated on 20 chickpea germplasm. For PCR analysis, a total volume of 20 reaction mix was used, which included 1 µL of genomic DNA, 1 µL of 10× PCR buffer, 2.5 mM MgCl<sub>2</sub>, 0.3 µL of 10 mM dNTP mix, 0.3 µL of each primer (10 µM), 0.2 µL of Taq DNA polymerase (5 U/µL), and ddH<sub>2</sub>O. The PCR reaction was conducted with the following conditions: 95 °C for 5 min; followed by 29 cycles of 95 °C for 20 s, 60 °C for 40 s, and 72 °C for 40 s; 7 cycles of 95 °C for 20 s, 55 °C for 40 s, and 72 °C for 40 s; and extension at 72 °C for 7 min (Sari et al., 2023). The PCR products were visualized on 3% agarose gels and recorded as codominant data, with genotypes by fragment size.

**3. Results**

A total 349.86 M raw sequence reads of 20 chickpea accessions with the mean of 3.68 M was generated from the Illumina HiSeq platform. The guanine-cytosine (GC) content of the reads was 38%. Using variant calling pipeline, 1,396 microsatellites were identified among the accessions, with an average of 530 SSR/Mb. Motifs

ranged from 2 to 17 bp in length. Considering different types of repeats, dinucleotide motifs were the most frequent type corresponding to 62.03% of the total SSR regions identified, followed by trinucleotides (25.50%) and tetranucleotides (4.58%), while octa-nucleotide motifs were the rarest repeat (1.46 %) (Figure 1).



**Figure 1.** Distribution of SSRs in different classes.

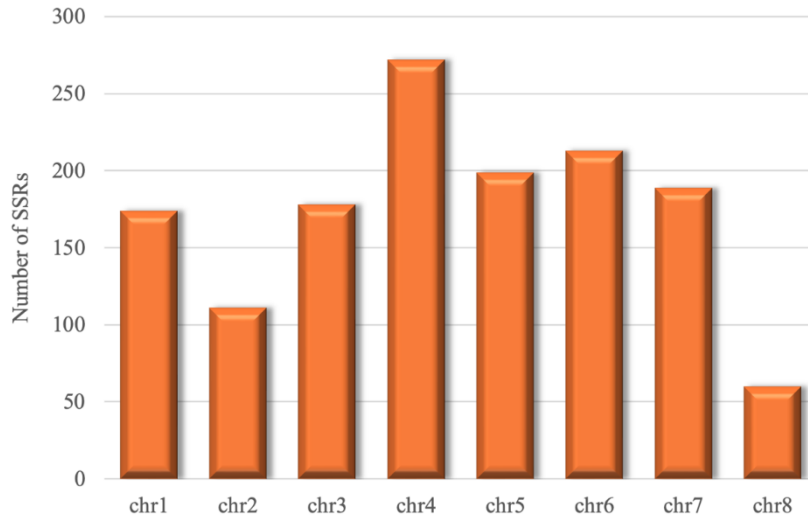
All the SSRs distributed across all 8 chromosomes (Figure 2), with a correlation between the frequency of microsatellite loci and the chromosome size. For instance, chromosome 4 had the greatest frequency of 19.48 %, it was also one of the largest chromosomes. The greatest number of microsatellites was occurred on chromosome 4 (272), followed by chromosomes 6 (213) and 5 (199), and the smallest number of SSR was observed on chromosome 8 (60) (Figure 2).

The investigation of nucleotide composition characteristics showed that some motifs were more common than others. For instance, the AT/TA motif greatly characterized among dinucleotide repeats, and it was also the most common type in the chickpea genome accounting for 36.5% of the total SSR regions identified, followed by AG/GA (139) and TC/CT (135) among dinucleotide motifs. Trinucleotides (25.50%) and tetranucleotides (4.58%) were other abundant repeat types in chickpea genome. Among trinucleotides, the AAT/ATT (184) was the most abundant followed by GAA/CTT (68), whereas, among tetranucleotides, TAAA/TTTA (28) were most abundant type.

Considering their genomic distribution and simple visualization on agarose gels, we examined SSR regions of 10 bp and longer for identification of SSR markers (Table 2). A total of 10 SSR regions was successfully designed. The SSRs were dispersed across the 8 chromosomes. The chromosomal position, size information and repeat size of SSR regions were shown in Table 2. The longest polymorphic repeat (28 bp) was in chromosome 7 (physical position: 3887904), followed by chromosome 1 (physical position: 26908497), chromosome 2 (physical position: 35938514) and chromosome 5 (physical position: 38571762) with 18, followed by chromosome 2 (physical position:

36011349) with 16. Figure 3 showed the PCR aplicons which were generated with these primers. Annotation analysis of SSRs showed their highest frequency in

intergenic regions (66.6%), followed by coding sequences (CDS) (22.2%), and exons (22.2%) (Table 3).



**Figure 2.** Distribution of all SSRs on 8 chromosomes (chr1-chr8) of chickpea.

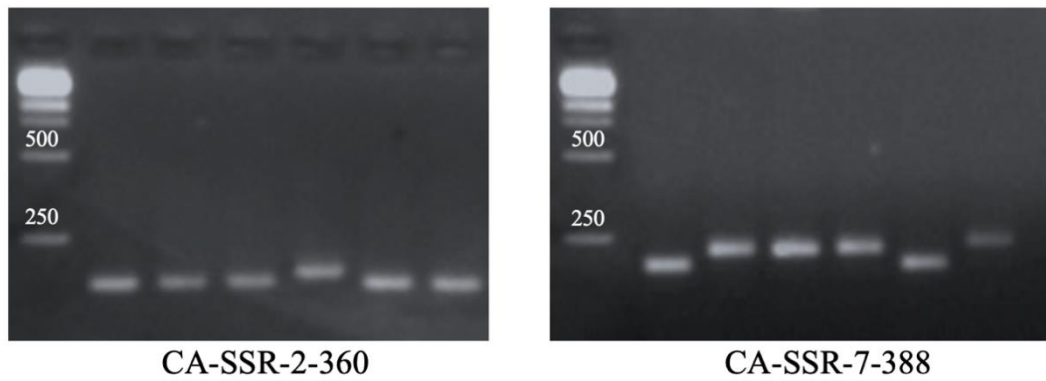
**Table 2.** Information about SSRs identified in this study

Marker Name	Chromosome	Physical Position	Size (bp)	Repeat
CA-SSR-1-269	chr1	26908497	18	(ATA)
CA-SSR-1-221	chr1	2218745	14	(AATTTTC)
CA-SSR-2-359	chr2	35938514	18	(AGA)
CA-SSR-2-360	chr2	36011349	16	(CT)
CA-SSR-4-343	chr4	34309035	15	(TTA)
CA-SSR-5-385	chr5	38571762	18	(TG)
CA-SSR-7-388	chr7	3887904	28	(CT)
CA-SSR-7-630	chr7	6301545	15	(AATAT)
CA-SSR-7-165	chr7	16500535	12	(TAAAAA)
CA-SSR-8-101	chr8	10131288	12	(TAACCC)

**Table 3.** The primer sequences of the 10 SSR markers developed and used in this study

Marker Name	CHR	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	PL	Locus Location *
CA-SSR-1-269	chr1	AATGAATGAATTTATGGAACAATTT	GGCACTCCCTCCATTAGAA	258	intergenic region
CA-SSR-1-221	chr1	CAAGATGTGCTAAAGCTTACAAA	CCAAGGAATGGGAAAGGACT	180	intergenic region
CA-SSR-2-359	chr2	TTTCATTGCTAGGACCACCA	CTTTGTTCCTTCCGGTCTG	191	CDS
CA-SSR-2-360	chr2	AACGCCATCCCTAATCGTC	TGAGGAACCCCTAAGCATACAAA	180	exon
CA-SSR-4-343	chr4	TCTCTCATTATTATTCTTCCGACA	ATGGTCGTTTTCGGAACCTG	302	intergenic region
CA-SSR-5-385	chr5	TTCCTTGCTTTGCAGATCTTT	TCCGGTAGGGATAAAAAGCAA	183	CDS
CA-SSR-7-388	chr7	GAAAGCGCAGGGAATATAACA	CACAACACAACGGAATGGAG	173	intergenic region
CA-SSR-7-630	chr7	TCTTCAAACAATGGTCTCAGA	TCCACCGGTTAGTCTTTCT	442	intergenic region
CA-SSR-7-165	chr7	GCTTACCGGAATCAGACCAA	AAAATCGAGAAAATGCTAATATCAAAA	169	intergenic region
CA-SSR-8-101	chr8	TAATGGCGAACAGAACACGA	CCGTACGGTTGGTAAGGAAA	199	exon

CHR= chromosome, PL= product length (bp).



**Figure 3.** Amplification of chickpea DNAs with use of selected markers (Ladder 1 kb).

#### 4. Discussion

Extreme weather events such as rains, floods droughts, heat waves, freezes and, acidification, and emergence of new infectious diseases appears as a result of climate change (Singh et al., 2023). Exploiting the natural variations from germplasm resources to develop climate-resilient crops is one of the main objects of plant breeding. Marker-assisted breeding is a rapid and efficient tool to characterize genetic diversity in plants (Hasan et al., 2021). During recent decades, the development of molecular markers based on PCR such as RAPD, SSR, AFLP, STS, SNP, etc. have led to genetic resources utilization in chickpea (Shan et al., 2007; Talebi et al., 2008; Sari et al., 2022). Among the markers, SSRs play an important role as molecular markers owing to their high polymorphic, co-dominant and multi-allelic nature (Khajuria et al., 2015). In addition, the advent of NGS facilitated SSR identification in chickpea. ddRADseq is a popular tool used for SSR discovery and genotyping. It is based on the genome reduced representation by digestion with two restriction enzymes (Peterson et al., 2012). In this study, we used ddRADseq to identify SSRs markers. As a result of the study, 1,396 SSR regions with an average of 530 SSR/Mb in the whole genome (1,396 SSRs in a genome size of 740 Mbp) were successfully identified. Our SSR frequency was similar to cucumber (552 SSR/Mb; Cavagnaro et al., 2010), lower than rice 807 SSR/Mb; Lawson and Zhang, 2006), and higher than melon (109 SSR/Mb; Zhu et al., 2016), and wheat (163 SSR/Mb) (Huo et al., 2008). Sequencing method, the number of genotypes, or bioinformatic parameters during the variant calling might cause these differences in the SSR frequency.

Among the 1,396 SSRs identified in chickpea genome, dinucleotides were the most frequent type (62.03%). Trinucleotides were the second most common type account for 25.50% and followed by tetranucleotides (4.58%). These results were similar to those found in *Stevia rebaudiana* by Kaur et al. (2015). Interestingly, it was reported that trinucleotide repeats were the most abundant in safflower (Ahmadi and Ahmadikhah, 2022), pea (Gong et al., 2010), soybean (Hisano et al., 2007). Mononucleotide repeats were reported to be the most abundant in lentil (Bhati et al., 2015), *Brachypodium*

(Sonah et al., 2011) and faba bean (Abuzayed et al., 2017).

Overall, The AT/TA motif was largely characterized among dinucleotide repeats, and it was the most common type in the chickpea genome, which result was similar to flax Cloutier et al., (2009). On the other hand, GC motif was very limited among all repeats. This result agreed with that of Wang et al. (1994) and Tangphatsornruang et al. (2009), who indicated GC-rich repeats as the rarest type in several plant species.

All the SSRs distributed across all 8 chromosomes, with a correlation between the chromosome size and the frequency of microsatellite loci. For instance, chromosome 4 had the largest frequency SSRs, while it was one of the greatest chromosomes. Chromosome 8 had the smallest size in chickpea, the least density of SSRs was obtained from chromosome 8. These findings are confirmed by previous studies of chickpea describing a large number of markers in chromosome 4 (Varshney et al., 2014; Jaganathan et al., 2015; Srivastava et al., 2016; Thudi et al., 2016). In contrast, Singh et al. (2023) reported that there was no correlation between the frequency of SSRs and the chromosome size in pomelo.

