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Econometric Analysis of Corn Production in Türkiye

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Abstract: In this research, within the specified years, corn in Türkiye by years; It is aimed to determine the existence of a causality between the production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables. For this purpose, corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables between 1995 and 2020 published by the Turkish Grain Board (TMO; 2021). In addition, the crisis years were added to the data set as a dummy variable to see the crisis effect. In the research, Augmented Dickey Fuller method is the test that checks whether the series is stationary. After deciding that the series is non-stationary with the ADF test, the most appropriate lag length was determined with the VAR (Variance Autoregressive Model) model, and the analysis was carried out with the help of the VAR Granger Causality method. According to the estimation results, corn in the statistical tables between 1995-2020; In the data set, in which the crisis years were added as a dummy variable to see the effects of the crisis in terms of production, cultivation area, yield, export amount and import amount; First difference is stationary, Akaike Information Criterion is the smallest, 2nd lag length is the most suitable lag length, and corn; corn; It has been determined by the analysis that production amount, import amount and economic crises are a one-way cause of exports, and corn cultivation area is a one-way cause of economic crises.

Keywords: Corn, corn yield, econometrics, economic crises, years.

Türkiye'de Mısır Üretiminin Ekonometrik Analizi

Öz: Bu araştırmada, belirtilen yıllar içerisinde Türkiye'de mısırın yıllara göre; üretim miktarı, ekim alanı, verim, ihracat miktarı, ithalat miktarı ile ekonomik krizler arasında kukla değişken olarak eklenen üretim miktarı, ekim alanı, verim, ihracat miktarı, ithalat miktarı arasında nedensellik ilişkisinin varlığının belirlenmesi amaçlanmıştır. Bu amaçla, Türkiye Toprak Mahsulleri Ofisi (TMO; 2021) tarafından yayımlanan 1995-2020 yılları arasındaki istatistiki tablolarda yer alan mısır üretimi, mısır ekim alanı, mısır verimi, mısır ihracat miktarı ve mısır ithalat miktarı verileri kullanılmıştır. Ayrıca kriz etkisini görmek için kriz yılları da kukla değişken olarak veri setine eklenmiştir. Araştırmada, serilerin durağan olup olmadığını kontrol eden test, Augmented Dickey Fuller yöntemidir. Serinin ADF testi ile durağan olmadığına karar verildikten sonra VAR (Varyans Otoregresif Model) modeli ile en uygun gecikme uzunluğu belirlenmiş ve analiz, VAR Granger Nedensellik yöntemi yardımıyla gerçekleştirilmiştir. Tahmin sonuçlarına göre, 1995-2020 yılları arasındaki istatistiki tablolarda yer alan mısır; Üretim, ekim alanı, verim, ihracat miktarı ve ithalat miktarı açısından krizin etkilerini görmek için kriz yıllarının kukla değişken olarak eklendiği veri setinde; Birinci fark durağan, Akaike Bilgi Kriteri en küçük, 2. gecikme uzunluğu en uygun gecikme uzunluğu olup; mısırda; üretim miktarı, ithalat miktarı ve ekonomik krizlerin ihracatın tek yönlü nedeni olduğu, mısır ekim alanının ise ekonomik krizlerin tek yönlü nedeni olduğu yapılan analizlerle belirlenmiştir.

Anahtar Kelimeler: Mısır, mısır verimi, ekonometri, ekonomik krizler, yıllar.

1. Introduction

Corn is a cereal plant product that has been cultivated for thousands of years. It is estimated that the first place where agriculture was practiced was the American continent. In historical research conducted in the American state of New Mexico, it was determined that corn cob remains and grains found in caves and shelters built from rocks date back to approximately 5000 years ago. In addition, in historical research conducted in the capital of Mexico in 1954, corn flower dust was found 50-60 meters below the ground, which was determined to be approximately 7000 years old. According to the findings obtained in all historical researches, it has been determined that corn grain has a history of approximately 8,000 to 10,000 years (Babaoğlu, 2004).

Corn in the world; It is one of the three grains with the highest production, cultivation area, trade and usage. Its use in animal production as silage, green and concentrated feed has contributed greatly to the rapid development of corn in the world. In the last 20-25

years, corn yield has increased significantly in the world and in Türkiye, making silage the main feed component in the rations of dairy cattle (Korkmaz et al., 2019). The use of corn grains in industrial fields such as biofuel, edible oil, corn syrup, alcohol production and ethanol has increased. In human consumption, it has uses such as roasted, boiled, popcorn, snacks, flour, oil and starch (Özcan, 2009).

Global corn production in the world market in 2020 is 1.21 billion tons, while America is the leader in exports (63.5 million tons) and China ranks first in imports (185.5 million tons) (TMO, 2021).

The production amount of grain products in Türkiye is 38.7 million tons. Among grain products production, corn grain production ranks second with 8.5 million tons. In the 2020 market, corn production was 6.5 million, and Konya ranked first in exports (3.20 million tons) (TMO, 2021).

According to the literature review; According to the literature review; He examined the econometric analysis of soybean agriculture in Türkiye and the effects of the economic crises in Türkiye on soybean agriculture. As a result of the analysis, no significant relationship was found between the crises in Türkiye and the amount of imports, import prices and soybean yield (Unakıtan and Aydın, 2012); The study aimed to reveal the relationships between agricultural R&D expenditures and agricultural growth. Within the scope of the research, an average annual growth of 0.14% in technical efficiency and 0.38% in technological change was determined in the period 1990-2010 in Turkish agriculture.

As a result, total factor productivity increased by 0.51% during the period (Subaşı and Ören, 2013); This study aims to evaluate the sensitivity of corn producers to the environment and human health in terms of the use of seeds, chemical fertilizers and pesticides in Adana province. In the study, 95 producers were interviewed by face-to-face survey method and Chi-square independence test analysis was performed. As a result of the research, it was determined that corn producers in Adana province are generally not sensitive to the environment and human health in terms of the use of seeds, chemical fertilizers and pesticides (Özalp and Güldal, 2017); This study aims to investigate the economic development of corn, which is an important source of food and industrial raw materials in the world and in Türkiye. For this purpose, field values, production amount, efficiency, production-consumption balance, import-export values, domestic and international prices of corn grown from secondary data were analyzed. As a result of the research, it is seen that the USA is successful in domestic consumption and plays an important role in corn exports as well as domestic consumption. The qualification rate, which was determined in the qualifications of corn in the 2016/17 production year in Türkiye and was determined as 87.80% as of the year, has now been determined. It is expected that the increase in food demand due to population growth in the world, the search for alternative energy sources and the widespread use of corn will lead to an increase in demand and therefore an increase in economic value in the coming years (Bayramoğlu and Bozdemir, 2018); The study was conducted to determine the socio-economic status of corn producers in Diyarbakır province and the sources of information on production techniques and economic issues related to corn agriculture. In addition, the marketing structure and problems of corn in the province are revealed. As a result of the research, it was determined that there was a relationship between the producers' first sources of information about corn agriculture and their business size groups. It has also been revealed that there is a relationship between business size groups and marketing channels (Yaşa, 2019);

It is aimed to analyze the sustainability of agricultural activities in corn production, the solution of problems encountered in the process from planting to harvest, and the expectations of farmers. As a result of the research, it was determined that agricultural operators have future concerns about input costs, storage facilities and consumption of water resources (Bozdemir et al., 2019); In this study, the amounts of protein fractions in the grain of 120 genotypes of local maize populations were determined. In addition, protein fractions of 30 selected genotypes were determined according to their molecular weights by SDS-PAGE analysis, and a dendrogram revealing statistical relationships between them was obtained by the clustering method. As a result of the data obtained, it was revealed that local maize populations have a wide variation, and genotypes that can be used in breeding studies to develop varieties with different protein quality characteristics were identified (Akbulut et al., 2021). It is considered important in terms of repeating existing studies and developing solutions to problems.

In the years determined in this research, corn in Türkiye according to years; It is aimed to determine the existence of a causality situation between production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables.

2. Materials and Methods

The data set in this study was obtained from the fields of corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables between 1995-2020 published by the Turkish Grain Board (TMO; 2021). In addition, the crisis years were added to the data set as a dummy variable to see the crisis effect. In Table 1. and Table 2., corn production, corn cultivation area, corn yield, corn export amount and corn import amount are given according to years.

Table 1. Corn by years; production, cultivation area, yield, export amount and import amount.
Çizelge 1. *Yıllara göre mısır; üretim, ekim alanı, verim, ihracat miktarı ve ithalat miktarı.*

Years	Corn Planting Area (Ha)	Corn Production Amount (Ton)	Corn Yield (Kg/Da)	Corn Export Amount (Ton)	Corn Import Amount (Ton)
1995	515.000	1.900.000	369	6132	49.239
1996	550.000	2.000.000	364	73.995	239.807
1997	545.000	2.080.000	382	162.752	453.776
1998	550.000	2.300.000	418	481.751	695.782
1999	518.000	2.297.000	443	629.400	381.780
2000	555.000	2.300.000	414	502.139	28.509
2001	550.000	2.200.000	400	26.603	9
2002	500.000	2.100.000	420	10	78.596
2003	560.000	2.800.000	500	80.763	381.193
2004	545.000	3.000.000	550	362.133	474.302
2005	600.000	4.200.000	700	207.360	660.985
2006	536.000	3.811.000	711	540.870	0
2007	595.000	3.535.000	683	282.446	0

Table 2. Corn by years; production, cultivation area, yield, export amount and import amount.
Çizelge 2. *Yıllara göre mısır; üretim, ekim alanı, verim, ihracat miktarı ve ithalat miktarı.*

Years	Corn Planting Area (Ha)	Corn Production Amount (Ton)	Corn Yield (Kg/Da)	Corn Export Amount (Ton)	Corn Import Amount (Ton)
2008	595.000	4.274.000	718	346.564	832.378
2009	592.000	4.250.000	718	524.654	183.467
2010	594.000	4.310.000	726	90.572	83.491
2011	589.000	4.200.000	713	362.508	47.632
2012	622.609	4.600.000	739	24	125.962
2013	659.998	5.900.000	894	67.607	1.373.444
2014	658.645	5.950.000	903	1.229.771	173.541
2015	688.170	6.400.000	930	280.296	1.752.453
2016	680.019	6.400.000	941	1.465.880	1.756.906
2017	639.084	5.900.000	923	2.539.964	204.757
2018	591.900	5.700.000	963	638.119	184.247
2019	638.829	6.000.000	939	-	-
2020	691.632	6.500.000	940	-	-

The data set obtained from the fields of corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables between 1995-2020, published by the Turkish Grain Board (TMO; 2021), was used in 2000, 2001, 2008, 2018, 2019 and The economic depression and crises that occurred in 2020 are included in the model as dummy variables.

In this research, unit root tests were used to make the time series stationary in the given data set. If there is a unit root, the time series is not stationary. Dickey-Fuller (DF) and Augmented Dickey-Fuller (ADF) tests, which are used to detect the presence of a unit root, are the most well-known methods.

γ_t The relationship of the variable to its value one period ago is formulated as follows.

$$\gamma_t = \beta_{\gamma_{t-1}} + u_t \quad (1)$$

The hypotheses are based on the model;

$H_0 = \beta = 1$ (the series contains a unit root, the series is not stationary)

$H_1 = \beta < 1$ (there is no unit root in the series, the series is stationary).

Here, u_t independent identically distributed (iid) has constant variance and zero mean

is assumed. The error term with these properties is called white noise and the equation is shown as follows

$$u_t \approx iid(0, \sigma^2 \quad (2)$$

$\beta = 1$ on the other hand, the series is under the effect of one period previous value and random shocks. If so it can be said that the series contains a unit root. $\beta < 1$ whereas γ_{t-1} of γ_t Its effect on β will gradually decrease depending on the value of β.

Here, the 'τ' (tau) statistic, which emerged in Dickey-Fuller's Monte Carlo application, is used. If τ the absolute value of the statistic exceeds the absolute value of the Dickey-Fuller critical value, it accepts the hypothesis that the time series is stationary, and in general the Dickey-Fuller test is applied to the following regression patterns:

1)Dickey-Fuller equation with no constant term and no trend:

$$\Delta_{\gamma_t} = \delta_{\gamma_{t-1}+} u_t \quad (3)$$

2)Dickey-Fuller equation with constant term and no trend:

$$\Delta_{\gamma_t} = \beta_0 + \delta_{\gamma_{t-1}} + u_t \quad (4)$$

3) Dickey-Fuller equation with constant term and trend:

$$\Delta_{\gamma_t} = \beta_0 + \beta_1 t + \delta_{\gamma_{t-1}} + u_t \quad (5)$$

As a result of the Dickey-Fuller test, if the stationarity of the series is not mentioned, it is retested by taking the difference of the dependent variable. If the series becomes stationary as a result of the first difference operation, the first difference is said to be stationary. If the series does not become stationary as a result of the first difference, the second difference of the series is tested and continued. The series that becomes stationary at this stage is called second-order difference stationary. It is continued in this way for further difference taking operations. However, since the interpretation of the coefficients will be difficult and the

degree of freedom will decrease, in practice, the difference is usually stopped after the second difference (Dickey and Fuller, 1979; 1981). In case of autocorrelation in the estimated regressions, the DF test results are invalid. Extended DF test is applied to fix this problem. Simply put, the lagged values of the dependent variable are to the right of the equation. The equations to be estimated in the ADF test are as follows.

$\Delta_{\gamma_t} = \delta Y_{t-1} + \sum_{j=1}^{k} \alpha \Delta Y_{t-j} + u_t$ (without a fixed term) (6)

$\Delta_{\gamma_t} = \beta_0 + \delta Y_{t-1} + \sum_{j=1}^{k} \alpha \Delta Y_{t-j} + u_t$(constan term) (7)

$\Delta_{\gamma_t} = \beta_0 + \delta Y_{t-1} + \sum_{j=1}^{k} \alpha \Delta Y_{t-j} \alpha_t + u_t$ (constant term and trend variable added) (8)

$H_0 = \delta = 0$ If there is a unit root in the series, the series is not stationary.

$H_1 = \delta < 0$ There is no unit root in the series and the series is stationary. (Dickey ve Fuller, 1981).

The VAR (Variance Autoregressive Model) model was used to find the most appropriate lag length in the research. In VAR (Variance Autoregressive Model) model econometric studies, it is inevitable to use the simultaneous equation system in case the links between the link size are multilateral and complex. One of the methods developed as a solution method of simultaneous equations is Vector Autoregressive Models (VAR). The VAR model can be represented by the following equation for the p-value:

$$\gamma_t = A_1 \gamma_{t-1} + \cdots + A_p \gamma_{t-1} \beta_{x_t} + \varepsilon_t \quad (9)$$

Here, $y_t p \times 1$ the value vector of dimension, $x_t\ d \times 1$ dimensional deterministic variables, $A_p p \times p$ dimensional parameter matrices and error terms vector (Johansen, 1995).

VAR models are used for time series as they do not impede the systematic model and do not need to distinguish between extrinsic and intrinsic values. In addition, since there are lagged values of dependent values in VAR models, it makes it possible to make better and stronger predictions for the future. Since the coefficients calculated with the VAR model are very complex and difficult to interpret, more variance decomposition and impulse-response analysis methods are used (Gacener, 2005). While variance decomposition tries to explain how many % of the change in the variance of each of the analyzed values has its own delay and what percentage of the other values are excluded, impulse-response analysis tries to explain what happens when the other value or values cause a one-unit effect on any of the values. tries to explain how much he is affected (Tari, 2012).

In the following years, corn in Türkiye according to the years; The VAR Granger Causality test was used to determine the existence of a causality between the production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables. The VAR Granger Causality test showed that using the current and past values of Xt in addition to the past values of this series while performing the prediction of Granger Yt, Xt and Yt being two stationary time series, yielded better results than only the values of Yt in the past. Causality relationships between Xt and Yt depending on time $\alpha, b_1, \beta_1\ and\ \theta_1$ lag coefficients m same lag length for all variables $\varepsilon_{1t}, \varepsilon_{2t}$ with independent error terms;

$$Y_t + b_0 X_t = \sum_{i=1}^{m} \alpha_i Y_{t-i} + \sum_{i=1}^{m} b_i X_{t-i} + \varepsilon_{1t} \quad (10)$$

$$X_t + b\theta_0 Y_t = \sum_{i=1}^{m} \beta_i X_{t-i} + \sum_{i=1}^{m} \theta Y_{t-i} + \varepsilon_{2t} \quad (11)$$

is in the form. Here

- It shows that Xt causes Yt, provided that the bi values are different from zero at a certain significance level.
- θ_i It shows that Yt causes Xt, provided that its values are different from zero at a certain significance level.
- Both bi and θ_i It shows that there is a bilateral causality relationship between Xt and Yt, provided that they are different from zero at a certain significance level.
- Both bi and θ_i it shows that there is no causality between Xt and Yt if it is not different from zero at a certain significance level (Granger, 1969).

3. Results

The data set in this study was obtained from the fields of corn production, corn cultivation area, corn yield, corn export amount and corn import Amount in the statistical tables between 1995-2020 published by the Turkish Grain Board (TMO; 2021). In addition, the crisis years were added to the data set as a dummy variable to see the crisis effect. In the obtained data set, as a first step, the stationarity of the series was tested with the help of Augmented Dickey Fuller (ADF) method.

In Table 3, the first differences were taken with the help of the unit root test and the data set was made stationary.

Table 3. Unit root test result.
Çizelge3. *Birim kök testi sonucu.*

Variables	ADF t-statistics	Probabilities
X1t	-3.540331	0.0176
X2t	-5.838442	0.0001
X3t	-4.333990	0.0025
X4t	-3.030384	0.0483
X5t	-6.891581	0.0000
X6t	-5.805353	0.0001

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

In Table 4., it was determined that '*' was the most and the 2nd lag length with the smallest Akaike Information Criterion was the most appropriate lag length.

Corn by years; The existence of a causality between production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables was tested with the help of the VAR Granger Causality.

Table 4. Model lag length selection criteria.
Çizelge 4. *Model gecikme uzunluğu seçim kriterleri.*

Lag	LogL	LR	FPE	AIC	SC	HQ
0	-1417.585	NA	2.33e+46	123.7900	124.0862*	123.8645
1	-1371.048	64.74741	1.05e+46	122.8738	124.9473	123.3952
2	-1309.235	53.75092*	2.43e+45*	120.6291*	124.4799	121.5976*

* indicates lag order selected by the criterion, LR: sequential modified LR test statistic (each test at 5% level)
FPE: Final prediction error, AIC: Akaike information criterion, SC: Schwarz information criterion, HQ: Hannan-Quinn information criterion

Table 5. The result of the test when the dependent variable is the amount of corn production.
Çizelge 5. *Bağımlı değişken mısır üretim miktarı olduğunda testin sonucu.*

Values	Probabilities
X2t	0.9612
X3t	0.6955
X4t	0.7918
X5t	0.9138
X6t	0.2360

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

In Table 5. corn; It has been determined by the analysis that there is no reason for the amount of corn production because the cultivation area, yield, export amount, import amount and probability values of economic crises are greater than 0.05.

Table 6. Result of the test when the dependent variable is maize planting area.
Çizelge 6. *Bağımlı değişken mısır ekim alanı olduğunda test sonucu.*

Values	Probabilities
X1t	0.2818
X3t	0.2118
X4t	0.5003
X5t	0.9182
X6t	0.9688

*X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

Corn in Table 8. Since the cultivation area and yield probability values are greater than 0.05, there is no reason for the amount of corn export, while corn; Since the production amount, import amount and probability values of the economic crises that have occurred are less than 0.05, it has been determined by the analysis that there is a one-way reason for the amount of corn export.

In Table 9. corn; Since the production amount, yield, cultivation area, export amount and probability values of economic crises are greater than 0.05, it has been

Table 7. Result of the test when the dependent variable is corn yield.
Çizelge 7. *Bağımlı değişken mısır verimi olduğunda yapılan testin sonucu.*

Values	Probabilities
X1t	0.8553
X2t	0.8154
X4t	0.8936
X5t	0.9825
X6t	0.6773

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

Corn in Table 6. Since the production amount, yield, export amount, import amount and probability values of economic crises are greater than 0.05, it has been determined by the analysis that there is no reason for the corn cultivation area.

Table 8. The result of the test when the dependent variable is the amount of corn export.
Çizelge 8. *Bağımlı değişken mısır ihracat miktarı olduğunda yapılan testin sonucu.*

Values	Probabilities
X1t	0.0534
X2t	0.5086
X3t	0.2488
X5t	0.0000
X6t	0.0151

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

determined by the analysis that there is no reason for the amount of corn import.

Table 9. Result of the test when the dependent variable is corn import amount.
Çizelge 9. *Bağımlı değişken mısır ithalat miktarı olduğunda test sonucu.*

Values	Probabilities
X1t	0.6914
X2t	0.2829
X3t	0.0764
X4t	0.3930
X6t	0.2628

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

Table 10. The result of the test when the dependent variable is the dummy variable.
Çizelge 10. *Bağımlı değişkenin kukla değişken olduğu durumda testin sonucu.*

Values	Probabilities
X1t	0.6831
X2t	0.0303
X3t	0.9832
X4t	0.1791
X5t	0.9839

* X1t = Corn Production Amount * X2t= Corn Planting Area *X3t= Corn Yield *X4t=Maize Export Amount * X5t= Corn Import Amount * X6t= Economic Crises (dummy variable)

Corn in Table 10. It has been determined by the analysis that while the probability values of production amount, yield, export amount and import amount are greater than 0.05, there is no cause of the experienced economic crises, while the probability values of the corn cultivation area are a one-way cause of the economic crises experienced because the probability values are less than 0.05.

The difference between this research and the studies in the literature (Unakıtan and Aydın, 2012; Subaşı and Ören, 2013; Özalp and Güldal, 2017; Bayramoğlu and Bozdemir, 2018; Yaşa, 2019; Bozdemir et al., 2019; Akbulut et al., 2021) is in Turkey. corn according to years; The aim of this study is to determine the existence of a causality situation between production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables.

4. Discussion

In this research, within the specified years, corn in Türkiye by years; It is aimed to determine the existence of a causality between the production amount, cultivation area, yield, export amount, import amount and economic crises added as dummy variables. For this purpose, corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables between 1995 and 2020 published by the Turkish Grain Board (TMO; 2021). In addition, the crisis years were added to the data set as a dummy variable to see the crisis effect. In the research, Augmented Dickey Fuller method is the test that checks whether the series is stationary. After deciding that the series is non-stationary with the ADF test, the most appropriate lag length was determined with the VAR (Variance Autoregressive Model) model, and the analysis was carried out with the help of the VAR Granger Causality method.

According to the results of the research, the series are stationary because the probability values of the data set, whose first differences were taken by using the Argument Dickey-Fuller (ADF) test statistic in the created data set, are less than 0.05. In addition, with the help of the VAR (Variance Autoregressive Model) model, it was determined that the '*' was the most and the 2nd lag length with the smallest Akaike Information Criterion was the most appropriate lag length.

In the statistical tables published by the Turkish Grain Board (TMO; 2021), corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables and crisis years are added as a dummy variable to see the crisis effect. sweetcorn; corn; It has been determined by the analysis that production amount, import amount and economic crises are a one-way cause of exports, and corn cultivation area is a one-way cause of economic crises.

Data from the fields of corn production, corn cultivation area, corn yield, corn export amount and corn import amount in the statistical tables between 1995 and 2020 published by Turkish Grain Board (TMO; 2021) and crisis years are added as dummy variables to see the crisis effect. In order to obtain better results in the data set, it should be taken into account that the number of observations in the data set should be kept wider, economic and climatic changes should not be ignored in the selected years, and the decrease in efficiency due to natural events and global warming that has occurred in Türkiye in recent years. In order to avoid these and similar problems in future articles or thesis research, the deficiencies mentioned should not be ignored.

Author Contributions
The authors declare that they have contributed equally to the article.
Conflict of Interest
The authors of the article declare that there is no conflict of interest between them.

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Evaluation of 'Yomra' Hazelnut (*Corylus avellana* L.) Clones

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Abstract: This research was carried out in order to select superior clones with breeding aims in terms of late leafing, nut quality and yield in the Yomra hazelnut population of Düzce province and Akçakoca districts in 2020-2021. In the study, 98 Yomra hazelnut clones were examined and 20 clones were selected. Average yield per trunk cross-sectional area of selected clones changed from 83.25 to 32.43 g/cm^2, kernel ratio varied between 48.89 and 55.90%. It was determined that the nut weight, nut size and nut shape index, shell thickness, kernel weight , kernel size, and good kernel ratio of selected clones were respectively: 1.82 g to 3.90 g , 16.45 to 20.06 mm, 0.96 to 1.06, 0.87 mm to1.25 mm; 0.97 g to 1.91 g, 12.52 mm–14.88 mm, and 91.38% - 99.14%. It was determined that 5 clones (MZ 06, MZ 24, MZ 30, MZ 42 and 35 MZ 47) were superior to other clones. As a results, a significant genetic difference was observed in the Yomra hazelnut population.

Keywords: Clonal Selection, *Corylus avellana* L., Late leafing, Nut quality, Yield

Yomra Fındık Klonlarının (*Corylus avellana* L.) Değerlendirilmesi

Öz: Bu araştırma, 2020-2021 yıllarında Düzce ili ve Akçakoca ilçesi Yomra fındık popülasyonunda geç yapraklanma, meyve kalitesi ve verim ıslah amaçları yönünden üstün özellikli klonların seçilmesi amacıyla yürütülmüştür. Çalışmada 98 adet Yomra klonu incelenmiş ve 20 adet klon seçilmiştir. Seçilen klonların birim gövde kesit alanına düşen ortalama verimi 83.25 g/cm^2 ile 32.43 g/cm^2 arasında iç randımanı ise % 48.89 ile % 55.90 arasında değişmiştir. Seçilen klonların meyve ağırlığı, meyve iriliği, meyve şekil indeksi, kabuk kalınlığı, iç ağırlığı, iç iriliği ve sağlam iç oranı değerleri sırasıyla 1.82-3.90 g; 16.45-20.06 mm; 0.96-1.06; 0.87-1.25 mm; 0.97-1.91 g, 12.52-14.88 mm ve % 91.38-99.14 arasında bulunmuştur. Çalışmada; 5 klonun (MZ 06, MZ 24, MZ 30, MZ 42 ve 35 MZ 47) diğer klonlara göre daha üstün nitelikte olduğu tespit edilmiştir.

Anahtar Kelimeler: *Corylus avellana* L., Geç Yapraklanma, Meyve Kalitesi, Klonal Seleksiyon, Verim.

1. Introduction

Hazelnut production in the world is 1220427 tons. Türkiye ranks first in world hazelnut production with 765000 tons. Italy ranks 2nd with 98670 tons, Azerbaijan ranks 3rd with 72104 tons, and the USA ranks 4th with 70 310 tons. Although Türkiye ranks first in the world in hazelnut production, its yield is low. The average yield is 255.50 kg/da in the USA, 116.87 kg/da in Italy, 140.18 kg/da in Azerbaijan, and the average yield in Türkiye is 102.82 kg/da (Anonymous, 2024a). The amount of hazelnut production in Düzce province is 83052 tons in an area of 63 454 hectares. 16661 tons of hazelnuts were produced in an area of 12999 hectares in the central district and 29207 tons of hazelnuts were produced in an area of 21887 hectares in Akçakoca district (Anonymous, 2024b).

The most important hazelnut cultivars used in hazelnut cultivation in Türkiye are Cavcava, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kara, Kargalak, , Mincane, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem, Yuvarlak Badem, Okay 28, Giresun Melezi, Allahverdi, Çetiner and Yomrali.

Turkish and European hazelnut cultivars were independently selected from local types grown over centuries (Thompson et al., 1977). Commercial hazelnut nurseries do not exist in Türkiye. The plants of selected genotypes ("grower selections") have been used for a long time for establishing new orchards since the plants are readily propagated clonally with rooted suckers from neighboring orchards in Türkiye. Thus, plantations in the region are composed of mixtures of clones, which provide an opportunity to select the superior genotypes in terms of yield and nut quality. Among the existing hazelnut cultivars, it is important to select clones that are productive, resistant to diseases and pests, and have little or no alternance.

On the other hand, there are many studies in Türkiye aiming to develop new cultivars using different breeding

methods. Selection breeding studies initiated in the 1970s continue (Balık et al., 2018; Bilgen et al., 2018; Çetiner, 1976; İslam, 2003; İslam & Çayan, 2019; Kan, 2019; Karakaya, 2021; Şahin, 2019). From these studies, many clones with superior yield and nut quality have been identified. Some of these clones have been registered as new cultivars. 'Okay 28' and 'Giresun Melezi' varieties were developed in the hybridization breeding program that started in 1980 (Balık, 2018; Okay, 1999). Türkiye's special hazelnut variety breeding studies continue. New cultivars with high yield are needed to increase the comparative competitive conditions of hazelnut cultivation in Türkiye. In this context, it is important to find clone candidates with high yield values through clonal selection within the cultivars.

In previous hazelnut breeding studies, genetic diversity was not investigated in the Yomra hazelnut population of Düzce province and Akçakoca district. The aim of this research is to identify promising clone candidates within the Yomra hazelnut populations. In our research, clone candidates were selected based on leafing time, yield and nut quality parameters.

2. Material and Method

2.1. Material

This research was conducted in Düzce Central District and Akçakoca District between 2020 and 2021 (Figure 1). A total of 98 Yomra hazelnut clone candidates were examined and 52 clone candidates were identified.

2.1.1. Some characteristics of Yomra hazelnut cultivar

Yomra is widely found in the hazelnut orchards of Trabzon and Düzce provinces. The average nut weight of the variety is 2.06 g, the kernel weight is 1.28 g, and the kernel ratio is 57.6% (Köksal, 2018). The time of beginning of leaf budburst of the Yomra cultivar is later than Tombul, Mincane and any other Turkish cultivars. Yomra variety leaves 3-5 days earlier than Çakıldak variety. Yield characteristics are similar to other varieties (Balık et al., 2016).

2.1.2. Climate characteristics of the research area

It was reported that the annual average temperature, the annual average rainfall, the relative humidity and the daily average temperature were respectively: 13.7ºC, 829.90 mm, 75.28%, and 14.2 °C (Anonymous, 2024c).

2.2. Method

2.2.1. Determination of time of beginning of leaf budburst

Time of beginning of leaf budburst was determined according to Descriptors for Hazelnut (*Corylus avellana* L.) criteria (Köksal and Güneş, 2008).

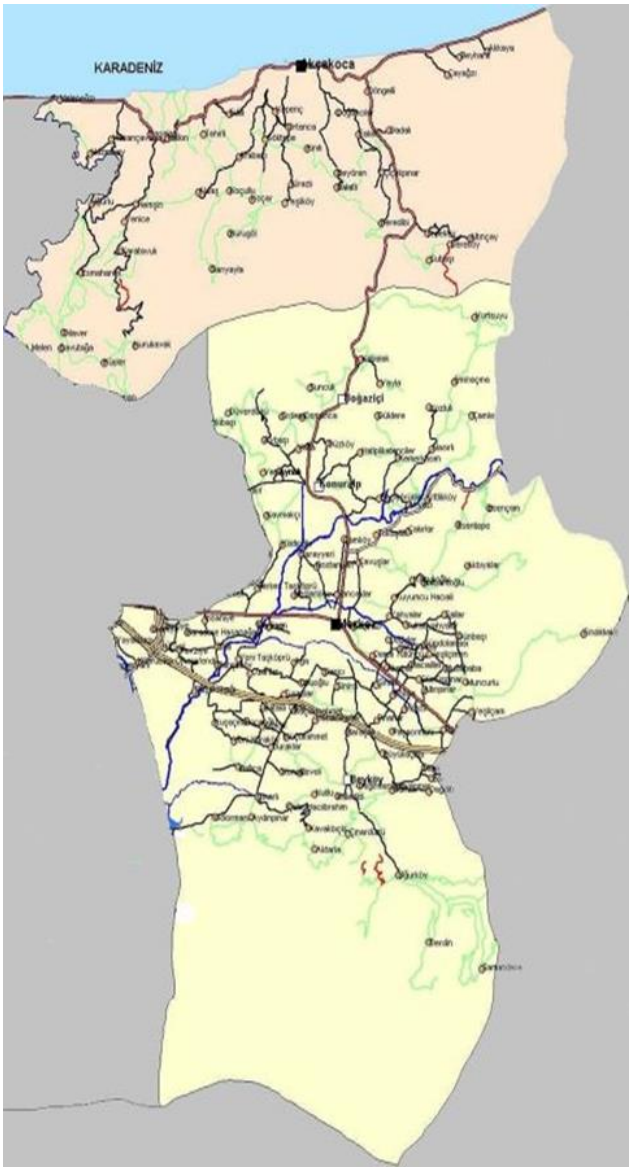

Figure 1. Map of the region where the research was conducted.
Şekil 1. *Araştırmanın yapıldığı alanın haritası*

2.2.2. Preselection of clones

Orchards were selected based on, yield, disease and pest observations (*Xanthomonas coryline, Phyllactinia guttata, Alaninus nucum L., Xyleborus dispar, Palemona prasine).* In the orchards where the research was conducted, care was taken to have 5-6 plants in the 'Ocak'. Late leafing plants were marked and selected in these orchards.

2.2.3. Yield (g/cm² and g/plant)

It was determined by weighing all nuts harvested from a plant (one branch). The yield per unit trunk cross-sectional area and the yield per branch of genotypes with late leafing time were determined. The yield was calculated based on formula (F_1) (Köksal & Güneş, 2008).

F_1 (g/cm²) = Total yield per branches (g) / $\pi.r^2$ (1)

2.2.4. Pomological characteristics

All nuts were collected from one branch selected from the southern side of the plants. These nuts were counted. The nuts used in pomological characteristics were dried until the kernel nut moisture content was reduced to 6%. Nuts samples were placed in 1 kg paper bags and stored in the refrigerator at ~4°C. Pomological characteristics were examined in 30 nuts randomly taken from selected branch according to Descriptors for Hazelnut (*Corylus avellana* L.) criteria In the measurements, a digital caliper sensitive to 0.01 mm and an electronic scale sensitive to 0.01 g were used. The nut size (F_2), kernel size (F_3), good kernel (F_4) and kernel percentage (F_5) were calculated based on formulas respectively (Köksal & Güneş, 2008).

$F_2 = \sqrt[3]{a.b.c}$ (2)

(a:Nut length; b:Nut width, c:Nut thickness)

$F_3 = \sqrt[3]{a.b.c}$ (3)

(a:Kernel length; b:Kernel width, c:Kernel thickness)

F_4= (%): ([Number of good kernels/Total number of kernels]) x100 (4)

F_5= ([Kernel weight/Nut weight]x100) (5)

2.2.5. Harvest time

The nuts were harvested when the moisture value dropped below 30%, and when 3/4 of the husks turn red or brown.

2.2.6. Selection of superior clones

The "Modified Weighted Rating Method" was used for the selection of clones. The importance levels and class ranges of the characters used in the weighted rating method are presented in Table 1.