In molecular breeding, agarose gel-based markers with breeder-friendly genotyping appear to be better more than SNP or KASP markers from NGS technologies (Hu et al., 2020). For this reason, we developed 10 agarose-resolvable markers resulting successful polymorphic bands among chickpea genotypes. Consequently, this provides an effective method for ddRADSeq library preparation and scripts for SSR identification, resulting in 100% efficiency in PCR amplicons. Annotation analysis revealed the highest frequency of SSRs in intergenic regions (66.6%) (Table 3), similar to the results in different crops (Grover et al., 2007; Parida et al., 2009; Liu et al., 2013). Parida et al. (2015) indicated the efficiency of polymorphic SSRs derived from non-coding areas in chickpea.

#### 5. Conclusion

The development of NGS technologies has prompted the discovery of high-quality genome-derived markers. SSRs are one of the most popular molecular markers in breeding studies due to its worthy desirable genetic

features. In the present study, we developed 10 SSR markers using ddRADSeq that might play an important role for chickpea genetic and genomic studies. Efficiency of these markers has also been tested on 20 different chickpea accessions.

**Author Contributions**

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	D.S.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## PREDICTION OF CANOPY COVER FOR AGRICULTURAL LAND CLASSIFICATION IN LAND PARCEL IDENTIFICATION SYSTEM (LPIS) DATA USING PLANET-SCOPE MULTISPECTRAL IMAGES: A CASE STUDY OF GELENDOST DISTRICT

Sinan DEMİR<sup>1\*</sup>


<sup>1</sup>Isparta University of Applied Sciences, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 32200, Isparta, Türkiye

**Abstract:** Determining canopy cover (CC) temporal variation is critical for sustainable management of natural resources and environmental protection efforts. Data analysis and interpretation methods for remote sensing are important for understanding these changes and adapting to natural systems. In this study used the Parcel Identification System (LPIS) database physical blocks as field ground data. In the study area, agricultural areas were determined from LPIS data, including classes A0, A1, A3, A4, S1, T0, and T1, and a total of 8424 physical blocks and an area of 14651.9 hectares were evaluated. CC estimates were made using 3-m spatial resolution Planet Scope multispectral satellite images of July and August 2023, and it was determined that there were significant differences in parcel-based distinctions, especially in parcels A0, A1, T0, and T1 ( $P < 0.05$ ). According to the study results, it was determined that using the estimated CC data, the A0 (69.27%) and T0 (30.43%) land cover types could be successfully used to determine the changes in the phenological period caused by environmental impact assessment such as climate change. At the same time, this study contributes to the rapid monitoring of agricultural production areas caused by climate change by using physical blocks of agricultural land classes within the LPIS data, the rapid determination of agricultural land management, and support payments with remote sensing data. In this regard, the use of modern technologies and data analysis methods will contribute to increasing agricultural sustainability.

**Keywords:** Land use/land cover, Canopy cover, NDVI, LPIS, Climate change, Remote sensing

\*Corresponding author: Isparta University of Applied Sciences, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 32200, Isparta, Türkiye

E mail: sinandemir@isparta.edu.tr (S. DEMİR)

Sinan DEMİR  <https://orcid.org/0000-0002-1119-1186>

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### 1. Introduction

Population increase and climate change have a significant impact on the dynamics of natural ecosystems and agricultural areas (Demir, 2024). Understanding and monitoring these changes are crucial for preserving natural ecosystems and ensuring agricultural sustainability. Remote sensing techniques offer effective methods for monitoring these changes (Demir, 2023).

Multispectral remote sensing programs, such as Landsat, Sentinel, Spot, IKONOS, WorldView, GeoEye, KOMPSAT, SkySat, MODIS, Gaofen, Pleiades, and PlanetScope, provide crucial spectral data across various regions of the electromagnetic spectrum (Vos et al., 2019). These data offer insights into plant properties such as leaf pigment concentration, water content, and internal structure, contributing to the effectiveness of remote sensing applications in agricultural and biodiversity research (Selim and Sönmez, 2015; Damm et al., 2018; Hatfield et al., 2019; Berger et al., 2022; Selim et al., 2022; Esetlili et al., 2022; Le et al., 2023; Demir et al., 2024; Aljanabi et al., 2024; Demir and Başayığit, 2024).

Vegetation dynamics play a pivotal role in agricultural

productivity, providing insights into plant health and growth. Canopy cover (CC), which represents the proportion of ground covered by photosynthetically active vegetation, is a key indicator of plant growth and health (Tucker, 1979; Pei et al., 2018). This metric is widely utilized in various applications, including crop canopy growth measurement, radiation interception, and evapotranspiration partitioning in hydrological and agricultural modeling (Trout et al., 2008; Talsma et al., 2018; Ghat et al., 2021; Tenreiro et al., 2021; Qin et al., 2023; Oliveira et al., 2024).

The Normalized Difference Vegetation Index (NDVI) is a commonly used tool for defining CC and is employed in both proximal and remote sensing methods (Tenreiro et al., 2021; Carella et al., 2024; Theime et al., 2024). In addition, several other vegetation indices have been developed alongside the NDVI to characterize vegetative diversity. Many indices have been created to characterize vegetative diversity in addition to the NDVI (Rouse et al., 1974; Huete, 1988; Clevers, 1989; Baret and Guyot, 1991; Pinty and Verstraete, 1992; Kaufman and Tanre, 1992; Rondeaux et al., 1996; Basso et al., 2004; Gitelson, 2013; Hassan et al., 2018; Kumar et al., 2018). Despite



theoretical promises of improvement over NDVI in addressing soil background and atmospheric influences, NDVI remains widely used due to its accessibility and user-friendliness across satellite and remote sensing platforms (Rondeaux et al., 1996; Gitelson, 2013; Hassan et al., 2018; Gong et al., 2023; Kumar et al., 2024; Demir et al., 2024). Remote sensing-based CC estimation is becoming increasingly useful for calibrating models in spatial analysis of cropping systems. Effective CC estimation models can be created using the multispectral image's NDVI and other vegetation indices. This approach is less expensive and requires less time than standard in situ measurements. Trout et al. (2008) used a handheld multispectral digital camera to measure the canopy cover of 11 different horticultural crops in 30 fields on the west side of California's San Joaquin Valley. They compared the results with NDVI values computed from Landsat 5 satellite images. The study found a strong correlation ( $R^2=0.95$ ,  $P<0.01$ ) between NDVI and canopy cover, with an average absolute error of 0.047 up to complete coverage. Tsakmakis et al. (2021) established an effective model for assessing canopy cover (CC) in maize fields. They examined the link between the NDVI values obtained from the Sentinel satellite images and the on-site CC, obtaining an  $R^2$  greater than 0.98. Thieme et al. (2024) studied the comparability between ground-based and spaceborne sensors for assessing the biophysical characteristics of winter cover crops. Their research focused on measuring biomass and fractional vegetative groundcover using SPOT 5, Landsat 7, WorldView-2 satellite imagery, and handheld multispectral proximate sensors. They found that surface reflectance imagery demonstrated greater associations with proximate sensors than with top-of-atmosphere data. Surface reflectance NDVI showed high agreement with proximate sensor-derived fractional green cover and biomass, with modified  $R^2$  values of 0.96 and 0.95. Studies have repeatedly revealed a strong association between CC and NDVI, although this relationship may differ among crop species. Standardized correlations are consequently required to reduce uncertainty when

forecasting CC using the NDVI. Despite these limitations, NDVI remains a valuable tool for estimating vegetation characteristics.

In summary, NDVI-derived vegetation indices using multispectral remote sensing data provide a viable method for quantifying canopy cover and understanding vegetation dynamics. These indexes help improve the accuracy of agricultural and environmental responses to climate change by supporting the development of canopy cover prediction models.

The aim of this study was to determine the possibility of merging Parcel Identification System (LPIS) data with high-resolution satellite images to evaluate canopy cover in LPIS-based subsidy programs. In addition, we intend to investigate these inconsistencies at the parcel level by using canopy cover data to address variations in the phenological stages in response to weather differences. The results of this study can have a substantial impact on agricultural policies and encourage the adoption of sustainable farming practices.

## 2. Materials and Methods

### 2.1. Field Description

The study area is located within the borders of the Gelendost district in the province of Isparta in Türkiye's Lakes Region. It extends between coordinates 310725–340359 east and 4202796–4232165 north (Zone 36, UTM-m) (Figure 1). The Gelendost district, which covers the study area, has a surface area of 610.95 km<sup>2</sup>, according to the General Directorate of Maps. The district is located 81 kilometers from Isparta's center at an elevation of 913–2213 m. Positioned on the eastern side of the Eğirdir lake, the district experiences a transition between Mediterranean and Central Anatolian climates. Mediterranean climate effects are prominent in low-lying areas because of the lake effect, transitioning to a cooler and rainy climate with increased altitude toward the mountains. The study area, located near the Eğirdir lake, has experienced an average total precipitation of 433.2 mm and an average temperature of 14.5 °C for many years (1990–2020) (MGM, 2024).

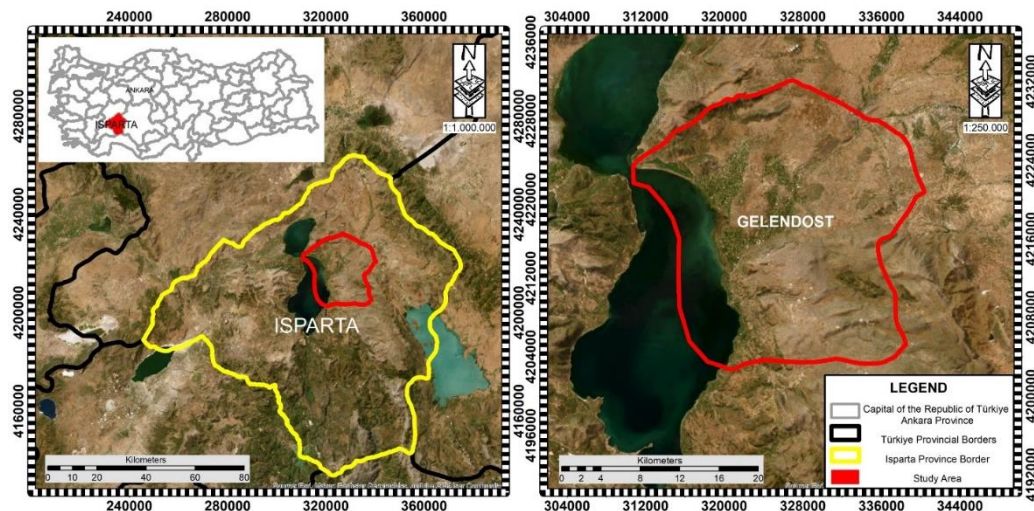


Figure 1. Study area location map.



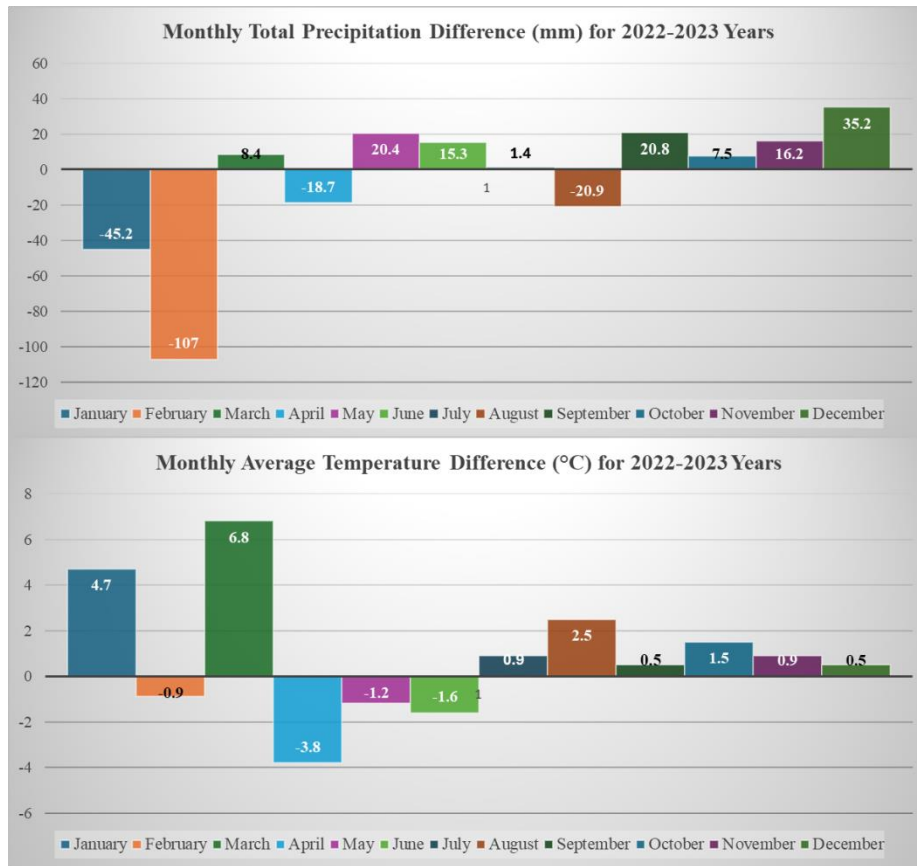


Figure 2. Changes in precipitation and temperature regimes for the years 2022-2023 in the study area.

The distribution of agricultural land use in the study area in 2023 by product group was determined. Within the farmland of fruits, beverages, and spice plants, apples are produced at 91.7%. In the farmland of vegetables, garlic accounts for 41.9%, with 12.6% being tomatoes, 11.2% being melons, 8.9% being cucumbers, and 5.6% being bean production. In the farmland of grains and other plant products, 42% consists of durum wheat, 35% barley, and 10.5% safflower production (TurkStat, 2024).

**2.2. Climate Data**

Climate data plays a crucial role in agricultural production, affecting various aspects of crop cultivation. It influences phenological dates, delaying the maturation of annual crops and affecting the flowering period in fruit orchards, thereby influencing fruit set and quality (Çakır et al., 2021; Yalçın et al., 2021; Yılmaz et al., 2021; Kazemi et al., 2023; Ličina et al., 2024). The impact of climate parameters on agriculture directly influences the plant growth cycle, harvest timing, and productivity. The monthly average temperature and precipitation data for the study area in 2022 and 2023 were obtained from station number 18114 of the General Directorate of Meteorology (MGM, 2024). Monthly variations are shown in Figure 2.

In 2022, there was 441.4 mm of precipitation overall; in 2023, there was 374.8 mm. As a result, 2023’s total precipitation was 66.6 mm less than 2022’s. Thus, the increase in temperature data is influenced by the decrease in precipitation. In 2022 and 2023, the annual

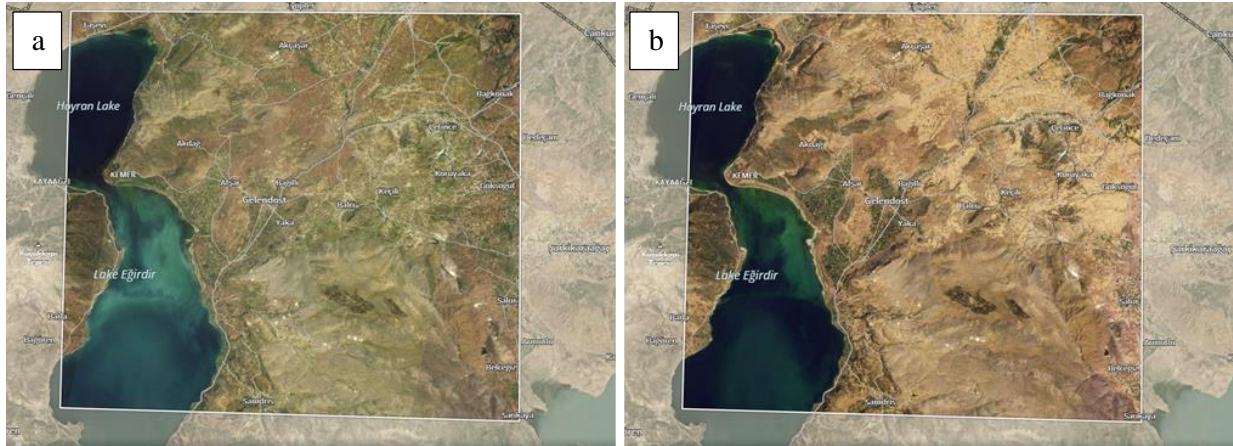
average temperature was 13.2°C and 14.1 °C, respectively. As a result, the average temperature in 2023 rose by 0.9 °C over 2022.

**2.3. Land Parcel Identification System Database**

One of the main components of the European Union’s IACS (Integrated Administration and Control System) is the Land Parcel Identification System (LPIS), a system that precisely defines each and every agricultural land parcel in member states. Up until 2003, member countries mandated its use. Under the scope of EU membership negotiations, Türkiye began implementing LPIS in 2003. In 2016, LPIS data for the entire nation were established using physical block reference systems (Anonymus, 2024). There are five-year updates to the LPIS data (Şimşek and Durduran, 2022). The administration of agricultural land, assistance payments, and the execution of environmental protection measures all heavily depend on this update. The parcel data used in this analysis were obtained from the LPIS database. The LPIS database’s agricultural land cover categories allowed us to choose classes that corresponded to arable areas for our study. The classes of land use status within the study area of the LPIS data are as follows: arable land (A0), arable land with sparse (scattered) trees (A1), mixed agricultural regions (A3), greenhouses (A4), continuous bush product: vineyards (S1), continuous wood products (T0), and permanent wood product: olive trees. Table 1 shows the distribution of classes in the study area within Türkiye’s borders (Anonymus, 2024).

**Table 1.** Spatial distribution of agricultural parcel classes according to LPIS data in Türkiye

Code	Name	Physical Block Count	Surface Area (Km <sup>2</sup> )
A0	Arable land	3598752	192953.41
A1	Arable land with scattered trees	24271	433.27
A3	Mixed agricultural areas	38271	31.22
A4	Greenhouses	76137	438.03
S1	Permanent shrub crops: Vineyards	157931	2695.61
T0	Permanent tree crop	943607	14545.74
T1	Permanent tree crop: Olive trees	146647	577.74



**Figure 3.** Orthorectified image (a) July 4, 2023 (b) August 24, 2023.

**2.4. Data Collection and Preprocessing**

With 120 satellites in orbit, the Planet-Scope constellation is the largest commercial satellite fleet in history, capturing images of the entire Earth’s surface every day (Ghuffar, 2018). With a resolution of 3–5 m, its sensors can capture images in four different multispectral bands: red, green, blue, and near-infrared. This makes it ideal for monitoring and assessing changes in the amount of plant and forest cover. Data from the commercial satellite Planet-Scope are available for purchase from Planet Inc. or can be downloaded for free for academic use (Team, 2017; Planet, 2024).

In our study, Planet-Scope imagery covering the study area, which extends between the coordinates 305526–351372 east and 4201685–4238913 (Zone 36, UTM-m), acquired on July 4 and August 24, 2023, was used. Figure 3 shows the product Level 3B images (Planet, 2024), which encompass the study area and were acquired on two different dates.

Satellite imagery is retrieved under different levels, with each level requiring necessary corrections before further processing. Our retrieved satellite imagery is ‘Surface Reflectance’ in the case of Planet-Scope Dove, already corrected for radiometric and atmospheric corrections (Planet, 2024). All the data have the same pixel size of 3 m.

Vegetation index, such as NDVI, is a measure of the health of a plant based on how the plant reflects light at certain frequencies (Rouse et al., 1974). The NDVI was calculated for the Planet-Scope imagery using Erdas Imagine software (Erdas, 2024), according to the equation given

in Equation 1. The canopy cover was then calculated according to the model proposed by Trout and Johnson (2007), as given in Equation 2.

$$NDVI = \frac{(NIR - RED)}{(NIR + RED)} \tag{1}$$

$$Canopy\ Cover\ (\%) = (1.22 * NDVI - 0.21) * 100 \tag{2}$$

After calculating the canopy cover, the determined LPIS physical block parcels within the study area were analyzed using the Zonal Statistics tool in ArcGIS software (Demir et al., 2024). A dataset for 8388 parcels was created, including pixels’ data. Canopy cover pixel values were determined for the LPIS dataset within land cover types such as arable land (A0), arable land with sparse (scattered) trees (A1), mixed agricultural regions (A3), greenhouses (A4), continuous bush product: vineyards (S1), continuous wood products (T0), and permanent wood product: olive trees (T1).

**2.5. Statistical Analyses**

The study was conducted based on the different types of land uses for LPIS parcels, and a frequency distribution analysis was carried out. The study used the Global Moran's I statistic to examine spatial autocorrelation and calculated a Moran's Index of 0.833 (Figure 4). This index, along with a z-score of 78.285 and a p-value of 0.000, signifies a clustered pattern with a probability of less than 1% of occurring randomly. These results point to a nonrandom spatial distribution within the dataset, indicating that underlying variables may be influencing observable clustering patterns. In addition, a one-sample Kolmogorov–Smirnov test of normality confirmed the

null hypothesis of normal distributions. In this study, box-plot statistics were computed on the basis of land cover types in the LPIS dataset using satellite imagery collected in two periods. The estimated canopy cover values for each period were determined at the parcel scale using the Zonal Statistics tool. Descriptive statistical results were then derived. Within the study area, mean canopy cover values for different periods were calculated on the basis of LPIS data corresponding to agricultural land cover types A0, A1, A3, A4, S1, T0, and T1. Levene’s test of homogeneity of variance for zone types revealed significant differences ( $P < 0.05$ ); hence, the conservative Tukey test, with significance measured at  $\alpha = 0.05$ , was employed for post hoc comparisons. This analysis involved 8388 different parcel scale observations across seven parcel types. ArcGIS software (ArcGIS, 2024) was used for geographic data processing, Erdas Imagine software (Erdas, 2024) for processing 3-m spatial resolution Planet-scope imagery, and statistical analyses of the resulting database were performed using the Minitab software package (Minitab, 2024).

ground data for the study area LPIS data. Table 2 shows the number of parcels and their spatial distribution in the research region for land cover classes A0, A1, A3, A4, S1, T0, and T1. Parcels labeled A0 represent the agricultural areas with the largest area in the research region. It has the largest area of 10149.07 ha, accounting for 69.27% of the total research area. The number of physical blocks indicates that such lands are broad and dispersed over large areas. The relatively high standard deviation indicates that such plots differ in size. General agricultural lands are broad areas where herbaceous crops, including cereals, legumes, and oilseeds, are farmed (Table 2). Parcels coded A1 are among the smallest farmlands in the study area. They account for only 0.08% of the study area, totaling 12.30 hectares. The lower number of physical blocks indicates that this sort of land is less common. The low standard deviation (0.601) indicates that the plot sizes are reasonably comparable (Table 2). A3 coded parcels are one of the farmlands with the smallest surface area, totaling 4.30 hectares and accounting for only 0.03% of the total area. The low number of physical blocks indicates that such regions are uncommon. The standard deviation is relatively low, indicating that such plots are very similar in size (Table 2). A4 coded parcels cover a small area of 4.23 hectares or 0.03% of the total area. The small number of physical blocks indicates that these farmlands are scarce. The low standard deviation indicates that the plot sizes are quite similar. Greenhouses mitigate the detrimental consequences of climate change by creating controlled environments (Table 2). Parcels coded S1 covers 1.06% of the research area, totaling 20.36 ha. These farmlands, with a physical block number of 20, are clustered in specific locations (Figure 3). The standard deviation number (1.064) indicates that the sizes are quite close together (Table 2). Parcels coded T0 covers a considerable area, totaling 4458.87 ha, or 30.43% of the total area. The large number of physical blocks indicates that such areas are relatively frequent. The relatively high standard deviation indicates that such plots vary in size (Table 2). Parcels code T1 covers a small area of 2.73 ha or 0.02% of the total area. The small number of physical blocks indicates that these territories are scarce. The low standard deviation indicates that the plot sizes are quite similar. In total, the study area includes 8388 physical blocks and 14,651.9 hectares of agricultural land. The largest area consists of agricultural lands with code A0, and the smallest area consists of special-use agricultural fields with codes A3 and A4. This distribution demonstrates that agricultural activities are primarily focused on large, general agricultural businesses. In addition, different land use forms appear to differ greatly in size and scope. Figure 5 shows the spatial distribution of the LCT code and physical block data from the LPIS dataset in the study area.