Table 1. Treatments, importance levels, class intervals and score table of the modified weighted grading method
Çizelge 1. *Değiştirilmiş tartılı derecelendirme yönteminde kullanılana özellikler, önem düzeyleri, sınıf aralıkları ve puanları*

Characters	Importance level (%)	Class	Class intervals			Score
			2020	2021	Means	
Yield (g/cm²)	30	High	48.78–70.27	68.69–97.18	58.33–83.25	5
		Medium	27.28–48.77	40.20–68.68	34.41–58.32	3
		Low	5.79–27.27	11.71–40.19	9.99–34.40	1
Kernel ratio (%)	15	High	47.45–54.66	51.49–54.30	49.85–54.17	5
		Medium	40.23–47.44	48.67–51.48	45.53–49.84	3
		Low	33.02–40.22	45.86–48.66	41.21–45.52	1
Nut weight (g)	15	High	3.18–3.88	3.20–3.92	3.19–3.90	5
		Medium	2.48–3.17	2.48–3.19	2.48–3.18	3
		Low	1.78–2.47	1.76–2.47	1.77–2.47	1
Shell thickness (mm)	10	High	1.11–1.26	1.47–1.79	1.22–1.41	1
		Medium	0.97–1.10	1.14–1.46	1.02–1.21	3
		Low	0.83–0,96	0.82–1.13	0,83–1.01	5
Good kernel ratio (%)	10	High	88.89–100	94.64–100	92.41–99.14	5
		Medium	77.78–88.88	90.24–94.63	85.69–92.40	3
		Low	66.67–77.77	85.85–90.23	78.96–85.68	1
Kernel cavity (mm)	15	High	3.73–4.87	3.76–4.91	3.75–4.89	1
		Medium	2.60–3.72	2.60–3.75	2.60–3.74	3
		Low	1.46–2.59	1.45–2.59	1.45–2.59	5
Blanching (%)	5	High	80.33–100	80.33–100	80.33–100	5
		Medium	60.67–80.32	60.67–80.32	60.67–80.32	3
		Low	41.00–60.66	41.00–60.66	41.00–60.66	1

Based on the 2020 and 2021 modified weighted rating total scores, clones with scores higher than 350 were selected as promising clones. Others were eliminated. In addition, all clones with fluctuations in their weighted rating scores in 2020 and 2021 have been eliminated. Again, clones that showed fluctuations in yield values over the years despite having high weighted rating scores were also eliminated.

It was determined that the Yomra hazelnut population examined in our research had negative characteristics in terms of yield, kernel percentage, nut weight, shell thickness, good kernel cavity and blanching. These characters were given priority in the pre-selection of clones. Thus, clones with superior characteristics in terms of these characters were selected from the population with disadvantageous characters.

2.2.7. Cluster analyses

The yield (g/cm²), nut weight, nut size, nut shape, shell thickness, kernel weight, kernel size, kernel shape, kernel cavity, good kernel and blanching characteristics of 98 clones were used in the cluster analysis. NTSYSpc21 statistical program was used in cluster analyses.

Table 2. Distribution of genotypes examined within the Yomra hazelnut population according to their characters.
Çizelge 2*. Yomra fındık populasyonunda incelenen genotiplerin karakterlerine göre dağılımı.*

Characters	Class	Values	The Number of Clones	Rate (%)
Yield (g/cm²)	High	58.33–83.25	**5**	**5,10**
	Medium	34.41–58.32	32	32,65
	Low	9.99–34.40	61	62,24
Kernel percentage (%)	High	49.85–54.17	**68**	**69,39**
	Medium	45.53–49.84	28	28,57
	Low	41.21–45.52	2	2,04
Nut weight (g)	High	3.19–3.90	**1**	**1,02**
	Medium	2.48–3.18	12	12,24
	Low	1.77–2.47	85	86,73
Shell thickness (mm)	High	1.22–1.41	7	7,14
	Medium	1.02–1.21	45	45,92
	Low	0,83–1.01	**46**	**46,94**
Good kernel ratio (%)	High	92.41–99.14	**71**	**72,45**
	Medium	85.69–92.40	23	23,47
	Low	78.96–85.68	4	4,08
Kernel cavity (mm)	High	3.75–4.89	14	14,29
	Medium	2.60–3.74	38	38,78
	Low	1.45–2.59	**46**	**46,94**
Blanching (%)	High	80.33–100	**57**	**58,16**
	Medium	60.67–80.32	30	30,61
	Low	41.00–60.66	11	11,22

3. Results and Discussion

3.1. First Year Results

A total of 98 clones candidates were selected in 2020. Time of beginning of leaf budburst of the selected clones were observed between 19 March and 9 April. We recorded the yield (g/cm²) between 5.79 and 70.27; kernel ratio between 54.66 and 33.02%; nut weight 1.78±0.27 g–3.88±0.30 g. Average shell thickness is between 0.83±0.10 mm and 1.26±0.16 mm; rate of good kernel observed between 100% and 41.00% and kernel cavity was determined to between 1.46±0.68 mm and 4.87±1.18 mm (Table 1).

3.2. Second Year Results

According to 2021 data, time of beginning of leaf budburst was determined between 21 March and 7 April (Table 2). We observed the yield (g/cm²) 11.71 and 97.18; kernel ratio was determined between 45.86 and 54.30%; nut weight varied between 1.76±0.27 g and 3.92±0.31 g. We recorded the good kernel between 85.85 and – 100.00% and the blanching was determined between 41.00 and 100.00% (Table 1).

The proportion of clones with high yield and high nut weight is low in the Yomra hazelnut population examined in our research (Table 2). It is important to select clones that are productive and have nut weight from the Yomra hazelnut population, which has a later leafing time than other Turkish hazelnut cultivars.

Table 3. Weighted rating scores of superior clones
Çizelge 3. *Üstün klonların tartılı derecelendirme puanları*

Clones	2020	2021	Mean	Altitude	Leafing date (2020-2021)
MZ06	420	420	420	210	March 22-23
MZ24	420	410	415	364	March 25-25
MZ47	390	390	390	515	March 25-28
AA02	390	380	385	25	March 23-29
MZ42	370	390	380	503	March 22-26
AA05	380	380	380	52	March 25-22
AA08	380	380	380	75	March 27 -28
AA10	380	380	380	85	March 29-2 April
AA13	380	380	380	93	March Mart
AA15	380	380	380	95	March Mart
AA24	380	380	380	162	April 1-5
AA17	360	380	370	100	April 1-4
MZ30	370	360	365	410	April 1-9
MZ01	360	360	360	169	April 1-2
MZ34	350	370	360	433	April 1-5
MZ50	360	350	355	530	March 29
AA23	360	350	355	155	March 29-30
AA25	360	350	355	165	March 29-30
MZ09	360	340	350	220	March 26-28
MZ21	350	350	350	315	March 23-3 April

3.3. Superior Clones

In our research, superior clones were selected from the Yomra hazelnut population in terms of yield, kernel ratio, nut weight, shell thickness, good kernel, kernel cavity and blanching. It is observed that the 20 clones candidates selected from the 98 clones that were pre-selected from the populations (Table 3). The weighted rating scores of superior clones ranged between 350-420. The altitudes of the selected clones were determined between 25 and 530 m above sea level. The bud burst time of the superior clones selected according to years were observed between 22 March and 1 April (Table 3.)

The superior clones of are described in Table 4. Yield is an important breeding aim. After high-yield genotypes are selected, the nut quality criteria of these genotypes are evaluated. There is a negative correlation between high yield and nut size. If genetic diversity is high in the population, the probability of selecting promising genotypes increases. In this research, genetic diversity was found quite high in the Yomra hazelnut population where our research was conducted. MZ 06, MZ 24, MZ 30, MZ 42 and MZ 47 clones were found to be more valuable than other clone candidates in terms of yield, nut weight and kernel percentage.

Most of the hazelnut orchards in Türkiye were established according to the 'ocak system'. In other countries, the single stem planting system with grafting is common. In Türkiye, the yield of hazelnut varieties is determined on one branch selected from one 'Ocak'. Differences in training systems make it difficult to compare the yield of cultivars between countries.

The yield value (g/cm²) of the superior clones ranged 58.33 to 83.25, and average yield was calculated as 47.82 g/cm^2 (Table 4). Superior clones MZ06 and MZ24 have the highest yield value. The yield value of Palaz clones was between 8.2 (P-50) and 363.4 g/cm^2 (P-27) and the average yield value was 83.77 g/cm^2. In the Çakıldak population, yield values varied between 24.5 (Ç-80) and 323.5 g/cm^2 (Ç-11). The average yield value is given as 67.2 g/cm^2 (Karakaya, 2021). The yield values of selected clones was found lower than other Turkish hazelnut clone candidates. Low yield may be caused by insufficient rainfall. As a matter of fact, the total rainfall in Ordu (1045 mm) and Giresun (1290 mm) is higher than Düzce province (829 mm).

Across the clones studied here, nut weight ranged from 1.82 to 3.90 g, while kernel weight ranged from 0.97 to 1.91 g with clones MZ42 and MZ47 showed highest kernel and nut weight (Table 5). For nut weight a heritability estimate of 0.63 was determined by Yao and Mehlenbacher (2000). Islam, (2003) determined nut weights between 1.69 and 2.28 g for 'Tombul', between 2.05 and 2.71 g for 'Palaz', and between 1.56 and 2.34 for 'Uzunmusa'. In the high range of reported values for several cultivars: 3.89 g 'Gunslebert' and 3.82 g Grada de Viseu (Ferrão et al., 2021), 2.9 g 'Willamette'(Mehlenbacher et al., 1991); 2.10-2.60 g 'Tonda Gentile Romana clones (Monastra et al., 1997), 1.98–2.25g 'Gironell Clones, 1.40-2.01 g, 1.40-2.01 g 'Negret' (Rovira et al., 1997), 2.9 g 'Lewis' (Mehlenbacher et al., 2000), 2.50 g 'Clark' (Mehlenbacher et al., 2001), 2.29-2.50 g 'Palaz clones', 1.88-2.01 g 'Çakıldak clones' (İslam & Özgüven, 2001),

3.70g 'Jefferson' (Mehlenbacher et al., 2011), 2.75-2.88g 'Pollyo' (Mehlenbacher et al., 2019), 2.08 g 'Somerset' (Molnar et al., 2020); 2.06 g 'Yomra'(Köksal, 2018), 2.13-2.27 g 'the clones candidate of Palaz' (Balık&Beyhan 2014), 3.90 g 'Barcelona' and 3.40 g 'Dorris' (Mehlenbacher et al., 2013). MZ47 has a much higher nut weight than other hazelnut cultivars.

Table 4. Yield and nut characteristics of selected clones
Çizelge 4*. Seçilen klonların verim ve kabuklu meyve özellikleri*

Clones	Yield (g/plant)	Yield (g/cm²)	Kernel percentage (%)	Nut weight (g)	Nut size (mm)	Nut length (mm)	Nut width (mm)	Nut height (mm)	Nut Shape index	Bleaching (%)	Fibrousness
AA02	375.10	66.84	53.67bcde*	2.21±0.24fgh	18.11	17.75±0.77efg	19.26±0.79cd	17.37±0.59efg	0.97	86	Low fibrous
AA05	483.30	42.20	54.22abcde	1.99±0.26jk	17.40	16.94±1.30hı	18.52±0.86efg	16.80±0.81hıj	0.96	91	Low fibrous
AA08	336.09	42.26	55.90a	2.20±0.20fgh	17.98	17.54±0.68fgh	19.05±0.83cde	17.40±0.50defg	0.96	91	Low fibrous
AA10	539.26	46.31	54.96abc	2.16±0.25ghıj	17.95	17.56±0.75fgh	19.13±0.87cde	17.21±0.94fgh	0.97	100	Low fibrous
AA13	531.35	46.01	52.34e	2.36±0.28def	18.76	18.23±2.16de	20.12±2.03ab	18.01±2.03bc	0.96	95	Low fibrous
AA15	425.85	39.79	55.39ab	2.14±0.16hıj	17.44	17.03±0.76hı	18.27±0.58fgh	17.04±0.58fghı	0.96	94	Low fibrous
AA17	314.70	42.06	54.35abcde	2.18±0.25gh	17.74	17.74±0.86efg	18.82±0.75def	16.74±0.76hıj	1.00	89	Fibrous
AA23	450.26	42.81	52.69de	2.56±0.23d	19.10	18.93±0.76bc	20.03±0.73ab	18.37±0.59b	0.99	82	Unfibrous
AA24	497.61	42.04	54.94abc	2.11±0.21ıj	17.34	17.79±0.72efg	17.74±0.65hı	16.52±0.65ıj	1.04	99	Unfibrous
AA25	415.97	32.43	52.34e	2.40±0.14cde	17.59	17.29±0.60ghı	18.64±0.60defg	16.90±0.63ghıj	0.97	91	Low fibrous
MZ01	445.67	35.86	54.16abcde	2.31±0.27efg	16.90	17.49±0.86fgh	16.91±0.70j	16.36±0.60j	1.06	92	Low fibrous
MZ06	478.28	63.91	53.87abcde	2.30±0.28efgh	17.77	18.31±0.75cde	18.12±0.81gh	16.93±0.72ghı	1.05	81	Unfibrous
MZ09	182.35	34.10	52.45e	2.24±0.27efgh	17.40	18.03±0.80def	17.34±0.80ıj	16.87±0.77ghıj	1.06	54	Unfibrous
MZ21	335.20	47.70	54.56abcd	2.39±0.32de	18.23	18.59±1.44bcd	18.53±1.21efg	17.59±1.14cdef	1.03	81	Unfibrous
MZ24	467.21	83.25	54.58abcd	2.26±0.28efgh	17.81	18.27±0.86de	18.07±0.87gh	17.12±0.62fgh	1.04	93	Low fibrous
MZ30	360.32	62.70	53.90abcde	2.16±0.29ghıj	17.50	17.72±0.85efg	17.77±0.95hı	17.03±0.69fghı	1.02	85	Unfibrous
MZ34	369.11	44.09	54,81abc	2.51±0.31cd	18.42	18.32±0.70cde	19.10±1.02cde	17.85±0.74bcde	0.99	73	Unfibrous
MZ42	334.54	67.40	49.33f	2.86±0.32b	18.90	19.20±1.00b	19.61±0.94bc	17.94±0.72bcd	1.02	88	Fibrous
MZ47	803.96	39.00	48.89f	3.90±0.31a	20.06	20.43±0.89a	20.61±1.29a	19.18±0.98a	1.03	92	Fibrous
MZ50	318.60	35.65	53.02cde	1.82±0.24k	16.45	16.75±0.88ı	16.89±0.89j	15.75±0.79k	1.03	95	Fibrous
Range	182.35: 803.96	32.43: 83.25	48.89: 55.90	1.82: 3.90	16.45: 20.06	16.75: 20.43	16.89: 20.61	15.75: 19.18	0.96: 1.06	54.00: 100.00	-
Mean	423.24	47.82	53,52	2.35	17.94	18	18.63	17.25	1.01	86	-

*Each data value is represented as means ± standard deviation. Means within columns followed by different letters differ significantly ($p \leq 0.05$)

Yao and Mehlenbacher, (2000), reported that the heritability of kernel weight in hazelnuts, was 0.67. Average kernel weight in selected clone candidates was 0.97 g (MZ50)–1.91 g (MZ47). Values reported as kernel weight for some hazelnut varieties: 1.45 g 'Willamette' (Mehlenbacher et al., 1991), 1.01–1.11 g, Tonda Gentile Romana' (Monastra et al., 1997), 0.82–0.94 g, 'Negret', (Rovira et al., 1997), 0.99–1.43 g Palaz superior clones (Bostan & İslam 1999), 1.23–1.44 1.01–1.09 g Çakıldak clones (İslam & Özgüven, 2001). The kernel weight of MZ47 clone was found higher than 'Barcelona', 'Clark', 'Dorris', 'Lewis' and 'York' (Mehlenbacher et al., 2013). Also, MZ47 clone has higher kernel weight than other Turkish hazelnut cultivars. Although the nut weight of the MZ47 clone is similar to the Barcelona variety, its kernel weight higher than 'Barcelona' (Mehlenbacher et al., 2013).

Shell thickness ranged from 0.87 mm to 1.25 mm across our clones. It was seen that the another studies determined that it varied between the given values; Kan and İslam (2023) 0.89-1.34 mm, İslam & Çayan (2019), 0.60-1.24 mm, Uzun (2021) 0.71-1.42 mm, Petriccione et. all, (2010),1.0 and 1.8, İslam (2003) 0.75–0.93 mm. These values indicate that the selected clones have a shell thickness comparable to other cultivars described in the literature. Kernel percentage is one of the most important nut trait (Table 5).

Heritability estimates of 0.92 (Thompson, 1977) and 0.87 (Yao and Mehlenbacher, 2000) have been reported for this trait. In our research, the kernel percentage of clones varied between 48.89% (MZ47) and 55.90% (AA08) and was above the average of several hazelnut cultivars. The kernel percentage value was found to be lower than 50% in only two clones. The kernel percentage was determined as 51.59–57.31% (P-60) in Palaz clones and 49.62–58.84% in Çakıldak clones (Karakaya, 2021). In our research, the kernel percentage values of the selected clones were observed similar to the Palaz and Çakıldak populations. In our research, the kernel percentage of the selected clones were found to

be similar to the Willamette', Tonda Gentile Romana', 'Gironell' clones, Palaz, 'Lewis' and Clark (Mehlenbacher et al., 1991; Monastra et al., 1997; Rovira et al., 1997; Bostan & İslam 1999; Mehlenbacher et al., 2000; Mehlenbacher et al., 2001; Mehlenbacher et al., 2011). We recorded the blanching (%) between 54.00 -100.00% (Table 4).It was determined that the good kernel (%) ranged between 91.38 and 99.14 ((Table 4). Good kernel (%) values given by some researchers: İslam, (2003) 69.90-92.15, Turan &Beyhan, (2009) 32.00-98.00, Pekdemir, (2019) 57.00-59.00, Karakaya, (2021) 73.70-90.70, Aydemir et al., (2023) 53.00-95.00. As a matter of fact, the good kernel ratio (%) values of our clones are higher than Willamette, 'Negret', 'Lewis', 'Clark', 'Jefferson', Okay 28' varieties. It is especially important that our MZ47 clone has a high value. The kernel nuts of the 7 clones selected as promising are unfibrous (Table 4).

Table 5. Kernel characteristics of selected clones
Çizelge 5. *Seçilen klonların iç meyve özellikleri*

Clones	Kernel weight (g)	Kernel size (mm)	Kernel Length (mm	Kernel width (mm)	Kernel height (mm)	Kernel Shape Index	Shell thickness (mm)	Kernel cavity (mm)	Good Kernel (%)
AA02	1.19±0.15fgh*	14.13	13.24±0.76defg	14.96±1.40bcd	14.25±0.82bcd	0.98	0.98±0.07hı	2.62±0.96cd	95.60
AA05	1.08±0.15ı	13.56	12.73±0.76g	14.40±1.15defg	13.61±0.73efgh	0.90	0.90±0.06k	1.61±0.78e	97.79
AA08	1.23±0.10efgh	14.21	13.29±0.69cdef	14.96±0.76bcd	14.44±0.39abc	0.90	0.87±0.07k	2.17±0.81cde	97.38
AA10	1.19±0.15fgh	13.98	13.29±0.73cdef	14.82±0.93cde	13.87±0.98defg	0.89	0.89±0.08k	2.33±0.82cd	97.24
AA13	1.23±0.11efgh	14.20	13.18±0.73efg	15.50±0.86ab	14.02±0.85cde	0.89	0.97±0.06ıj	2.35±0.83cd	98.64
AA15	1.18±0.08fgh	13.66	13.10±0.65efg	14.18±0.57fg	13.72±0.50defgh	0.91	0.91±0.06	2.37±1.05cd	95.04
AA17	1.18±0.13fgh	14.07	13.30±0.91cdef	15.27±0.85abc	13.71±0.91defgh	0.97	0.97±0.06ıj	2.53±0.82cd	96.93
AA23	1.35±0.12bcd	14.88	14.12±0.68b	15.61±0.91a	14.93±0.67a	0.93	1.03±0.06fgh	3.65±1.10a	95.86
AA24	1.16±0.11hı	13.66	13.74±0.61bcd	14.06±0.75fgh	13.21±0.81hı	1.01	0.91±0.06jk	2.57±0.74cd	97.96
AA25	1.26±0.10def	13.81	13.39±0.50cdef	14.44±0.79def	13.61±0.82efgh	0.96	0.96±0.05ıj	2.31±1.02cd	93.52
MZ01	1.25±0.17efg	12.86	13.18±1.16efg	12.57±0.85k	12.88±0.81ıj	1.04	1.19±0.11bc	2.18±0.77cde	99.14
MZ06	1.24±0.17efgh	13.55	13.63±0.82bcde	13.37±0.80ıj	13.67±0.87efgh	1.01	1.13±0.07cd	2.58±1.20cd	95.16
MZ09	1.17±0.16fghı	13.21	13.04±0.85fg	13.26±1.11ıj	13.34±1.09ghı	0.98	1.07±0.13ef	2.06±0.88de	94.11
MZ21	1.30±0.16cde	13.93	13.78±1.08bc	13.79±0.89ghı	14.25±0.86bcd	0.98	1.06±0.08ef	3.74±1.24a	97.15
MZ24	1.23±0.15hı	13.56	13.75±0.80bcd	13.47±1.18hıj	13.45±0.90fgh	1.02	1.05±0.07ef	2.42±0.90cd	96.93
MZ30	1.16±0.16hgı	13.29	13.39±0.62cdef	12.89±1.05jk	13.61±0.74efgh	1.01	1.07±0.13	2.75±1.07bc	90.69
MZ34	1.37±0.17bc	14.47	14.07±0.87b	14.63±1.09cdef	14.73±0.58ab	0.96	0.99±0.09ghı	3.33±1.02ab	98.14
MZ42	1.41±0.15b	14.37	14.97±0.87a	14.27±0.92efg	13.90±0.81cdef	1.06	1.25±0.16a	3.70±1.19a	96.25
MZ47	1.91±0.15a	13.42	14.78±1.23a	13.19±1.44ıjk	12.39±1.46j	1.16	1.23±0.11ab	1.99±0.98de	98.05
MZ50	0.97±0.16	12.52	12.14±0.67h	12.99±0.69jk	12.46±0.83j	0.96	1.05±0.07efg	2.05±0.97de	96.47
Range	0.97: 1.91	12.52: 14.88	12.14: 14.97	12.57: 15.61	12.39: 14.93	0.89: 1.16	0.87: 1.25	1.61: 3.74	91.38: 99.14
Mean	1.25	13.77	13.51	14.13	13.7	0.97	1.03	2.57	96.44

*Each data value is represented as means ± standard deviation. Means within columns followed by different letters differ significantly ($p \leq 0.05$)

3.4. Cluster Analysis

The population dendrogram is divided into 2 main groups and 2 subgroups in each group. In the analyses, the matrix correlation coefficient was found to be 0.74 at the 1% significance level. In the analyses, the matrix correlation coefficient was calculated as 0.76 at the 1% significance level. According to the dendrogram, clone 18 (81AA23) formed a different subgroup, and clones 13 (81AA08) and 14 (81AA10) were found to be identical between them (Figure 1).

4. Conclusions

A lot of clonal selection programs were performed in Türkiye and many cultivars were evaluated. 20 superior clones of 'Yomra' were identified in this study, although none of them were outstanding for all traits. Clones MZ06, MZ24, MZ30, MZ42 and MZ47 showed the best combination of percent pomological traits and yield. Studies are being carried out to preserve the promising clone candidates selected in our research and to use them as genetic resources in variety breeding.

Conflict of Interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

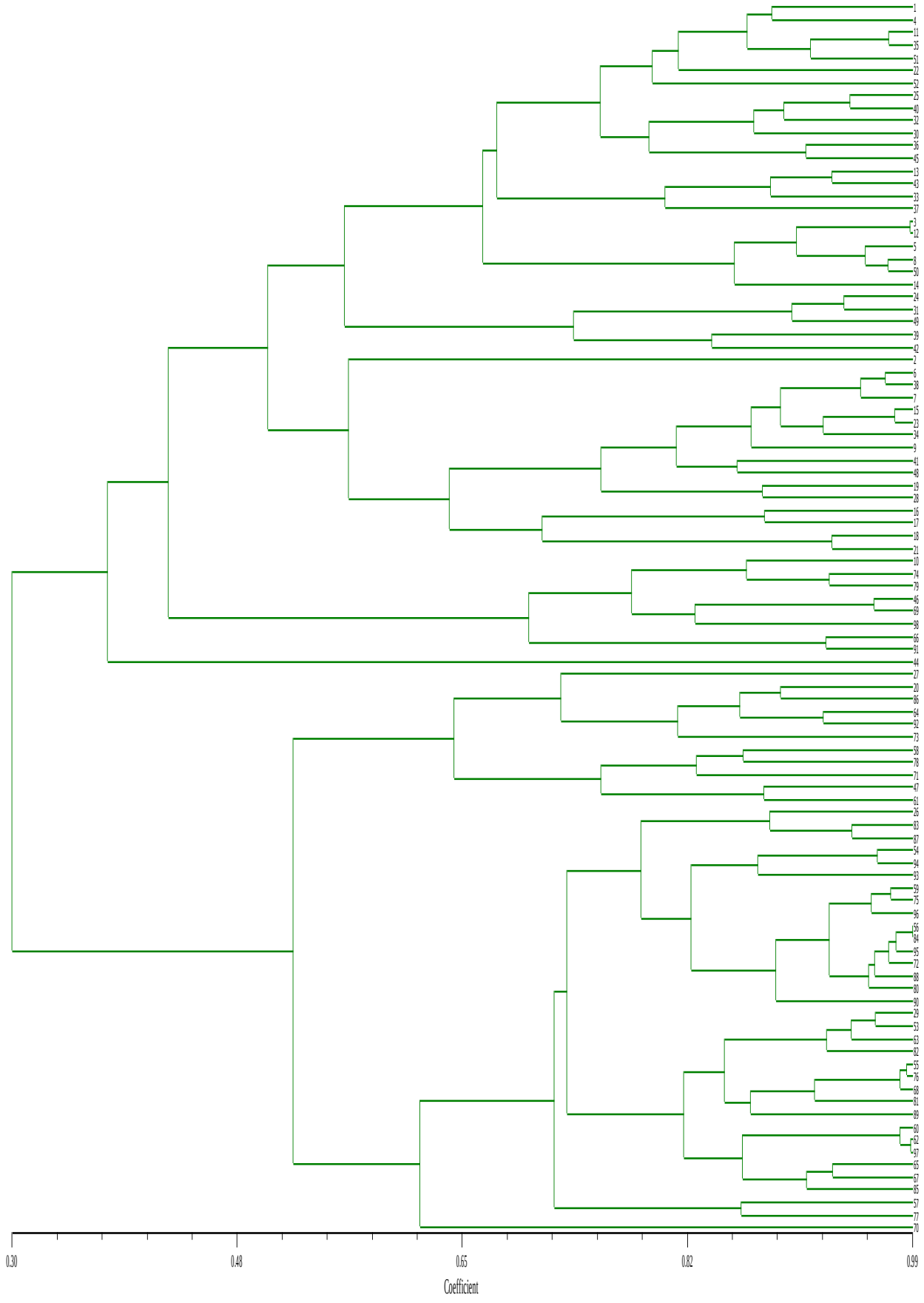

Figure 2. Dendrogram of Yomra clones according to the main nut characteristics
Şekil 2. *Yomra klonlarının başlıca meyve özelliklerine göre dendrogramı*

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Possibilities of Evaluation of Silage in Karacabey District, Bursa City

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Abstract: In this study, it was aimed to provide information about silage types, silage usage, feeding method of silage to dairy cows and solutions by organizing a survey in Karacabey district of Bursa city. A survey was applied to a total of 60 daiy cow breeders, including large, medium, and small-scale enterprises.
When asked "which type of silo do you use?", 82% of the animal enterprises declared that they use bulk-type silage, while 10% declared that they use bank-type silage and 8% declared that they use bale-type silage. When the the animal enterprises were asked what they use as a silage additive, 70% of the breeders stated that they did not use additives in silage making, while 20% stated that they used bacterial inoculants plus enzymes in silage making, and %5 stated that they used yeast and the rest stated that they used enzymes. When the breeders were asked about the method of feeding the silage to dairy cows, 73.0% reported that they gave silage to their cows mixed with roughage and concentrate feed, 25.0% reported that they gave silage to their cows mixed silage with dry roughage and 2.0% reported that they gave only silage to their cows. As a result, it is seen that most of the breeders are dependent on maize for silage production and prefer the bulk-type silage. On the other hand, it has been determined that basic information about silage production and animal nutrition of small-scale livestock enterprises is insufficient.
In this context, it can be recommented the small-scale enterprises to participate in training of organization which will increase their knowledges on subjects such as silage making and animal feeding. It may be suggested that they use other forage crops (food pulp residues and heat-resistant fodder crops) as a source of silage, and can also be fed the their animals daily by using a large bank type silage collectively.

Keywords: Survey, silage, Karacabey, dairy cow breeders

Bursa İli Karacabey İlçesinde Silaj Dğerlendirilme Olanakları

Öz: Bu çalışmada, Bursa'nın Karacabey ilçesinde anket çalışması düzenlenerek, silaj tipleri, silaj kullanımı, silajın süt ineklerine yemleme yöntemi ve çözüm yolları konusunda bilgi verilmesi amaçlanmıştır. Büyük, orta ve küçük ölçekli işletme olmak üzere toplam 60 süt ineği yetiştiricisine anket uygulanmıştır.
Sonuç, "ne tip silo kullanıyorsunuz?" sorusuna hayvan yetiştiricilerin %82'si yığın tipi silaj kullandıklarını beyan ederken, %10'u bank tipi silaj ve %8'i de balya tipi silaj kullandıklarını beyan etmişlerdir. "Yetiştiricilere silaj katkı maddesi olarak ne kullanıyorsunuz?" sorusuna, yetiştiricilerin %70'i katkı maddesi kullanmadıklarını ifade ederken, %20'si silaj yapımında bakteriyel inokulant artı enzim kullandıklarını %5'i maya ve geri kalanı ise enzim kullandıklarını bildirmişlerdir. Yetiştiricilere silajın süt ineklerine veriliş şekli sorulduğunda %73'ü ineklerine silajı kaba ve yoğun yemle karışık olarak verdiklerini, %25'i ineklerine silajı kuru kaba yemle karıştırarak verdiklerini %2'si ise silajı ineklerine tek başına verdiklerini bildirmişlerdir. Yetiştiricilerin büyük bir kısmı silaj yapımında mısıra bağımlı oldukları ve yığın tipi silajı tercih ettikleri görülmektedir. Diğer yandan küçük ölçekli hayvancılık işletmelerin silaj üretimi ve hayvan besleme ile ilgili temel bilgilerin yetersiz olduğu tespit edilmiştir.
Bu kapsamda, küçük ölçekli işletmelere silaj yapımı ve hayvan beslemeye yönelik deneyim ve bilgilerini artıracak tarımsal eğitim faaliyetlerin düzenlenmesi, silaj yem kaynağı olarak (gıda posası artıkları ve sıcağa dayanıklı) diğer yem bitkilerine yönelmeleri, kooperatif aracılığıyla büyük ölçekli silo yapılarak günlük silaj almaları önerilebilir.

Anahtar Kelimeler: Anket çalışması, Karacabey, silaj, süt işletmesi

1. Introduction

The most widely used plant in silage in the world is corn. In 2020/21, 196,982 areas (thousand ha) were allocated to corn production, and corn production is over 1.1 billion tons (1,143,555) and feed production is 725 million tons. Biofuel and other industrial uses also affect demand. As in previous years, the most important countries in corn production and cultivation in 2020/2021 are the USA, China, and Brazil (Anonymous, 2021). The export price of corn is 207.4 ($/ton) for food and agriculture organization of the United Nations (FAO) in 2021. As of 2019, 6.5 million tons of corn was used in feed production in Turkey. Karacabey is a district of Bursa, in the Southern

Marmara part of the Marmara Region. There are 98.454.605 decares of pasture area in the district, and the pastures are in the 3rd class position. Considering the presence of animals in the district, both the pasture area and forage crops are not sufficient for the roughage requirements of the animals. According to Turkish Statistical Institute (TUIK) data for 2020, Karacabey district; with 268 thousand 500 tons of silage corn production on 44 thousand decares of land, meets 22.30% of Bursa and 1% of our country. The starch content of corn grain is high compared to other grains. This is a sought-after feature in terms of silage fermentation (Mooi, 1991). Corn is preferred in dairy and fattening enterprises because it is a delicious, nutritious feed with energy value for ruminants as a silage feed source (FAO, 2013). The silage has a great potential in terms of meeting the vital needs of farm animal.

Economically, silage is cheaper than other feeds, with lower warehouse costs (Özhan, 2010) and labor requirements (Şahin & Zaman, 2010). On the other hand, in terms of the continuity of animal production, to increase the yield per animal and reduce the cost, it has great importance that the roughage given to the animal is of high quality and cheap (Yaylak & Alçiçek, 2003; Yıldırım, 2015). Paksoy & Ortasöz, (2018) The researcher states that the farmers prefer corn farming because of the state support and mechanization convenience. In Demir & Elmalı (2016) survey study, it was determined that one of the reasons why business owners use silage is the increase in the milk they provide from dairy cows. On the other hand, if the silage is contaminated with rot, mold, bad smell, or soil residues; it should not be fed to animals (FAO, 2022). When corn silage is also used in high amounts in the rations of ruminant animals, a decrease in milk fat can cause abomasum displacement, diarrhea, and acidosis. (Queiroz et al., 2018; Değirmencioğlu, 2020). In Çekiç (2017) survey study in Malkara district, he states that farmers use heap-type silos for silage production, they do not use additives, and they prefer corn for silage production, but farmers have difficulty in preparing a balanced ration with silage. In Akay & Dağdemir (2009) and Denli et al. (2014) studies, they stated that the producers experienced nutrient losses in the silage and were insufficient in feeding the animals.

With the increase in the number of animals in the future, the shortage of silage and other forage crops is expected to increase in Karacabey. In this study, it was aimed to provide information about silage types, silage usage, feeding method of silage to dairy cows, and solutions by organizing a survey in Karacabey district of Bursa city.

2. Material and Method

A survey was applied to a total of 60 daiy cow breeders, including large, medium, and small-scale enterprises in Karacabey district of Bursa province. In the research; the education level of dairy cow breeders, how many years they made silage, where they learned how to make silage, what type of silage they used, the feed they used in silage making, how much silage they gave to the animals per day and their thoughts on silage delivery times were evaluated. Questions answered (multiple choice, ordering according to the importance and yes or no) was used to determine the knowledge and thoughts of the breeders.

3. Research Findings and Discussion

As seen in Figure 1, 55% of the breeders attributed silage to being cheap as the reason for choosing it, 32% to increasing milk yield, 10% no response and 3% to consuming animals fondly. The rate of 32% participating in the survey partially parallels the positive effect of Demir & Elmalı (2016) on milk yield as the reason why farmers prefer silage.

When asked which type of silo you use, 82% of the animal enterprises declared that they use bulk-type silage, while 10% declared that they use bank-type silage and 8% that they use bale-type silage (Figure 2). Animal breeders in Karacabey prefer bulk-type silage for economic reasons. The fermentation losses in such silage are higher than the fermentation losses in all other silage types. The result obtained regarding the use of bulk type silage in Karacabey district is similar to the survey findings conducted in different regions of Turkey (Yıldız et al., 2008; Şahin & Zaman, 2010; Pınar & Dilek, 2016; Değirmencioğlu, 2016; Çekiç, 2017). Bench-type silage used by 10% of the respondents are the most suitable silage type to be used in Karacabey in terms of silage quality.