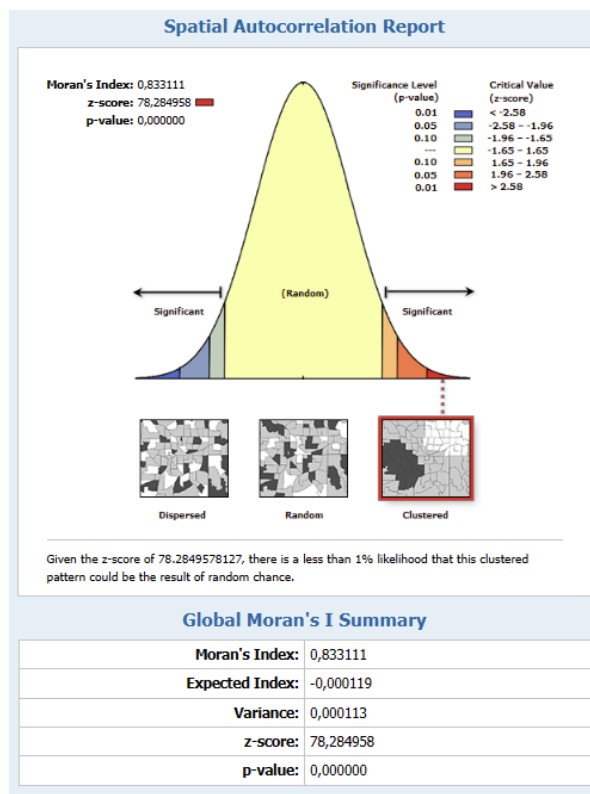


Figure 4. Moran I index of physical blocks in the study area.

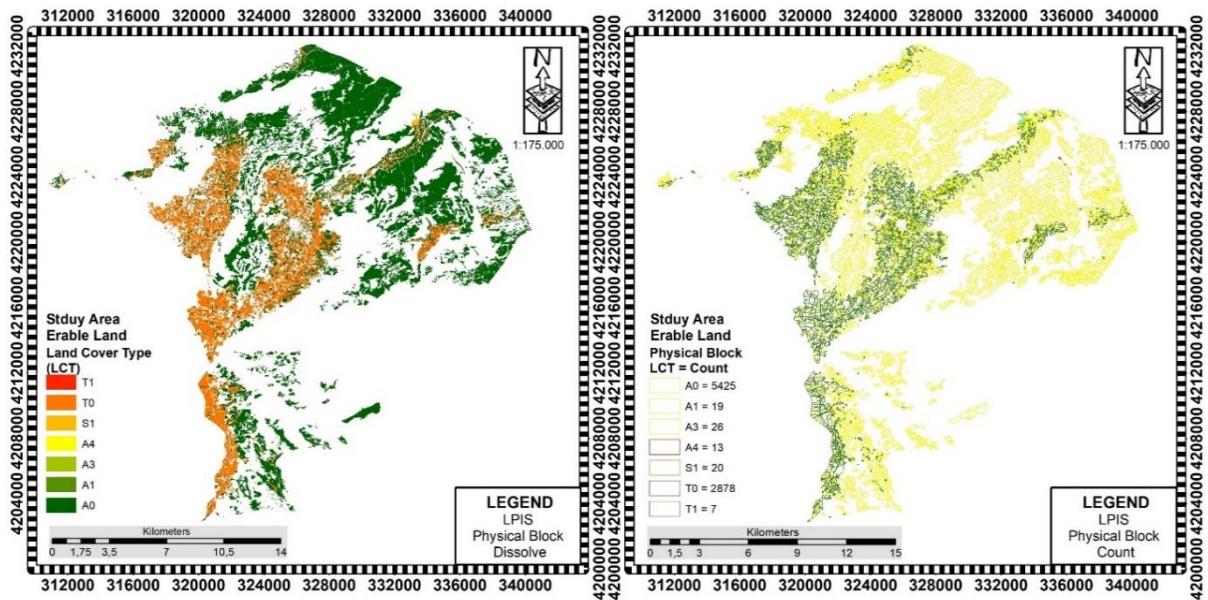
### 3. Results

#### 3.1. Study Area LPIS Database

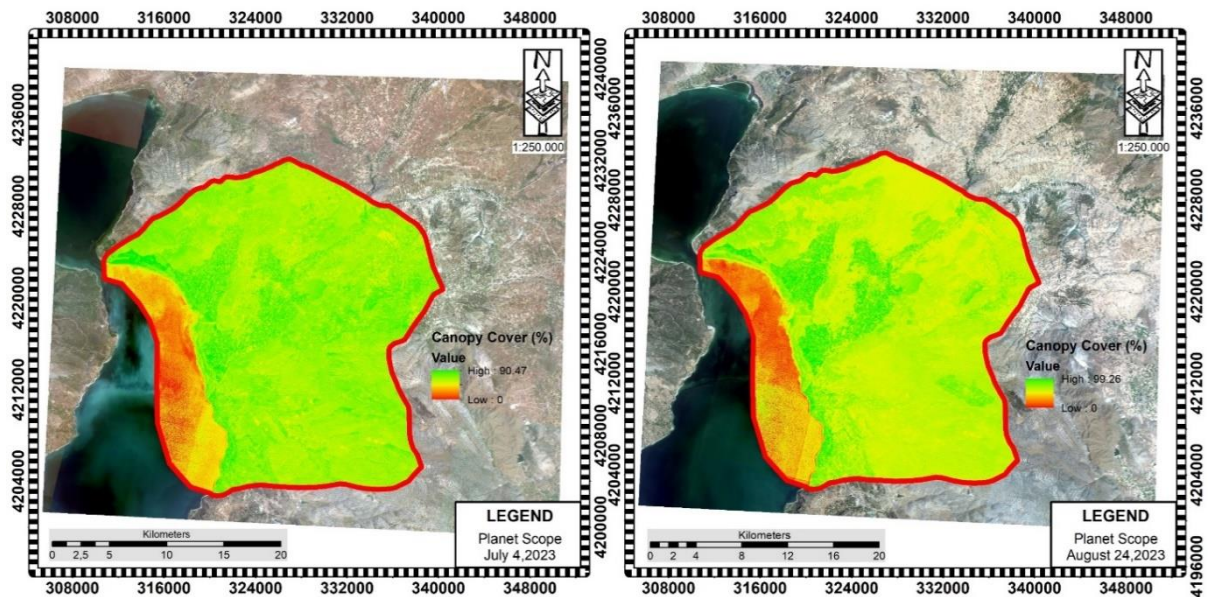
The analysis of the LPIS data indicated land use patterns that are crucial in enhancing agricultural output. Proper classification and usage of agricultural lands, particularly in high-productivity zones, aids in the optimization of production amounts. Furthermore, by tracking land use changes, these spatial data help promote sustainable agriculture practices. Enable land use data served as field

**Table 2.** Study area and agricultural parcel status according to the LPIS database

LCT Code	Physical Block Count	Area (Hectare)	Standard Deviation	Area (%)
A0	5425	10149.07	69.27	2.343
A1	19	12.30	0.08	0.601
A3	26	4.30	0.03	0.092
A4	13	4.23	0.03	0.305
S1	20	20.36	0.14	1.064
T0	2878	4458.87	30.43	2.246
T1	7	2.73	0.02	0.249
Total	8388	14651.9	-	100



**Figure 5.** Study area agriculture parcel physical blocks spatial distribution.



**Figure 6.** Study area canopy cover prediction at different temporal resolutions.

**3.2. Canopy cover prediction**

In this study, the CC of agricultural areas in the study area was estimated using high-resolution PlanetScope satellite images with varying temporal resolutions. Changes in the vegetation period due to changes in the

study area’s climatic circumstances resulted in considerable changes in the CC data (Figure 6). These changes were evaluated using the LCT (Land Cover Types) physical block types from the LPIS data, and the descriptive statistics results are shown in Table 3. Table

3 shows descriptive statistics for canopy cover percentages based on different LCT types, including minimum, mean, maximum, standard deviation, coefficient of variation, skewness, and kurtosis values. These statistics provide important information for understanding and evaluating the CC distribution of different LCT categories in the study area. The estimated CC values in the first and second periods show significant changes in different LCT codes. While in the first period, code A0 had the highest average CC value (33.53), this value decreased significantly (21.16) in the second period. In addition, the skewness and kurtosis values in the A0 code shifted, indicating that the distribution's asymmetry and kurtosis characteristics had changed. It is worth noting that in the A1 code, the average values are low in both periods, as are the skewness values; this indicates that the distribution has a long tail to the right, resulting in more extreme results. While the average values in the A3 and S1 codes are similar in both eras, the average and coefficient of variation in the A4 category are much lower and higher. In the T0 and T1 codes, a larger distribution and increased standard deviation values were observed in the second period, indicating that environmental variables and agricultural methods have considerable effects on canopy cover across time. As a result, the A0 and T0 codes show a more homogenous distribution, whereas the A1 and A4 codes show higher variability and skew. In the T0 code, the canopy cover percentage had a high average value and a homogeneous distribution with low skewness and kurtosis. This demonstrates that the T0 code contains dense and regular vegetation in the research area.

Figure 6 shows the spatial distribution of July and

August's estimated canopy cover levels. These spatial distribution maps depict the effects of changes in vegetation phase and meteorological conditions on canopy coverage. Data collected in July and August are crucial for a better understanding of temporal changes in CC estimates and the impact of agricultural operations in the study area. While CC values increase in well-structured covered orchards within the study area, Figure 6 shows the spatial distribution of the decline in canopy value in dry-farmed areas where annual plants are planted.

This study investigated the impact of canopy cover values estimated across different time periods on land cover in agricultural areas. The Kolmogorov-Smirnov normality test results indicated that the average results of the physical blocks for the estimated canopy cover values followed a normal distribution. Average canopy cover values for each LCT (Land Cover Type) type were investigated using post hoc tests such as analysis of variance and the Tukey test at a 95% confidence interval. Analyses revealed that canopy cover values varied significantly among vegetation periods (Table 4). These discrepancies enabled us to gain a better understanding of the periodic changes in vegetation in agricultural areas and their effects on canopy cover. In addition, the investigation attempted to assess the applicability of canopy cover estimations using high-resolution satellite images to distinguish between LCT types based on physical block ground truth (Table 4). There were significant changes in canopy cover rates between July 4, 2024, and August 24, 2024, for each land use type ( $P < 0.05$ ).