When asked what they use as plant material in silage making, 61% of the animal enterprises stated that they use corn as plant in silage making, while 32% stated that they use a mixture of grain and corn as plant, and the remaining 7% declared that they use a mixture of corn and legumes (Figüre 3). The usage of corn as a plant source in silage making is similar to the survey findings of (Değirmencioğlu, 2016;Çekiç, 2017). On the contrary, (Özdemir & Okumuş, 2021) state in their studies that corn, alfalfa and vetch varieties are used as plant in silage in Turkey.

According to the results of the 93% who participated in the survey, it is understood that other green fodder sources are not used sufficiently in silage production. It can be said that this situation is due to the fact that the studies on the silage usage in animal nutrition have not been transferred to farmers and remain at a limited level. The researcher stated that corn and some legume forage crops are grown together, and the silage made is higher

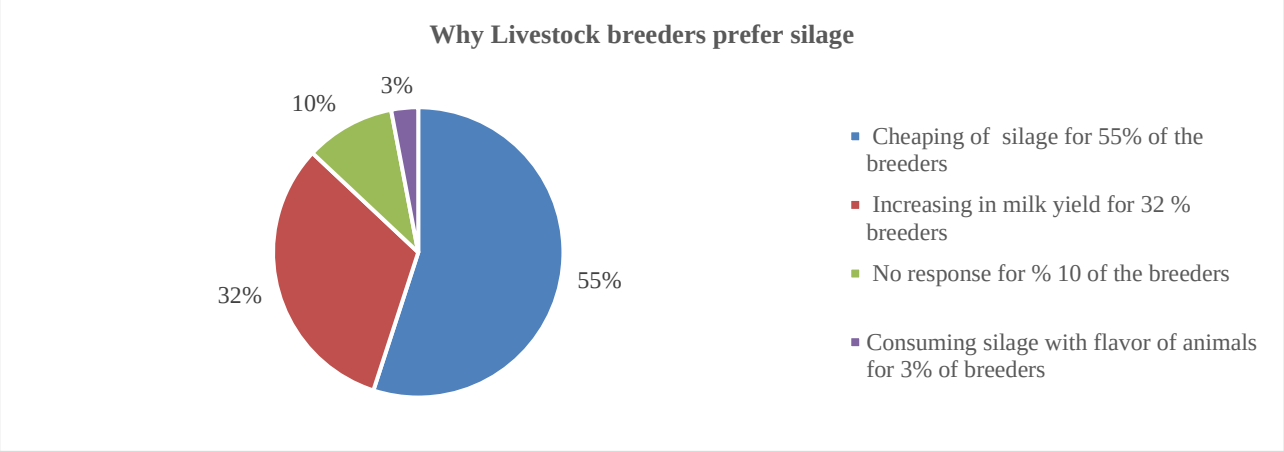

Figure 1. Why do you prefer silage?
Şekil 1. Silajı neden tercih ediyorsunuz ?

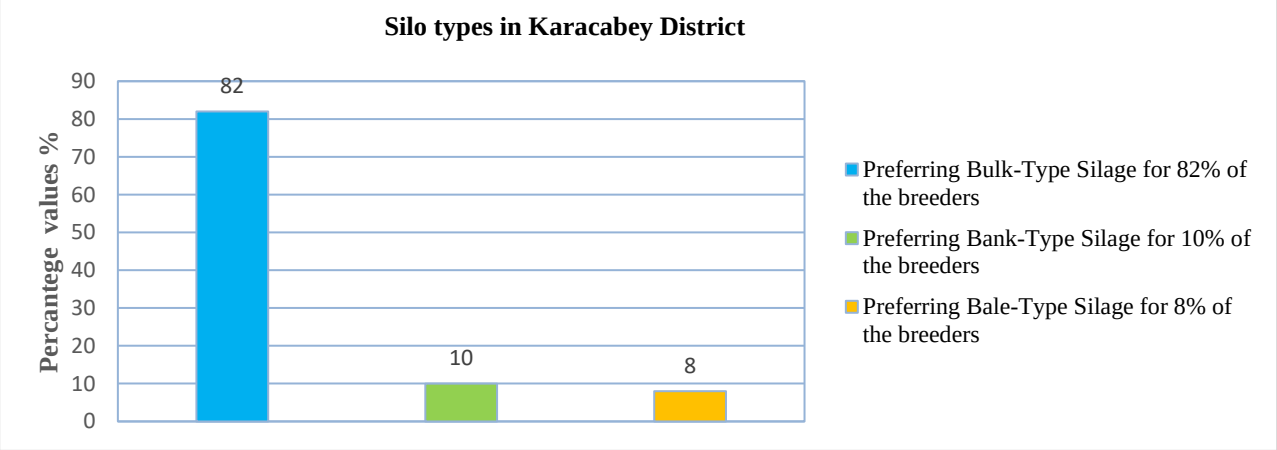

Figure 2. Which type of silo do you use in Karacabey?
Şekil *2.* Karacabey de hangi tip silo kullanıyorsunuz ?

in Dry Matter (DM) than corn silage and there is an increase in the crude prote rate (Kızılşimşek et al., 2020). There are many tomato processing factories in the Karacabey district. Tomatoes are mainly processed in the food industry and the rest (3.5-5%) is used in animal nutrition (Çapçı et al., 1995; Ergen, 1991). Karabulut et al. (1999) investigated the feed value of tomato pulp silage, which has undergone different physical (Un crushed and crushed) and chemical processes (NaOH %2.5 and Urea %3.5), and the possibilities of usage it in lamb fattening. As a result of that research, it was determined that processing the tomato pulp (Urea %3.5) had a positive effect on the feed value. Researchers have obtained similar results on body weight and body weight gain between groups. In another study, researchers fed cows in the 1st group (survival share needs + 10 kg milk yield requirements) with tomato pulp silage and molasses, and the other group with vetch-dried hay. The remaining yield share needs of the groups were met with milk feed. As a result of the research, it was determined that tomato pulp had a positive effect on dairy cows and reduced the cost of milk (Erdinc et al., 1992). Therefore, limited feed resources can increase areas if businesses are encouraged to turn to food pulp residues as plant silage material, both through incentives and demonstrations.

Silage quality is determined by the appropriate material to be selected and the correct silage applications. The additive helps to improve the ambient conditions where the silage material is in the silo. This improvement can be stated as creating an acidic environment in the silo, accelerating LAB development,

improving aerobic stability, reducing hygienic risks, increasing the feed value, and increasing the digestibility of the feed. (Karabulut, 1995). When the the animal enterprises were asked what they use as a silage additive, 70% of the breeders stated that they did not use additives in silage making, while 20% stated that they used bacterial inoculants plus enzymes as a silage additive, and %5 stated that they used yeast and the rest stated that they used enzymes (Figure 4). 70% of the breeders who participated in the survey stated that they do not use additives in silage making. These findings are also consistent with the reports of other researchers Değirmencioğlu, 2016; Çekiç, 2017). It can be said that this situation arises from the economic, educational, and preference differences in the regional structure. According to the results of the survey, it is understood that natural additives are not used enough in silage making.

In the studies have been found positive results on the silage quality of natural additives substances. Research stated that the essential oil (60 mg cinnamon + 60 mg flaxseed + 60 mg lemon seed essential oils/kg) additives increas the aerobic stability of the silage, can improves the quality and nutritive value of silage (Besharati et al., 2020). Thus, the usage of natural silage additives in farms throughout Turkiye should be encouraged, as they do not leave residues in animal products and do not pollute the nature.

As can be seen in Figure 5, 35% of the breeders stated that they did not use carbohydrate source in silage making, While 29% stated that they used wheat craker in silage making, 20% stated that they used molasses, 11% stated that they used barley crushed, and the remaining 5% said that they used concentrate feed in silage making (Figure 5). The usage of molasses and other carbohydrate sources in silage making was found to be compatible with the reports of (Özdemir & Okumuş 2001). Researchers added molasses as an additive to the silage of the lenox plant and determined that molasses improved the odor and DM ratio Gümüş et al. (2020).

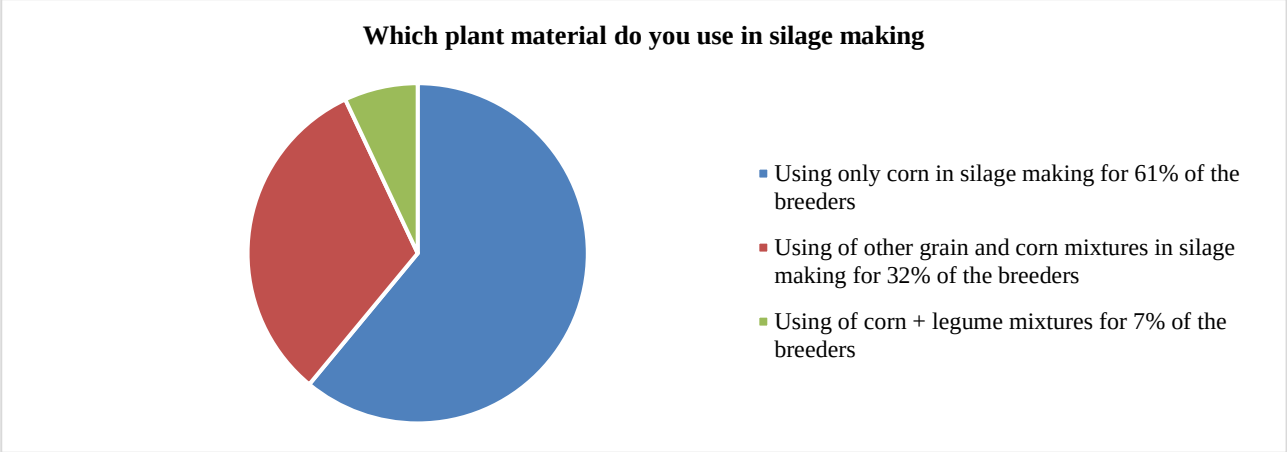

Figure 3. What do you use as plant material in silage making?
Şekil 3. Silaj yapımında bitkisel materyal olarak ne kullanıyorsunuz?

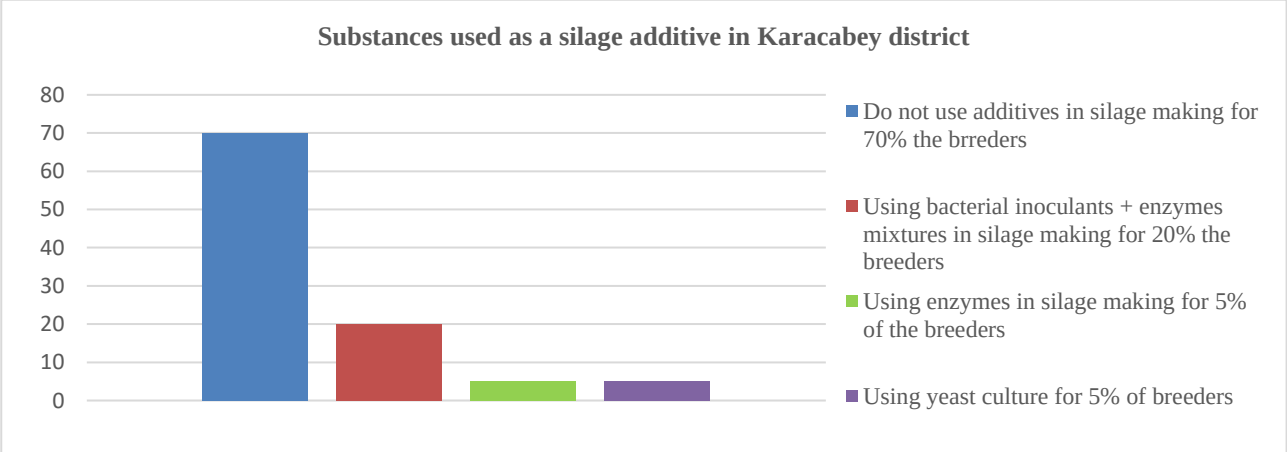

Figure 4. What do you use as a silage additive?
Şekil 4. Silaj katkı maddesi olarak ne kullanıyorsunuz?

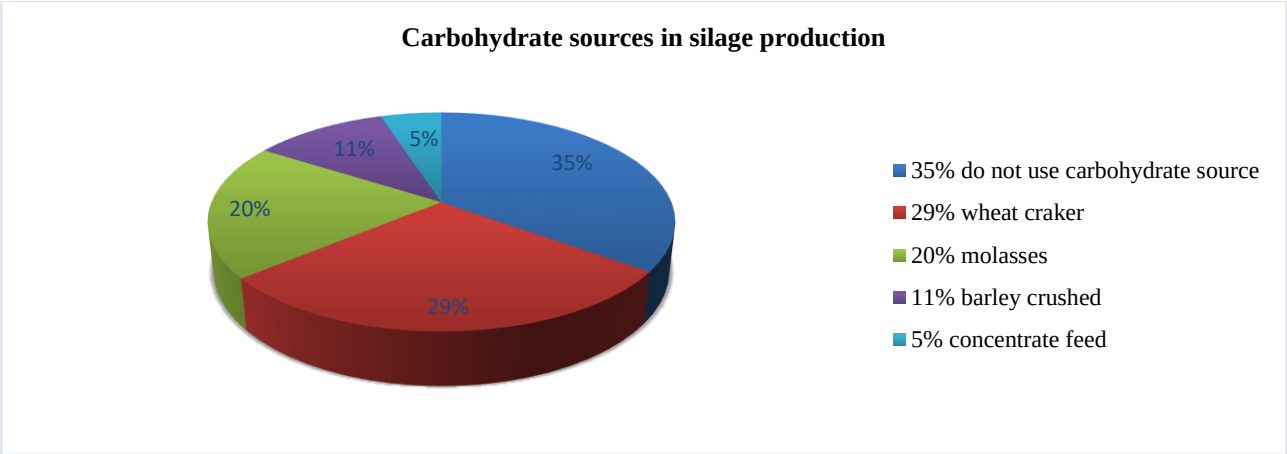

Figure 5. What do you use as a carbohydrate source?
Şekil 5. Karbonhidrat kaynağı olarak ne kullanıyorsunuz?

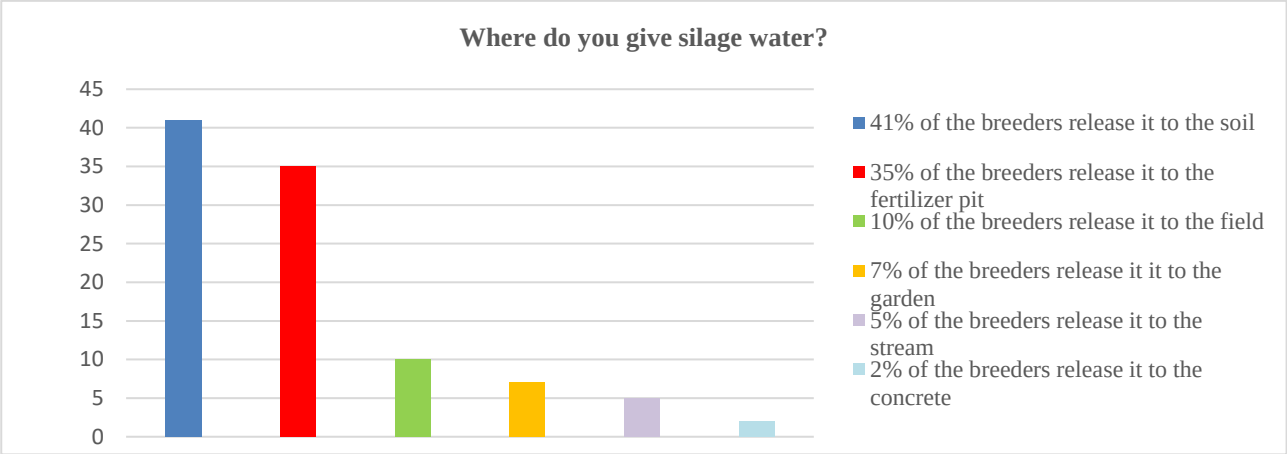

Figure- 6. Where do you release the silage water?
Şekil 6. Silaj suyunu nereye veriyorsunuz?

To the question of where do you release silage water, 41% said that they released it to the soil, while 35% stated that they released it to the fertilizer pit, 10% stated that they released it to the field, 7% stated that they released it to the garden, 5% stated that released it to the stream, and 2% said that they released it to the concrete (Figure 6). According to the survey results, it is understood that most of the animal breeders release silage water to the environment and do not pay due attention to the silage making stage. Silage making regulations should be applied at every stage of silage production. During the production and storage of silage, discharge of silage water into the soil or surrounding water resulting from poor drainage is harmful to the environment (Peterson et al., 1958). The Department for Environment Food & Rural Affairs (DEFRA) regulations numbered 1997/547 state at silage wastes are 100 times more polluting than untreated water. Therefore, care must be taken to minimize the risk of pollution by farmers (Aslım & Danış 2021).

When the breeders were asked about the daily milk yield of their cows, 30% of the the animal enterprises participating in the survey reported that they received 25 (lt / day) milk from their cows, while 28% reported that they received 20 (lt / day) milk from their cows, 24% reported that they received 15 (lt /day) of milk, and the remaining 18% stated that they received 30 and more (lt / day) of milk (Figure 7).

The survival rate of dairy cows can be met by ½ of the dry matter requirements from corn silage and the other half from quality legumes or grass hay. When asked how much silage they give to their milking cows per day, 70% of the breeders reported that they fed by 10-20 kg of silage/day to their cows, 27% of them reported that they fed by 20-30 kg of silage/day to their cows, and 3% of them reported that they fed by 30 or more kg of silage/day to their cows cows (Figure 8).

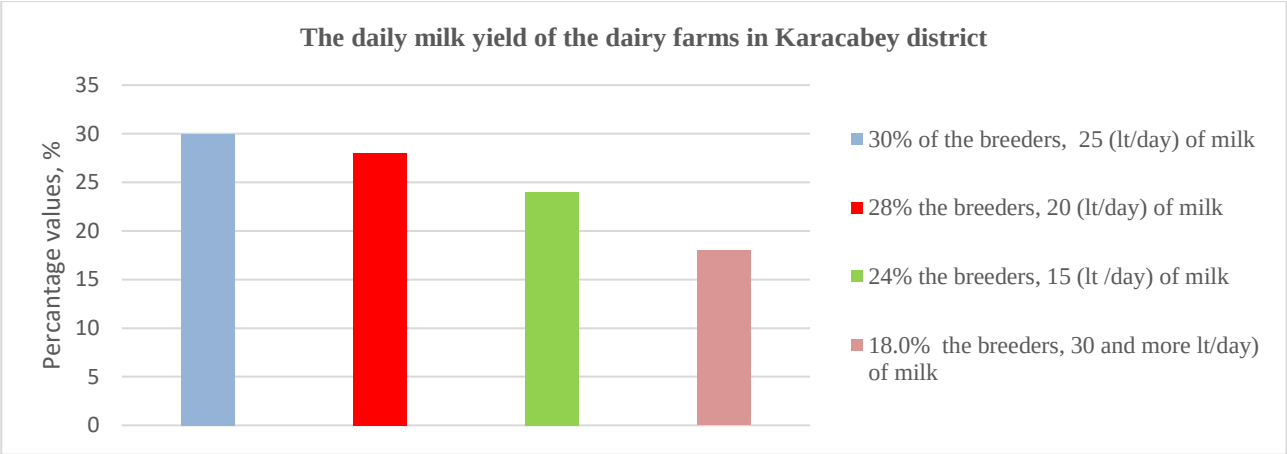

Figure 7. What does the daily milk yield of the cows in your business
Şekil 7. İşletmemizdeki ineklerin günlük süt verimi nedir?

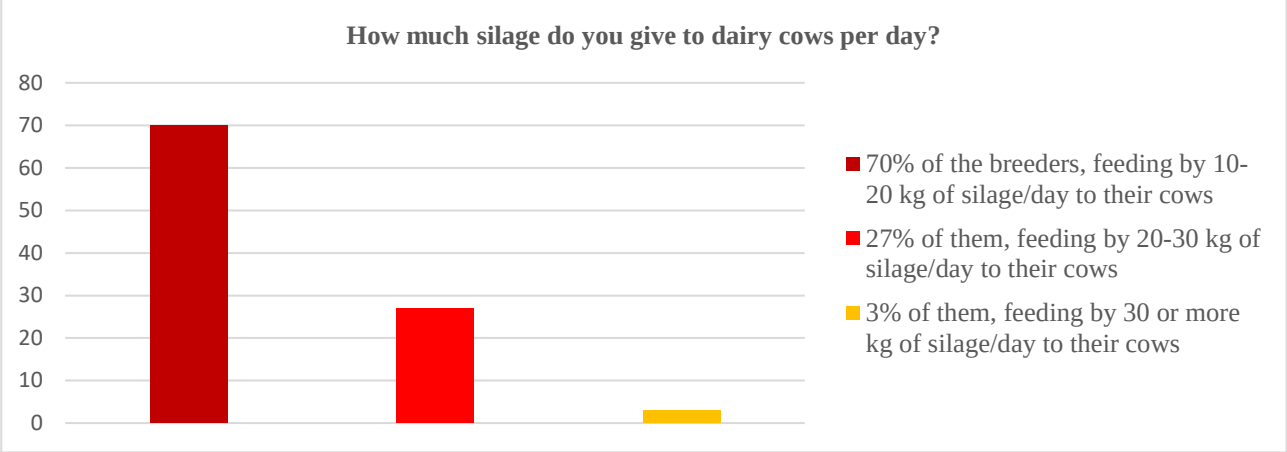

Figure 8. How much silage do you give dairy cows per day?
Şekil 8. Sağmal ineklerinize günde ne kadar silaj veriyorsunuz?

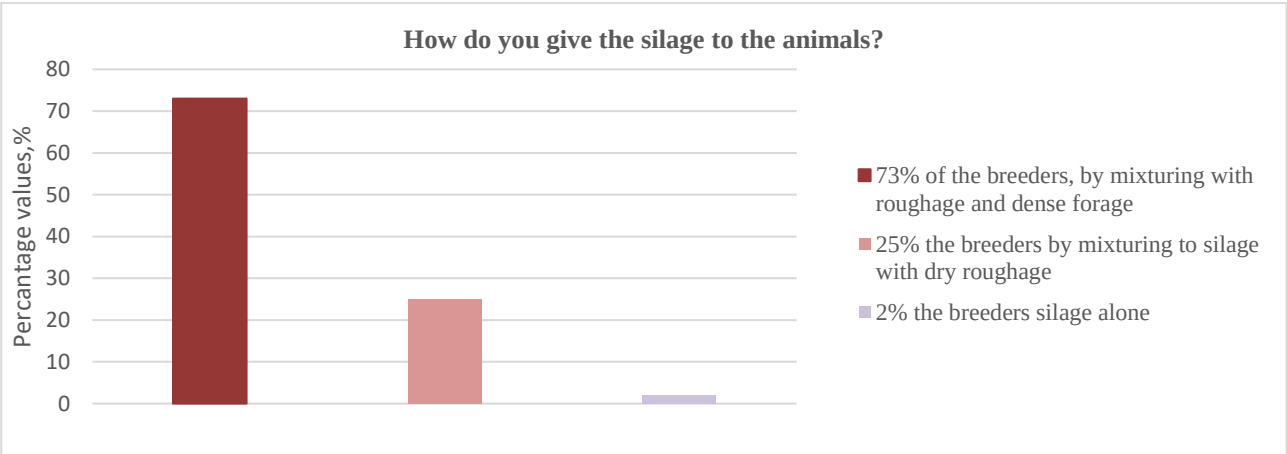

Figure 9. How do you give the silage to the animals
Şekil 9. Silajı süt ineklerinize nasıl veriyorsunuz?

When the breeders were asked about the method of feeding the silage to dairy cows, 73.0% reported that they gave silage to their cows mixed with roughage and concentrate feed, 25.0% reported that they gave silage to their cows mixed silage with dry roughage and 2.0% reported that they gave only silage to their cows. In the obtained questionnaire study, it is seen that the breeders mixed the silage with dry roughage and dairy feed Total Mixed Ration (TMR) for their cows. It is known that this mixture provides a more stable environment by

preventing pH fluctuations around the rumen (Görgülü, 2019; Değirmencioğlu, 2020). Thus, the evaluation efficiency of the feed by microorganisms increases. 30% of the breeders stated that they received 25 (lt /day) milk from their cows is in parallel with the results obtained. In general, in the Karacabey district, only 2% of the producers participating in the survey stated that they gave silage alone and 3% stated that they gave 30 or more kg of silage/per day. In contrast, (Akay & Dağdemir 2009; Denli et al., 2014) stated in their study that most of the producers misbehaved in the way of giving silage to animals. This may be due to interregional educational, and socio-economic differences.

Dairy cow nutritional diseases

When asked to rank the damage seen in animals as a result of excessive silage in your business, they stated that they were caught in first-degree stomach upset (abomasum displacement, second-degree diarrhea, and acidosis). It is known that it occurs when the abomasum is located on the right or left side of the rumen. With the birth of the calf and the throwing of the last, the movement area of the stomach parts in the abdomen expands. On the other hand, giving low-particle feeds such as corn silage to dry cows also increases the risk of abomasum slippage (Trimberger et al., 1972; Coppock, & Everett, 1973; Belyea et al., 1974; Görgülü, 2019). Acidosis causes a decrease in rumen pH, a decrease in the ratio of acetic acid and butyric acid in the rumen fluid, and an increase in the ratio of propionic acid, as a result of excessive amounts of easily soluble carbohydrates in cows. This suppresses healthy rumination, milk production and milk fat formation (Kleen et al., 2003; Maulfair et al., 2013). Similarly, the total feed mixture blended with 60% concentrated corn silage triggers the incidence of acidosis (Dänicke et al., 2020). As a matter of fact, the opinion of researchers (Trimberger et al., 1972; Coppock & Everett, 1973; Belyea et al., 1974; Görgülü, 2019; Dänicke et al., 2020) that abomasum displacement and incidence of acidosis increase in cows fed with corn silage is in line with the results of the survey.

4. Conclusion and Recommendations

As a result, it is seen that most of the breeders are dependent on maize for silage production and prefer the bulk-type silage. On the other hand, it has been determined that basic information about silage production and animal nutrition of small-scale livestock enterprises is insufficient.

In this context, it can be recommented the small-scale enterprises to participate in training of organization which will increase their knowledges on subjects such as silage making and animal feeding. It may be suggested that they use other forage crops (food pulp residues and heat-resistant fodder crops) as a source of silage and can also be fed their animals daily by using a large bank type silage collectively.

First of all, medium-sized enterprises can survive against large-scale enterprises in their commercial activities, depending on their reliability and awareness of animal products.

Forage production in Karacabey district is insufficient and dependent on outside sources. Encouraging breeders to produce forage crops can reduce the forage problem in livestock farming. Essentialy, complying with legal procedures when granting licenses to livestock farming during the establishment phase, destroying the waste genarated in accordance with the feed regulations, choosing natural additives when making silage, and thus teaching a lifestyle without harming nature; it is important for the protection of nature.

According to the results obdained in the surveys, the negative effects of silage on animals can also be seen. In such cases, problems may occur in the rumen and the microbial composition. On the other hand, negative effects may also be observed in the milk fat and reproductive fuctions of animals. The negative factors of silage affecting farm animals should be minimized. For this purpose, when feeding silage to dairy cows, supporting with quality roughage, the Neutral Detergen Fiber (NDF) range of ration and feeding in the form of TMR should be taken into consideration. Taking the necessary precautions against the negativities created by the survey results in dairy farms located in Karacabey; it is important in terms of bringing Turkish farmers and livestock into the economy.

Declaration

I declare that the author, familiar with the content of this article, has given permission for the article to be published in the format presented in the Journal of Gaziosmanpasa University Faculty of Agricultural.

Conflict of Interest

The author declares that he has no competing interests in this section.

Ethics committee certificate

A study was carried out in Karacabey enterprises in 2019. In the years before 2020, the ethics committee document was not requested in the journals, so it was not received.

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The Effects of Bacterial Inoculation on Agricultural and Quality Characteristics of Fenugreek (*Trigonella foenum-graecum* L.) Cultivars

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Abstract: This study was conducted in Kayseri ecological conditions in 2022 to determine the effects of bacterial inoculation on some agricultural and quality characteristics of fenugreek cultivars. In the study, the experiment was set up according to the experimental design of randomized blocks split plots with four replications. Bacterial inoculation (with and without bacteria) was placed in the main plots, and cultivars (Güraslan, Çiftçi and Berkem) were placed in the sub-plots. In the experiment, plant height varied between 43.73-46.38 cm, first pod height varied between 17.10-21.08 cm, pod length varied between 11.59-12.69 cm, number of pods per plant varied between 11.50-16.38 pods plant^{-1}, number of seeds per pod varied between 7.70-9.06 seeds pod^{-1}, thousand seed weight varied between 17.93-19.80 g, biological yield varied between 375.73-473.49 kg da^{-1}, seed yield varied between 75.98-109.62 kg da^{-1}, harvest index varied between 19.04-25.35% and crude oil content varied between 5.26-5.74%. At the end of the study, bacterial inoculation was found to be statistically insignificant. In addition, cultivars were found to be statistically significant in thousand seed weight and biological yield, and bacterial inoculation x cultivar interaction was found to be statistically significant in seed yield and harvest index. Eight different fatty acid components were identified and the main component of these was linoleic acid. Consequently, it can be said that the cultivation of Berkem cultivar without bacterial inoculation in terms of seed yield, and the cultivation of Gürarslan cultivar inoculated with bacteria in terms of UFA (unsaturated fatty acids) are the most suitable practices for fenugreek cultivation in Kayseri conditions.

Keywords: Fenugreek, bacterial inoculation, seed yield, fatty acid composition, linoleic acid

Çemen (*Trigonella foenum-graecum* L.) Çeşitlerinin Tarımsal ve Kalite Özellikleri Üzerine Bakteri Aşılamasının Etkileri

Öz: Bu çalışma, 2022 yılında bakteri aşılamasının çemen çeşitlerinin bazı önemli tarımsal ve kalite özellikleri üzerine etkilerinin belirlenmesi amacıyla Kayseri ekolojik koşullarında yürütülmüştür. Çalışmada deneme tesadüf bloklarında bölünmüş parseller deneme desenine göre dört tekerrürlü olarak kurulmuştur. Ana parsellere bakteri aşılaması (bakterili ve bakterisiz), alt parsellere ise çeşitler (Güraslan, Çiftçi ve Berkem) yerleştirilmiştir. Denemede bitki boyu 43.73-46.38 cm, ilk bakla yüksekliği 17.10-21.08 cm, bakla boyu 11.59-12.69 cm, bitkide bakla sayısı 11.50-16.38 adet bitki^{-1}, baklada tohum sayısı 7.70-9.06 adet bakla^{-1}, bin tohum ağırlığı 17.93-19.80 g, biyolojik verim 375.73-473.49 kg da^{-1}, tohum verimi 75.98-109.62 kg da^{-1}, hasat indeksi %19.04-25.35 ve sabit yağ oranı ise %5.26-5.74 arasında değişmiştir. Çalışma sonunda bakteri aşılaması istatistiki olarak önemsiz bulunmuştur. Ayrıca çeşitler bin tohum ağırlığı ve biyolojik verim özelliklerinde ve bakteri aşılaması x çeşit interaksiyonu ise tohum verimi ve hasat indeksi özelliklerinde istatistiki olarak önemli bulunmuştur. Bununla beraber ana bileşen linoleik asit olan, 8 farklı yağ asidi bileşeni tespit edilmiştir. Sonuç olarak, tohum verimi açısından bakteri aşılaması yapılmayan Berkem çeşidi ekimi ve UFA (doymamış yağ asitleri) açısından ise bakteri aşılaması yapılan Gürarslan çeşidi ekiminin Kayseri koşullarında çemen yetiştiriciliği için en uygun uygulamalar olduğu söylenebilir.

Anahtar Kelimeler: Çemen, bakteri aşılaması, tohum verimi, yağ asidi kompozisyonu, linoleik asit

1. Introduction

Fenugreek, whose species name is *Trigonella foenum graecum* L., is an annual medicinal and aromatic plant belonging to the legume (Fabaceae) family (Arslan et al., 1989). The plant grows approximately 60 cm tall, and its leaves are in triple form. The stems are hard and hollow. Petals may be pinkish or white. Its fruit is called pod, and it has 10-20 seeds in each pod. Its seeds can have colors ranging from dirty yellow to dark brown (Baytop, 1984; Köroğlu, 1985).

It is evaluated for both the seeds and the vegetative parts of the fenugreek plant. Its seeds contain minerals, oil, protein and vitamins etc. Due to this rich content, its seeds are consumed as a meal flavoring and its leaves are consumed as vegetables. It also has forage plant value due to its high protein content (Acharya et al.,

2008; Dutta et al., 2011).

There are three cultivars of the plant registered in Türkiye as a result of breeding studies. These cultivars are Gürarslan cultivar registered by Ankara University, Faculty of Agriculture, Berkem cultivar registered by Dicle University, Faculty of Agriculture and Çiftçi cultivar registered by Transitional Zone Agricultural Research Institute (Anonymous, 2023).

When the latest TUIK data was examined, fenugreek was grown in Afyonkarahisar, Amasya, Ankara, Karaman, Kastamonu, Kayseri, Konya, Samsun, Sivas, Tokat, Yozgat and Çorum provinces in Türkiye. In total of these provinces, the fenugreek cultivation area was approximately 8900 decares in 2022. In addition, approximately 1040 tons were produced from this area and a yield of 117 kg da^{-1} was obtained (TUIK, 2023).

Fenugreek, as a legume plant, performs nitrogen fixation. Nitrogen fixation is a process that occurs as a result of the symbiotic relationship of *Rhizobium* bacteria with legume plants and is carried out through the nodules formed by the bacteria. In this process, the fenugreek plant both meets its own nitrogen needs and enriches the soil with nitrogen. The effective bacteria for fenugreek was reported to be *Rhizobium meliloti* in studies, so this bacteria was applied in this study (Sarıoğlu et al., 1993; Tunçtürk, 2010). This study was carried out to determine the changes in the agronomic and quality characteristics of different fenugreek cultivars due to bacterial inoculation.

2. Material and Methods

2.1. Material

Three different cultivars of fenugreek (Çiftçi, Berkem and Güraslan) were used in the study. In addition, *Rhizobium meliloti* bacteria used in the inoculation of seeds in the study were obtained from Soil, Fertilizer and Water Resources Central Research Institute.

2.2. Climate and soil characteristics of the experimental site

The climate characteristics of the study area and the properties of the study soil taken from a depth of 30 cm are given in Table 1. When the climate data were examined, in the months when the experiment was conducted, the highest temperature was in August (26.1 °C), the highest relative humidity was in May (62.1%) and the highest rainfall was in June (56.7 mm). When the soil properties were examined, it was determined that the trial soil had a neutral pH value, no salt, medium organic matter content, calcareous content, high phosphorus content and clayey texture (Table 1).