**Table 3.** Descriptive statistics results for canopy cover effects on land cover in different periods

Variable	LCT	N	Minimum	Mean	Maximum	StDev	CoefVar	Skewness	Kurtosis
Canopy Cover (%)	A0	5425	3.187	33.531	73.659	11.967	35.69	0.46	-0.38
	A1	19	18.70	28.97	53.12	8.27	28.54	1.52	2.88
	A3	26	26.99	40.97	57.30	8.45	20.63	0.34	-0.62
	A4	13	7.91	28.39	43.05	11.93	42.02	-0.72	-0.79
	S1	20	25.43	39.37	53.41	8.12	20.62	0.02	-0.81
	T0	2878	8.632	45.226	74.677	10.634	23.51	-0.45	-0.17
	T1	7	39.19	53.87	65.78	11.49	21.32	-0.34	-1.80
Canopy Cover(%)	A0	5425	-3.163	21.157	78.132	16.974	80.23	1.03	0.01
	A1	19	3.18	18.08	40.81	10.33	57.13	0.90	-0.19
	A3	26	17.14	38.12	55.76	9.90	25.96	-0.24	-0.60
	A4	13	-0.65	26.97	51.37	15.48	57.42	-0.28	-0.79
	S1	20	13.36	35.28	66.14	13.45	38.14	0.37	-0.13
	T0	2878	2.020	45.768	76.189	15.163	33.13	-0.60	-0.41
	T1	7	26.82	45.26	64.57	14.51	32.07	0.11	-1.80

**Table 4.** ANOVA and Tukey test results for canopy cover effects on land cover in different periods

LCT	Physical Blocks	4 July 2024 CC (%) (Mean±SE)	24 August 2024 CC (%) (Mean±SE)
A0	5425	53.87±0.16 <sup>B</sup>	21.16±0.23 <sup>C</sup>
A1	19	45.23±1.90 <sup>B</sup>	18.08±2.37 <sup>C</sup>
A3	26	40.97±1.66 <sup>B</sup>	38.12±1.94 <sup>AB</sup>
A4	13	39.37±3.31 <sup>B</sup>	26.97±4.29 <sup>BC</sup>
S1	20	33.53±1.82 <sup>AB</sup>	35.28±3.01 <sup>AB</sup>
T0	2878	28.97±0.199 <sup>A</sup>	45.77±0.28 <sup>A</sup>
T1	7	28.39±4.34 <sup>A</sup>	45.26±5.49 <sup>AB</sup>

\* Capital letters indicate the difference between canopy cover averages for each land use (P<0.05).

Table 4 shows a significant difference between the LCT groups in canopy cover estimates on July 4, 2024 and August 24, 2024 (P<0.05). A substantial difference was found between "T0" and "A0 and A1" in both times (P<0.05). A substantial difference was found between "T1" and "A0 and A1" in both times (P<0.05). There was no statistically significant difference found between the plant species grown in groups A3, A4, and S1 and those grown in groups A0, A1, T0, and T1. This could also be due to parallel plant growth processes, which are expected to result in similar canopy cover levels. In addition, it is believed that this is related to the fact that the number of physical blocks in the research region for the A3, A4, and S1 land cover groups is less than that of the A0 and T0 groups.

#### 4. Discussion

The temperature and rainfall in the study area significantly varied between 2022 and 2023. Precipitation trends tend to fluctuate. Significant decreases were observed in January and February, whereas significant increases were observed in May, September, November, and December. These oscillations can be used to predict seasonal and climate changes. Temperatures vary similarly, with considerable increases in January and March and decreases in April and May. Temperatures rose modestly throughout the second half of the year. These changes reflect the climate's dynamic structure and are crucial data to consider for future climate analyses and environmental planning. Climate change also has a significant impact on agriculture. While changes in phenological periods cause shifting growing seasons and fluctuations in productivity in annual plants, precipitation and temperature changes during the flowering period in perennial plants have a negative impact on development and productivity due to issues with fruit set and quality. This situation is of critical importance in terms of agricultural production and sustainability (Talsma et al., 2018; Nhemachena et al., 2020; Revzi et al., 2023; Kazemi Garajeh et al., 2023; Qin et al., 2023; Carealla et al., 2024). Climatic changes in the research area in 2023 reduced apple production, which was grown in 91.7% of fruit farming areas by 20,521 tons. This increased 11503 tons in wheat and barley plant yields throughout 78.82% of grain fields (TurkStat, 2024). This circumstance stresses the importance of

changing agricultural production patterns in response to global climate change or switching to agricultural products appropriate for phenological times. In studies conducted with different apple varieties grown in Isparta province and its districts, it has been reported that full flowering dates are distributed in April and May (Uçgun and Gezgin, 2017; Eskimez et al., 2020; Küçükyumuk, 2021; Küçükyumuk and Erdal, 2022). In the study area's agricultural land use, apple cultivation is practiced in the majority of the fruit-growing areas, and the drop in yield is attributed to an increase in precipitation and a decrease in temperature in May 2023, the full flowering time. It has been stated that under Isparta climatic conditions, the wheat plant is in its development period in March, April, and May; therefore, increased rainfall increases productivity (Akgün et al., 2011). The increase in precipitation during the development phase of wheat and barley plants, which are grown in most grain fields in March, April, and May, improved yield while delaying harvest. Other research findings corroborate the idea that changes in the study area's climate have varying effects on agricultural goods (Akgün et al. 2011; Uçgun and Gezgin, 2017; Eskimez et al., 2020; Küçükyumuk, 2021; Küçükyumuk and Erdal, 2022). Keeping track of these changes is critical for establishing sustainable food supplies, agricultural policies, and subsidies. As a result of the study conducted to determine the land cover change due to climate change using the high-resolution Planet Scope satellite image of the land use classes corresponding to agricultural lands in the LPIS database, it was determined that the LPIS physical block data can be used as field data. The CC estimation performed using an image of the research region obtained on July 4 revealed that the grain areas were not harvested because of climate change that occurred during the plant growth season, which delayed harvest maturity. It was discovered that CC values had dropped in grain fields harvested in August (Table 3). It has been reported in studies that machine learning and deep learning algorithms made with physical blocks can be determined with high accuracy in determining the land cover type of Türkiye from LPIS data (Şimşek and Durduran, 2022; Şimşek, 2023). As a result, land cover classes (A0, A1, A3, A4, S1, T0, T1) representing agricultural areas in the study area were employed as ground truth. The utility of this data in monitoring phenological changes in land cover caused by climate change was determined based

on a variance analysis of the average canopy values of the physical block values. The analysis results can be used to discriminate between fruit agricultural areas and grain areas based on canopy cover values estimated throughout both periods (Table 4). It has been established that canopy cover estimation can be used to determine land use. The results were found to be consistent with those from other investigations (Trouth et al., 2008; Tsakmakis et al., 2021; Thieme et al., 2024). The limited number of physical blocks in the A3, A4, and S1 land cover types identified in this study is assumed to be the cause of their low discrimination compared with other classes.

In the future, it will be of great importance to develop the necessary strategies for agricultural areas to adapt to climate change. These strategies include developing plant species that are resistant to climate change, improving irrigation techniques, and optimizing soil management practices. In addition, agricultural policies and subsidies need to be rearranged within the framework of adaptation to climate change to ensure the sustainability of agricultural production.

This study has shown that the use of the LPIS database and high-resolution satellite images is an effective method for determining the effects of climate change on agricultural land cover. Monitoring canopy cover values can be used as an important tool to monitor the effects of climate change on phenological changes. Thus, changes occurring in agricultural areas can be detected more quickly and accurately, and adaptation strategies can be implemented in a timely and effective manner.

## 5. Conclusion

In this study, canopy cover estimation of agricultural lands in the study area was performed using high-resolution PlanetScope satellite images at different temporal resolutions. Changes in climatic conditions and vegetation have led to significant differences in the canopy cover data. Analyses of images taken in July and August showed that canopy cover values vary significantly in different LCT categories. While a more homogeneous distribution was observed in the A0 and T0 categories, more variability and skewness were noted in the A1 and A4 categories.

Data obtained in July and August provided critical information for understanding temporal changes in canopy cover estimates and the effects of agricultural activities in the study area. The study results revealed that the canopy cover values of plant species in the A3, A4, and S1 categories did not differ significantly from those in the A0, A1, T0, and T1 categories. This situation can be explained by the impact of similar plant cultivation techniques. In addition, it was determined that the number of physical blocks in the A0 and T0 categories was the two highest groups, and discrimination could be made according to canopy cover estimation in both periods due to differences in land use and plant patterns.

CC estimations based on high-resolution satellite images can be useful for monitoring phenological changes in agricultural fields and designing agricultural policies. Therefore, constant monitoring and adaptation studies are critical for mitigating the effects of climate change on agricultural production and ensuring food security. In this regard, applying contemporary technologies and data analysis methodologies can help improve agricultural sustainability. It is also recommended that local and national remote sensing resources be rapidly deployed and made available as standard data types for monitoring and evaluation studies.

## Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

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	S.D.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

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C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

## Conflict of Interest

The author declared that there is no conflict of interest.

## Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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## MYOGENIC REGULATOR GENES RESPONSIBLE FOR MUSCLE DEVELOPMENT IN FARM ANIMALS

Godswill Arinzechukwu IWUCHUKWU<sup>1\*</sup>, Dilek GÖKCEK<sup>1</sup>, Zeynep ÖZDEMİR<sup>1</sup>

<sup>1</sup>Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun, Türkiye

**Abstract:** Breeding farm animals, especially poultry, helps meet global meat demand and boosts meat production efficiency. To meet high-quality meat demand, muscle growth and development must improve. Fetal skeletal muscle formation involves myogenesis, fibrogenesis, and adipogenesis. Kinase-encoding genes and myogenic regulatory factor genes regulate a complex network of intrinsic and extrinsic components in two or three stages. MYF5, MYOD, myogenin, and MRF4 are helix-loop-helix transcription factors that govern skeletal muscle cell specification and differentiation throughout embryogenesis and postnatal myogenesis. The transcription factors MYF5, MYOD, Myogenin, and MRF4 have been discovered to determine the skeletal muscle lineage and regulate myogenic differentiation during development. These factors also determine the muscle satellite cell lineage that becomes the adult skeletal muscle stem cell compartment. MYF5, MYOD, Myogenin, and MRF4 serve small functions in adult muscle, but they again direct satellite cell activity to regenerate skeletal muscle, linking genetic regulation of development and regeneration myogenesis. Understanding and identifying these genes helps increase meat yield and quality. This detailed review examines myogenic regulatory variables in satellite cell specification, maturation, and skeletal muscle regeneration.

**Keywords:** Meat, Myogenesis, Satellite cell, Myogenic differentiation, Regeneration, Skeletal muscle

\*Corresponding author: Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun, Türkiye

E mail: godswill2014.gi@gmail.com (G. A. IWUCHUKWU)

Godswill Arinzechukwu IWUCHUKWU



<https://orcid.org/0009-0001-3621-7055>

Dilek GÖKCEK



<https://orcid.org/0000-0001-8721-3380>

Zeynep ÖZDEMİR



<https://orcid.org/0009-0003-2726-4743>

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### 1. Introduction

The aim of every livestock breeder is to produce animals of sufficient body weight with optimum nutritional requirements at the least cost. More importantly, with the increase in global population and the need to meet the increasing human protein requirement for different age groups, the rapid growth rate and muscle development of livestock breeds have become essential characteristics for meat farmers and producers. According to some studies, myogenic regulatory factors (MRFs) and growth promoters are essential and crucial for muscle differentiation, growth and development in farm animals. It is also widely accepted that muscle development in both embryonic and postnatal stages of farm animals is affected by these factors. Therefore, it demonstrates the importance of examining and understanding muscle regulatory factors.

MRFs consist of *MRF4*, Myogenic Determination Factor 1 (*MYOD*), Myogenic Factor 5 (*MYF5*), Myogenin, also known as Herculin or Myf6. These regulatory factors direct myogenesis, that is, the formation of skeletal muscles, and as early as embryogenesis, these myogenic regulatory factors control different stages of developmental skeletal muscle formation. Two of the four MRFs, Myogenic Determination Factor 1 (*MYOD*) and Myogenic Factor 5 (*MYF5*), are regulators of

myogenesis progenitor specification. While *MRF4* and Myogenin (*MYOG*) are expressed much later in embryonic development, they play a crucial role in determining and differentiating embryonic stem cells to become committed myogenic cells. *MYOG* is the primary determinant of myoblast differentiation, while *MRF4* is expressed in mature myocytes (Nabeshima et al., 1993). Apart from these, a growth factor worth mentioning is Myostatin (*MSTN*). It is the most potent negative regulator of myogenesis, but is also expressed in adult muscles, indicating that it also inhibits postnatal muscle growth (McPherron et al., 1997; Lee and McPherron, 2001; Amthor et al., 2004).