Table 1. Climate and soil characteristics of the trial site
Çizelge 1. *Deneme alanının iklim ve toprak özellikleri*

Climate properties						
Months	**Monthly average temperature (°C)**		**Monthly average relative humidity (%)**		**Monthly total precipitation (mm)**	
	2022	**Long years**	**2022**	**Long years**	**2022**	**Long years**
April	13.8	10.7	46.6	58.9	13.2	46.9
May	14.2	15.1	62.1	59.0	46.0	57.9
June	20.3	19.3	59.2	54.5	56.7	40.6
July	22.0	22.7	48.2	46.8	0.6	11.9
August	26.1	22.6	39.6	46.7	-	9.5

Soil properties					
Texture	**EC (mmhos cm^{-1})**	**Organic matter (%)**	**Lime (%)**	**P_2O_5 (kg da^{-1})**	**pH**
Clayey soil	0.176	2.24	1.43	20.73	7.05

2.3. Bacterial inoculation of the seeds

Bacterial inoculation (*Rhizobium meliloti* bacteria) was applied to the fenugreek seeds before sowing, with the calculation of 1 kg of bacteria per 100 kg of seeds (Tunçtürk, 2010). The application was made in an environment without sunlight and the seed planting was carried out without wasting time.

2.4. Conducting field experiments

The field study was established on the land of Erciyes University Agricultural Research and Application Center on April 2, 2022, according to the experimental design of randomized blocks split plots. In the study, bacterial inoculation (with and without bacteria) was placed in the main plots, and different cultivars of fenugreek (Güraslan, Çiftçi and Berkem) were placed in the sub-plots. In the study, the parcels were formed in 6 rows in each parcel, with row spacing of 30 cm and parcel length of 4 m. In the trial, the sowing rate was 3 kg da^{-1} (Gökçe, 2015). Before sowing in the trial area, basic fertilization was made at 6 kg of pure phosphorus per decare (Gökçe, 2015). The water needs

of the plants were met with the help of drip irrigation pipes placed in each row, and weeds were constantly cleaned to prevent competition with the plants.

2.5. Plant harvests and obtaining data

In the study, harvest was carried out manually on August 2, 2022, by removing the first and last rows in each parcel as edge effect. All weighing, measurements and calculations were made on plants harvested from the middle four rows. At the end of the study, plant height, first pod height, pod length, number of pods per plant, number of seeds per pod were determined from 10 randomly selected plants in each plot. Biological yield was determined by taking the weight of all plants in each plot, and seed yield was determined by taking the weight of the seeds blended from all plants harvested in each plot. While calculating the thousand seeds weight, 4x100 groups of threshed seeds in each parcel were counted, their weights were averaged, and the resulting value was multiplied by 10. The crude oil content was determined as a percentage by analyzing the ground fenugreek samples (3 g) with petroleum ether in an automatic oil determination device. Fatty acid components were determined with the help of GC device (Schimadzu, GC 2010 plus). The fatty acid peaks obtained during the analysis were identified by comparison with the standard (Sigma Supelco 37 Fame Mix).

2.6. Statistical analysis

Variance analysis of the study findings was determined with the help of the MSTAT-C package program, in accordance with the experimental design of randomized blocks split plots with four replications. The significance level of differences between subjects was determined by the Duncan Test, and the significance level of differences in terms of bacterial inoculation was determined by the t-test (Düzgüneş et al., 1987).

3. Results

Variance analysis results in the characteristics of fenugreek cultivars as a result of bacterial inoculation were given in Table 2. In the study, bacterial inoculation was found to be statistically insignificant in all parameters examined ($p>0.05$). The cultivars were found to be statistically significant at 1% levels ($p<0.01$) in thousand seed weight and 5% levels ($p<0.05$) in biological yield. However, the bacterial inoculation x cultivar interaction was found to be statistically significant at the 1% levels ($p<0.01$) in seed yield and harvest index (Table 2).

Table 2. Variance analysis results in the characteristics of fenugreek cultivars as a result of bacterial inoculation
Çizelge 2. *Bakteri aşılaması sonucunda çemen çeşitlerinin özelliklerinde oluşan varyans analiz sonuçları*

Sources of variation	Bacterial inoculation	Cultivars	Bacterial inoculation x Cultivar interaction
Plant height	ns	ns	ns
First pod height	ns	ns	ns
Pod length	ns	ns	ns
Number of pods per plant	ns	ns	ns
Number of seeds per pod	ns	ns	ns
Thousand seed weight	ns	**	ns
Biological yield	ns	*	ns
Seed yield	ns	ns	**
Harvest index	ns	ns	**
Crude oil content	ns	ns	ns

ns: non-significant, **: Significant at 1% level, *: Significant at 5% level

3.1. Plant height (cm), First pod height (cm) and Pod length (cm)

In this study, the effects of bacterial inoculation, cultivars and bacterial inoculation x cultivar interaction in plant height, first pod height and pod length were found to be statistically insignificant ($p>0.05$) (Table 2). In average values, plant height varied between 43.73-46.38 cm. The averages of plant height in with and without bacterial inoculation were 45.17 and 45.03 cm, respectively. In addition, the averages of plant height in Çiftçi, Berkem and Gürarslan cultivars were 43.84, 45.83 and 45.64 cm, respectively. In average values, first pod height varied between 17.10-21.08 cm. The averages of first pod height in with and without bacterial inoculation were 19.98 and 19.42 cm, respectively. In addition, the averages of first pod height in Çiftçi, Berkem and Gürarslan cultivars were 18.95, 20.68 and 19.48 cm, respectively. In average values, pod length varied between 11.59-12.69 cm. The averages of pod length in with and without bacterial inoculation were 12.30 and 12.13 cm, respectively. In addition, the averages of pod length in Çiftçi, Berkem and Gürarslan cultivars were 12.60, 12.02 and 12.04 cm, respectively (Table 3).

Table 3. Average values of the examined parameters of different fenugreek cultivars as a result of bacterial inoculation

Çizelge 3. *Bakteri aşılaması sonucunda farklı çemen çeşitlerinde incelenen parametrelerin ortalama değerleri*

Bacterial inoculation	Fenugreek cultivars							
	Çiftçi	Berkem	Gürarslan	Mean	Çiftçi	Berkem	Gürarslan	Mean
	Plant height (cm)				First pod height (cm)			
With bacteria	43.73	45.40	46.38	**45.17**	20.80	20.28	18.88	**19.98**
Without bacteria	43.95	46.25	44.90	**45.03**	17.10	21.08	20.08	**19.42**
Mean	**43.84**	**45.83**	**45.64**	**-**	**18.95**	**20.68**	**19.48**	**-**
	Pod length (cm)				Number of pods per plant (pods plant^{-1})			
With bacteria	12.50	11.92	12.49	**12.30**	15.28	14.20	16.38	**15.28**
Without bacteria	12.69	12.11	11.59	**12.13**	15.48	15.10	11.50	**14.03**
Mean	**12.60**	**12.02**	**12.04**	**-**	**15.38**	**14.65**	**13.94**	**-**
	Number of seeds per pod (seeds pod^{-1})				Thousand seed weight (g)			
With bacteria	8.83	7.70	8.91	**8.48**	18.93	17.98	19.18	**18.70**
Without bacteria	9.06	8.46	8.77	**8.76**	19.80	17.93	19.55	**19.09**
Mean	**8.95**	**8.08**	**8.84**	**-**	**19.37 a**	**17.96 b**	**19.37 a**	**-**
	Biological yield (kg da^{-1})				Seed yield (kg da^{-1})			
With bacteria	375.73	383.28	473.49	**410.83**	89.77 ab	75.98 b	109.14 a	**91.63**
Without bacteria	388.28	421.98	447.40	**419.22**	94.14 ab	109.62 a	87.60 ab	**97.12**
Mean	**382.01 B**	**402.63 B**	**460.44 A**	**-**	**91.96**	**92.80**	**98.37**	**-**
	Harvest index (%)				Crude oil content (%)			
With bacteria	23.18 ab	20.10 b	23.12 ab	**22.13**	5.45	5.36	5.41	**5.40**
Without bacteria	23.23 ab	25.35 a	19.04 b	**22.54**	5.49	5.74	5.26	**5.50**
Mean	**23.20**	**22.72**	**21.08**	**-**	**5.47**	**5.55**	**5.33**	**-**

Lowercase letters indicate different groups at the 1% level, Capital letters indicate different groups at the 5% level

3.2. Number of pods per plant (pods plant^{-1}) and Number of seeds per pod (seeds pod^{-1})

In this study, the effects of bacterial inoculation, cultivars and bacterial inoculation x cultivar interaction in number of pods per plant and number of seeds per pod were found to be statistically insignificant (p>0.05) (Table 2). In average values, number of pods per plant varied between 11.50-16.38 pods plant^{-1}. The averages of number of pods per plant in with and without bacterial inoculation were 15.28 and 14.03 pods plant^{-1}, respectively. In addition, the averages of number of pods per plant in Çiftçi, Berkem and Gürarslan cultivars were 15.38, 14.65 and 13.94 pods plant^{-1}, respectively. In average values, number of seeds per pod varied between 7.70-9.06 seeds pod^{-1}. The averages of number of seeds per pod in with and without bacterial inoculation were 8.48 and 8.76 seeds pod^{-1}, respectively. In addition, the averages of number of seeds per pod in Çiftçi, Berkem and Gürarslan cultivars were 8.95, 8.08 and 8.84 seeds pod^{-1}, respectively (Table 3).

3.3. Thousand seed weight (g) and Biological yield (kg da^{-1})

In this study, cultivars were found to be statistically significant at 1% levels (p<0.01) in thousand seed weight and 5% levels (p<0.05) in biological yield. In addition, the effects of bacterial inoculation and bacterial inoculation x cultivar interaction were found to be statistically insignificant (p>0.05) (Table 2). In average values, thousand seed weight varied between 17.93-19.80 g. The averages of thousand seed weight in with and without bacterial inoculation were 18.70 and 19.09 g, respectively. In cultivars, the highest values in thousand seed weight were obtained from Çiftçi and Gürarslan cultivars (19.37 g) which are in the same statistical group. The lowest value in thousand seed weight was taken in the Berkem cultivar (17.96 g). In average values, biological yield varied between 375.73-473.49 kg da^{-1}. The averages of biological yield in with and without bacterial inoculation were 410.83 and 419.22 kg da^{-1}, respectively. In cultivars, the highest value in biological yield was obtained from the Gürarslan cultivar (460.44 kg da^{-1}). The lowest values were obtained from Berkem (402.63 kg da^{-1}) and Çiftçi (382.01 kg da^{-1}) cultivars, which are in the same statistical group.

3.4. Seed yield (kg da^{-1}) and Harvest index (%)

In this study, bacterial inoculation x cultivar interaction was found to be statistically significant at 1% levels (p<0.01) in seed yield and harvest index. In addition, the effects of cultivars and bacterial inoculation were found to be statistically insignificant (p>0.05) (Table 2). In average values, seed yield varied between 75.98-109.62 kg da^{-1}. Although the highest seed yield value was taken from the Berkem cultivar without bacterial inoculation (109.62 kg da^{-1}), no statistical difference was observed in all applications

except for the Berkem cultivar inoculated with bacteria. The lowest seed yield value was obtained from the Berkem cultivar inoculated with bacteria (75.98 kg da^{-1}). The averages of seed yield in with and without bacterial inoculation were 91.63 and 97.12 kg da^{-1}, respectively. In addition, the averages of seed yield in Çiftçi, Berkem and Gürarslan cultivars were 91.96, 92.80 and 98.37 kg da^{-1}, respectively. In average values, harvest index varied between 19.04-25.35%. The highest harvest index value was obtained from the Berkem cultivar without bacterial inoculation (25.35%) and the lowest value was obtained from the Gürarslan cultivar without bacterial inoculation (19.04%). The averages of harvest index in with and without bacterial inoculation were 22.13 and 22.54 %, respectively. In addition, the averages of harvest index in Çiftçi, Berkem and Gürarslan cultivars were 23.20, 22.72 and 21.08%, respectively (Table 3).

3.5. Crude oil content (%) and Fatty acid composition (%)

In this study, the effects of bacterial inoculation, cultivars and bacterial inoculation x cultivar interaction in crude oil content were found to be statistically insignificant (p>0.05) (Table 2). In average values, crude oil content varied between 5.26-5.74%. The averages of crude oil content in with and without bacterial inoculation were 5.40 and 5.50%, respectively. In addition, the averages of crude oil content in Çiftçi, Berkem and Gürarslan cultivars were 5.47, 5.55 and 5.33%, respectively. The average results of the fatty acid composition of different fenugreek cultivars as a result of bacterial inoculation are given in Table 4. Accordingly, 8 different fatty acid components were determined, and linoleic acid was determined as the main component of these components. In addition, apart from this component, α-linolenic acid, oleic and palmitic acid rates were found to be high. In the study, it was determined that the linoleic acid ratio varied between 43.83-45.19%, α-linolenic acid ratio varied between 23.78-25.77%, oleic acid ratio varied between 13.35-13.87% and palmitic acid ratio varied between 8.60-10.01%. The highest linoleic acid ratio was obtained from the Gürarslan cultivar inoculated with bacteria and the lowest value was obtained from the Çiftçi cultivar inoculated with bacteria. The highest α-linolenic acid ratio was obtained from the Çiftçi cultivar inoculated with bacteria and the lowest value was obtained from the Berkem cultivar without bacterial inoculation. The highest oleic acid ratio was obtained from the Berkem cultivar without bacterial inoculation, and the lowest value was obtained from Çiftçi cultivar inoculated with bacteria. In addition, the highest palmitic acid ratio was obtained from the Berkem cultivar inoculated with bacteria and the lowest value was obtained from the Gürarslan cultivar inoculated with bacteria. In the study, the SFA ratio varied between 14.5-15.7% and the UFA ratio varied between 84.3-85.5%. Accordingly, the highest SFA ratio was obtained from the Berkem cultivar inoculated with bacteria. The lowest value was obtained from the Gürarslan cultivar inoculated with bacteria. The highest UFA ratio was obtained from the Gürarslan cultivar inoculated with bacteria and the lowest value was obtained from the Berkem cultivar inoculated with bacteria (Table 4).

Table 4. Average values of fatty acid compositions (%) of different fenugreek cultivars as a result of bacterial inoculation

Çizelge 4. *Bakteri aşılaması sonucunda farklı çemen çeşitlerinin yağ asidi kompozisyonlarının (%) ortalama değerleri*

		With bacteria			Without bacteria		
Components	**Formula**	**Çiftçi**	**Berkem**	**Gürarslan**	**Çiftçi**	**Berkem**	**Gürarslan**
Palmitic acid	C16:0	9.63	10.01	8.60	9.51	9.87	8.70
Palmitoleic acid	C16:1	0.26	0.26	0.15	0.27	0.27	0.28
Stearic acid	C18:0	4.95	5.05	5.40	5.24	5.07	5.34
Oleic acid	C18:1	13.35	13.38	13.59	13.58	13.87	13.86
Linoleic acid	C18:2	43.83	44.19	45.19	44.45	44.70	44.78
γ-Linolenic acid	C18:3*n6*	1.61	1.67	1.67	1.78	1.77	1.66
α-Linolenic acid	C18:3*n3*	25.77	24.77	24.88	24.54	23.78	24.78
Behenic acid	C22:0	0.60	0.66	0.52	0.64	0.68	0.61
SFA		15.2	15.7	14.5	15.4	15.6	14.7
UFA		84.8	84.3	85.5	84.6	84.4	85.3

SFA: saturated fatty acid, UFA: unsaturated fatty acid

4. Discussion

Many studies have been conducted on bacterial inoculation of fenugreek (Tunçtürk and Çiftçi, 2011; Gendy, 2013; Żuk-Gołaszewska et al., 2015; Tunçtürk

et al., 2016; Tunçturk and Tunçturk, 2017; Rezaei-Chiyaneh et al., 2021). Tunçtürk and Çiftçi (2011) reported that the effect of bacterial application on plant height, number of pods per plant, number of seeds per pod, thousand seed weight and seed yield was statistically significant in the combined averages of the two years in fenugreek. Gendy (2013) emphasized that as a result of bacterial inoculation in fenugreek, there was no significant change in plant height in both growing seasons compared to the application without inoculation, but there was an increase in the number of pods per plant and crude oil ratio. However, it was reported that there was no significant effect on seed yield, which is an important feature, in the first year with bacterial inoculation, and that there was an increase in the second year. Żuk-Gołaszewska et al. (2015) inoculated fenugreek seeds with *Rhizobium meliloti* bacteria and reported that bacterial inoculation was ineffective on plant height, number of pods per plant, thousand seed weight and crude oil properties. In addition, it was reported that bacterial inoculation significantly reduced the number of seeds per pod and harvest index properties compared to the control. Tunçtürk et al. (2016) tested different bacterial strains on the fenugreek. This study reported that bacterial inoculation had no effect on plant height, first pod height, pod length, number of pods per plant and thousand seed weight in fenugreek. Tunçturk and Tunçturk (2017) reported that bacterial inoculation affected the plant height, number of pods per plant, number of seeds per pod and seed yield characteristics of fenugreek plants and noted that there were increases in these parameters compared to the control. However, it was reported that bacterial inoculation did not statistically affect the pod length, thousand seed weight and oil ratio. Rezaei-Chiyaneh et al. (2021) examined intercropping systems with fenugreek and black cumin plants and reported that the highest plant height, number of pods per plant, number of seeds per pod, thousand seed weight and seed yield values were obtained from the bacterial inoculation application in sole fenugreek sowing. In our study, results of plant height are similar with Tunçtürk et al. (2016), Gendy (2013), Żuk-Gołaszewska et al. (2015), results of thousand seed weight are similar with Tunçturk and Tunçturk (2017), Żuk-Gołaszewska et al. (2015) and Tunçtürk et al. (2016), results of pod length are similar with Tunçtürk et al. (2016), Tunçturk and Tunçturk (2017), results of crude oil ratio are similar with Tunçturk and Tunçturk (2017) and Żuk-Gołaszewska et al. (2015), results of number of pods per plant are similar with Żuk-Gołaszewska et al. (2015) and Tunçtürk et al. (2016), and results of first pod height are similar with Tunçtürk et al. (2016). No effects of bacterial inoculation were seen in this study. The reason for this may depend on many factors. In particular, soil temperature, rainfall conditions, and insufficient macro and micro element contents in the soil may limit the functioning of the bacteria.

Studies have been carried out in which cultivars were tested on the fenugreek plant. In previous studies, Aşkın (2021) reported the average of plant height as 58.61 cm in Çiftçi cultivar and 62.19 cm in Gürarslan cultivar; Evci (2019) as 35.40 cm in Gürarslan cultivar; Aslantaş (2023) as 53.20 cm in Gürarslan cultivar; Güzel and Özyazıcı (2021) as 78.70 cm and 71.70 cm in Berkem and Gürarslan cultivars, respectively. Also, Aşkın (2021) reported the average of first pod height as 26.03 cm in the Çiftçi cultivar and 27.30 cm in Gürarslan cultivar; Köksal (2021) as 25.40 cm in the Gürarslan cultivar; Güzel and Özyazıcı (2021) as 41.76 cm and 34.45 cm in Berkem and Gürarslan cultivars, respectively. In previous studies, Aşkın (2021) reported the average of pod length as 9.86 cm in the Çiftçi cultivar and 9.58 cm in the Gürarslan cultivar; Köksal (2021) as 14.45 cm in the Gürarslan cultivar; Bulut (2023) as 15.50 cm in the Gürarslan cultivar. In addition, Aşkın (2021) reported the average of number of pods per plant as 21.75 pods plant^{-1} in the Çiftçi cultivar and 18.95 pods plant^{-1} in the Gürarslan cultivar; Köksal (2021) as 20.16 pods plant^{-1} in the Gürarslan cultivar; Bulut (2023) as 26.44 pods plant^{-1} in the Gürarslan cultivar; Güzel and Özyazıcı (2021) as 14.80 ve 12.35 pods plant^{-1} in Berkem and Gürarslan cultivars, respectively. In previous studies, Aşkın (2021) reported the average of number of seeds per pod as 12.95 seeds pod^{-1} in the Çiftçi cultivar and 12.24 seeds pod^{-1} in the Gürarslan cultivar; Köksal (2021) as 13.67 seeds pod^{-1} in the Gürarslan cultivar; Güzel and Özyazıcı (2021) as 14.39 and 12.55 seeds pod^{-1} in Berkem and Gürarslan cultivars, respectively. Also, Aşkın (2021) reported the average of thousand seed weight as 16.30 and 16.39 g in Çiftçi and Gürarslan cultivars, respectively; Köksal (2021) as 18.29 g in the Gürarslan cultivar; Güzel and Özyazıcı (2021) as 16.74 and 17.45 g in Berkem and Gürarslan cultivars, respectively. In previous studies, Köksal (2021) reported the average of biological yield as 441.48 kg da^{-1} in the Gürarslan cultivar; Bulut (2023) as 416.00 kg da^{-1}; Beyzi (2016) as between 184.81-350.56 kg da^{-1} in first year and between 492.59-872.22 kg da^{-1} in the second year. In addition, Evci (2019) reported the average of seed yield as 70.6 kg da^{-1} in the

Gürarslan cultivar; Aslantaş (2023) as 101.2 kg da^{-1} in the Gürarslan cultivar; Köksal (2021) as 150.00 kg da^{-1} in the Gürarslan cultivar; Bulut (2023) as 171.0 kg da^{-1}. In previous studies, Aşkın (2021) reported the average of harvest index as 21.54 and 22.08 % in Çiftçi and Gürarslan cultivars, respectively; Köksal (2021) as 34.24% in Gürarslan cultivar; Bulut (2023) as 41.46 %. In previous studies, Evci (2019) reported the average of crude oil content as 5.1% in the Gürarslan cultivar; Aslantaş (2023) as 6.3% in the Gürarslan cultivar; Köksal (2021) as 6.59% in the Gürarslan cultivar; Bulut (2023) as 4.45%. In different recent studies, Ciftci et al. (2011) reported that the ratios of palmitic, oleic, linoleic and α-linolenic acid varied between 9.8-11.2, 12.6-17.1, 45.1-47.5 and 18.3-22.8%, respectively. Sulieman et al. (2008) reported in another study that the palmitic acid ratio was 11%, oleic acid ratio was 16.7%, linoleic acid ratio was 43.2% and linolenic acid ratio was 22%.

5. Conclusion

The aim of this study is to determine the changes in the agricultural and quality characteristics of fenugreek as a result of bacterial inoculation. In the study, bacterial inoculation was found to be statistically insignificant ($p>0.05$) on all characteristics. At the end of the study, cultivars were found to be significant in biological yield ($p<0.05$) and thousand seed weight ($p<0.01$), while bacterial inoculation x cultivar interaction was found to be significant in seed yield ($p<0.01$) and harvest index ($p<0.01$). Eight different fatty acid components were determined in the fatty acid composition analysis. Among these components, linoleic acid was found to be the main component. This component was followed by α-linolenic acid, oleic and palmitic acid, respectively. As a result, it can be said that the cultivation of the Berkem cultivar without bacterial inoculation due to its high seed yield value, and the cultivation of the Gürarslan cultivar inoculated with bacteria due to its high unsaturated fatty acid (UFA) ratio are the most suitable practices for fenugreek cultivation in Kayseri conditions. In addition, it is concluded that the experiment should be repeated for a few more years in order to better determine the effect of bacterial inoculation in fenugreek cultivation.

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Classification of Apple Diseases and Pests using The Google.com Powered Teachable Machine

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Abstract: Machine learning and deep learning methods are used in the classification of plant diseases. It takes a long time to extract features in machine learning. In deep learning, computers are required to process big data depending on the size of the data set. With Google Teachable Machine, faster results can be obtained without the need for feature extraction or very powerful computers. For this purpose, a model was created with four apple diseases using the data set related to apple diseases. In this model, results of over 95% were obtained in diseases.

Keywords: Plant Disease Detection, Machine learning, Teachable Machine.

Google.com Destekli Öğretilebilir Makine Kullanılarak Elma Hastalıklarının Sınıflandırılması

Öz: Bitki hastalıklarının sınıflandırılmasında makine öğrenimi ve derin öğrenme yöntemleri kullanılmaktadır. Makine öğreniminde özellikleri çıkarmak uzun zaman alıyor. Derin öğrenmede, veri kümesinin boyutuna bağlı olarak bilgisayarların büyük verileri işlemesi gerekir. Google öğretilebilir makina ile özellik çıkarımına veya çok güçlü bilgisayarlara ihtiyaç duymadan daha hızlı sonuçlar alınabilir. Bu amaçla elma hastalıkları ile ilgili veri seti kullanılarak dört elma hastalığı ile model oluşturulmuştur. Bu modelde hastalıklarda %95'in üzerinde sonuçlar elde edilmiştir.

Anahtar Kelimeler: Bitki Hastalığı Tespiti, Makine öğrenimi, Öğretilebilir Makine.

1. Introduction

The fruit sector creates positive value for countries in terms of economy and human nutrition. Since fruits are directly consumable after being harvested, their unprocessed form is also a source of income (Bashimov, 2016). China, the European Union (EU), the United States of America (USA), and Turkey come first in the world ranking of fresh fruit producer countries (Chammem et al., 2018). Plant disease is one of the most important problems encountered in the agricultural sector. This problem negatively affects the industry socially and economically. Plant pests and diseases are responsible for the loss of global food production of up to 40-45% with post-harvest losses. When plant disease is not diagnosed and detected early, it can affect not only one plant but also a large agricultural area (Akbas, 2019).

Farmers struggle to diagnose diseases in apples because the symptoms produced by different diseases can be similar and sometimes appear simultaneously. Machine learning approaches such as deep learning are proposed for the timely and accurate detection of apple diseases from plant leaves (Khan et al. 2021). In addition, this effect can last for many years. Apple, produced within the scope of fruit growing, is one of the fruits traditionally produced in agricultural enterprises (Branco et al. 2020; Chao et al. 2020). Apple adjusts the acid-base balance in the blood with the vitamins and organic acids it contains. It, which is rich in sugars, acids, proteins, fatty substances, vitamins and mineral salts, is also rich in vitamins A and C. It is a fruit that can be consumed in all seasons due to its annual storage possibilities (Kacar, 2019).

Under suitable conditions, apple saplings that have formed the branch infrastructure in nursery conditions begin to produce fruit economically in 2-3 years, while

apple seedlings that do not form branches when planted in the garden begin to produce fruit efficiently after 4-5 years (Boyaci, 2009). Losing and replanting existing apple plants and waiting for fruit harvest can cause substantial economic losses. It is both costly and time-consuming to understand whether there is a disease in the plant and to detect this disease type (Turkoglu et al., 2020). When there are any of the known diseases in fruits and vegetables and more plant diseases, the symptoms of diseases such as bacteria, fungi, viruses, molds, and mites are evident in the images and thus can be identified and categorized accordingly (Jasim, 2021).

Machine learning or deep learning methods are used to classify plant diseases (Odabas et al., 2015; Odabas et al., 2016; Caliskan et al., 2017) . Although machine learning methods are low in cost, feature extraction takes time (Senel, 2020; Dammer et al., 2019). Models developed using Deep Learning methods are more successful and eliminate the loss of time in feature extraction (Odabas et al., 2017).

In addition to these advantages, deep neural networks have a significant disadvantage. These powerful hardware resources are required in deep network training and testing because deep network models need high memory and powerful GPU cards to work effectively. Researchers researched disease detection of apple plants using deep convolutional neural networks and achieved an accuracy percentage of 99.54% with the ResNet-34 architecture (Aksoy et al., 2020). In another study, an accuracy rate of 99.30% was performed in the study for the detection of peach diseases (Aslan, 2021).

In the field of agriculture, machine learning and other soft computing methods have been widely employed for the identification and categorization of diseases (Bansal et al. 2021). Teachable Machine (Google 2022) allows us to train our datasets using a web application. Image and sound classification can be made (Google, 2023). There is no need for a high-performance computer and GPU card while training the data. With a mobile application developed, a corn plant recognition application was developed using the data sets of the corn plant, and they achieved an accuracy rate of 80.7% (Aqil et al., 2021). In another study, 97% accuracy rates were obtained in animal classification by the researchers (Agustian et al., 2021). In the research on insect classification, 2646 images were used, and 100% accuracy was achieved (Gupta, 2021).

In this study, real-time apple disease classification was made through a web application designed by training the datasets of the disease of the apples.

2. Material and Method

In this study, the Turkey-Plant Dataset dataset created by Turkoglu et al. was used (Turkoglu et al. 2021). All images in the dataset are 300 x 300 pixels in size (Table 1).

Table 1. Apple diseases examined in the study and the number of images related to them.
Çizelge 1. *Araştırmada incelenen elma hastalık ve zararlılarına ilişkin görsel sayısı.*

Apple diseases	Abbreviation	The number of data
Aphis Spp.	AS	162
Eriosoma lanigerum	EL	366
Monilinia laxa	ML	255
Venturia inaequalis	VI	633

The aphid species (*Aphis* Spp) in apple orchards are the green apple aphid, *Aphis pomi* de Geer, and the spirea aphid, Aphis spiraecola Patch (*Hemiptera: Aphididae*). Green apple aphids reduce tree growth and nonstructural carbohydrate concentration and fruit production in young apple trees. Severe infestation also increases the risk of winter death. The negative impact of green apple aphids is more significant on young trees than on mature trees (Fréchette et al., 2008). Woolly apple aphid (WAA), *Eriosoma lanigerum* is a worldwide pest of apple. It colonizes roots and sites on the trunk and branches previously injured and can also occupy undamaged current-year shoots (Lordan et al., 2015). *Monilinia* spp. is an economically important disease. *Monilinia laxa* is causing mainly blossom and twig blight. Under suitable weather conditions, the disease develops rapidly. That's why, Monilinia spp., before or during storage, is essential for crop losses on pome fruits, especially post-harvest (Spitaler et al., 2022). *Venturia inaequalis* (Cooke Wint) affects the leaves and fruit tissue of trees. The pathogen was placed into the genus Venturia by Winter in 1880. It leads to both a saprophytic and parasitic lifestyle. The pathogen ascospores on the leaves broke the thin surface epithelium of immature leaves when moisture was present. The germ tube differentiates into an appressorium upon coming into contact with a cuticle and releases sticky mucilaginous chemicals that are thought to aid in adhesion to the host surface. Once an infection is established, curative preparations are required to stop further development of the mycelium. In organic apple growing, sulfur, lime, and copper are used for scab disease (Doolotkeldieva and Bobusheva, 2017).

Images of four diseases of the apple plant were trained by a teachable machine. While training the

dataset, parameter values are 100 for epoch, 32 for batch size, and 0.001 for learning rate. There are no settings related to training and test datasets. Epoch is a term used to describe an iteration within the scope of training a model in which the model uses the entire training set to update its weights. Updating weights during the training phase usually does not rely on all training sets simultaneously due to computational complexities or a data point due to noise issues. Instead, the update step is done with mini-sets, where the number of data points in a batch is a hyperparameter that we can adjust. Data in mini-clusters is called batch (Amidi, 2022). The learning rate determines the rate at which weights are updated, usually denoted as alpha (α) or sometimes eta (η). It can be fixed or adapted. The most popular method available is called ADAM and it is a method that adjusts the learning rate.

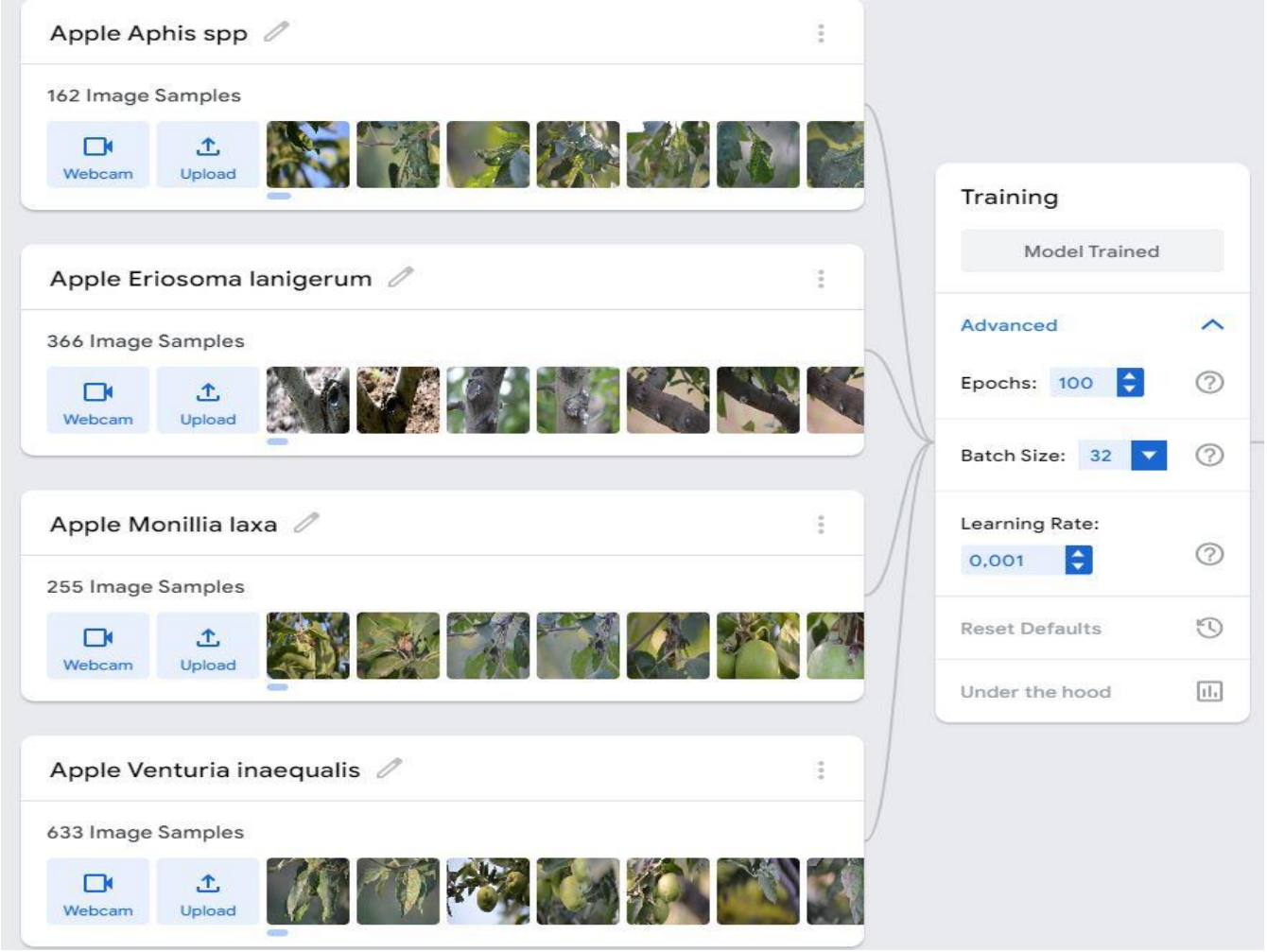

Figure 1. Performing machine learning based classification
Şekil 1. *Makine öğrenimi tabanlı sınıflandırma gerçekleştirme*

Figure2. Web interface
Şekil 2. *Web arayüzü*

The model formed in the study was designed as a web interface and classified apple disease and transferred to the web interface with the tensorflow.js file (Saka, 2022).