On the other hand, understanding the impact of these regulatory factors on skeletal muscle gene expression and its impact on meat quality and yield, as well as considering future perspectives such as regenerative myogenesis, is essential to successfully modify these genes.

To manipulate the myogenetic potentials of farm animals, we need to have complete information about MRFs, hence the purpose of this paper.

### 2. Muscles

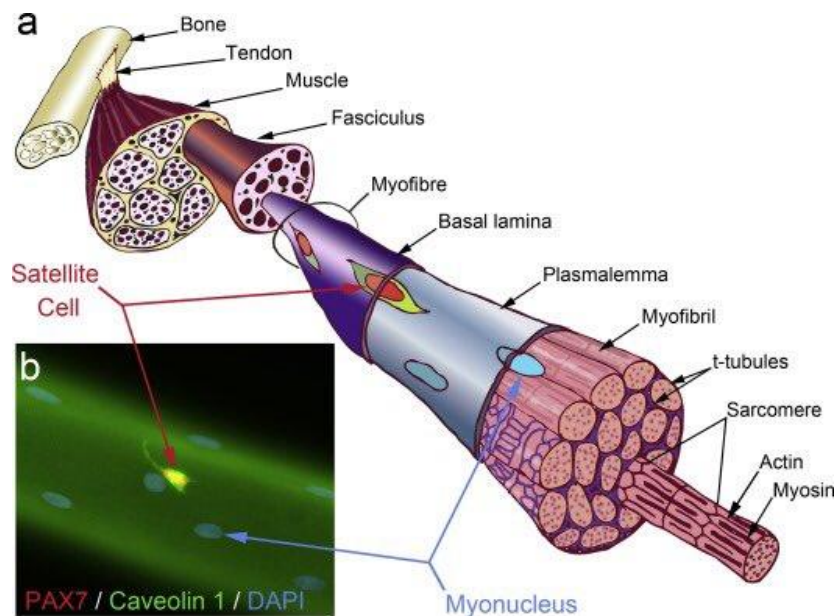
Skeletal system, with more than 600 separate muscles, is the body's most important tissue mass and is crucial for



movement and support. Skeletal and cardiac muscles constitute the two main forms of striated muscle. Skeletal muscle represents innervated, voluntary muscle cells that exhibit fatigue and have high energy requirements, whereas cardiac muscle functionally represents a set of self-exciting, non-fatiguing muscle cells with moderate energy requirements. The organism's ability to actively control skeletal muscles distinguishes them from cardiac and smooth muscles. Skeletal muscle is considered a very important organ for the muscular system because it is a complex and heterogeneous tissue (Bentzinger et al. 2012) (Figure 1). In vertebrates, this tissue is extremely abundant and performs a variety of vital metabolic functions. The amount of lean skeletal muscle controls how quickly the body burns calories (Mifflin et al. 1990; Nelson et al. 1992; Taguchi et al. 2011). According to theory, obesity can be prevented by increasing muscle mass and energy expenditure from muscle protein oxidation (Wolfe 2006). Skeletal muscle also has the highest insulin-stimulated glucose absorption, which helps keep the body's overall insulin sensitivity high (DeFronzo et al. 1981). High skeletal muscle development is very important in farm animals as it produces tissue that meets human requirements for meat consumption. Myogenesis (including myoblast

proliferation, differentiation and fusion), fibrogenesis and adipogenesis (Du et al. 2010) are involved in the formation of fetal skeletal muscle produced from mesenchymal stem cells (MSCs). Myogenesis is controlled by a complex network of intrinsic and extrinsic factors, typically divided into two or three phases, and is regulated by genes encoding kinases. Meat quality may also be improved by shifting MSC commitment from muscle to adipocyte formation with the addition of overlaying intramuscular fat. Proliferation and differentiation of myoblasts, the progenitors of muscle cells, play an important role in the formation of skeletal muscle. Growth promoters and myogenic regulatory factors (MRFs) are required for muscle development in agricultural animals. (Parakati and DiMario, 2013).

In general, fiber type position can influence muscle growth, which is considered an inherited trait, especially in terms of metabolism, contraction rate, temperature and food availability (Leatherland, 1994; Rehfeldt et al. 2011). In response to growth and injury, skeletal muscle has a remarkable capacity to renew and rebuild itself by activating muscle stem cells or satellite cells (Shi et al., 2006; Meadows et al. 2008).



**Figure 1.** Skeletal muscle structure (a) and satellite cell (b). (Relaix et al., 2012) (Adapted with permission from The Company of Biologist, Ltd).

**2.1. Myogenesis**

Myogenesis is the complex process by which skeletal muscles are built in various species, including farm animals. Myogenesis is generally aimed at producing multinucleated myofibers with contractile activity. Different species require different periods of time for each stage of development (Knight and Kothary 2011). During embryogenesis, the basic components and structure of skeletal muscle are modeled (Buckingham et al. 2014; Bentzinger et al. 2012; Tapscott 2005). During

early pregnancy, the locations and characteristics of the cells that will form the three germ layers (ectoderm, mesoderm and endoderm) are determined (Arnold SJ and Robertson, 2009). Depending on the distance from the midline/neural tube, the mesoderm is morphologically divided into paraxial, middle and lateral mesoderm. Paraxial mesoderm, a tissue that develops in the tail bud of embryonic axis elongation and subsequently in the primitive streak/blastopore during gastrulation, is the source of skeletal muscles. The

presomitic mesoderm at the posterior end of the embryo consists of the developing paraxial mesoderm. Presomitic mesoderm is a temporary tissue that can be divided into an immature posterior region and a specialized anterior region, the latter which divides to form somites. Skeletal myogenesis begins with the determination of premyogenic progenitors and skeletal myoblasts in the somites. Mononuclear myocytes fuse to form multinucleated myofibers after going through many stages of proliferation and differentiation. Myogenesis is typically controlled by a complex network of internal and external stimuli (Bentzinger et al. 2012) and is regulated at various stages by MRF genes and genes producing protein kinases (Knight and Kothary, 2011). The control of myogenesis is also significantly affected by nutrition. Both undernutrition and overnutrition during pregnancy inhibited fetal myogenesis, but only overnutrition promoted intermuscular fat accumulation (Zhao et al., 2019; Berri et al., 2006). Activation of the myogenic factor *MYF5* in cells in the dorsomedial part of the newly formed somite is the earliest indicator of myogenesis in mouse and chicken embryos (Ott et al., 1991; Pownall & Emerson, 1992).

According to studies carried out by Biressi et al. (2007) and Stockdale (1992), myogenesis can be divided into two stages throughout development: the early embryonic or primary stage (E10.5–E12.5 in mice; E3–7 in chicken) and the later fetal or secondary stage (E14.5–17.5 in mice; E8+ in chicken). The first myofibers are initially generated from *PAX3+/PAX7+* (in chickens) or *PAX3+/PAX3+* (in mice) dermomyotomal progenitors (Horst et al., 2006; Hutcheson et al., 2009; Otto et al., 2006). These early myotomes and limb muscles are made from these early myofibers, which serve as building blocks for adult muscles (Murphy and Kardon, 2011). Muscle development is mostly maintained during secondary myogenesis by cell fusion and addition of myonuclei from dividing *PAX7+* progenitors (White et al., 2010). Muscle satellite cells gradually function to support muscle growth after birth, while myogenic factors support and differentiate muscle throughout pregnancy. Farm animals undergo a series of biochemical processes, including protein deposition and muscle cell growth, to produce muscle (Du et al., 2010). Only a small fraction of the myotome's progenitor cells proliferate before differentiating into myoblasts. These myoblasts stop participating in the cell cycle and begin to differentiate and fuse with each other to form primary myofibers and myotubes (Buckingham et al. 2014).

Secondary muscle fibers are formed by proliferation and fusion of myoblasts in close proximity to primary muscle fibers (Beermann et al. 1978). The muscles of adult animals develop predominantly through secondary myogenesis. Satellite cells arise when some myogenic cells enter quiescence in the late fetal period. Therefore, in addition to influencing the number of muscle fibers, the number of myoblasts also influences the number of satellite cells present throughout postnatal development

(Zhao et al., 2019). Fetal myogenesis is required for effective muscle growth in farm animals because, in the majority of cases, the number of muscle fibers does not change after birth (Du et al., 2010). Postnatal hypertrophy, or size expansion, results from the differentiation and fusion of satellite cells with pre-existing muscle fibers after initial proliferation of satellite cells. Without exogenous cues (such as injury and activity), satellite cells in mature animal muscles are dormant. Injured muscle fibers are repaired or replaced with activated satellite cells. Some age-related diseases cause a decrease in satellite cells, which impairs regeneration and causes muscle deterioration (Fukada, 2018).

## **2.2. Myogenic Regulatory Factors**

### **2.2.1. Discovery of the bHLH myogenic regulatory factor.**

Myogenic regulatory factors MRFs (*MYF5*, *MYOD*, myogenin, and *MRF4*), *PAX7*, and *PAX3* are among the unique muscle-related transcription factors that primarily regulate myogenesis. These elements function as regulators of the final signaling process and help produce appropriate transcripts for each step. *MYOD*, a basic helix-loop-helix factor (bHLH), was first discovered in 1987 by state-of-the-art subtractive hybridization research using myoblast cDNA libraries. These studies have shown that *MYOD* can convert various cell types, including fibroblasts, into cells that can fuse into myotubes. An important advance in understanding the molecular mechanisms underlying the selection and differentiation of muscle progenitors was the identification of *MYOD* and associated proteins. Subsequently, three additional myogenic basic helix-loop-helix factors, *MYF5*, Myogenin, and *MRF4* (also known as Myf6) has also been found to be able to induce myoblast properties in non-muscle cell lines (Braun et al. 1989; Edmondson and Olson 1989; Rhodes and Konieczny 1989; Braun et al. 1990; Miner and Wold 1990). When *MYOD*, *MYF5*, Myogenin, and *MRF4* are ectopically expressed, they can transform various cell types into myogenic lineages, which is one of their most remarkable features (Edmondson and Olson 1993). Thus, myogenic regulatory factors (MRFs) are the highly conserved genes *MYOD*, *MYF5*, myogenin, and *MRF4* that are collectively expressed in the skeletal muscle lineage (Weintraub et al. 1991; Rudnicki and Jaenisch 1995). The four MRF genes are expressed early during development, when myogenic lineage commitment is established in somites and developing limbs, as expected by several studies. The basic helix loop helix (bHLH) domain is a highly conserved core region found in *MYOD*, *MYF5*, myogenin, and *MRF4* proteins. The helix-loop-helix motif, found in the promoters of many muscle-specific genes, is required for heterodimerization with E proteins that mediate recognition of genomic E-boxes. In contrast, the basic domain of MRFs facilitates DNA binding. The resulting heterodimer has a strong affinity for the *CANNTG* DNA motif known as the E-box. According to

Edmonson and Olson (1993), such binding is required for transcriptional activation of E-box-containing genes. This motif can be found in the promoters of many, but not all, skeletal muscle-specific genes. The first MRF to be produced during embryonic development is *MYF5*, which is briefly upregulated in the paraxial mesoderm before working with other MRFs to help establish the myotome (Ott et al. 1991; Buckingham 1992).