3. Result and Discussion

The appropriate division of a dataset is crucial in leveraging machine learning techniques for the identification and categorization of diseases and pests in apple plants (Thakur et al. 2022). Typically, datasets are segmented into three key sections: the training set, the

validation set, and the test set. The training set is utilized to train the model, while the validation set aids in fine-tuning parameters and optimizing the model's performance. Finally, the test set evaluates the model's overall effectiveness. Common ratios for this division include allocating 60-80% for the training set, 10-20% for the validation set, and another 10-20% for the test set. Maintaining randomness in this partitioning process ensures the dataset's diversity and representation. Initially, the dataset is divided into training and temporary subsets, which are further segmented into validation and test sets. Ultimately, this process yields the training set (X_train, y_train), validation set (X_val, y_val), and test set (X_test, y_test). These steps are essential in effectively utilizing machine learning approaches, enabling robust model performance and generalization (Mesías-Ruiz et al. 2023).

After the data set training process was completed, a report on the model consisting of the "Under the hood" menu was received. When this report was examined, it was seen that the data set was divided into 85% training and 15% test set, and the training process was carried out. The results of this training process are given in Table 2.

Table 2. Results obtained at the end of the training
Çizelge 2. *Eğitim sonunda elde edilen sonuçlar*

Class	Accuracy	The number of samples
Aphis Spp.	0.96	25
Eriosoma lanigerum	0.98	55
Monilinia laxa	0.95	39
Venturia inaequalis	0.98	95

According to the results, the highest accuracy value was seen in Apple Venturia inaequalis disease (AVI) at 98%, and the lowest accuracy value was seen in Apple Monillia laxa (AML) disease at 95%. In the disease prediction table, the accuracy values of the test sets belonging to the classes formed from apple diseases are included. Monillia laxa disease, with the lowest accuracy, was seen to be confused with Venturia inaequalis only (Table 3).

Table 3. Prediction table of apple diseases
Çizelge 3. *Elma hastalıklarının tahmin tablosu*

AAS	24	0	0	1
AEL	0	54	0	1
AML	0	1	37	1
AVI	1	0	1	93
	AAS	AEL	AML	AVI

After the training and test data are divided into two groups, this test set is tested as much as the determined epochs (100) value in the epochs process. Accuracy, and loss values are pivotal indicators in assessing the performance of a machine learning model during training. The accuracy graph tracks the model's precision at each epoch, ideally showing a steady increase and stabilization at high levels across both training and validation sets. Conversely, if accuracy peaks on the training set but declines on the validation set, overfitting may be occurring. Meanwhile, the loss graph demonstrates the model's error rate throughout training, with the expectation that it steadily decreases. A significant gap between training and validation set losses may signal overfitting. Evaluating these metrics together provides valuable insights for model refinement, such as considering model complexity adjustments or the acquisition of additional data to address overfitting issues (Burgkart et al. 2001). Accuracy and loss values are shown in Figure 3.

When the graphs are examined, the epoch value shows that stable results start to be obtained after 30, and it becomes the most stable after 40. After this value, there was a slight decrease in the accuracy value of the test set. In addition, there is a slight increase in the loss amount of the test set after this value.

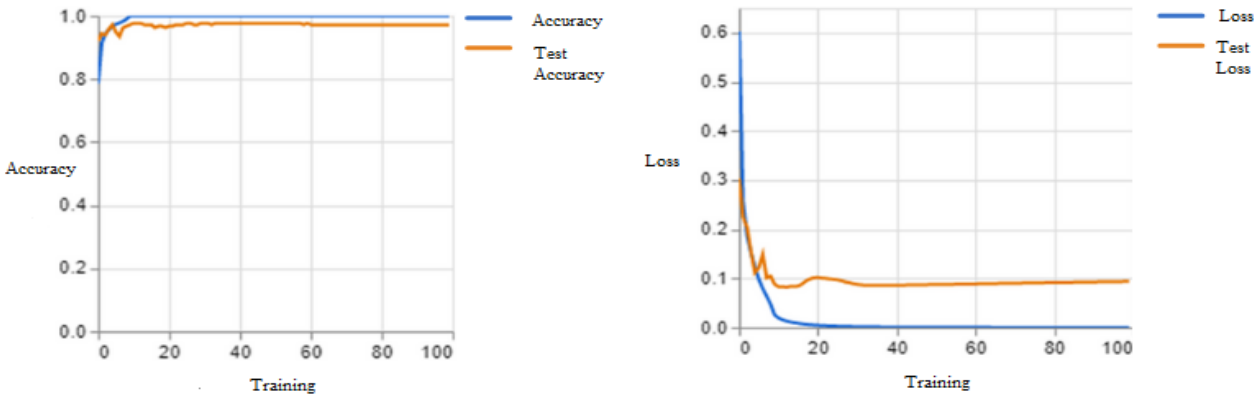

Figure 3. Epochs accuracy and loss values
Şekil 3. *Epoch doğruluğu ve kayıp değerleri*

4. Conclusion

Utilizing a teachable machine to develop a model for detecting four different diseases in apple plants represents a notable advancement in agricultural technology. The achieved accuracy rates, ranging from 95% to 98%, underscore the effectiveness of this approach in disease identification. Furthermore, the study suggests a positive correlation between accuracy rates and the volume of available data, indicating the potential for even greater accuracy with larger datasets.

Support from existing literature in agricultural technology reinforces the importance of accurate disease detection methods. For instance, research conducted by (Kala et al. 2023), emphasizes the crucial role of advanced technology in mitigating the adverse effects of plant diseases on crop yields. Similarly, studies such as (Storey et al. 2022) highlight the significance of employing machine learning techniques for precise disease diagnosis and timely intervention in agricultural contexts. These findings align with the outcomes of the current study, further validating the efficacy of utilizing a teachable machine for disease detection in apple plants.

In the domain of machine learning methodologies, the feature extraction process is acknowledged for its time-consuming nature. However, it is worth noting that accuracy values tend to increase proportionally with the expansion of features. Conversely, deep learning methods, often requiring high-performance computers equipped with GPUs, offer impressive accuracy levels. In contrast, the implementation of a teachable machine proves to be both time-saving and cost-effective, making it a viable solution for disease detection in apple plants.

The findings of this study underscore the feasibility of developing high-accuracy models through the application of appropriate methods and techniques, particularly when employing a teachable machine for processing image datasets. This highlights the potential for widespread adoption of such technologies in the agricultural sector, leading to improved disease management practices and ultimately contributing to enhanced crop yields and agricultural sustainability.

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Improving the Soil Physical and Hydraulic Properties by Irrigation with Wastewater under Different Soil Tillage Management

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Abstract: Irrigation with recycled wastewater increases the organic substance of the soil. Thus, the effect of the increased organic substance on the physical and hydraulic properties of the soil can be developed with different irrigation water quantities and soil tillage treatments. In this study, the effect of increased organic matter was determined after a two-year study carried out on a silage maize field irrigated at varying irrigation water levels of recycled wastewater (RWW) (100%, 67%, and 33% irrigation levels with RWW) and freshwater (FW) (100% irrigation level with FW) under direct sowing (DS) and conventional tillage (CT). RWW is compared to FW, the bulk density at 100% irrigation level was 1.5% lower, while porosity, aggregate stability, field capacity, wilting point, and available water were significantly higher by 1.9%, 12.0%, 2.8%, 2.2%, and 3.6%, respectively. Bulk density, aggregate stability, field capacity, wilting point, and available water were 1.5%, 4.3%, 3.3%, 2.2%, and 4.2% were significantly higher in DS according to CT, respectively, while porosity was 1.5% lower. These effects can be attributed to the RWW irrigation under DS due to the organic matter content in DS which was 1.1% higher than with CT, while RWW increased the organic matter content by 17% according to FW between full irrigations. As a result of the study, it was concluded that 100% irrigation levels using RWW directly within the scope of DS may be a practical approach to improve the physical and hydraulic properties of the silage maize field.

Keywords: Conventional tillage, Direct sowing, Irrigation, Recycled wastewater, Soil organic matter

Farklı Toprak İşleme Yönetimi Kapsamında Atık Su ile Sulama Yapılarak Toprağın Fiziksel ve Hidrolik Özelliklerinin İyileştirilmesi

Öz: Geri dönüştürülmüş atık su ile sulama yapmak, toprağın organik maddesini artırmaktadır. Böylece artan organik maddenin toprağın fiziksel ve hidrolik özelliklerine etkisi farklı sulama suyu miktarları ve farklı toprak işleme uygulamaları ile geliştirilebilir. Bu çalışmada, artan organik maddenin etkisi, doğrudan ekim (DS) ve geleneksel toprak işleme (CT) altında değişen geri dönüştürülmüş atık su (RWW) (RWW ile %100, %67 ve %33 sulama seviyeleri) ve temiz suyla (FW) (FW ile %100 sulama seviyesi) sulama seviyelerinde sulanan bir silajlık mısır tarlasında gerçekleştirilen iki yıllık bir çalışmanın ardından belirlenmiştir. %100 sulama düzeyinde; RWW, FW ile karşılaştırıldığında, hacim ağırlığı %1.5 daha düşük olmuşken, porozite, agregat stabilitesi, tarla kapasitesi, solma noktası ve kullanılabilir su kapasitesi sırasıyla %1.9, %12.0, %2.8, %2.2 ve %3.6 oranında önemli ölçüde artış göstermiştir. Hacim ağırlığı, agregat stabilitesi, tarla kapasitesi, solma noktası ve kullanılabilir su kapasitesi CT'ye göre DS'de sırasıyla %1.5, %4.3, %3.3, %2.2 ve %4.2 seviyesinde anlamlı derecede artış göstermişken, porozite %1.5 daha düşük olmuştur. Bu etkiler, DS'deki organik madde içeriğinin CT'ye göre %1.1 daha yüksek olması nedeniyle DS altında RWW ile sulamaya ilişkin açıklanabilirken, tam sulamalar arasında; RWW, FW'ye göre organik madde içeriğini %17 arttırmıştır. Çalışma sonucunda DS kapsamında RWW kullanılarak %100 sulama seviyelerinin silajlık mısır tarlasının fiziksel ve hidrolik özelliklerini iyileştirmede pratik bir yaklaşım olabileceği sonucuna ulaşılmıştır.

Anahtar Kelimeler: Geleneksel toprak işleme, Doğrudan ekim, Sulama, Geri dönüştürülmüş atık su, Toprak organik maddesi

1. Introduction

Although provoking various environmental and health problems, sustaining the agricultural or drinking water requirements of the ever-increasing population on a global scale in an environment exposed to increasing freshwater scarcity encourages producers to reuse

wastewater in crop production, especially in areas with severe arid, because of the many essential inorganic and organic nutrients it contains (Shahid et al., 2020). While the untreated, often diluted, partly reclaimed wastewater in agriculture areas covers 30 million hectares worldwide, agriculture areas irrigated with reclaimed wastewater are estimated to be about one million hectares (Drechsel et al., 2022). The reuse of recycled wastewater in irrigation water is seen as one of the main ways to avoid future water scarcity and to reduce the damage caused by water pollution to the environment.

Recycled wastewater improves the structural properties of soil with its high organic matter content because organic matter is an effective binding matter for increasing soil aggregation (Tunc & Sahin, 2016). Cakmakci and Sahin (2021) determined that the physical properties of the soil improved with the contribution of dissolved organic matter in recycled wastewater irrigation, supporting productivity in silage maize. Similarly, Dogan Demir and Sahin (2019) reported that increasing the organic matter of soil by irrigating with recycled wastewater increased the aggregate stability of soil by about 4% compared to irrigation with freshwater.

The mechanism of soil water retention, which indicates the balance of the water in the profile of the soil including field capacity and permanent wilting point, shows how much water the crop will consume from the soil. Water retention which is one of the general hydraulic soil properties could be increased by increasing organic matter content. Water retention in the soil occurs as a result of soil organic matter improving the soil pore size distribution and structure (Ors et al., 2015). Mujdeci et al. (2017) stated that organic matter increases porosity in favor of useful water retention by increasing the space rates among soil aggregates.

Soil tillage can cause differences in the hydraulic properties of soil by changing the structural properties of the soil. In intensive soil tillage conditions, the soil bulk density decreases with decreased soil compaction. However, since no interference is made with the soil in direct sowing, the bulk density may increase, and thus porosity can decrease (Gozubuyuk et al., 2014). However, organic matter stocks in the soil can be increased in direct sowing conditions, both physically due to less interference with the soil and biochemically due to less mineralization as a result of less oxygen input compared to intensive tillage treatments. The intensive size of tillage can increase the decomposition rate of crop residues and cause significant decreases in soil organic matter content, while the oxidation of organic matter is reduced in direct sowing since the soil is handled less (Malhi et al., 2018). Thus, increasing organic matter in the soil can support the increase of soil and crop productivity by improving the physical and hydraulic properties of the soil.

In previous studies, the effects of either irrigation with recycled wastewater or different tillage practices on the physical and/or hydraulic properties of the soil have been investigated and discussed. However, no integrated study has been found in the literature examining the physical and hydraulic properties of soil irrigated at changed levels with recycled wastewater under different tillage practices. With a significant contribution of soil organic matter that may be increased in these conditions, further improvement in soil physical and hydraulic conditions can be expected. Thus, this study aimed was to evaluate and discuss the changes in the physical and hydraulic properties of silage maize soil irrigated at varying irrigation water levels with recycled wastewater under conventional and direct sowing practices compared to fully irrigation with freshwater. Therefore, this study hypothesizes that full irrigation with recycled wastewater under direct sowing practice can provide considerable contributions to improve the physical and hydraulic properties of the soil.

2. Materials and Methods

2.1. Experimental area climate and soil properties

This experiment was carried out in the experimental area of Van Yuzuncu Yil University Faculty of Agriculture (38°34′35″ N, 43°17′26″ E) in East Turkey during two silage maize (*Zea mays* L. cultivar OSSK-644) crop vegetation periods between May and September in 2020-2021. The area where the experiment was conducted has a semi-arid climate with an annual average precipitation of 410 mm for many years (1991-2020) (TSMS, 2022). According to the experimental area weather station (iMETOS-2) data (Cakmakci & Sahin, 2021; Yerli et al., 2023 and 2024), the mean temperature and total precipitation in 2020 (May 15 - September 13) in 2021 (May 11 - September 4) were 22.4°C and 37.0 mm – 22.8°C and 52.1 mm, respectively.

The soil texture in the surface layer of 0–30 cm of the experimental field is classified as sandy clay loam. The determined main properties in the surface layer, as the mean of three replicates were: pH 8.2, electrical conductivity (EC) 0.34 dS m^{-1}, organic matter 1.4%, total nitrogen 0.08%, $CaCO_3$ 11%, field capacity 0.384 $m^3\ m^{-3}$, permanent wilting point 0.225 $m^3\ m^{-3}$, available water content 0.159 $m^3\ m^{-3}$, particle density 2.7, bulk

density 1.31 g cm^{-3}, total porosity 52%, and aggregate stability 44%.

2.2. Experimental design and treatments

The main treatments used in the study were conventional tillage (CT) and direct sowing (DS), while the sub treatments were 100% (RWW100), 67% (RWW67), and 33% (RWW33) irrigations with recycled wastewater and %100 (FW100) irrigation with freshwater (Figure1). The three-replication experiment was designed according to the split-plots study design, and randomized blocks study design. The total number of plots in the experimental field was 24, each plot was organized with a size of 3.5 × 7.2 m and five rows (Figure 1).

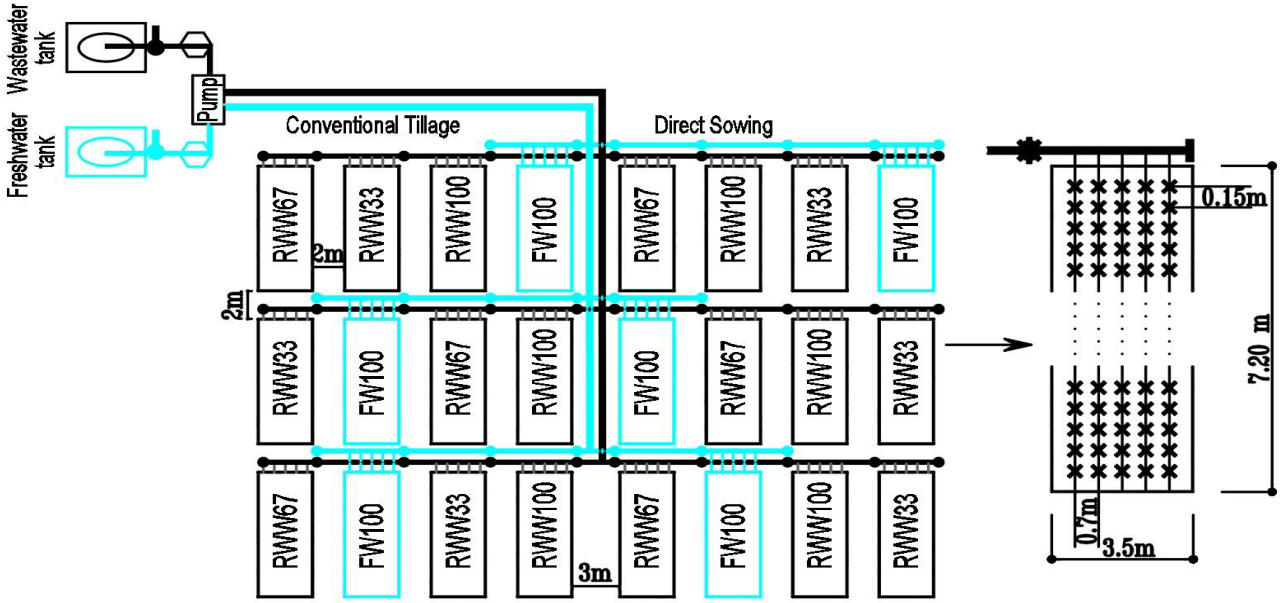

Figure 1. The experimental design. FW100: irrigation at 100% level with freshwater, RWW100: irrigation at 100% level with recycled wastewater, RWW67: irrigation at 67% level with recycled wastewater, RWW33: irrigation at 33% level with recycled wastewater.

Şekil 1. *Deneme deseni. FW100: Temiz su ile %100 düzeyinde sulama, RWW100: Geri dönüştürülmüş atık su ile %100 düzeyinde sulama, RWW67: Geri dönüştürülmüş atık su ile %67 düzeyinde sulama, RWW33: Geri dönüştürülmüş atık su ile %33 düzeyinde sulama.*

2.3. Irrigation water

While freshwater was delivered from the tap water network, the recycled wastewater was transferred to tanks in the experimental field from the Biological Treatment Plant of wastewater positioned in the Edremit district of Van province, Turkey before each irrigation via a water tanker with 20 tones. The applied waters were sampled to determine their characteristics each month during irrigation periods. The properties of the freshwater and recycled wastewater used are given in Table 1.

Considering the guidelines of the Food and Agriculture Organization of the United Nations (FAO) on the interpretation of water quality for irrigation purposes, according to the pH, which should be between 6.5-8.4, and the electrical conductivity (EC) classification values (low < 0.7 dS m^{-1}, medium 0.7-3.0 dS m^{-1}, high > 3.0 dS m^{-1}), the pH and EC values of the used fresh water and recycled wastewater were in the non-problematic class (Pescod, 1992; Ayers & Westcot, 1994). SAR values of the applied waters less than 3, when evaluated together with EC, do not pose any risk in terms of soil degradation considering FAO guidelines. Suspended solid matter content, which can cause clogging in the drip irrigation equipment (e.g. driplines, drippers), had no restriction on the use since it did not exceed the limit value of 50 mg L^{-1} given by Ayers and Westcot (1994). Boron content with less than 0.7 mg L^{-1} was also no toxicity problem according to the same guideline. The heavy metal contents of recycled wastewater were below the maximum allowable values considering the phytotoxic threshold levels of trace elements mentioned by FAO resources (Pescod, 1992; Ayers & Westcot, 1994). Total nitrogen and phosphorus contents of recycled wastewater, which have important contributions to soil fertility and crop development, were high. However, total nitrogen content in wastewater was appropriate considering the threshold value (10 mg L^{-1}) in the agricultural reuse mentioned in many international regulations and guidelines (Shoushtarian & Azar, 2020). Biochemical oxygen demand (BOD) reflecting organic pollutants that can be

degraded by microorganisms indicates organic content load in water, and is mostly used as five-day biochemical oxygen demand (BOD_5). Chemical oxygen demand (COD) as another parameter in the measurement of organic pollution shows the organic compound oxidized by the oxidant (Zhao et al., 2022). While EPA (2012) did not suggest a value for COD, the wastewater quality was appropriate considering the BOD_5 value, which should be less than 30 mg L^{-1} in irrigation waters for non-food crops. The wastewater contained only domestic waste since there is no major industrial facility in the region. As a result, it was concluded that there is no harm in using irrigation water for irrigation (Yerli & Sahin, 2022).

Table 1. The properties of freshwater and recycled wastewater used in the study
Çizelge 1. *Çalışmada kullanılan temiz su ve geri dönüştürülmüş atık suyun özellikleri*

Properties	Freshwater		Recycled wastewater	
	2020	2021	2020	2021
pH	8.10 ± 0.08	8.20 ± 0.05	7.44 ± 0.04	7.72 ± 0.07
EC (dS m^{-1})	0.348 ± 0.01	0.358 ± 0.02	1.108 ± 0.05	1.139 ± 0.01
Sodium adsorption rate	0.89 ± 0.02	0.75 ± 0.05	2.54 ± 0.05	2.51 ± 0.08
Total phosphorus (mg L^{-1})	–	–	1.69 ± 0.10	1.18 ± 0.03
Total nitrogen (mg L^{-1})	–	–	10.9 ± 0.9	10.8 ± 0.5
SSM (mg L^{-1})	–	–	21.9 ± 1.5	29.9 ± 1.4
COD (mg L^{-1})	–	–	36.3 ± 0.6	38.7 ± 3.2
BOD_5 (mg L^{-1})	–	–	22.0 ± 0.5	24.3 ± 1.5
B (mg L^{-1})	–	0.55 ± 0.03	-	0.46 ± 0.04
Fe (mg L^{-1})	0.054 ± 0.005	0.412 ± 0.009	0.053 ± 0.005	0.419 ± 0.007
Cu (mg L^{-1})	–	0.011 ± 0.001	-	0.011 ± 0.001
Mn (mg L^{-1})	0.009 ± 0.001	0.071 ± 0.006	0.007 ± 0.001	0.095 ± 0.004
Zn (mg L^{-1})	–	0.015 ± 0.001	–	0.015 ± 0.000
Pb (mg L^{-1})	–	0.002 ± 0.001	–	0.002 ± 0.001
Cd (mg L^{-1})	–	0.001 ± 0.001	–	-
Cr (mg L^{-1})	–	0.001 ± 0.000	–	0.001 ± 0.001
Ni (mg L^{-1})	–	0.038 ± 0.001	–	0.047 ± 0.002

– : not determined, ± : standard error of mean, EC: electrical conductivity, SSM: suspended solid matter COD: chemical oxygen demand, BOD_5: biological oxygen demand

2.4. Irrigation treatments

Considering the root development and water need of silage maize vegetation period, the irrigations were carried out in two separate periods 1st (until the crop height 40-50 cm of silage maize, that is, until the 4-6 leaf period) and 2nd (after the period of 4-6 leaves) (Yerli et al., 2023). The irrigation dates in the 1st and 2nd periods were determined with an approach that the sum of the difference between crop evapotranspiration (ETc) and precipitation (P) values. The irrigations were made when this sum value formulated as the $\sum(ETc - P)$ was reached to 40% of the available water at a soil layer of 0.30 m (19 mm) in 1st period and 0.90 m (60 mm) in 2nd period (Allen et al., 1998). ETc was calculated by multiplying ($ETc = kc \times ETo$) crop coefficient (kc) and reference evapotranspiration (ETo) values. While kc was obtained from Crop Water Consumption Guide for Irrigated Crops in Turkey, ETo was calculated by using daily climate data measured at the weather station (Imetos 2) in the study area with the CROPWAT program. In the 1st period, the depleted moisture at the 0.3 m soil layer in the freshwater plots in each irrigation was completed to the field capacity by freshwater applied equally to all plots with a 30% wetting percentage. The irrigations in the 2nd period were carried out with different irrigation quantities (RWW100, RWW67, RWW33, FW100) using a 65% wetting percentage (Cakmakci & Sahin, 2021). In each irrigation during this period, water amounts sufficient to replenish the decreasing moisture amount in the 0.90 m soil layer in the freshwater plots of each tillage sowing treatments to the field capacity were applied to the full irrigation plots. RWW67 and RWW33 plots were irrigated at a rate of 67% and 33% of the full irrigation amounts, respectively.

Field capacity, wilting point, and available water values in the experimental plots were determined according to the approach principles specified in the soil sampling and analysis section after the study. The soil moisture measurements at certain times during the vegetation period (sowing, before each irrigation, and harvesting) were carried out at a distance of about 15-20 cm from the drippers, between two crops in the middle of plots. While the water content at 0-30 cm soil layer was directly measured with a portable TDR (Trime-Pico, IPH/T3, IMKO) calibrated to experimental field conditions, gravimetric sampling was applied in the soil layers of 30-60 and 60-90 cm. The soil moisture content

in gravimetric sampling was expressed in terms of weight as the ratio of weight difference between wet and dry soil to the weight of dry soil. Equation 1 was used to determine the volumes of irrigation water applied, and the confirmation of the water volumes was also provided by the readings on the water meters located at the beginning of each plot.

$$V = (FC - CM) \times BD \times SD \times WP \times IP \times PA \quad (1)$$

Where V is the irrigation quantity (L), FC and CM are the field capacity and current moisture (% of weight), BD is the bulk density of the soil (g cm^{-3}), SD is the soil depth (0.30 m and 0.90 m for 1st and 2nd periods, respectively), WP is the wetting ratio (0.30 and 0.65 for 1st and 2nd periods, respectively), IP is the irrigation ratio (1.0, 0.67, and 0.33 for 100%, 67% and 33% irrigation levels, respectively), PA is the plot area (25.2 m^2). Seasonal irrigation quantities as a two-year average were between 351-327 mm, 242-227 mm, and 129-122 mm for 100%, 67%, and 33% irrigation treatments in conventional tillage, while the quantities in direct sowing were 319-294 mm, 220-204 mm, and 118-111 mm.

2.5. Tillage sowing treatments and cultural processes

In the conventional tillage, the field was plowed, a cultivator-rotary harrow was used, and finally, seeding was done with a pneumatic seeder, respectively. However, plots without tillage were seeded with a direct sowing machine. In conventional tillage, the hoeing was carried out at 2 separate times when the crop height was 15-20 cm and 40-50 cm (4-6 leaf stage), while in direct sowing, an herbicide for weed removal was applied without hoeing. During the 1st year, 100 and 150 kg ha^{-1} of urea and TSP were applied together with the sowing, and the 2nd urea fertilization was carried out equally to the first dose in the 4-6 leaf period. In the 2nd year, fertilization was applied only in the freshwater plots to supplement the missing nitrogen and phosphorus considering the first-year residual effect incurred by recycled wastewater.

2.6. Soil sampling and analysis

The disturbed and undisturbed soil samples were taken from three layers (0-30 cm, 30-60, and 60-90 cm) in each plot. Organic matter content, particle and bulk densities, wet aggregate stability, field capacity, and permanent wilting point were determined during the harvest periods of the two experimental years. The Walkley-Black method was applied to determine soil organic matter content (Nelson & Sommers, 1982). The particle density was determined with the pycnometer method (Blake & Hartge, 1986a). The bulk density was obtained by dividing the undisturbed soil samples with dry weight (g) taken with a cylinder after drying in the oven to the volume (100 cm^3) of the cylinder (Blake & Hartge, 1986b). Wet aggregate stability was obtained according to the wet sieving method using soil fraction with a diameter of 1-2 mm (Kemper & Rosenau, 1986). Field capacity corresponds to the upper limit of available water in the soil and indicates the moisture of the soil after drainage of the water retained in the macro pores by gravity effect, and represents the moisture balanced with tension of practically 0.033 MPa suction. The permanent wilting point represents the inferior limit of available water for crop in the soil and corresponds to the moisture balanced with tension usually around 1.5 MPa. Therefore, using undisturbed soil samples taken with an approximate volume of 100 cm^3 soil core sample rings for field capacity and disturbed soil samples sieved through a 2 mm mesh for wilting point were used. The amounts of moisture retained at field capacity and wilting point were determined by applying a tension of 0.033 MPa and 1.5 MPa to the saturated samples, respectively with a pressure plate apparatus in the laboratory (Klute, 1986). The available water (AW) was calculated as the difference between the water content at field capacity and wilting point. Porosity was calculated via Equation 2 (Danielson & Sutherland, 1986).

$$P = [(1 - (BD / (PD \times \gamma_w))] \times 100 \quad (2)$$

Where P is the porosity (% of volume), BD is the bulk density of the soil (g cm^{-3}), PD is the particle density of the soil, and γ_w is the volume weight of pure water at +4°C (1 g cm^{-3}).

2.7. Statistical analysis

The statistical analyses of all data were carried out with the SPSS program. ANOVA analysis was performed for all parameters to determine the differences between soil layers (0–30 cm, 30–60 cm, and 60–90 cm), and the results showed that there was a general similarity for all parameters in the soil layers. In addition, considering that the surface soil layer is more critical in crop production (Yerli et al., 2024), the evaluations were carried out in the 0-30 cm soil layer. Thus, by accepting the variables of tillage sowing and irrigation treatments as constant, the results were evaluated with the General Linear Model, and significant means were classified with the Duncan

multiple comparison test at a 5% probability level. In addition, the RStudio program was used to show correlative relationships.

3. Results and Discussion

The results showed that all physical and hydraulic properties (except particle density) and organic matter content values were significantly ($p < 0.01$) affected by irrigation and tillage sowing treatments in the 0-30 cm soil layer, considering the two-year averages (Table 2).

Table 2. Variance analysis results
Çizelge 2. *Varyans analizi sonuçları*

Year	Source		Particle density			Bulk density		
		df	mean square	F	P	mean square	F	P
2020	tillage sowing	1	0.000	1.029	0.326	0.002	33.333	0.000
	irrigation	3	0.000	1.524	0.247	0.000	8.222	0.002
	tillage sowing × irrigation	3	6.111E-005	0.419	0.742	1.111E-005	0.222	0.880
	error	16	0.000			5.000E-005		
2021	tillage sowing	1	0.000	0.000	1.000	0.003	16.447	0.001
	irrigation	3	0.000	2.222	0.125	0.001	6.763	0.004
	tillage sowing × irrigation	3	1.111E-005	0.178	0.910	0.000	1.079	0.386
	error	16	6.25E-005			0.000		
2020 2021	tillage sowing	1	6.667E-005	1.000	0.332	0.002	44.000	0.000
	irrigation	3	7.778E-005	1.167	0.353	0.001	15.636	0.000
	tillage sowing × irrigation	3	1.111E-005	0.167	0.917	5.000E-005	1.091	0.381
	error	16	6.667E-005			4.583E-005		
Year	Source		Porosity			Wet aggregate stability		
2020	tillage sowing	1	2.982	23.216	0.000	25.010	240.100	0.000
	irrigation	3	0.878	6.837	0.004	32.017	307.364	0.000
	tillage sowing × irrigation	3	0.034	0.268	0.848	0.510	4.900	0.013
	error	16	0.128			0.104		
2021	tillage sowing	1	3.550	12.599	0.003	26.670	110.551	0.000
	irrigation	3	1.394	4.949	0.013	66.083	273.918	0.000
	tillage sowing × irrigation	3	0.278	0.985	0.425	0.744	3.083	0.057
	error	16	0.282			0.241		
2020 2021	tillage sowing	1	3.241	47.417	0.000	25.834	340.665	0.000
	irrigation	3	1.036	15.152	0.000	47.463	625.665	0.000
	tillage sowing × irrigation	3	0.080	1.171	0.352	0.630	8.313	0.001
	error	16	0.068			0.076		
Year	Source		Field capacity			Permanent wilting point		
2020	tillage sowing	1	1.127	58.783	0.000	0.184	11.919	0.003
	irrigation	3	1.407	73.391	0.000	0.738	47.883	0.000
	tillage sowing × irrigation	3	0.013	0.696	0.568	0.008	0.532	0.667
	error	16	0.019			0.015		
2021	tillage sowing	1	2.100	11.457	0.004	0.240	3.600	0.076
	irrigation	3	5.212	28.427	0.000	0.583	8.750	0.001
	tillage sowing × irrigation	3	0.050	0.275	0.843	0.008	0.117	0.949
	error	16	0.183			0.067		
2020 2021	tillage sowing	1	1.550	28.189	0.000	0.220	12.595	0.003
	irrigation	3	3.025	54.997	0.000	0.629	35.960	0.000
	tillage sowing × irrigation	3	0.017	0.311	0.817	0.007	0.405	0.752
	error	16	0.055			0.018		
Year	Source		Available water content			Organic matter		
2020	tillage sowing	1	16.467	62.156	0.000	0.008	10.243	0.006
	irrigation	3	0.431	1.627	0.223	0.235	298.878	0.000
	tillage sowing × irrigation	3	0.389	1.468	0.261	0.001	1.256	0.323
	error	16	0.265			0.001		
2021	tillage sowing	1	33.112	8.864	0.009	0.002	1.449	0.246
	irrigation	3	24.282	6.500	0.004	0.323	281.820	0.000
	tillage sowing × irrigation	3	1.912	0.512	0.680	0.000	0.203	0.893
	error	16	3.735			0.001		
2020 2021	tillage sowing	1	24.080	24.779	0.000	0.004	9.062	0.008
	irrigation	3	7.404	7.619	0.002	0.276	585.404	0.000
	tillage sowing × irrigation	3	0.921	0.948	0.441	0.000	0.637	0.602
	error	16	0.972			0.000		

df – degree of freedom, F – F-ratio score, P – P-value

3.1. Soil organic matter content

Organic matter content increased in all irrigation and tillage sowing treatments compared to the pre-experiment value (1.36%), and the highest values were observed in the RWW100 and direct sowing treatments (Figure 2), and the 2nd year values were found to be higher than the first-year values. In the 2nd year, the RWW100 and RWW67 treatments increased organic matter content by 19.0% and 1.7%, respectively compared to the FW100 treatment, while the RWW33 treatment resulted in a 12.6% lower content with decreasing in the irrigation quantity.

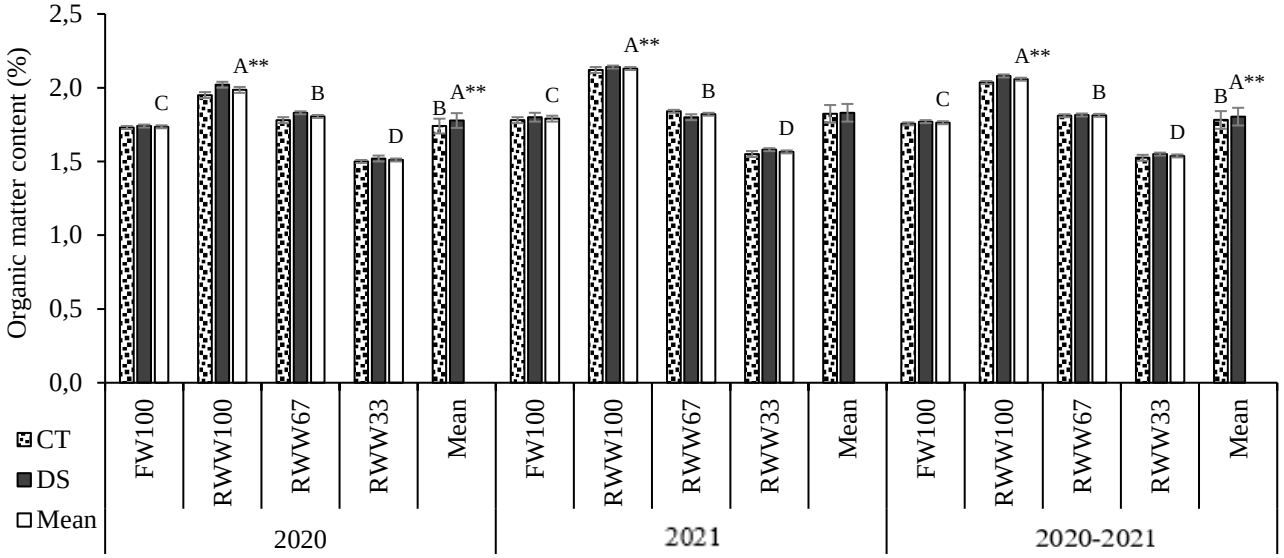

Figure 2. Organic matter contents in 0-30 cm soil layer in different tillage sowing and irrigation treatments. CT: conventional tillage, DS: direct sowing, FW100: irrigation at 100% level with freshwater, RWW100: irrigation at 100% level with recycled wastewater, RWW67: irrigation at 67% level with recycled wastewater, RWW33: irrigation at 33% level with recycled wastewater; **: $p < 0.01$; the significance comparisons are between both irrigation treatments and tillage sowing treatments in each experiment year.

Şekil 2. *Farklı toprak işleme ekim ve sulama uygulamalarında 0-30 cm toprak tabakasındaki organik madde içerikleri. CT: geleneksel toprak işleme, DS: doğrudan ekim, FW100: temiz su ile %100 düzeyinde sulama, RWW100: geri dönüştürülmüş atık su ile %100 düzeyinde sulama, RWW67: geri dönüştürülmüş atık su ile %67 düzeyinde sulama, RWW33: geri dönüştürülmüş atık su ile %33 düzeyinde sulama; **: p < 0,01; istatistik karşılaştırmalar her iki deneme yılı için hem sulama hem de toprak işleme ekim uygulamaları arasındadır.*

The significant ($p < 0.001$) positive correlation between the irrigation quantity and soil organic matter content (Figure 3) showed that the RWW33 treatment with less wastewater quantity limited the effect of organic matter on the soil from wastewater (Figure 2). This also explained the high organic matter content in the RWW100 treatment irrigated with a high quantity of wastewater. Bedbabis et al. (2015) have determined that organic matter in the soil increases with the effect of high chemical oxygen demand and biological oxygen demand contents of wastewater. Therefore, the suspended organic matter based on high chemical oxygen demand and biological oxygen demand values (37.5 and 23.2 mg L^{-1} as two-year averages, respectively) found in the wastewater used in this study resulted in a significant increase in organic matter of the soil especially in the RWW100 treatment (Figure 2). Many researchers have also reported that with the reuse of wastewater, there are significant increases in the organic matter content of the soil due to the organic components of the water (Tunc & Sahin, 2016; Abd-Elwahed, 2019; Dogan Demir & Sahin, 2020; Cakmakci & Sahin, 2021).

Minimal stirring of soil in direct sowing can protect organic matter stock because the intensive process in conventional soil tillage increases decomposition of organic matter because of oxidation in highly aerated soil (Malhi et al., 2018). In addition, crop residues left on the soil in direct sowing contribute to soil organic matter (Gozubuyuk et al., 2020). Denardin et al. (2019), Yang et al. (2019), and Kan et al. (2020) have also reported that the organic matter content of the soil was enriched in direct sowing according to intensive tillage practices.

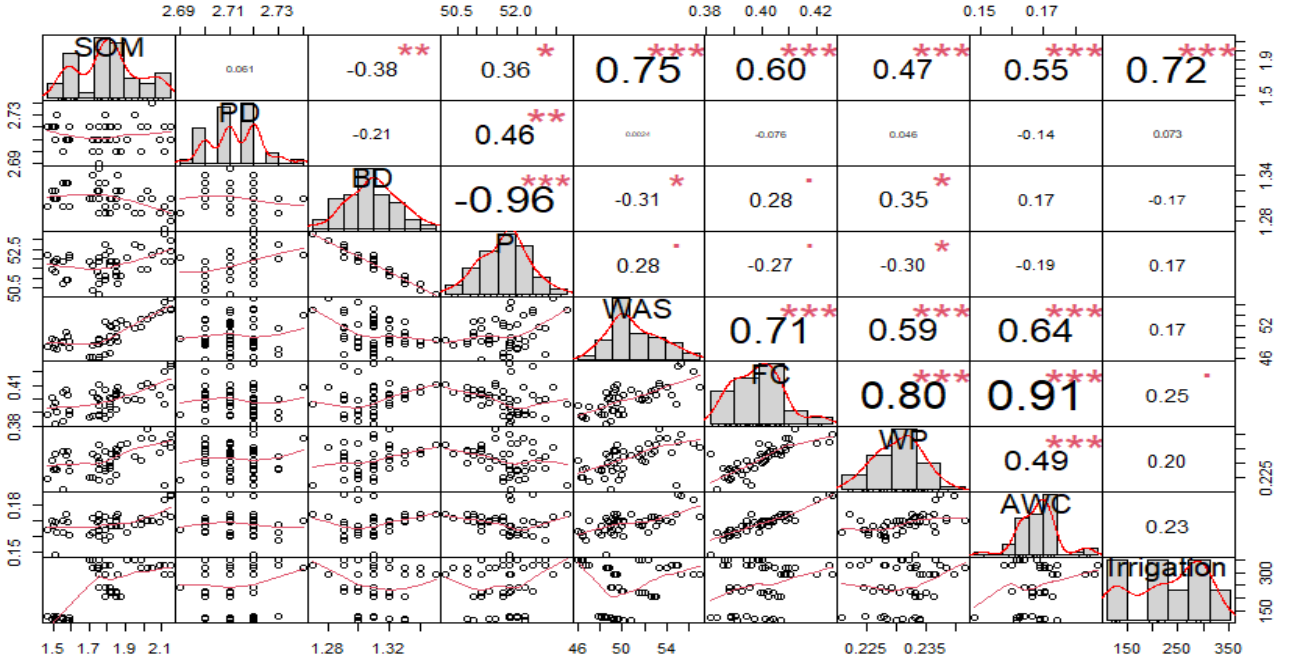

Figure 3. Correlation matrix for data in 0-30 cm soil layer. SOM: soil organic matter content, PD: particle density, BD: bulk density, P: porosity, WAS: wet aggregate stability, FC: field capacity, WP: permanent wilting point, AWC: available water content, Irrigation: irrigation quantity; ***, **, *: significant at 0.001, 0.01 and 0.05 level, respectively.

Şekil 3. *0-30 cm toprak tabakasındaki veriler için korelasyon matrisi. SOM: toprağın organik madde içeriği, PD: özgül ağırlık, BD: hacim ağırlık, P: porozite, WAS: ıslak agregat stabilitesi, FC: tarla kapasitesi, WP: devamlı solma noktası, AWC: kullanılabilir su kapasitesi, Sulama: sulama miktarı; ***, **, *: sırasıyla 0,001, 0,01 ve 0,05 düzeyinde önemlidir.*

3.2. Particle and bulk densities, and porosity

Bulk density in the RWW100 in the 2nd year was found to be less in both tillage sowing practices compared to the pre-experiment value (1.31 g cm^{-3}) (Figure 4). Considering the significant ($p < 0.01$) changes in bulk density between treatments (Table 2), it has been observed that porosity significantly ($p < 0.01$) increased in treatments in which bulk density values were low (Figure 3). Therefore, a significant ($p < 0.001$) negative correlation between porosity and bulk density was determined (Figure 3). Furthermore, the linear increase of porosity with particle density was determined to be statistically significant ($p < 0.01$).

Since the variation in particle density is mostly related to soil organic carbon, it could be said that the organic matter content did not reach a level that affected particle density considering the non-significant relationship among organic matter content and particle density in the study (Figure 3). Moreover, as a general approach, it is stated that particle density may not change significantly in short periods. In general, the formation of more stable aggregates and a lower bulk density are attributed to the presence of organic matter (Ramezani et al., 2019). Therefore, the limitation in the decrease in bulk density in the RWW67 and RWW33 treatments was attributed to less organic matter content in the soil (Figure 2). A significant negative correlation ($p < 0.01$) of bulk density with soil organic matter content also confirmed these findings (Figure 3). Moreover, it could be said that strong aggregation decreases bulk density considering the significant ($p < 0.05$) negative correlation between bulk density and wet aggregate stability (Figure 3). Similarly, many researchers have also stated that the bulk density of soils irrigated with the reuse of the wastewater decreased due to the enriched in organic matter (Biswas et al., 2017; Dogan Demir & Sahin, 2019; Cakmakci & Sahin, 2021).

Bulk density was low in conventional tillage due to the loose soil structure and was found to be higher with the effect of soil compaction with the direct sowing treatment. Similarly, Gozubuyuk et al. (2014) determined that conservation tillage practices cause soil compaction and thus increase bulk density according to conventional tillage. Many researchers have also stated that bulk density was higher in direct sowing according to intensive tillage or conventional tillage (Gozubuyuk et al., 2014; Blanco-Canqui & Ruis, 2018).

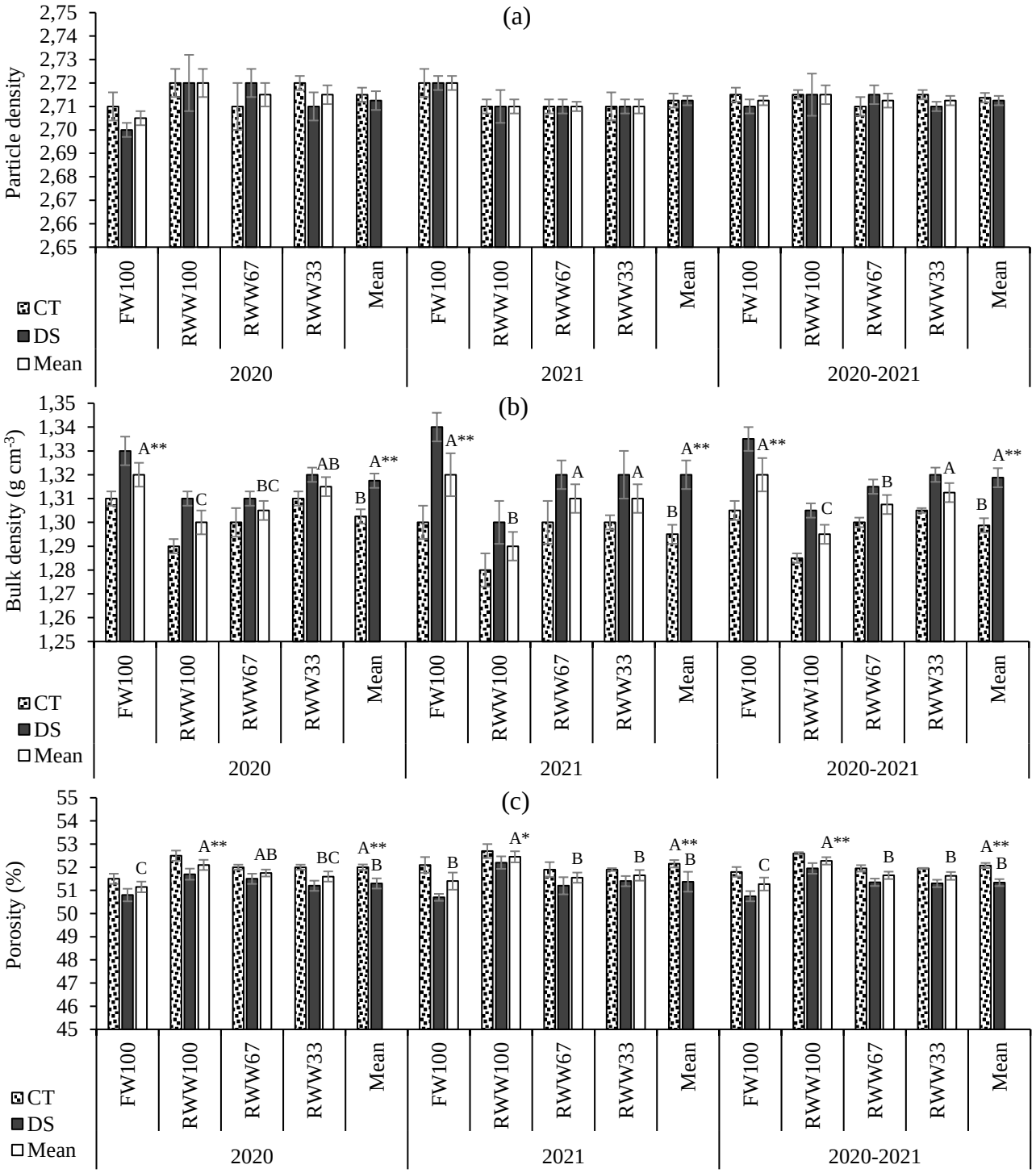

Figure 4. Particle density (a), bulk density (b), and porosity values (c) in 0-30 cm soil layer in different tillage sowing and irrigation treatments. CT: conventional tillage, DS: direct sowing, FW100: irrigation at 100% level with freshwater, RWW100: irrigation at 100% level with recycled wastewater, RWW67: irrigation at 67% level with recycled wastewater, RWW33: irrigation at 33% level with recycled wastewater; **: $p < 0.01$; the significance comparisons are between both irrigation treatments and tillage sowing treatments in each experiment year.

Şekil 4. *Farklı toprak işleme ekim ve sulama uygulamalarında 0-30 cm toprak tabakasındaki özgül ağırlık (a), hacim ağırlık (b) ve porozite değerleri (c). CT: geleneksel toprak işleme, DS: doğrudan ekim, FW100: temiz su ile %100 düzeyinde sulama, RWW100: geri dönüştürülmüş atık su ile %100 düzeyinde sulama, RWW67: geri dönüştürülmüş atık su ile %67 düzeyinde sulama, RWW33: geri dönüştürülmüş atık su ile %33 düzeyinde sulama; **: p < 0,01; istatistik karşılaştırmalar her iki deneme yılı için hem sulama hem de toprak işleme ekim uygulamaları arasındadır.*

The porosity values in the RWW100 treatment under conventional tillage and direct sowing in the 2nd year were higher by 1.7% and 0.8% than the pre-experimental value (51.8%), respectively. The approach that organic matter added to soil increases porosity under irrigation conditions with wastewater is supported by Biswas et al. (2017) who stated that porosity in irrigation with wastewater was 6% higher than with freshwater. Similarly, increases in porosity have been detected in wastewater irrigation conditions in many studies (Tunc & Sahin, 2015; Dogan Demir & Sahin, 2019; Cakmakci & Sahin, 2021). The porosity decreased with the increase in bulk density due to the decrease of organic matter entering the soil in deficit irrigation treatments (Figures 2 and 4). Compared to conventional tillage, the higher bulk density in direct sowing also revealed lower porosity values. Kucukalbay and Akbolat (2015) indicated that low porosity values were determined in direct sowing (53.1%) among different tillage practices, while high porosity values were determined in conventional tillage (56.7%) and reduced tillage (53.7%).

3.3. Wet aggregate stability

RWW100 under direct sowing resulted in significantly ($p < 0.01$) higher wet aggregate stability values by 14.2% and 17.3% compared to the FW100 treatment under direct sowing and conventional tillage, respectively, considering 2nd year values which were higher than for the 1st year (Table 2 and Figure 5). While all treatments increased wet aggregate stability compared to the pre-experimental value (43.8%), RWW100 treatment in the 2nd year resulted in a 26.9% higher value. Direct sowing in the 2nd year also increased this value by 18.9%.

Soil organic matter mediates better aggregation by flocculation and cementation of particles (Alhassan et al., 2018). It has been clarified that adding organic matter to the soil with recycled wastewater irrigation contributes positively to aggregate stability, and therefore, the decrease in the organic matter with the decrease in the amount of irrigation also reduces aggregate stability. The significant ($p < 0.001$) positive correlation between wet aggregate stability and organic matter content also supports this (Figure 3). Dogan Demir and Sahin (2020) stated that the aggregate stability increased under the conditions of using wastewater for irrigation. Cakmakci and Sahin (2021) confirmed a similar situation and reported that aggregate stability increased at lower levels due to deficit irrigation with wastewater.

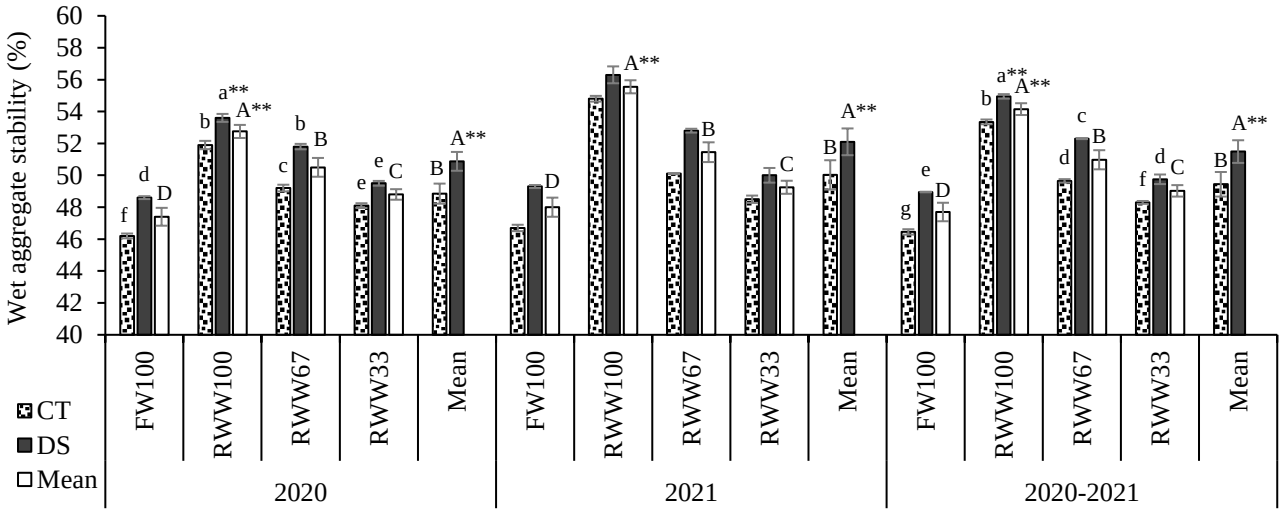

Figure 5. Wet aggregate stability values in 0-30 cm soil layer in different tillage sowing and irrigation treatments. CT: conventional tillage, DS: direct sowing, FW100: irrigation at 100% level with freshwater, RWW100: irrigation at 100% level with recycled wastewater, RWW67: irrigation at 67% level with recycled wastewater, RWW33: irrigation at 33% level with recycled wastewater; **: $p < 0.01$; the significance comparisons are between both irrigation treatments and tillage sowing treatments in each experiment year.

Şekil 5. *Farklı toprak işleme ekim ve sulama uygulamalarında 0-30 cm toprak tabakasındaki ıslak agregat stabilitesi değerleri (c). CT: geleneksel toprak işleme, DS: doğrudan ekim, FW100: temiz su ile %100 düzeyinde sulama, RWW100: geri dönüştürülmüş atık su ile %100 düzeyinde sulama, RWW67: geri dönüştürülmüş atık su ile %67 düzeyinde sulama, RWW33: geri dönüştürülmüş atık su ile %33 düzeyinde sulama; **: p < 0,01; istatistik karşılaştırmalar her iki deneme yılı için hem sulama hem de toprak işleme ekim uygulamaları arasındadır.*

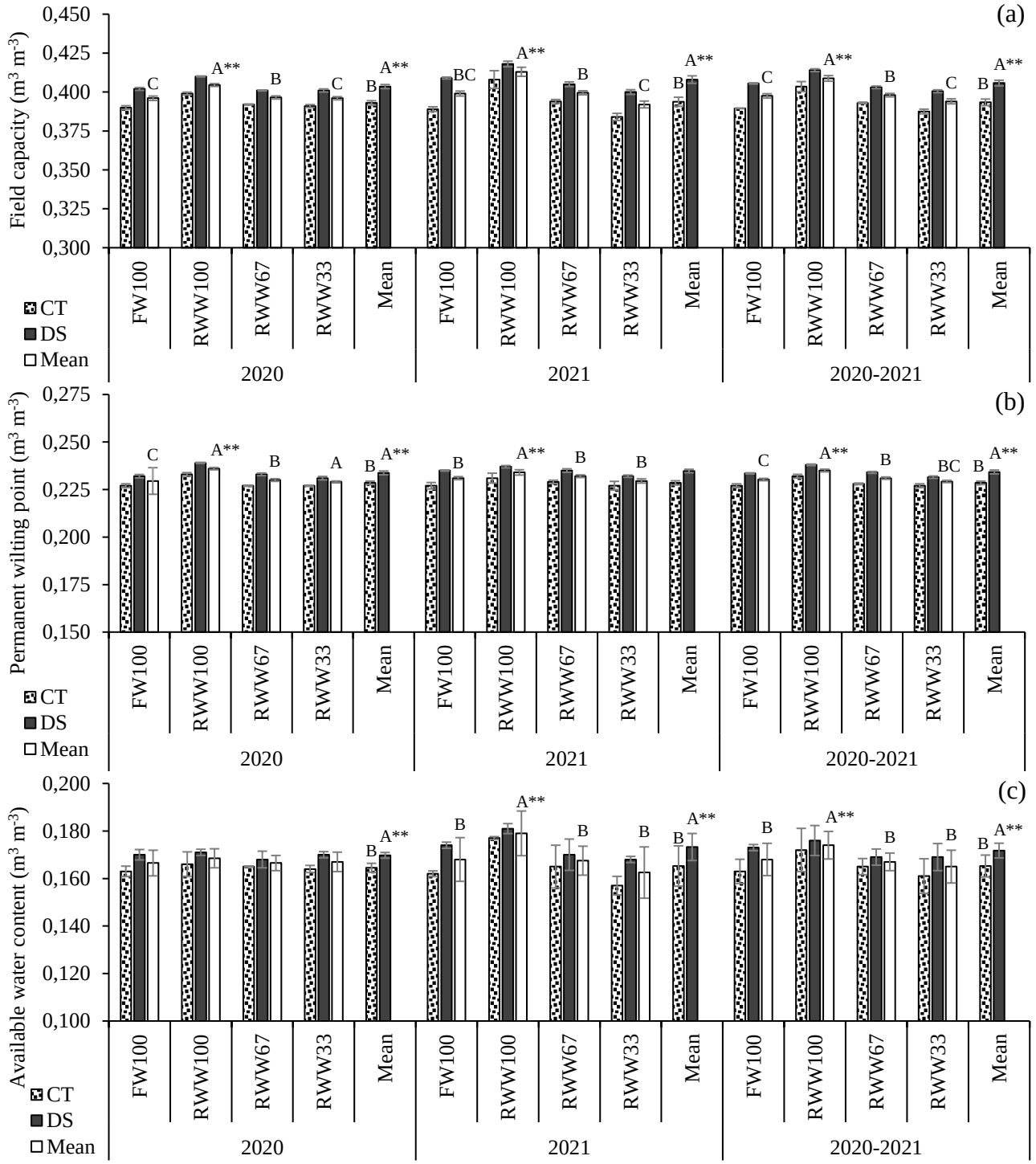

Figure 6. Field capacity (a), permanent wilting point (b), and available water content values (c) in 0-30 cm soil layer in different tillage sowing and irrigation treatments. CT: conventional tillage, DS: direct sowing, FW100: irrigation at 100% level with freshwater, RWW100: irrigation at 100% level with recycled wastewater, RWW67: irrigation at 67% level with recycled wastewater, RWW33: irrigation at 33% level with recycled wastewater; **: $p < 0.01$; the significance comparisons are between both irrigation treatments and tillage sowing treatments in each experiment year.

Şekil 6. *Farklı toprak işleme ekim ve sulama uygulamalarında 0-30 cm toprak tabakasındaki tarla kapasitesi (a), devamlı solma noktası (b) ve kullanılabilir su tutuma kapasitesi değerleri (c). CT: geleneksel toprak işleme, DS: doğrudan ekim, FW100: temiz su ile %100 düzeyinde sulama, RWW100: geri dönüştürülmüş atık su ile %100 düzeyinde sulama, RWW67: geri dönüştürülmüş atık su ile %67 düzeyinde sulama, RWW33: geri dönüştürülmüş atık su ile %33 düzeyinde sulama; **: p < 0,01; istatistik karşılaştırmalar her iki deneme yılı için hem sulama hem de toprak işleme ekim uygulamaları arasındadır.*

The higher aggregate stability of direct sowing according to conventional tillage can be attributed to residues of the crop adding organic matter to the soil in direct sowing and organic matter conservation is better since the soil is less disturbed. Similarly, Sithole et al. (2019) reported that aggregate stability was found to be higher in direct sowing and this was associated with the longer preservation of organic matter and slower mineralization in direct sowing according to intensive tillage. In addition, many researchers have stated that aggregate stability is higher in direct sowing according to conventional tillage (Du et al., 2013; Gozubuyuk et al., 2014; Nouwakpo et al., 2018).

3.4. Field capacity, permanent wilting point, and available water

The RWW100 and the direct sowing treatments significantly ($p < 0.01$) increased field capacity, permanent wilting point, and available water content compared to the FW100 treatment and conventional tillage (Table 2 and Figure 6) and were also higher than pre-experiment values: field capacity 0.384 $m^3\ m^{-3}$, permanent wilting point 0.225 $m^3\ m^{-3}$, available water content 0.159 $m^3\ m^{-3}$.

The physical properties of the soil such as bulk density and porosity highly influence water retention with hydraulic behavior changes in the soil (Hartmann et al., 2020). Many studies have reported that positive developments in soil hydraulic properties occur with the improvements in soil properties brought about by irrigation with recycled wastewater (Tunc & Sahin, 2015; Musazura et al., 2019; Badaou & Sahin, 2021). The significant ($p < 0.05$) correlations of bulk density and porosity values with field capacity and permanent wilting point in this study also confirmed this opinion (Figure 3). Moreover, more significant ($p < 0.001$) correlations of field capacity, permanent wilting point, and available water content with organic matter content were determined. Therefore, it could be said that the higher level of field capacity and permanent wilting point values in recycled wastewater irrigation conditions compared to freshwater irrigation can be related to the organic matter contribution of recycled wastewater to soils. This also explains the reducing effect of declining content of organic matter on the water holding capacity in deficit irrigation treatments. Mujdeci et al. (2017) stated that the increase in voids and stabilization of soil aggregates with the addition of organic matter increases porosity in favor of water holding capacity. Ors et al. (2015) indicated that the water holding capacity of the soil is directly dependent on the pore distribution of the soil and soil organic matter can improve the pore size distribution in the soil in favor of better water retention. In addition, Abdelfattah (2013) reported that the high amount of organic matter in the soil, especially in areas suffering from drought, supports the increase in the amount of available water. Many studies have indicated that the addition of organic matter to soil improves field capacity and wilting point (Ors et al., 2015; Kadioglu & Canbolat, 2019; Alaboz & Cakmakci, 2020).

As a similar approach, the increases in water holding capacity in direct sowing according to conventional tillage can be explained by the contribution of organic matter by direct sowing into the soil (Figure 2). Gozubuyuk et al. (2014) stated that higher available water values in the surface soil layer were obtained in direct sowing according to conventional and reduced soil tillage treatment. In addition, the increase in the amount of available water in no-tillage conditions may be related to the micro and macro pore distribution. This study also indicated the possible positive effects of higher aggregate stability values on the pore size distribution and thus water retention in direct sowing practice. Furthermore, another study examining the effects of tillage sowing practices on the hydraulic properties of soil has reported that better values were obtained in no-till conditions (Somasundaram et al., 2018).

4. Conclusion

As a result of the study, it was concluded that full irrigation with recycled domestic wastewater under direct sowing can be good practice and contribute to the development of the soil, considering that full irrigation with wastewater under direct sowing improves the physical and hydraulic properties of the soil in silage maize cultivation and that the use of domestic wastewater in irrigation, saving freshwater, as well as reducing the risk of environmental pollution by waste disposal. Thus, it can be suggested that the silage maize field be operated under direct sowing by irrigation with recycled domestic wastewater, but it should be considered that short-term findings should be supported by long-term data to see the sustainable effect on soil.

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Bioinformatic Analysis of LEA Genes in Stout Camphor Tree (*Cinnamomum micranthum* f. *Kanehirae*)

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Abstract: LEA proteins have an important role in the response of plants to abiotic stresses. *Cinnamomum micranthum* f. *kanehirae*, a medicinal and aromatic plant belonging to the *Lauraceae* family. The genome sequence of the Kanehirae or Stout Camphor tree was recently completed. Although there are studies on its genome, there are no studies on LEA genes.

57 LEA genes (CmiLEA) were identified in the Stout Camphor genome. CmiLEA was divided into 8 distinct clusters based on phylogenetic analysis. When the subcellular localizations of CmiLEA were examined, they were found to be localized mostly in the cytoplasm. A total of 13 genes targeting only one miRNA were identified. In CmiLEA, a total of 23 genes were found to have only exon regions and no introns. In total, 35 conserved motifs were identified, while there was only one conserved motif in CmiLEA-42. Consistent with the 3D structure results, CmiLEA-21, CmiLEA-31, CmiLEA-44, CmiLEA-45, and CmiLEA-57 from the LEA-2 subfamily showed over 90% accuracy.

The present study was the first *in-silico* analysis of LEA genes in *Cinnamomum micranthum* f. *Kanehirae*. It is thought that it may form a base for advanced functional analysis in *Cinnamomum* in future.

Keywords: Bioinformatics, LEA genes, LEA proteins, miRNA, Stout Camphor Tree

Stout Kafur Ağacında LEA Genlerinin Biyoinformatik Analizi (*Cinnamomum micranthum f. Kanehirae*)

Öz: LEA proteinleri bitkilerin abiyotik streslere karşı tepkilerinde önemli bir role sahiptir. *Lauraceae* ailesine ait tıbbi ve aromatik bir bitki olan *Cinnamomum micranthum* f. *Kanehirae* veya Stout Kafur ağacının genom dizisi yakın zamanda tamamlanmıştır. Stout Kafur genomunda çalışmalar olmasına rağmen LEA genleri ile alakalı herhangi bir çalışma bulunmamaktadır. Bu nedenle bu çalışmada biyoinformatik araçlar kullanılarak Stout Kafur genomunda yer alan LEA genlerinin genom çapında analizinin yapılması amaçlanmıştır.

Stout Kafur genomunda 57 LEA geni (CmiLEA) tanımlandı. CmiLEA filogenetik analize göre 8 ayrı kümeye ayrılmıştır. CmiLEA'nın subselüler lokalizasyonları incelendiğinde daha çok sitoplazmada lokalize oldukları ve sadece bir miRNA hedefleyen toplam 13 gen tanımlanmıştır. CmiLEA' da toplam 23 genin yalnızca ekzon bölgelerine sahip olduğu ve intronsuz olduğu tespit edilmiştir. Toplamda 35 korunmuş motif belirlenirken, CmiLEA-42'de yalnızca bir korunmuş motif bulunmuştur. 3B yapı sonuçlarına uygun olarak LEA_2 alt ailesinden CmiLEA-21, CmiLEA-31, CmiLEA-44, CmiLEA-45 ve CmiLEA-57 %90'ın üzerinde doğruluk göstermiştir.

Bu çalışma, *Cinnamomum micranthum* f. *kanehirae* bitkisinde LEA genleri ile ilgili yapılmış ilk biyoinformatik çalışma olup, *Cinnamomum* cinsinde gelecekte ileri fonksiyonel analizler için bir temel oluşturabileceği düşünülmektedir.

Anahtar Kelimeler: Biyoinformatik, LEA genleri, LEA proteinleri, miRNA, Stout Kafur Ağacı

Introduction

Lauraceae is a family of tropical plants consisting of approximately 2500 species of trees and shrubs in 55 genera. Since members of the *Cinnamomum* are rich in essential oils, they are used in perfume making, spices production and compound for alternative medicine around the world. Studies have shown that they are rich in terpenoids and phenylpropanoids (Dong Wang, 2022). *Cinnamomum micranthum* f. *Kanehirae* or Stout Camphor tree is a medicinal and aromatic plant and is also a valuable tree for forestry due to its rot-resistant trunk. It is a plant of ecological, agricultural and economic importance that grows in the Far East and is endemic in Taiwan. Stout camphor tree is also the only host of *Taiwanofungus camphoratus* which is used in traditional medicine. Studies in high-fat-fed mice reported the anti-inflammatory, anti-obesinogenic and antidiabetic effects of this rare fungus (Chung and Hsieh, 2023; Chang et al., 2018).

Although studies have been carried out to determine the composition of essential oils and to determine genetic relationships in the *Cinnamomum* by sequencing genes such as chloroplast genes, studies at the whole genome level are rare (Dong Wang, 2022). Li et al. (2023) constructed a high-quality reference genome in *Cinnamomum camphora* by whole genome resequencing and made a genomic comparison with *C. Kanehirae*. They identified phenylpropanoid metabolism genes related to cold stress and terpene synthases (TPSs) genes related to defense response. In genomic studies conducted in *Cinnamomum camphora*, it was determined that there are 36,411-24,883 proteins, number of functional annotations are 97.06%-82.71%, and the number of TPS genes is ranged between 72-85 (Shen et al. 2022; Sun et al. 2022; Jiang et al. 2022; Wang et al., 2022; Li et al., 2023).

Late embryogenesis abundant (LEA) proteins were first found in cotton seeds during dehydration and maturation period (Cheng et al., 2021). Then, LEA proteins were detected in many plants such as *Arabidopsis*, poplar, peanut, tobacco, watermelon and melon. These proteins were also identified in organisms such as mosses and fungi (Hundertmark and Hintcha, 2008; Bies-Etheve et al., 2008; Altunoglu et al., 2017; Cheng et al., 2021; Huang et al., 2022; Geng et al., 2022). It has been determined that LEA proteins, which are involved in the response to abiotic stress factors, are found in many different parts of the cell, including the inner and outer membranes, cytoplasm and organelles (Altunoglu et al., 2017). It has been observed that LEA proteins, which are divided into 8 subfamilies ((LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, LEA_6, DHN (Dehydrin) and SMP (seed maturation protein)) according to their conserved domains, are rapidly expressed in plant tissues in the face of stress factors such as drought, saline, or cold stress (Bies-Etheve et al., 2008). The studies show that LEA proteins are not tissue specific and are produced at different expression levels in tissues throughout developmental processes. These proteins are also known to have a highly hydrophilic structure and are thought to be intrinsically disordered proteins under normal physiological conditions, for example. It is estimated that these ordered structures serve as molecular chaperones and have an important role in ensuring cellular homoestasis by binding to molecules such as enzymes, ions, ROS, etc. (Hong-Bo, et al., 2005; Lin et al., 2021).

Abiotic stress factors like drought and salinity are important environmental factors affecting crop production. Examining the response to these stress factors plays an essential role in organizing the necessary breeding studies to develop resistant plants. At the same time, abiotic stress factors are important in the protection of endemic or endangered plants due to these stress factors's environmental impact. In this study, it was aimed to examine LEA genes and LEA proteins in the stout camphor tree with bioinformatic tools, classify them and determine their predicted functions.

Material and Methods

Material

The data related with Stout camphor tree retrieved from NCBI (The National Center for Biotechnology Information) database (NCBI, 2024).