**2.2.2. Paired Homeobox Transcription (PAX) Factors**

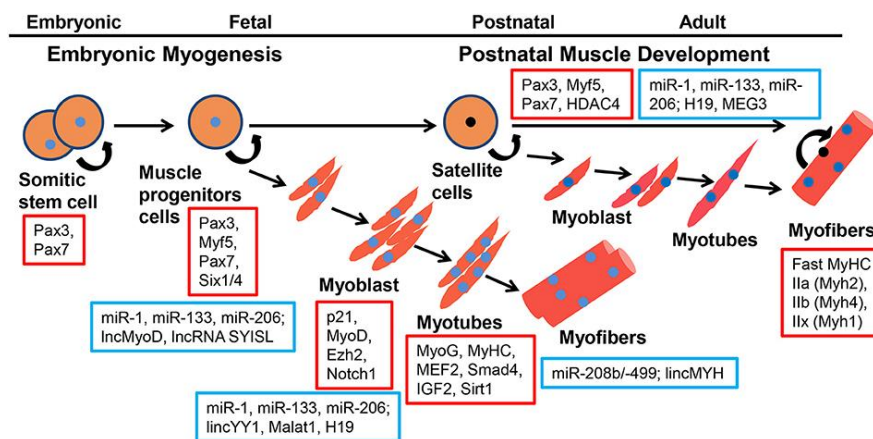
The next rung of the genetic ladder controlling myogenesis is dominated by the paired homeobox transcription factors *PAX3* and *PAX7*. Transcription factor paired box 7 (*PAX7*) is upregulated during myoblast differentiation but downregulated in proliferating myoblasts (Seale et al., 2000). In adult muscles, *PAX7* is expressed in both quiescent and proliferating satellite cells. (Zammit et al., 2004). Since all vertebrates appear to share at least one of these genes, it has been suggested that duplication of a particular gene from a common ancestor gave rise to these genes. (Noll, 1993).

Paired box transcription factors *PAX3* and *PAX7* are expressed by embryonic myogenic progenitors derived from the nuclear region of the somitic dermomyotome. (Seale et al., 2000, Relaix et al.). *MYF5* and *MYOD* are fully hierarchically induced at this stage of embryogenesis, followed by myogenin and *MRF4* and *MYOD*. Myogenesis is regulated by these three myogenic factors. Prior to the production of *MYF5* and *MYOD*, the paired box transcription factors are initially expressed in mesoderm cells (Buckingham, 2001). *PAX3* production during skeletal myogenesis upregulates *MYOD* expression, which is essential for skeletal muscle formation. In addition to regulating *MYF5* expression, *PAX7* maintains satellite cells in a quiescent state and is required for the growth of activated myoblasts (Knight et al.; Ridgeway et al.) Precursors of adult satellite cells that do not exhibit MRFs but still express *PAX3* and *PAX7* are thought to be a subset of myogenic precursor cells.

**2.3. Function of MRFs in Muscle Development and Differentiation**

**2.3.1. MYF5, MYOD, and MRF4 overlap in directing myogenic specification, whereas Myogenin is indispensable for myogenic differentiation.**

Myogenic differentiation is hierarchically controlled by many transcriptional gene regulatory networks, each of which is precisely regulated by a master regulator located at specific temporal and geographical developmental stages (Buckingham et al. 2014) (Figure 2). The natural gene regulatory program for a non-muscle cell to become a myogenic-like cell can be overridden by ectopic expression of any of the MRFs that serve as master regulators of myogenesis. However, during development, the location, timing, and expression levels of MRFs are precisely modulated to ensure the correct progression of the developmental process. In cultured myogenic cells, sequential activation of bHLH myogenic regulators suggests that these elements have distinct functions in the regulation of myogenesis. Quiescent satellite cells do not express MRF at all. Myogenin and *MRF4* transcript increases are only seen when cells begin to differentiate, whereas *MYOD* and/or *MYF5* are the first MRFs produced in active muscle satellite cells (Smith et al., 1994; Yablonka-Reuveni and Rivera, 1994; Cornelison and Wold, 1997). The four MRFs express in a specific spatiotemporal pattern during mouse embryogenesis. (Currie and Ingham, 1998). *MYF5* is initially expressed in the dorsomedial cells of the dermomyotome that give rise to myogenic progenitors that develop into epaxial muscles. The ventrolateral dermomyotome cells, which form the progenitors of the hypaxial muscles, then begin to express the *MYOD* gene. According to Rehfeldt et al., myogenin and *MRF4* are required to support the differentiation and development of muscle fibers. *MYOD* and *MYF5* are very important for the emergence of different types of muscle cells. *MYF5* and *MYOD* are found earlier than myogenin-expressing cells during myotome development. Genes required for muscle stem cell proliferation are typically stimulated and activated by *MYF5*, *MYOD*, and *MRF4* (Knight and Kothary 2011). In addition, differentiation and fusion of myoblasts into myotubes depend on these elements.



**Figure 2.** The process of embryonic and postnatal myogenesis is regulated by coding genes and noncoding RNAs. Red squares represent coding genes, while blue squares represent non-coding RNAs (Luo H. et al., 2021).

Myogenin and Myocyte Enhancer Factor 2 (*MEF2*), which work together to promote differentiation, are required for the differentiation of active myoblasts (Shi et al., 2006). According to Pownall and Emerson, *MYOD* has the power to activate additional MRFs, resulting in the production of muscle-specific proteins in avian species. Expression of *MYF5* was significantly downregulated in Wagyu × Angus relative to Angus cattle, and samples from 6-month-old Angus cattle compared to Hereford and age-matched cattle. There was a higher myoblast proliferation rate at 5–20 h in in vitro cultures than samples from Wagyu × Angus cattle (Coles et al., 2015). On the day of hatching, the pectoralis major muscle of low-weight selected (LWS) chickens expressed more *PAX3*, *MYOD*, and *MRF4* than the pectoralis major muscle of high-weight selected (HWS) chickens, and on day 28, *PAX3*, *PAX7*, *MYF5*, *MYOD1*, *MYOG*, and *MRF4* expression was higher in HWS animals than in LWS animals (Yin et al., 2014). On the day of hatching, *PAX3*, *MYF5*, *MYOD* and *MYOG* expressions were higher in LWS chickens than in HWS chickens, and on the 28th day, *PAX7*, *MYF5*, *MYOD1* and *MRF4* expressions were higher in LWS than HWS chickens (Yin et al., 2014). According to a quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) study on samples from Dzhalginsky Merino sheep, *MYOD1* was one of 17 of the 48 genes studied and had the greatest expression in the loin muscle (Trukhachev et al., 2016). In pigs and cattle, *MYF5*, another important myogenesis regulator, has been associated with traits affecting meat quality (Ujan et al., 2011; Liu et al., 2008). Various regulatory transcription factors, such as *MYOD1*, have been discovered in the biceps femoris and longissimus dorsi muscles (LDMs) of purebred (IB) and Duroc-hybrid (IB DU) pigs (Ayuso et al. 2016). *MYOD1* was expressed during two developmental periods (birth and growth) and may consequently have a significant impact on phenotypes. This makes pigs' gene an important candidate gene for the ability to build muscles.

Gene disruption in mice has also been used to clarify the function of bHLH myogenic regulators (Arnold and Winter, 1998). *MRF4* can initiate only a limited amount of myogenesis during embryonic development in the absence of both *MYF5* and *MYOD* (Kassar-Duchossoy et al., 2004). In the absence of *MYF5*, *MYOD*, and *MRF4*, a complete failure of myoblast differentiation and muscle formation occurs (Rudnicki et al., 1993). Consequently, these elements work together in transcriptional networks that are only partially redundant to control the fate of myoblast cells during embryonic and fetal development. However, while *MRF4* and *MYOD* can support some differentiation during embryogenesis, fetal myogenesis largely fails in Myogenin-null animals, with only a few differentiated myofibers present (Hasty et al., 1993; Nabeshima et al., 1993; Venuti et al., 1993)., 1995). This is where myogenin plays a unique function in fetal myocardial development. In cases where *MYOD* or *MYF5* null mutations are present, muscle growth is

approximately normal. Careful examination revealed that early limb and branchial arch muscle development was delayed in *MYOD* null embryos, whereas trunk muscle development was slowed in *MYF5* null embryos. The total lack of skeletal myocytes or myofibers in mice with null mutations in both *MYOD* and *MYF5* genes suggests that *MYF5* or *MYOD* is required for the development and/or survival of myoblasts. Due to the already identified interference with the development of myogenic cells, the myogenin null mutation significantly reduces the amount of skeletal muscle tissue. Targeted silencing of the *MRF4* gene leads to largely normal muscle development, demonstrating that *MRF4* is not required to develop or maintain differentiated, functional skeletal muscle. *MRF4/MYOD* double mutants exhibit severe muscle deficiency equivalent to that conferred by myogenin gene deletion, although *MRF4* and *MYOD* single mutations have no effect on myogenesis. This suggests that when the *MYOD* gene is inactivated, myogenin alone cannot maintain proper muscle development. Based on the findings of null mutation studies, MRFs have unique but overlapping roles.

Muscle-related transcription factors play a role in the complex signaling cascades that trigger myogenesis, but they do not specifically control it. A crucial step in myogenesis is the essential and reversible phosphorylation process performed by the family of enzymes known as protein kinases. Numerous protein kinases have been shown to participate in various stages of myogenesis; therefore, activating or inhibiting them can directly alter the activity of muscle cells (Knight and Kothary 2011). Protein kinase A (PKA) is required for the formation of myogenic precursors in the dermomyotome at various stages of muscle development. In the case of PKA, myogenic factors such as *PAX3*, *MYOD*, and *MYF5* can form myotomes in dermomyotome cells (Chen et al., 2005). Wnt1 and Wnt7a, both produced by dorsal neural tubes outside the ectoderm, are involved in the initial phase of this activity. Myogenesis and adipogenesis are controlled by up- and down-regulation of (Wnt)/-catenin cascade signaling, respectively (Du et al. 2010). In addition to promoting the production of myogenic factors such as *MYF5*, *MYOD* and *PAX3*, PKA increases proliferation by phosphorylating MEF2 and inhibiting its effect (Knight and Kothary 2011). Retinoblastoma protein (Rb) is phosphorylated by cyclin-dependent kinases 2 and 4 (CDK2, 4) to prevent its binding to E2 factor (E2F), which maintains the expression of genes involved in the cell cycle and allows for its continued expression. Cell cycle progression is governed by this mechanism. In addition, phosphorylated Rb is unable to bind *MYOD*, which initiates S phase entry and allows CDK2 and CDK4 to suppress differentiation (Skapek et al, 1996; Gu et al, 1993). The presence of growth factors (GFs), such as fibroblast growth factor (FGF) and insulin-like growth factor (IGF), is required for extracellular signal-regulated kinase (ERK) activation. ERK1/2 activation is necessary for the prevention of myoblast

proliferation and myoblast differentiation in the early stages of myogenesis and for correct myocyte fusion in the late stages (Knight and Kothary, 2011). In addition to promoting proliferation, Akt1 also inhibits the expression of genes linked to cell cycle exit by phosphorylating FOXO1 (Nagata et al., 1998 ; Morooka et al., 1998 ; Bhat et al., 2007). Phosphorylation of MEF2 and E47 in myogenesis results in cell cycle exit of myogenic precursor cells. All of these elements work in concert to induce differentiation along with phosphorylated RNA polymerase II triggered by *MYOD* and CDK9.