Methods

Conserved domains in PFAM database were found by using CLC Genomic Workbench 21 (Qiagen, 2022). The sequences screened with the BLASTP tool (NCBI, 2024), and LEA proteins were identified and named. The characteristics of LEA proteins (isoelectric point, protein length, physical position in chromosomes, instability, etc.) were found through the ExPasy ProtPARAm tool (Gasteiger et al., 2005) Exon-intron regions of LEA protein genes were determined and visualized using Gene Structure Displayer Server (GSDS) 2.0 (Hu et al., 2015). The 3D structures of the detected proteins were determined using the Hidden Markov Model (HMM) algorithm in Protein Fold Recognition Server 2 (Phyre2), and 3D protein modeling was performed (Kelley et al., 2015). Sequences of LEA proteins were aligned using the ClustalW in the MEGA 11 program using default options (Tamura and Kumar, 2021). Conserved motifs in amino acid sequences and 3-dimensional structures of proteins were determined with the MEME Suite program (Bailey et al., 2021). Molecular function, subcellular localization and biological processes (Gene ontology) analyzes of LEA proteins were performed by using the Blast2Go (Conesa et al., 2005).

Micro RNA (miRNA) targeting LEA transcripts data retrieved from miRBase (*Arabidopsis thaliana*) (Kozomara and Griffith-Jones, 2013) and LEA genes in stout camphor tree were evaluated using the Plant Small RNA Target Analysis Server, psRNATarget (Dai et al. 2018).

Table 1. Characteristics of *Cinnamomum micranthum* f. *Kanerihae* LEA proteins
Tablo 1. *Cinnamomum micranthumf. KanerihaeLEA proteinlerine ait özellikler*

ID	Cinnamomum micranthum Genomics Database Identfier	Startposition (bp)	EndPosition (bp)	Proteinlength (aa)	pI	Molecular weight (Da)	Instability index	Stable or unstable	Subfamily	GRAVY	Hydropathy	Alifatic Index	Subcellular Localization
CmiLEA-01	RWR97808.1	14,925	20,172	228	9.75	24891.08	33.02	Stable	LEA_2	0.143	Hydrophobic	102.94	InnerMembrane
CmiLEA-02	RWR98064.1	24,801	30,831	165	5.87	17495.89	46.51	Unstable	LEA_5	-1.332	Hydrophilic	33.82	Extracellular, Cytoplasmic, Periplasmic
CmiLEA-03	RWR97823.1	40,810	41,605	209	9.57	22964.80	29.12	Stable	LEA_2	0.076	Hydrophobic	97.32	Cytoplasmic, Periplasmic
CmiLEA-04	RWR97684.1	158,790	160,389	107	5.90	11724.83	42.16	Unstable	LEA_6	-1.036	Hydrophilic	44.77	Extracellular
CmiLEA-05	RWR97695.1	329,333	357,397	188	4.74	20294.58	29.68	Stable	LEA_6	-0,469	Hydrophilic	78.40	Cytoplasmic
CmiLEA-06	RWR93166.1	2,196,823	2,198,729	232	9.23	25384.70	43.40	Unstable	LEA_2	0,033	Hydrophobic	102.50	OuterMembrane, Cytoplasmic
CmiLEA-07	RWR94634.1	3,169,570	3,170,099	95	9.86	10458.11	53.68	Unstable	LEA_3	-0,215	Hydrophilic	83.05	Cytoplasmic, Periplasmic
CmiLEA-08	RWR90147.1	4,913,657	4,914,889	225	9.49	24814.96	40.21	Unstable	LEA_2	0,142	Hydrophobic	101.24	InnerMembrane, Periplasmic
CmiLEA-09	RWR78722.1	5,396,142	5,397,052	252	9.97	27999.78	39.51	Stable	LEA_2	-0,1	Hydrophilic	101.27	OuterMembrane
CmiLEA-10	RWR96356.1	6,195,381	6,196,184	144	5.29	15485.97	25.87	Stable	LEA_4	-1,165	Hydrophilic	44.44	Periplasmic
CmiLEA-11	RWR78857.1	7,768,911	7,769,440	79	9.96	8501.28	51.88	Unstable	LEA_5	-1,358	Hydrophilic	38.35	Cytoplasmic
CmiLEA-12	RWR90377.1	8,575,238	8,586,288	445	5.91	50415.56	57.70	Unstable	LEA_2	-0,527	Hydrophilic	85.84	Cytoplasmic
CmiLEA-13	RWR84490.1	9,743,858	9,744,653	187	9.28	20810.37	31.46	Stable	LEA_2	-0,136	Hydrophilic	106.26	Cytoplasmic
CmiLEA-14	RWR82192.1	10,105,899	10,106,296	108	6.06	11361.74	31.84	Stable	SMP	-0,253	Hydrophilic	75.19	Periplasmic, Extracellular, Cytoplasmic
CmiLEA-15	RWR82193.1	10,108,755	10,117,797	313	4.72	32423.09	42.50	Unstable	SMP	-0,413	Hydrophilic	71.50	Periplasmic
CmiLEA-16	RWR72447.1	11,010,992	11,015,343	306	9.54	33583.29	59.07	Unstable	LEA_2	-0,344	Hydrophilic	75.13	OuterMembrane
CmiLEA-17	RWR82287.1	11,800,766	11,801,561	199	9.51	22673.34	55.08	Unstable	LEA_2	0,102	Hydrophobic	108.69	InnerMembrane
CmiLEA-18	RWR82288.1	11,808,328	11,815,810	209	9.67	23744.66	46.42	Unstable	LEA_2	-0,028	Hydrophilic	85.89	InnerMembrane
CmiLEA-19	RWR88431.1	12,126,011	12,126,806	187	8.55	20550.31	25.88	Stable	LEA_2	0,444	Hydrophobic	113.10	InnerMembrane, Cytoplasmic
CmiLEA-20	RWR82316.1	12,177,214	12,179,889	182	9.30	20054.09	50.70	Unstable	LEA_2	-0,005	Hydrophilic	101.92	Cytoplasmic
CmiLEA-21	RWR96700.1	12,212,253	12,230,276	398	5.42	44448.15	30.01	Stable	LEA_2	-0,309	Hydrophilic	97.66	Cytoplasmic
CmiLEA-22	RWR82451.1	14,709,244	14,710,366	132	9.43	13804.19	31.68	Stable	Dehydrin	-1,049	Hydrophilic	51.74	Cytoplasmic, Periplasmic, Extracellular
CmiLEA-23	RWR93426.1	18,311,369	18,312,164	185	9.08	20332.85	36.67	Stable	LEA_2	0,401	Hydrophobic	110.05	OuterMembrane, Extracellular
CmiLEA-24	RWR93427.1	18,337,243	18,338,038	185	9.08	20332.85	36.67	Stable	LEA_2	0,401	Hydrophobic	110.05	OuterMembrane, InnerMembrane, Extracellular
CmiLEA-25	RWR93452.1	19,621,530	19,622,346	226	9.39	26175.93	39.05	Stable	LEA_2	-0,28	Hydrophilic	75.09	Cytoplasmic, InnerMembrane
CmiLEA-26	RWR82748.1	19,647,065	19,648,003	260	10.51	28235.88	53.68	Unstable	LEA_2	-0,196	Hydrophilic	84.00	OuterMembrane, Extracellular, Periplasmic
CmiLEA-27	RWR93456.1	19,880,530	19,881,325	211	9.49	23938.68	33.16	Stable	LEA_2	-0,146	Hydrophilic	77.16	Periplasmic, Extracellular, OuterMembrane
CmiLEA-28	RWR82951.1	23,165,577	23,165,974	104	4.79	10912.80	53.26	Unstable	LEA_6	-1,004	Hydrophilic	46.92	Extracellular, Periplasmic, Cytoplasmic
CmiLEA-29	RWR97091.1	24,384,561	24,385,374	225	9.24	25852.89	33.88	Stable	LEA_2	-0,264	Hydrophilic	86.22	Cytoplasmic, InnerMembrane

Table 1. Characteristics of *Cinnamomum micranthum* f. *Kanerihae* LEA proteins

Tablo 1. *(Devam) Cinnamomum micranthumf. KanerihaeLEA proteinlerine ait özellikler*

ID	Cinnamomum micranthum Genomics Database Identfier	Startposition (bp)	EndPosition (bp)	Proteinlength (aa)	pI	Molecular weight (Da)	Instability index	Stable or unstable	Subfamily	GRAVY	Hydropathy	Alifatic Index	Subcellular Localization
CmiLEA-30	RWR97092.1	24,459,404	24,460,199	205	9.30	22663.15	55.80	Unstable	LEA_2	0,108	Hydrophobic	100.78	OuterMembrane, InnerMembrane
CmiLEA-31	RWR83165.1	27,151,221	27,154,379	314	4.94	34561.31	16.38	Stable	LEA_2	-0.279	Hydrophilic	94.30	Cytoplasmic
CmiLEA-32	RWR92255.1	28,749,904	28,753,959	435	4.94	47980.19	41.69	Unstable	LEA_4	-1.371	Hydrophilic	42.46	Cytoplasmic, Extracellular, Periplasmic
CmiLEA-33	RWR92404.1	33,140,885	33,143,809	222	5.14	24864.70	59.50	Unstable	Dehydrin	-1.364	Hydrophilic	51.80	Cytoplasmic
CmiLEA-34	RWR95843.1	36,270,638	36,271,721	236	9.71	26828.53	35.39	Stable	LEA_2	0.149	Hydrophobic	99.87	OuterMembrane, InnerMembrane
CmiLEA-35	RWR94184.1	36,359,405	36,360,200	102	7.90	10846.32	44.12	Unstable	LEA_3	-0.331	Hydrophilic	82.25	Periplasmic
CmiLEA-36	RWR92640.1	37,837,684	37,838,591	251	9.83	28205.10	46.78	Unstable	LEA_2	-0.067	Hydrophilic	98.61	OuterMembrane
CmiLEA-37	RWR85244.1	39,510,036	39,510,849	225	9.24	25221.13	35.03	Stable	LEA_2	-0.095	Hydrophilic	90.49	OuterMembrane
CmiLEA-38	RWR85246.1	39,547,897	39,548,976	198	9.94	22718.71	49.87	Unstable	LEA_2	-0.013	Hydrophilic	109.19	InnerMembrane
CmiLEA-39	RWR94357.1	39,965,933	39,967,980	193	5.17	20953.01	48.76	Unstable	LEA_6	-0.891	Hydrophilic	51.09	Extracellular, Periplasmic
CmiLEA-40	RWR94359.1	40,152,225	40,152,622	95	5.27	10302.32	35.69	Stable	LEA_6	-1.125	Hydrophilic	41.16	Periplasmic, Cytoplasmic
CmiLEA-41	RWR89543.1	43,806,867	43,807,662	210	9.73	23303.20	45.63	Unstable	LEA_2	0.061	Hydrophobic	104.38	Periplasmic, OuterMembrane, InnerMembrane
CmiLEA-42	RWR76501.1	45,645,122	45,652,333	131	9.91	14070.92	33.56	Stable	LEA_3	-0.256	Hydrophilic	87.71	Periplasmic, Cytoplasmic
CmiLEA-43	RWR87982.1	48,225,122	48,225,917	209	9.53	23079.04	27.63	Stable	LEA_2	0.097	Hydrophobic	99.19	Cytoplasmic
CmiLEA-44	RWR80213.1	50,510,906	50,530,057	151	4.81	16361.70	12.66	Stable	LEA_2	0.007	Hydrophobic	101.99	Cytoplasmic
CmiLEA-45	RWR80217.1	50,671,714	50,689,053	151	4.81	16373.76	11.84	Stable	LEA_2	0.041	Hydrophobic	104.57	Cytoplasmic, Periplasmic
CmiLEA-46	RWR80282.1	53,670,451	53,671,246	193	9.77	21062.14	40.20	Unstable	LEA_2	0.160	Hydrophobic	80.26	Extracellular, InnerMembrane, OuterMembrane
CmiLEA-47	RWR80488.1	58,855,002	58,861,298	191	10.71	21715.28	57.16	Unstable	LEA_5	-1.142	Hydrophilic	48.48	Cytoplasmic, Extracellular, Periplasmic
CmiLEA-48	RWR80547.1	59,952,002	59,952,797	210	9.84	23089.12	44.40	Unstable	LEA_2	0.187	Hydrophobic	101.57	InnerMembrane
CmiLEA-49	RWR80548.1	59,955,049	59,963,042	240	10.33	26599.21	39.60	Stable	LEA_2	-0.124	Hydrophilic	99.83	OuterMembrane
CmiLEA-50	RWR73823.1	61,242,699	61,243,494	129	9.22	13693.15	26.97	Stable	LEA_1	-0.999	Hydrophilic	49.30	Periplasmic
CmiLEA-51	RWR80844.1	65,778,793	65,779,588	203	9.42	22242.25	42.94	Unstable	LEA_2	0.499	Hydrophobic	103.99	InnerMembrane
CmiLEA-52	RWR80845.1	65,791,299	65,792,094	210	9.63	23339.98	39.22	Stable	LEA_2	-0.024	Hydrophilic	90.95	OuterMembrane, Extracellular, Periplasmic
CmiLEA-53	RWR77559.1	66,813,066	66,823,071	225	7.73	25799.60	45.25	Unstable	LEA_2	-0.116	Hydrophilic	99.16	Cytoplasmic
CmiLEA-54	RWR81329.1	74,132,580	74,133,109	98	9.15	10514.45	47.81	Unstable	LEA_5	-1.393	Hydrophilic	33.98	Cytoplasmic
CmiLEA-55	RWR78006.1	74,571,090	74,574,233	108	9.05	12415.03	34.82	Stable	LEA_3	-0.807	Hydrophilic	70.46	Cytoplasmic
CmiLEA-56	RWR78102.1	76,142,313	76,143,259	262	10.19	28821.31	45.90	Unstable	LEA_2	-0.227	Hydrophilic	85.92	OuterMembrane
CmiLEA-57	RWR81477.1	76,528,885	76,529,931	290	5.09	31166.17	23.32	Stable	LEA_2	0.338	Hydrophobic	107.55	OuterMembrane

Results

LEA genes in *Cinnamomum micranthum* f. *Kanehirae* have been named, and their characteristics such as starting and ending positions of genes in base pairs, isoelectric points, protein lengths, molecular weights, stability, hydropathy have been determined and are given in Table 1. When the results were evaluated, 57 LEA genes were found and named. The chromosome locations of all of these genes were found as scaffolds. LEA genes are divided into 8 subfamilies according to their sequence homologies and conserved domains in the PFAM database. It is classified as LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, LEA_6, dehydrin and SMP. While it was found that there was 1 gene belonging to the LEA_1 subfamily and 37 genes in the LEA_2 subfamily, 2 genes were detected in the LEA_4, SMP and Dehydrin subfamilies, 3 genes in the LEA_3 gene family, 4 genes in LEA_5 and 5 genes in the LEA_6 gene family, respectively. Since the chromosomal distribution was determined as scaffolds, the exact location of LEA genes on chromosomes has not been revealed. The largest protein length was detected as 445 aa in CmiLEA-12, the shortest protein length was determined as 79 aa in CmiLEA-11, similarly the highest molecular weight (50415 Da) and the lowest molecular weight (8501 da) were calculated in CmiLEA-12 and CmiLEA-11. According to the isoelectric points of the proteins, the lowest isoelectric point was observed in CmiLEA-15 with 4.72, and the highest isoelectric point was observed in CmiLEA-47 with 10.70. The instability index values showed 27 of proteins were stable and 30 of proteins were unstable. The results of hydropathy properties revealed 40 of proteins were hydrophilic and 17 of proteins were hydrophobic. In the Aliphatic index, which is suggested to increase the thermostability of globular proteins, the lowest value was found in CmiLEA-2 with 33.82, and the highest value was found in CmiLEA-19 with the value of 113.10.

The longest upstream/downstream region was found in CmiLEA-40 as the gene was also the longest gene, and total of 23 genes has only exon regions and they were found to be intronless. A total of 35 conserved motifs were determined while CmiLEA-42 had only one conserved motif. Although there are common motifs in general, the motifs showed differences in LEA subfamilies. The highest number of motifs was detected in CmiLEA-43 and CmiLEA-03, the constructed dendrogram shows consistency with the motif patterns. The distribution of exons and introns, conserved motifs and the phylogenetic tree of CmiLEAs are given in Figure 1, Figure 2 and Figure 3, respectively.

In the phylogenetic tree of CmLEA genes, different subfamilies are shown in different colors. It has been observed that it is divided into eight subfamilies. While all families are grouped among themselves; CmLEA 42 and CmLEA-55 genes, which are belonged to the LEA_3 subfamily, are placed in the LEA_2 group. It was determined that the LEA_2 subfamily formed a separate cluster in the phylogenetic tree, LEA_3 was a separate group with a single branch, and the remaining gene subfamilies grouped in a different cluster.

When subcellular localizations were examined, total of 13 proteins localized in cytoplasm (CmiLEA-33, CmiLEA- 12, CmiLEA-13, CmiLEA-20, CmiLEA-21, CmiLEA-31, CmiLEA-43, CmiLEA-44, CmiLEA-53, CmiLEA-55, and CmiLEA-11). Only CmiLEA-04 from the LEA_6 family was found to localize extracellularly. CmiLEA-01, CmiLEA-17, CmiLEA-18, CmiLEA-38, CmiLEA-48, and CmiLEA-51 observed in the inner membrane and all of them were members of the LEA_2 family. It has been found that there are 7 members of the LEA_2 subfamily in the outer membrane (CmiLEA-09, CmiLEA-16, CmiLEA-36, CmiLEA-37, CmiLEA-49, CmiLEA-56, CmiLEA-57), 6 of which are hydrophilic and 1 is hydrophobic. CmiLEA-50, CmiLEA-35, CmiLEA-10, CmiLEA-15 from LEA_1, LEA_3, LEA_5 and SMP subfamilies were found to be located in periplasm. To accordance with structure results, CmiLEA-21, CmiLEA-31, CmiLEA-44, CmiLEA-45 and CmiLEA-57 from the LEA_2 subfamily showed accuracy above 90%. The 3-dimensional structure of these proteins were given in Figure 4.

In total, 220 miRNAs were associated with 52 CmiLEA genes. A total of 13 genes were identified with only one miRNA (CmiLEA-5, CmiLEA-6, CmiLEA-9, CmiLEA-14, CmiLEA-15, CmiLEA-17, CmiLEA-22, CmiLEA-23, CmiLEA-28, CmiLEA-35, CmiLEA-41, CmiLEA-51 CmiLEA-56; ath-miR842, ath-miR5632-5p, ath-miR447c-5p, ath-miR5652, ath-miR773a, ath-miR5658, ath-miR472-3p, ath-miR863-5p, ath-miR399c-5p, ath, respectively. -miR414, ath-miR8168, ath-miR156c-3p and ath-miR5657, respectively). There is no association with any miRNAs for 5 CmiLEA genes (CmiLEA-4, CmiLEA-20, CmiLEA-40, CmiLEA-49, CmiLEA-50). The association between CmiLEAs and miRNAs are given in Figure 5.

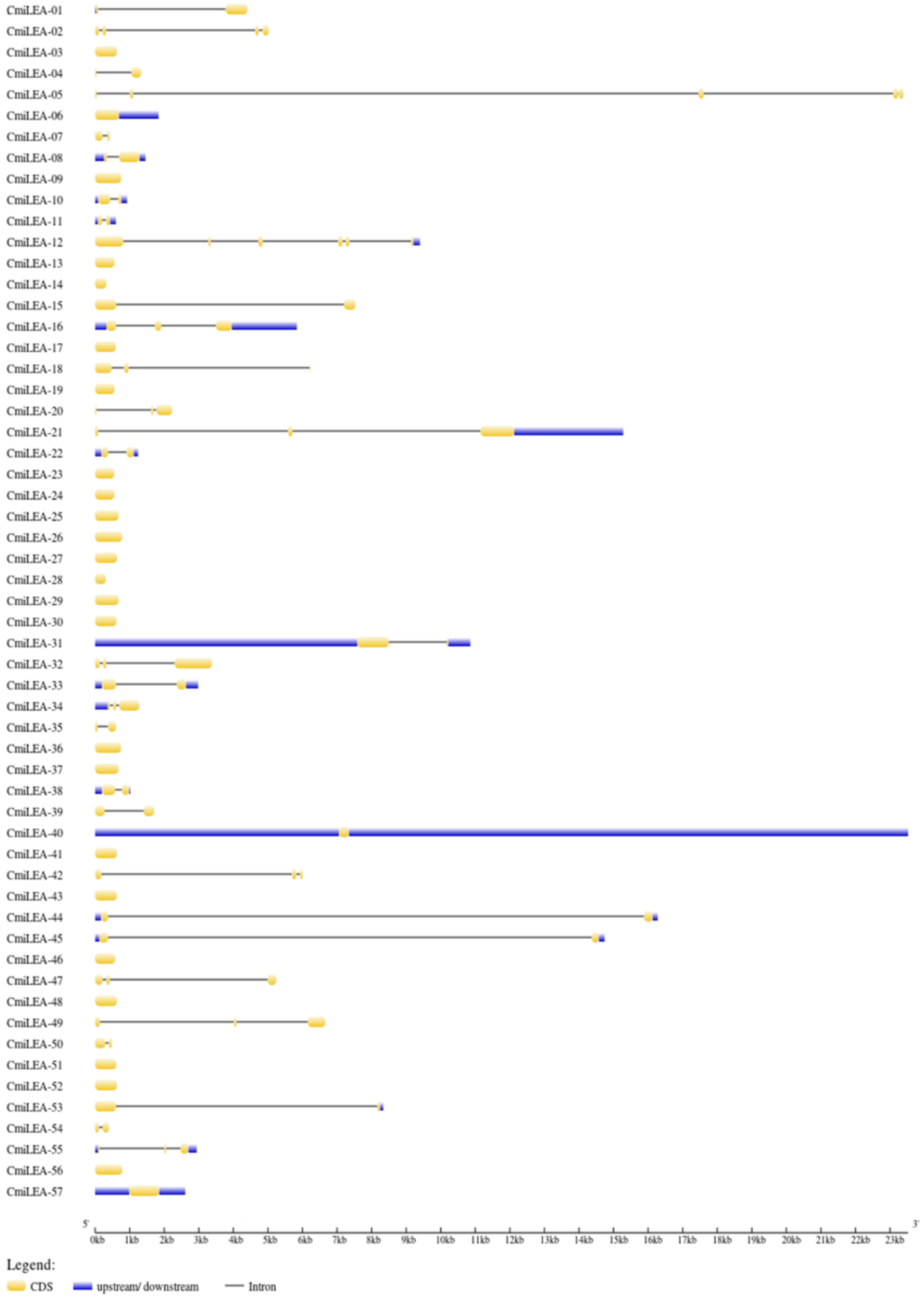

Figure 1. Exon and intron distributions of CmiLEA genes
Şekil 1. *CmiLEA genlerinde ekzon intron dağılımları*

Discussion

LEA proteins are linked with seed development and abiotic stress response. It has been reported that overexpression of LEA genes in transgenic plants shows an increased resistance to abiotic stress factors (Bies-Etheve et al., 2008). LEA genes are divided into 8 groups in plants according to their conservative PFAM

domains, these are LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, LEA_6, DHN (Dehydrin) and SMP (seed maturation protein) (Geng et al., 2022). In our study, 57 CmiLEA genes divided in 8 subfamilies. However, the classification made according to repeated conserved domains, 9 subfamilies were detected in *Arabidopsis thaliana*, while 8 subfamilies were found in many other studies. In linseed flax (*Linum usitatissimum* L.) fifty LEA genes (LuLEA) were determined and these genes were divided into 8 classes (Li et al., 2021).

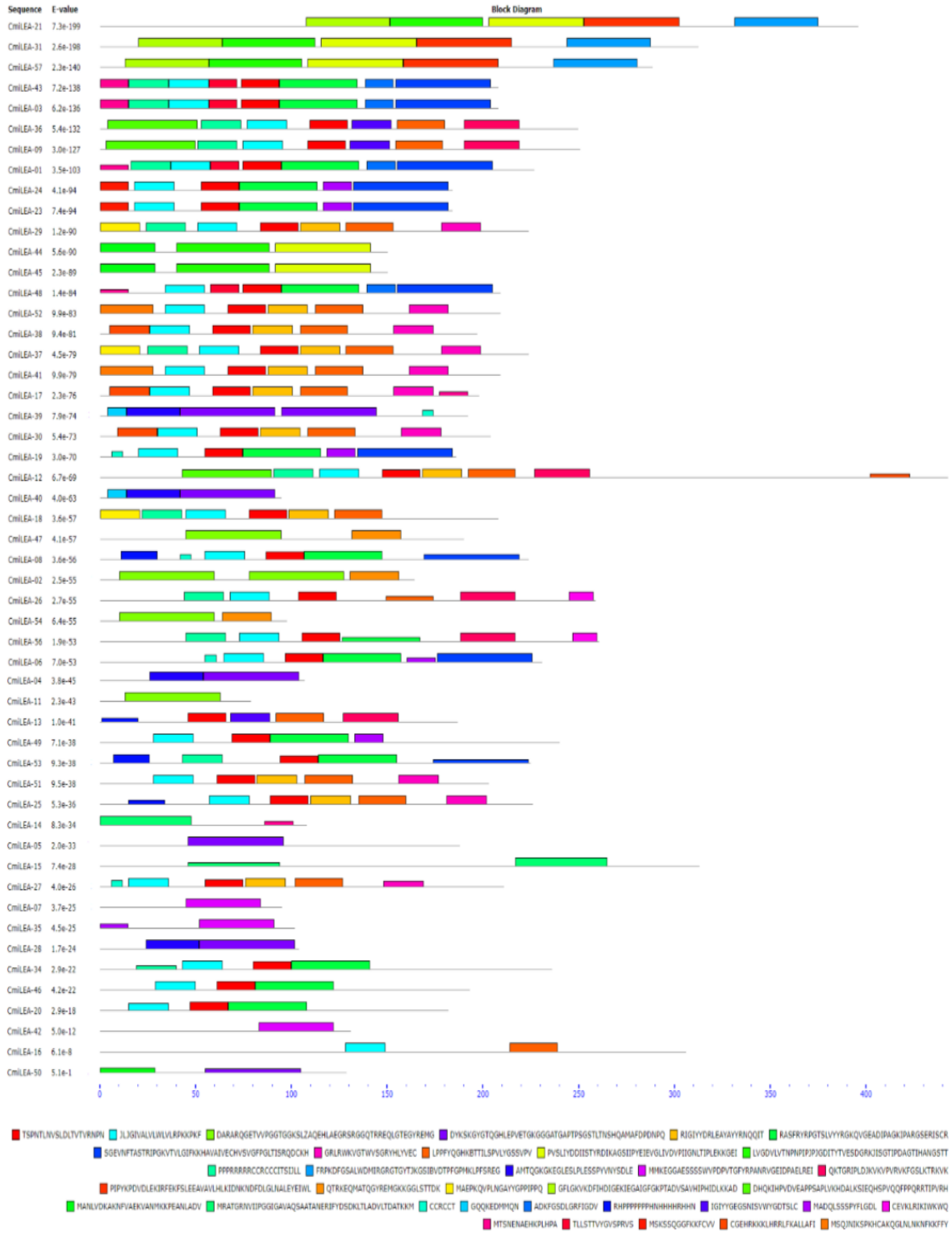

Figure 2. Conserved motifs of CmiLEA genes
Şekil 2. *CmiLEA genlerinde korunmuş proteinler*

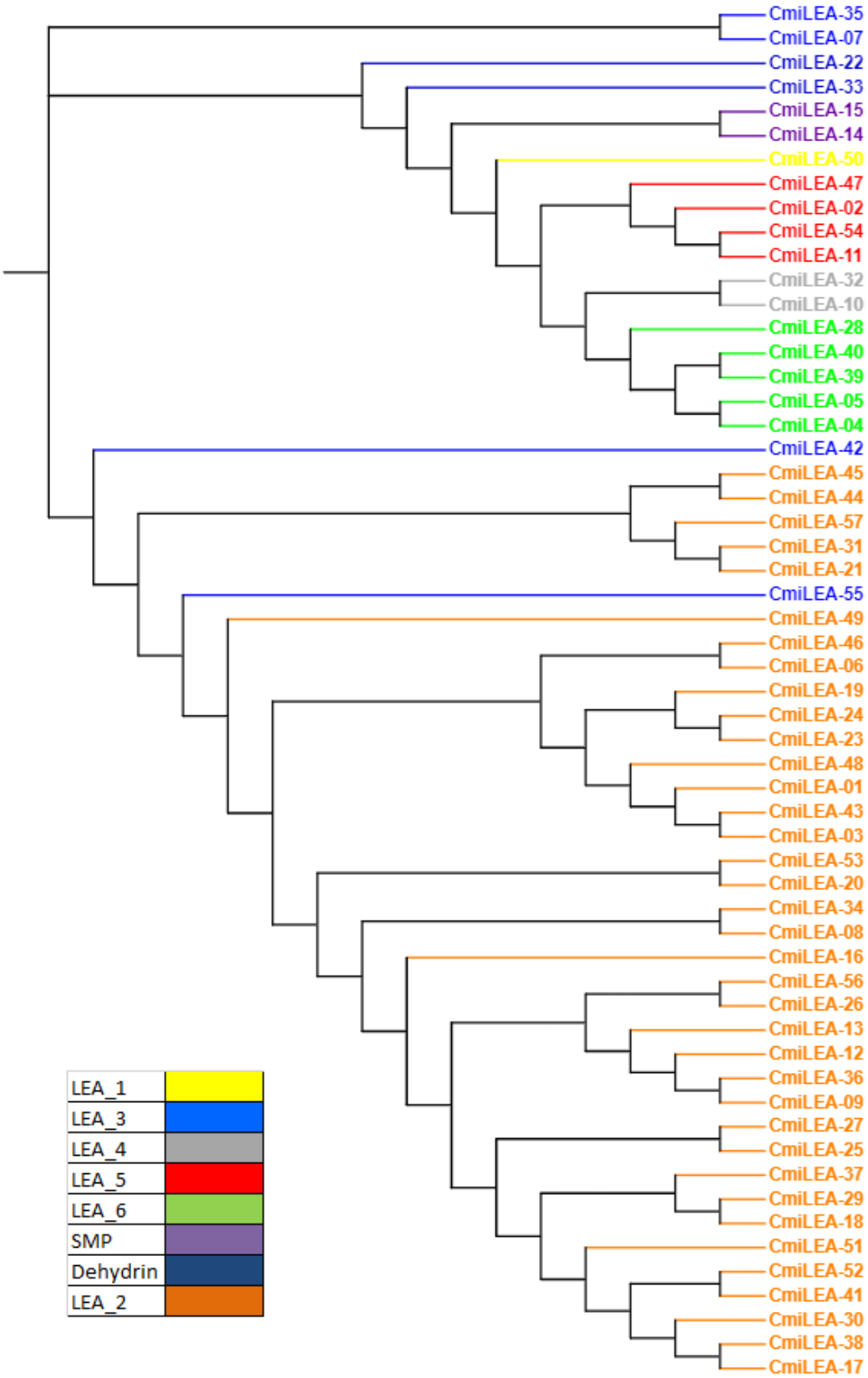

Figure 3. Phylogenetic tree of CmiLEA genes
Şekil 3. *CmiLEA genlerine ait filogenetik ağaç*

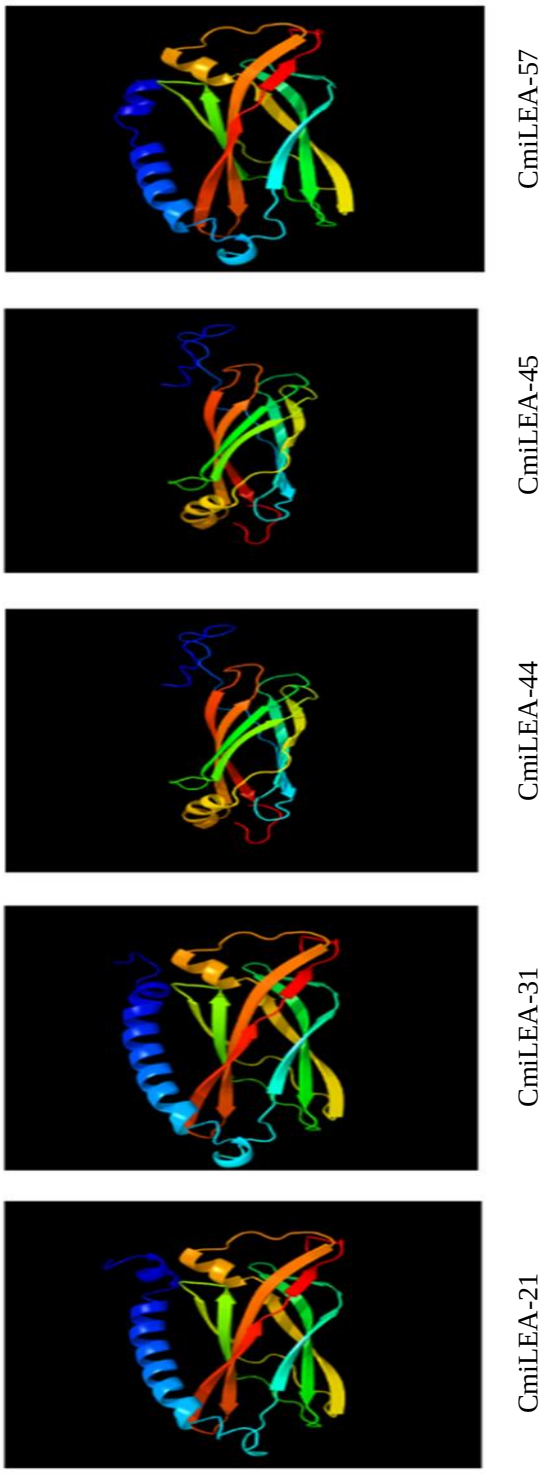

Figure 4. Three dimensional structure of some CmiLEA proteins
Şekil 4. *Bazı CmiLEA proteinlerinin 3 boyutlu yapısı*

Among these, the highest number of genes (10) were detected in the dehydrin subfamily. Lin et al. (2021) identified 84 LEA genes (CrLEA) in *Canavalia rosea*. They found 60 genes in LEA_2 and the fewest genes were found to be in LEA_4, LEA_5, LEA_6 subfamilies with 2 genes. Similarly, Li et al. (2023) reported 79 LEA genes were detected in sweetgum hybrids (*Liquidambar styraciflua* × *Liquidambar formosana*) and the most abundant in 8 subfamilies was the LEA_2 (57 genes). As a result of their study on tobacco, Geng et al. (2022) observed 123 NtLEA genes in *Nicotiana tabacum* L.and reported that the LEA_2 was the most abundant group. In consistent with previous studies, the present study identified the most abundant genes as the members of LEA_2 subfamily

In general, the results showed LEA protein lengths are relatively short, they are basic in terms of isoelectric points, and their molecular weights are low. In our study, it was revealed that 49 LEA proteins weighed less than 30 kDa, 38 of them were basic in character and their lengths were 79-455 aa long. When the average hydropathy values of LEA proteins were examined, it was found that the values were generally below 0 and the proteins mostly had hydrophilic character. These results are also similar with the findings of linseed flax, tobacco, lotus, and *Canavalia rosae* (Li et al., 2021; Lin et al., 2021; Geng et al., 2022; Chen et al., 2023). According to the hydropathy (GRAVY) values, we observed 18 proteins from the LEA_2 subfamily were hydrophilic and 19 proteins were hydrophobic, and all hydrophobic proteins were in LEA_2 subfamily.