### **2.3.2. Function of myogenic regulatory factors in mature muscle: In mature, healthy muscle, *MRF4* is the most expressed MRF.**

Muscle progenitors at the postnatal satellite cell stage are identified by *PAX3* and *PAX7* proteins located beneath the basal lamina of adult myofibers (Kassar-Duchossoy et al., 2005). In the postnatal myofiber, all satellite cells express *PAX7*, but not all satellite cells express *PAX3*. Gene expression studies in primary myoblasts in conjunction with ChIP-seq research on *PAX7* and *PAX3* revealed that *PAX7* has a greater affinity for homeodomain binding motifs than *PAX3*, even though both transcription factors recognize the same DNA patterns. While *PAX7* specifically activates genes involved in the maintenance of the phenotype of adult satellite cells, from regulation of proliferation to inhibition of differentiation, *PAX3* binds a subset of *PAX7* target genes that are mainly involved in the regulation of embryonic functions and the maintenance of an undifferentiated phenotype (Soleimani et al., 2012). Research has predominantly focused on understanding how *MYF5* and *MYOD* expression is regulated in satellite cells and how this affects the cells' commitment to the myogenic lineage. Recent studies have shown that adult satellite cells do not express *MYOD* at rest, but use of a *MYOD*-iCre mouse strain with a lineage-tracing reporter allele shows that all progenitors derived from satellite cells express *MYOD* prenatally, regardless of their anatomical location and embryological origin (Kanisicak et al., 2009). To induce expression of *MYF5*, the histone methyltransferase complex Wdr5-Ash2l-Mll2 (Kmt2) must be recruited to the *MYF5* locus. This promotes transcriptional activation of *MYF5* through asymmetric muscle stem cell divisions (McKinnell et al., 2008). In addition, it was shown that satellite cells actually produce the *MYF5* gene, but the transcript is retained in mRNP granules by a process mediated by miR31, maintaining these cells in a quiescent state. Release of trapped transcripts and rapid translation of *MYF5* mRNAs occurs as a result of mRNP granule separation during satellite cell activation (Crist et al., 2012). The transcription factors FoxO3, Six1/4, *PAX3*, and *PAX7* stimulate *MYOD* expression in proliferating myoblasts (Grifone et al., 2005; Hu et al., 2008). As differentiation progresses towards the formation of myotubes, the *MYOD* locus migrates to the lumen of the nucleus, where the transcription factors TAF3/TRF3 promote *MYOD* expression (Yao et al., 2011). *MYOD*

stimulates Myogenin production and inhibits *MYF5* expression in these conditions (Deato et al., 2008). The switch from *MYF5* to myogenin occurs simultaneously with cell cycle exit and the differentiation decision (Liu et al., 2012). Expression of the *MRF-4* gene and other late muscle differentiation genes results from the combined activities of *MYOD* and Myogenin and drives the development of multinucleated fibers. *MYOD* and Myogenin expression is then downregulated in mature muscle fibers, but *MRF4* is still produced at high levels to serve as the major MRF in adult differentiated muscle (Hinterberger et al., 1991). In adult rodent muscle, *MRF4* transcript levels are the highest among MRFs, and mice do not show a clear preference for any particular muscle or fiber type (Hughes et al., 1993; Voytik et al., 1993). *MRF4* mRNA is transiently produced in fetal mice and exhibits a biphasic expression pattern during muscle development, in contrast to previous reports in mice that *MRF4* expression is restricted to adult skeletal muscle (Rhodes and Konieczny, 1989; Hinterberger et al., 1991). *MRF4* mRNA was consistently expressed in growing chicken breast muscle, and subsequent studies using Northern blot hybridization found comparable results (Fujisawa-Sehara et al., 1992). *MRF4* mRNA expression has been found in adult pectoral muscle. There was no significant change in expression levels between ALD, PLD, and both. However, the *MYF5* expression level in these mature skeletal muscles was quite low.

### **2.4 Regulation of MRFs by signaling molecules**

Direct expression of MRFs is synergistically induced by a combination of signaling molecules secreted from the neural tube and surrounding structures, which tightly control vertebrate myogenesis during embryonic development to identify myogenic progenitors in somites and drive their differentiation (Bryson-Richardson et al., 2008). Wnts, Sonic hedgehog (Shh), Notch receptor, and bone morphogenetic proteins (BMPs) are among the chemicals that can trigger myogenic specification (Bentzinger et al., 2012; Marcelle et al., 1997). A large family of glycoproteins known as Wnt proteins has been revealed to have multiple members that are essential for early myogenesis in somites (Rudnicki et al., 2015). In addition to Wnt proteins, Sonic hedgehog (Shh) produced from the notochord and dorsal neural tube functions in somitic tissue to promote myogenesis in vitro (Münsterberg, 1995). Shh signaling maintains *MYF5* and *MYOD* expression in mouse limb buds during the development of hypaxial muscles, and there is a significant deficiency in hypaxial limb muscles in Shh  $-/-$  animals (Krüger et al., 2001). Shh is an important protein found in the *MYF5* enhancer to identify myogenic progenitor cells. Gli- directly stimulates the expression of *MYF5* through its binding sites (Anderson et al., 2012). These findings suggest that Wnts and Shh may affect the myogenic potential of unknown cells.

It shows that they are working collaboratively to determine BMPs and the Notch receptor suppress the production of MRFs, while Wnt and Shh proteins

positively control the properties of myogenic progenitors (Hirsinger et al., 1997; Hirsinger et al., 2001; Schuster-Gossler et al., 2007). They prevent cells from differentiating, which promotes progenitor cell growth instead of differentiation. BMPs, members of the Transforming growth factor (TGF) superfamily, work through serine-threonine kinase receptors to activate SMAD proteins and their translocation to the nucleus, resulting in activation or repression of target genes (Hinck, 2012).

### **2.5. Myogenic Regulatory Factor Functions in Satellite Cells during Regenerative Myogenesis**

To test satellite cell functionality *in vivo*, acute or chronic regeneration can be initiated. Acute regeneration models involve intramuscular injections of myotoxins, such as cardiotoxin or notexin, by freezing or crushing, which are more synchronous and traumatic (Hardy et al., 2016). Chronic regeneration is often evaluated using muscle disease models that experience repeated regenerative/degenerative episodes, such as the *mdx* mouse (Bulfield et al., 1984). Hepatocyte growth factor, sphingolipids, nitric oxide, and other signals are just a few of the signals that can activate satellite cells (Comai et al., 2014; Dumont et al., 2015a; Dumont et al., 2015b). In addition to *MYF5* protein, *MYF5* mRNA is released from mRNP granules in quiescent satellite cells to promote rapid translation (Crist et al., 2012). *MYOD* and Myogenin expression can be detected in mononuclear cells before DNA synthesis begins, which occurs 4–8 h after acute crush injury in a mouse. Expression levels in myotubes then begin to decrease after 8 days and return to pre-injury values (Grounds et al., 1992; Rantanen et al., 1995). When damage is combined with denervation, in which case *MYOD* is expressed more strongly and over a longer period of time, *MYOD* is detectable after only 12 h *in vivo* in mice and is only present momentarily in some nuclei of the least developed myotubes (Koishi et al., 1995; Rantanen et al., 1995). Therefore, if an acute injury is caused by muscle excision, marcaine HCl immersion, and regrafting (Fuchtbauer et al., 1992), denervation is likely to cause *MYOD* to express in both mononuclear cells and explains the subsequent discovery of entire nuclei of newly formed myotubes. Approximately 12 hours after injury, myogenin appears in mononuclear cells and later also in myotubes (Fuchtbauer et al., 1992; Rantanen et al., 1995). Adult muscle contains myonuclei where *MRF4* is located and is increased when muscle injury occurs (Zhou et al., 2001). Although *MRF4* has a limited role in establishing the myogenic lineage during embryogenesis, it is expressed in adults only after myoblasts have fused into myotubes and undergo maturation (Kassar-Duchossoy et al., 2004). Neither *MRF4* transcript nor *MRF4* protein is present during satellite cell activation and proliferation or even during early myogenic differentiation and fusion (Hinterberger et al., 1991; Zhou et al., 2001; Pavlath et al., 2003). Serum growth factors such as transforming growth factor- $\beta$  (Vaidya et al., 1989; Heino et al., 1990), fibroblast growth

factor (Vaidya et al., 1989; Brunetti et al., 1990) and insulin-like growth factor (Florini et al., 1991) has been shown to be involved in myogenic determination and differentiation by controlling the expression of myogenic factors in *in vitro* myogenesis systems. The mechanisms by which myogenic factors regulate muscle growth are currently not fully understood. However, previous research suggests that innervation regulates the development of chicken breast muscle. According to previous studies, isoform transition of myofibrillar proteins from the neonate to the adult state is prevented by denervation of neonatal chicken breast muscle (Obinata et al., 1984). In addition, these studies show that denervated adult muscle reexpresses neonatal isoforms such as slow C-protein, muscle-type f-tropomyosin and neonatal versions of troponin T (Obinata et al., 1984; Obinata et al., 1986). Therefore, it can be speculated that the different pattern of expression of myogenic factors may be vital in supporting muscle development from embryonic to adult fast or slow muscle, and innervation may play a crucial role in controlling this process.

### **2.6. Role of Growth Factors (GFs) in Skeletal Muscle Growth**

Various types of GFs affect the differentiation and proliferation of skeletal muscle growth. Hepatocyte growth factor (HGF) has been shown to improve the surface elasticity of bovine satellite cells *in vitro* (Lapin et al., 2013) and to stimulate the proliferation and migration of myogenic cells (Bandow et al., 2004). In chickens, Fibroblast growth factor FGF2 has been discovered to inhibit cell differentiation and stimulate the proliferation of satellite cells and myoblasts, the two types of muscle precursor cells (Velleman, 2007). Therefore, FGF2 expression is essential for the normal development of muscle fibers throughout the embryonic stage. However, this substance also prevents the proper development of myotubes by inhibiting myogenin transcription (Brunetti et al., 1990). IGFs (insulin-like growth factor) control and promote cell proliferation, differentiation, hypertrophy, and protein synthesis associated with myogenesis (Kamanga-Sollo et al., 2003; Knight and Kothary 2011). Transforming growth factor (TGF) and myostatin (GDF-8) have opposing effects on differentiation (Shahjahan, 2015); consequently, their expression in agricultural animals for meat purposes should be restricted. IGF-1 mRNA expression in chicken muscle decreased during development, increased after hatching, and decreased once again after day 7 post-hatching (Wu et al., 2011). It was also significantly higher in embryonic muscle than in embryonic liver. IGF-I and IGF-II increased during differentiation in porcine satellite cells (Theil et al., 2006). IGF-II mRNA is most increased at gestational day 85 in fetal sheep, highlighting its importance for leg myogenic fiber development during this period (Fahey et al., 2005), while double-muscled Gerrard (DM) cattle show a delay in IGF-II expression and it developed more muscle fibers as a result of a mutation in the *MSTN* gene (Gerrard et al., 1994).



Another key element is growth hormone (GH), which plays an important role in the GH-IGF axis and influences skeletal muscle development in farm animals both genetically and environmentally (Rehfeldt et al. 2011).

### 3. Conclusion

The next phase of research should focus on finding the reasons why adult skeletal muscle stem cells as well as the many muscle lineages throughout embryonic development are present. There are still unanswered questions about the roles of myogenin and *MRF4* in adult skeletal muscle and regeneration myogenesis. In the field of developmental biology, single-cell approaches and lineage tracing experiments are currently being used to uncover new mechanistic insights into upstream regulatory networks in the embryo and link these to our current biochemical understanding of muscle differentiation. This will provide a satisfactory and comprehensive understanding to create new therapeutic techniques to treat skeletal muscle disorders such as muscular dystrophies and age-related regeneration difficulties. In farm animals, selection can significantly improve the complex but continuous process of muscle growth, and the discovery of associated candidate genes can improve it further. Understanding the processes underlying muscle growth and development has advanced significantly over the past few years. In addition, important regulators, including transcription factors and GFs, have been identified and their functions related to many aspects of muscle development have been examined. Identification of such important regulators and genes provides a great help for marker-assisted selection, is crucial for the goal of increasing meat yield and helps breeders in maximizing meat quantity and quality. Moreover, gene sets typically associated with muscle growth and development may be helpful in applied investigation of mammalian muscle growth. However, the principles underlying muscle growth and development in agricultural animals, particularly sheep and cattle, require further research.

### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	G.A.I.	D.G.	Z.Ö.
C	40	30	30
D	40	30	30
L	40	30	30
W	40	30	30
CR	40	30	30
SR	40	30	30

C=Concept, D= design, L= literature search, W= writing, CR= critical review, SR= submission and revision.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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