By gene structure analysis, it was determined that there are very few introns (≤ 2) or no introns in LEA genes in lotus (Chen et al., 2023). It has been reported that the majority of genes have either no introns or 1 intron in *Canavelia rosae*. Li et al. (2021) stated that there are genes without introns in the LuLEA_2, LuLEA_3 and LuLEA_4 gene families in linseed flax, and the majority of genes have 1 or more introns. Li et al. (2023) were found there were very few introns in the LsfLEA genes in hybrid sweetgum. Our findings are compatible with other studies, and 12 genes in LEA_2 subfamily were determined to be intronless.

Among the target genes of related miRNAs of LEA_2 genes were detected as 2-phosphoglycerate kinase, GRAS, ERF, C2H2 transcription factors, phosphofructokinase family protein, squamosa promoter binding protein-like (SPL), and their possible functions were included nutrient deficiency response, carbohydrate metabolism, and drought response. It was found that there were in leaf and root development processes. For example, it was found that ath-miR447c-5p, associated with CmiLEA-09, was induced under C deficiency conditions but was suppressed under N deficiency conditions (Breakfield et al., 2012; Vidal et al., 2013; Shao et al., 2013; Liang et al., 2015; Thatcher et al., 2015; Rakhmetullina et al., 2021).

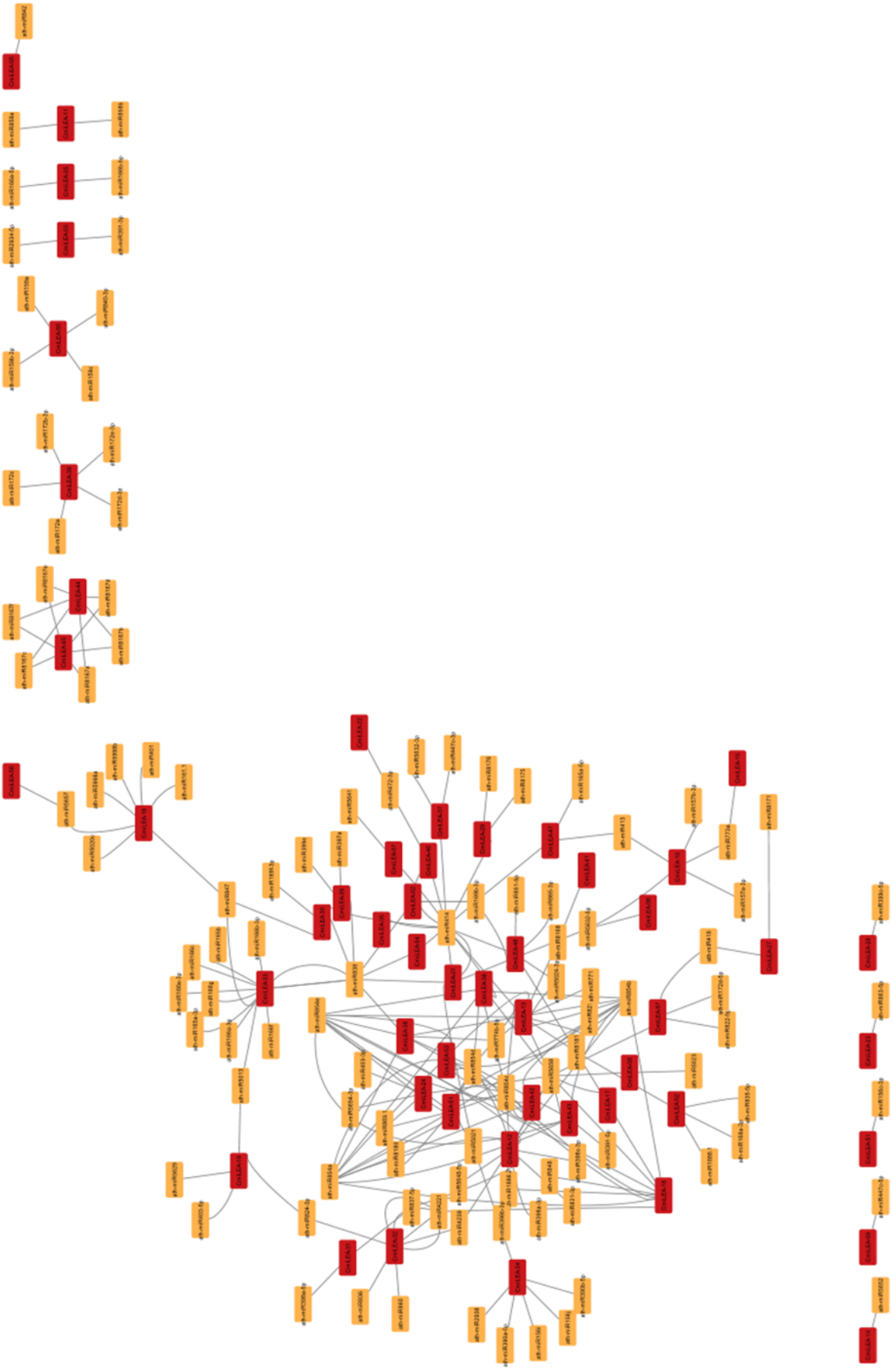

Figure 5. CmiLEA-miRNA interactions
Şekil 5. *CmiLEA-miRNA etkileşimleri*

The target genes of ath-miR842 and ath-miR399c-5p miRNAs, which are associated with CmiLEA-05 and CmiLEA-28 from LEA_6 subfamily, were found as Jacalin lectin family protein, copper superoxide dismutases, Ubiquitin conjugating enzyme (UCE) and vesicle-associated membrane protein and its possible functions can be nitrogen deficiency response and oxidative stress response (Jones-Rhoades et al., 2004; Sunkar and Zhu, 2004; Liang et al., 2012; Liang et al., 2015).

The lack of introns or the low number of introns in genes suggested the expression of LEA proeins are rapid under abiotic stress conditions. Thus, it shows that members of the LEA_2 subfamily are rapidly expressed in response to abiotic stress and may have important roles in the abiotic stress response. Previous studies also viewed that LEA_2 proteins have an ability to perform as molecular chaperones and they are involved in different stress responses such as ROS scavenging, membrane protection or preserving molecule structures under abiotic stress conditions (Aziz et al., 2023). Considering the possible target genes and functions of the CmiLEA proteins and miRNAs we found in present study, it can be suggested that some CmiLEA members from the LEA_2 subfamily are related to the stress response caused by nutrient deficiency.

Conclusion

In conclusion, the present study revealed LEA genes of Stout camphor tree (*Cinnamomum micranthum* f *Kanerihae*). for the first time. Total of 57 genes were identified and classified in subfamilies. Gene structure and miRNA analysis suggested the important functions of LEA genes in abiotic stress response. These findings suggest that it would be useful to analyze stress-sensitive expression patterns of these genes in *Cinnamomum* genus, which shows differences in tolerance to abiotic stress factors. The future studies can be revealed the relationship between gene induction and stress tolerance. Manipulating the survival of these genes in *Cinnamomum* may also lead to increased stress tolerance.

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Presence of Tomato Spotted Wilt Virus Between Cress and Pepper Intercropped in Kumluca District of Turkiye

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Abstract: Tomato spotted wilt virus (TSWV) transmits via thrips and causes significant diseases in *Solanaceae* species. In this study, samples were collected to identify TSWV and determine its frequency in cress (*Lepidium sativum*) and pepper (*Capsicum annum*) grown in 27 different greenhouses at Kumluca, Antalya province. More than 102 plant samples of cress and pepper plants were collected in Autumn 2023, these samples showing symptoms of virus-like ring spot and leaf decay, they were tested for the presence of TSWV using RT-PCR and qRT-PCR. Positive cress and pepper samples were discovered and TSWV cases in cress plants were common in 5 greenhouses. Based on the result, this is the first report of TSWV infection in cress. In conclusion, the role of thrips species in virus epidemiology in Turkey should be focused on with the potential of the tested TSWV isolates to break the resistance mechanisms in their hosts increases interspecies epidemic risks.

Key Words: Cress, TSWV, Pepper, Permaculture, PCR

Türkiye'nin Kumluca İlçesinde Yetiştirilen Tere ve Biber Arasında Domates Benekli Solgunluk Virüsünün Varlığı

Öz: Domates benekli solgunluk virüsü (TSWV), thrips yoluyla bulaşmakta ve *Solanaceae* bitkilerinde önemli hastalıklara neden olmaktadır. Bu çalışmada, Antalya ilinde Kumluca'da bulunan 27 farklı serada yetiştirilen tere (*Lepidium sativum*) ve biberde (*Capsicum annum*) TSWV'nin tespit edilmesi ve görülme sıklığının belirlenmesi amacıyla örnekler toplanmıştır. 2023 Sonbaharında tere ve biber bitkilerinden 102'den fazla bitki örneği toplanmıştır. Virüs benzeri halkalı leke ve yaprak bozulması belirtileri gösteren örnekler, TSWV'nin varlığı açısından RT-PCR ve qRT-PCR kullanılarak test edilmiştir. Pozitif saptanan tere ve biber örnekleri ayırt edilmiş ve tere bitkilerinde TSWV vakası 5 serada yaygın olarak görülmüştür. Bulgular ışığında, bu çalışma teredeki TSWV enfeksiyonuna ilişkin ilk rapordur. Sonuç olarak, Türkiye'deki virüs epidemiyolojisinde thrips türlerinin rolüne odaklanılmalı, test edilen TSWV izolatlarının konukçularındaki dayanıklılık mekanizmalarını kırma potansiyeli türler arası epidemik riskleri arttırmaktadır.

Anahtar Kelimeler: Tere, TSWV, Biber, Permakültür, PCR

1. Introduction

Cress (*Lepidium sativum*) is a spicy plant species from the cruciferous family, with its leaves are commonly consumed as a salad. It is massively cultivated in Anatolia, but its origin is native to Egypt and Southwest Asia (Shah et al.2021).

Cress is an important crop in Kumluca province located in the western part of Antalya, Turkiye. Total production of leafy or edible stem vegetables in Turkiye was 2,012,801 tons and Kumluca contributed to 10,222 tons of the production in 2023 (Anonymous, 2023).

One of the most widely spread plant viruses, Tomato spotted wilt virus (*Bunyaviridae, Tospovirus,* TSWV), is economically devastating with reference to yield losses in many vegetable productions. Although TSWV has a wide host range, it has more than 900 plant host (Peters, 1998) consisting of ornamental, vegetable (tomato, pepper, lettuce etc.) and weed. It is mainly transmitted by western flower thrips (*Frankliniella occidentalis*) (Antignus et al., 1997) in a propagative-persistent manner (Wijkamp *et al*., 1993). There are several TSWV incidences reported on tomato (Tekinel, 1973; Azeri, 1994), tobacco (Azeri, 1981), pepper (Yurtmen, 1998) and lettuce (Kamberoglu and Alan, 2011) in Turkiye.

To date, there is limited information about TSWV presence and incidence in cress. The objectives of this

study are to conduct molecular examinations for detecting TSWV, determining incidence in cultivated cress plants through RT-PCR and qPCR analysis and highlighting epidemiology at Kumluca, Antalya, Türkiye.

2. Material and Methods

2.1. Surveys and sample collection

Preliminary examinations in field were conducted in randomly selected cress and pepper growing as mix-cultivated greenhouses in Kumluca region in the autumn of 2023 (Table 1). Samples of leaves from each plant were placed in plastic bags and tagged then placed in a cool box. Sampling process was conducted on affected plants exhibiting both typical TSWV symptoms with distorted leaves, yellowing, brown spots, and wilting and generic virus symptoms, minor mosaic, crinkling, and deformation (Figure 1).

Table 1. All collected samples and positive found samples with TSWV, they were collected from different greenhouses located in Antalya province.
Çizelge 1*. Antalya'nın farklı lokasyonlarından toplanan örnekler ve bu örneklerde TSWV pozitif bulunanlar.*

Antalya Locations	Collected Samples	TSWV (+) Pepper	TSWV (+) Cress
Kumluca	27	11	5
Finike	11	6	-
Gazipaşa	13	4.	-
Serik	14	6.	-
Aksu	12	3	-
Total	**77**	**30**	**5**

Figure 1. Minor mosaic and spots on pepper (left side), crinkling and leaf deformation on cress (right side) due to TSWV infection.
Şekil 1. *TSWV enfeksiyonuna bağlı biberde küçük mozaik ve lekeler (solda), tere yaprağında buruşma ve deformasyon (sağda).*

2.2. Molecular tests

The RT-PCR and qRT-PCR molecular analyses were used to detect viruses on pepper and cress (testing was repeated thrice). Pepper viruses were not the focus of this study, however, Tomato yellow leaf curl virus (TYLCV), Tomato mosaic virus (ToMV), Tomato mottle mosaic virus (ToMMV), Pepper mild mottle virus (PMMoV), Tobacco mosaic virus (TMV), Tobacco rattle virus (TRV), Cucumber mosaic virus (CMV), Potato virus Y (PVY) and Alfalfa mosaic virus (AMV) were tested for in cress.

2.3. Total RNA extraction

Total RNA was extracted from fresh leaves of TSWV infected cress and pepper plants using GeneJet RNA Purification Kit (Thermo Fisher Scientific, USA), according to manufacturer's instructions.

2.4. RT-PCR

One step RT-PCR reactions were performed as described below; the complementary DNA (cDNA) strands of a portion of cDNA were synthesized with specific amplification of the TSWV N (nucleocapsid) gene by one-step RT-PCR using the TSWV N gene specific primers, TSWV L1 AATTGCCTTGCAACCAATTC; TSWV L2 ATCAGTCGAAATGGTCGGCA (Mumford et al., 1996). They were amplified with expected product size of 276 bp.

A total of 25 µL mixture was used including of 2 µL of RNA template, 1 µL of each specific primer, 0.25 µL of Verso enzyme mix, 1.25 µL of RT Enhancer, and 12.5 µL 1-Step PCR Hot-Start Master Mix (Thermo Science) and 7 µL of nuclease-free water were used for amplification. The cDNA stage was performed at 50 °C for 15 minutes and the reaction was terminated at 95 °C for 2 minutes. Then PCR steps were followed with 35 cycles of denaturation at 95°C for 45 seconds, 52 °C annealing for 30 seconds, 72 °C extension for 45 seconds, and then final elongation at 72 °C for 10 minutes (Fidan and Koç, 2019; Fidan et al., 2019). The amplified products were run on 1.5% agarose gel during gel electrophoresis. The Verso 1-step RT-PCR Kit (Thermo Fisher Scientific, USA) was used to conduct a one-step qRT-PCR test with a final volume of 25 µL, adhering to the manufacturer's recommendations. Using the Bio-Rad Realtime PCR Detection System (Bio-Rad, Germany), 2 µL of total RNA isolated from collected samples was used as a template for each reaction.

A probe and primary designs required for qRT-PCR were carried out using TSWV-F (5'-GCTTGTTGAGGAAACTGGGAATT- 3') as forward, and TSWV-R (5'-AGCCTCACAGACTTTGCATCATC-3') as reverse

primer. The fluorescent dye-labelled probe (5' 6FAM-AAATCTAAGATTGCTTCCCACCCTTTGATTCAA -TAMRA 3') was also used in qPCR reactions (Roberts et al., 2000).

The reporter dye's (FAM) and quencher dye's (TAMRA) fluorescence intensities were measured during the amplification process. The threshold cycle (Ct value) refers to the number of amplification cycles required for a significant increase in the reporter's fluorescence. The Bio-Rad (Hercules, California /USA) Real Time PCR Detection System Program was used to examine their data.

3. Results

The survey and sample collection were carried out in a total of 27 greenhouses in Kumluca where mainly cress growing fields with 3800 plants per decare (da). More than 273,600 cress plants were monitored in 27 greenhouses where these 5 da of them were showing virus-like symptoms were tested using RT-PCR and qPCR analyses. The rate of incidence of TSWV in collected cress samples was found as 18.5% at Kumluca district. The expected fragment size of 276 bp was obtained after electrophoresis of RT-PCR products in 1.5% agarose gel (Figure 2). plants. The qRT-PCR analyzes exhibited Ct values in 8th cycles for pepper and 14th cycles for cress plants respectively (Figure 3). A Ct value was obtained at 24th cycles for positive control but there was no Ct value for negative control (Figure 3).

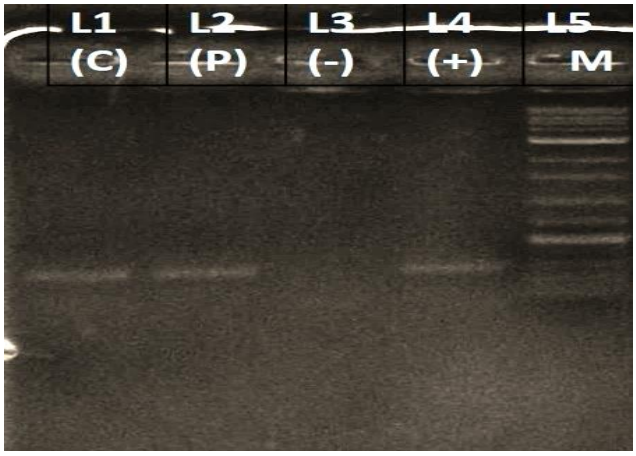

Figure2. A 276 bp RT-PCR product of TSWV was amplified and visualized on agarose from collected samples at Kumluca district. Lane 5- M: contain 100 bp DNA marker; Lane 1, 2: typical symptomatic cress and pepper plants respectively; Lane 3: negative control, Lane 4: positive control.

Şekil 2. *TSWV'nin RT-PCR ürünü olan 276 bp fragment Kumluca ilçesinden toplanan örneklerden elde edildi ve agaroz jel elektroforezinde görüntülendi. Kulvar 5-M:100 bp DNA moleküler marker içermektedir; Kulvar 1, 2: sırasıyla tipik semptomatik tere ve biber bitkileri; Kulvar 3: negatif kontrol; Kulvar 4: pozitif kontrol içermektedir.*

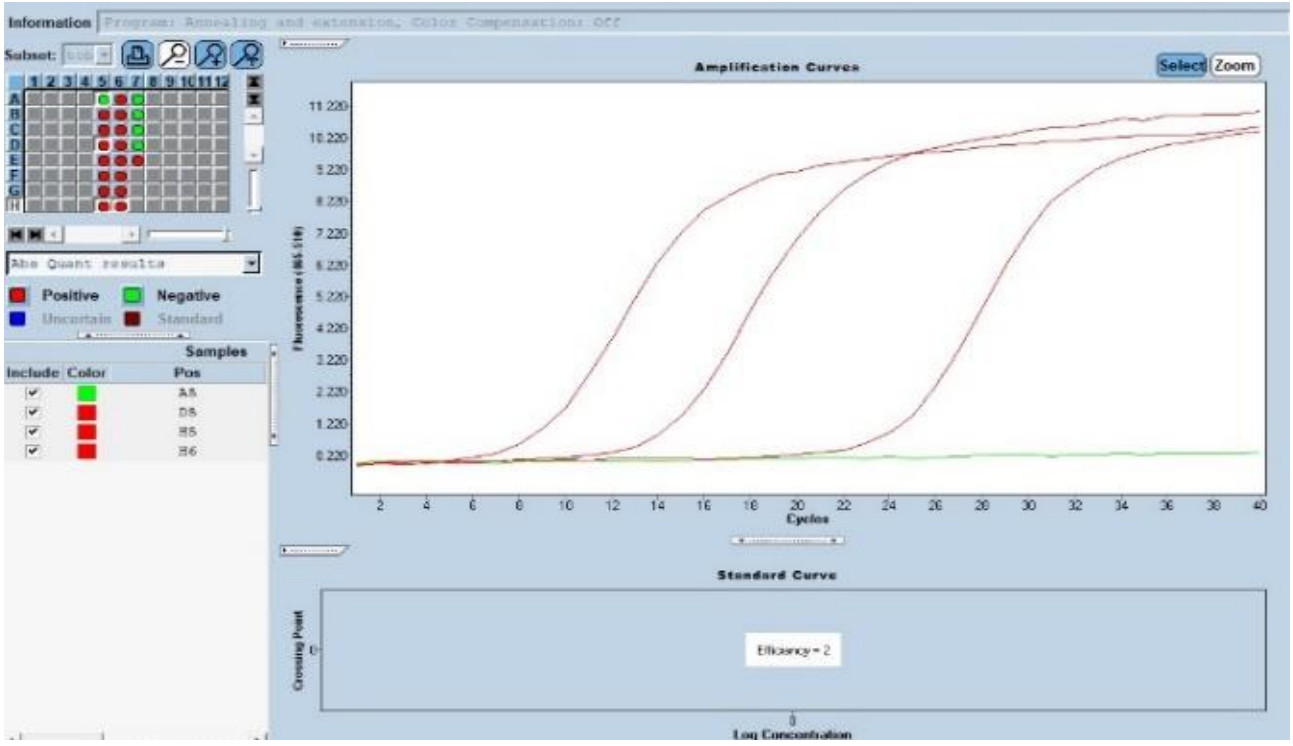

Figure 3. Bio-Rad RealTime PCR Detection System has revealed amplification curves from cress, pepper plants and positive control but there was not any curve for negative control.

Şekil 3*. Bio-Rad RealTime PCR Tespit Sistemiyle analiz edilen tere, biber bitkileri ve pozitif kontrolde parobol eğrisi üretiliyorken negatif kontrolde herhangi bir parabolik eğri elde edilmemiştir.*

A portion of small RNA of TSWV was targeted to amplify using total RNA extracted from symptomatic plants with virus specific primers in qRT-PCR analyses. The qRT-PCR analyzes confirmed the presence of TSWV infection in interspecies and also confirmed transmissibility of the virus between cress and pepper

After eradicating the diseased plants in the affected greenhouses, they were sterilized through solarization and exposed to disinfectant applications during preparations for the next crop season.

4.Discussion

Cress is one of the important crops cultivated in winter and early spring in Kumluca district, Antalya, Türkiye. The surveys were conducted in greenhouses close to main pepper growing areas during the early autumn in Kumluca. Cho et al. (1987) reported that high incidence of TSWV was attributed to elevating population of *Thrips tabaci* L and *Frankliniella occidentalis* species influenced by local microclimatic conditions like rain fall, minimum and maximum temperature. For example, in research conducted by Atakan and Sarı (2010), *F. occidentalis* and *T. tabaci* were detected on lettuce with reduced and winter time (October-March) in the Cukurova region where has similar climatic condition to Kumluca. Atakan and Sarı, (2010) also indicated that the collected thrips species were female and no larval thrips belonging to either thrips species were collected from lettuce fields. The male population of thrips transmitted TSWV at a higher rate compared to females (Rotenberg et al., 2009; Van de Wetering et al., 1998) also reported that a higher proportion of females in the thrips population has a negative impact on plant damage and virus transmission, and thus spread of TSWV. This is attributed to the lower mobility and higher consumption rate of females in comparison to their male counterparts. The low presence of thrips and the predominance of female thrips during the winter and early spring could explain the low occurrence of Tomato spotted wilt virus (TSWV) in other cress like leafy vegetables as lettuce plants in the Cukurova region (Kamberoglu and Alan, 2011). Wilson (1998) indicated that the percentage of TSWV-infected lettuce plants varied depending on the farm location and season, with more infections more prevalent in late summer and early fall, resulting in losses of 25-65% during fall harvests in southern Tasmania. Moreno et al. (2004) reported that TSWV epidemics were much more frequent in autumn compared to spring, which they attributed to the absence of virus vectors.

Real-time diagnosis of local thrips species should be detailed in such local or limited production areas, especially in places where perm culture is carried out. Thus, the role of local subspecies of thrips on local or regional epidemics will be better understood. In this way, control methods can be shaped more effectively. The role of culture of aromatic plant species, which serve as intermediate reservoir host potential, on virus ecology and the effect of auxiliary arguments are among the main topics that need to be investigated. Distribution of reservoir plants for the virus and insect vectors, efficiency of transmission from these hosts to cultivated crops, determination, and characterization of Turkish local isolates of TSWV from different locations needs to be investigated in Turkey. Thus, the collection of TSWV isolates originating from a wide range of both wild and cultivated forms through different vectors in common host pools at certain periods may constitute a step for the formation of new sub-isolates that can break the resistance in plants. Both the impact factor on agro-ecology and the economic damage to agricultural areas of new races that can develop based on mixed infections and mutation cannot be predicted. To follow effective control strategies, the potential for the formation of new strains and their compatibility with vectors should be reviewed in detail. The effects of global warming on both vectors and pathogen behavior have not yet been predicted, and host plant adaptations are an unknown phenomenon. For this reason, it is considered exclusive to focus on changes in virus ecology and isolate behavior according to host species. In addition to variable global warming, changes in the ability of viral agents and vectors to expand the host spectrum, which can be triggered by regional droughts, are a separate threat. In recent past, local outbreaks of Tomato brown rugose fruit virus (ToBRFV), Tomato Yellow Leaf Curl New Delhi Virus and similar diseases have turned into continental threats in similar ways. Furthermore, genetic recombinant TSWV and ToBRFV isolates were shown to have ecologically selective advantage over the original virus. The challenges of virus disease control are becoming increasingly difficult with the constant emergence of new breeds of existing viruses or completely new viruses. Viruses have a great potential to adapt to the pressure of natural selection for reasons such as large populations, the absence of repair mechanisms that facilitate genetic variation in their genomes, and their ability to reproduce in a short time.

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Conflict of interest All authors declare that they have no conflict of interests.

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Analysis of Reasons of Breakage in Bag Production Machine for Agricultural Materials

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Abstract: In a globalizing world, the borders on the map have started to disappear economically and customers in the world economy expect high levels of quality from their suppliers. Only companies that can respond to customer expectations in the most accurate way will be able to survive in the future. One of the main goals of today's companies is to offer the best quality product at the most affordable price in line with the needs of customers. At this point, the Six Sigma approach is one of the most effective methods that companies can try.
In this study, by applying the Six Sigma methodology, the root causes of PE (Polyethylene) film breaks in plastic bags were investigated by taking plastic bag production machines, processes and control steps as an example, necessary measurements were made by considering the production lines in a sample company and improvements were made in the production process by analyzing with Minitab program. At the end of the study, 24.012 TL/year savings were achieved by reducing downtime due to film breaks in the PE Film line, and 13.938 TL/year savings were achieved financially by reducing the amount of waste.
In this way, the plastic bag industry benefited. In six sigma projects, it has been determined once again that the only element that is as important as making the change is that all the personnel of the company should keep up with and support this change.

Keywords:, 6 Sigma, plastic fertilizer bag, production line

Tarımsal Materyaller İçin Torba Üretim Makinesinde Kopma Nedenlerinin Analizi

Öz: Globalleşen dünyada haritadaki sınırlar ekonomik anlamda yok olmaya başlamıştır ve dünya ekonomisinde müşteriler, tedarikçilerinden yüksek düzeyde kalite beklentisi içerisindedir. Müşterilerin beklentilerine ancak en doğru şekilde cevap verebilen şirketler, gelecekte varlıklarını sürdürebileceklerdir. Müşterilerin ihtiyaçları doğrultusunda en kaliteli ürünü en uygun fiyattan piyasaya sunabilmek, günümüz şirketlerinin temel hedeflerinden biridir. İşte tam da bu noktada Altı Sigma yaklaşımı, şirketlerin deneyebilecekleri en etkili yöntemlerden biridir.
Bu çalışmada, altı sigma metodolojisi uygulanarak, plastik torba üretim makineleri, prosesleri, kontrol adımları örnek alınarak, torbadaki PE (Polyetlen) film kopmalarının kök nedenleri araştırılmış, örnek bir firmadaki üretim hatları dikkate alınarak gerekli ölçümler yapılmış ve Minitab programı ile analizler yapılarak, üretim prosesinde iyileştirmeler yapılmıştır. Çalışmanın sonunda, PE Film hattı film kopmaları kaynaklı duruş sürelerinin azaltılarak 24.012 TL/yıl tasarruf sağlanmış, telef miktarının azaltılması ile mali yönden 13.938 TL/yıl tasarruf elde edilmiştir.
Bu vesileyle plastik torba sektörüne fayda sağlanmıştır. Altı sigma projelerinde, değişimin yapılması kadar önemli olan yegane unsurun şirketin tüm personeli tarafından bu değişime ayak uydurulması ve desteklenmesi gerekliliği bir kez daha tespit edilmiştir.

Anahtar Kelimeler: 6 Sigma, plastik gübre torbası, üretim hattı

1. Introduction

In today's increasingly competitive market conditions, there is only one solution for businesses to survive, which is to listen to their customers, in other words, to understand the real and current needs of the customer correctly and to produce quality products with optimum cost. Accordingly, it is aimed to produce products that meet the criteria and relevant standards that meet customer needs, on the specified date, at the appropriate quality, at the appropriate cost. Although it seems very simple at the basic point, one of the most critical parameters that especially large enterprises miss in their daily work intensity is the opinion and satisfaction level of their customers about the service or product they receive. Customers are like a mirror held up to the company itself. In line with the recommendations or criticisms from their customers, companies may realize a feature that they have never

seen before or close their missing points. Although there are methods such as Benchmark product price comparisons, Six Sigma methods are a powerful way of listening to the voice of the customer, which provides effective success in this part. In this way, companies have the opportunity to examine, reorganize and reorganize their processes based on the basic points that their customers need and move to a much better stage.

The most important criterion for the implementation and success of the six sigma perspective in a business is the support of the top management, the establishment of a team with relevant critical personnel, the belief of all employees and their best efforts. When brainstorming and improvements are made, it is of great importance that they become widespread and sustainable. The idea of change often scares people and causes resistance. However, if one employee in the team or even in the company does not believe in this approach and change, it negatively affects the whole system. In addition, although the cost of implementing a six sigma approach is not so critical, it is very important to choose logical projects that fit the economic structure of the company and respond directly to customer demand.

Of course, it is not easy to survive in the globalized competitive market and to be preferred. Companies that make a difference in every sector have a say. Being able to manufacture more products at once, using less data, at a rate closest to zero error, means more profit for companies and more resources for the next generation. There are many methods to improve processes. The Six Sigma approach is one of the methods that has been applied by many companies around the world and has been recognized as effective. The goal of the Six Sigma approach is to minimize the defect rate in a company's products or services as much as possible and to ensure sustainability. Six Sigma is a set of customer-oriented methods that takes into account the primary needs of the customer and selects them as the most effective parameter for solving the problem and analyzes them according to these assumptions. With the help of improvement steps and approach, it performs a radical revision by re-evaluating and questioning the existing processes and workflow within the company (Avunduk, 2019).

In this research, how businesses can solve problems within the framework of six sigma methods is investigated, and the concepts of identification, i.e. identifying the problem, measurement, analysis, development, validation and control, which are the stages of this methodology, are evaluated. This is also referred to as the Shewhart Cycle or the Deming Cycle. According to this cycle, it consists of these four stages that enable the elimination of recurring errors and the improvement of processes: (Işığıçok, 2005)

Plan: Identifying a problem or opportunity for improvement, understanding the opportunity/risk and developing a plan to achieve the goal.

When planning the problem, the possible causes of the problem are considered. The most important goal of the step is to identify the root causes of the problem in the process and to eliminate them. Various statistical methods to be used in analyzing the problem must be fast, effective and reliable. As a result of the analyses, the parts to be improved are clarified and changes are made in production. With the help of changes in operations, equipment or materials, products can be manufactured with less cost, minimum defects and high quality.

Implement: This is the stage where the plan is implemented.

Check: Measure the end products or service to understand whether the plan has achieved the objective and report the results.

Take Measures: This is the stage of re-planning and implementing the necessary changes to improve performance based on data analysis. After this stage, the Plan-Implement-Check-Take Measures cycle starts again.

In addition, 5S (Seiri (sorting), Seiton (order), Seiso (cleanliness), Seiketsu (standardization), Shitsuke (discipline)), a business philosophy that focuses on improving working conditions in terms of safety, cleanliness, comfort and performance, is one of the process improvement methods. It is used to prevent or minimize clutter, damage and accidents in a business (Lınderman, 2003).

Gemba, which in Japanese means the real place or the place where the work is done, is another continuous improvement technique. Accordingly, managers make visits to the workplace. The aim is for managers to see what is going on in the real work environment, to develop trust-based communication with field workers and to discuss current problems.

Value stream mapping is a method that mirrors processes by visually depicting a business process from start to finish.

In addition, customer satisfaction surveys, when conducted at the right time and in the right way, contribute greatly to the process by providing a clearer view of customer expectations and criticisms. In addition, the Benchmarking method gives the company an idea about that product by comparing it with the best

in the sector.

There are articles and theses about 6 sigma methodology in the literature. A few of these are described below. Improvements in production and process improvements have been made using this approach. However, there are no 6 sigma studies on plastic bags. It is necessary to prevent polyethylene film breaks, which is a major problem especially for companies producing plastic bags.

Ozveri and Cakır (2012) made improvements in the pulley production process, which has the highest number of errors, taking into account the project prioritization structure of Six Sigma. As a result of the improvements, it was observed that other problems were encountered within the scope of percentage inaccuracy in the pulley production process. At the end of the measurements, based on the results such as the inaccuracies caused by calibration decreased and the inaccuracies caused by the relay increased, it was determined that the next project role would be on improvement in the operation. Customers were asked to make pairwise comparisons in the identified demands. He concluded that companies should listen not only to the voices of the customers but also to the voices of the business within the scope of project selection and implementation.

Evren (2006), in his thesis on six sigma methods and its application in a selected company, examined the six sigma approach and the development actions it suggests in detail and clearly revealed the issues to be encountered in the process and the preparation of companies for six sigma steps. He summarized the framework picture of the related methodology through the six sigma applications he sampled.

Ozkan (2006), in his thesis on six sigma applications in industry, has seen in his studies that companies applying six sigma methods analyze their errors better, keep their processes under control and ensure customer satisfaction by producing better quality products.

Altuğ (2010), in his comparative analysis study in terms of the gains of companies in six sigma project implementation, the six sigma project implementation examples of 11 companies operating in the manufacturing industry were examined through a survey and the analysis of these examples were included. As a result of these analyzes, it was determined that the companies gained economically and technically by making a comparison before and after the six sigma project implementations. The finalization times of the projects implemented in the companies, the measurement of the gains from the project in the balance sheet and whether there are independent departments that advance the six sigma activities are also examined. In this context, it was concluded that the ability to meet the expenses of the project, to bring profit, and the measurability of these results in the balance sheet are strictly related to the completion of the relevant projects within the estimated time. Taking into account the top management's perspective, the relationship between six sigma activities and strategic business objectives was evaluated and it was concluded that the most critical objectives would be to satisfy the customer and to gain profit and benefit.

Pande and Holpp (2002) list the Six Sigma itinerary in five points as follows:

- Identifying the main workflow and the most important customer
- Correct identification of buyer requirements
- Measuring the performances that are possible
- Prioritization of improvements
- Integrating the idea and belief of six sigma to all employees, adapting it to the whole project, verifying it through analysis and ensuring its sustainability

In this study, plastic bag production lines and process stages are detailed. How businesses can solve problems within the framework of six sigma methods has been investigated, and the concepts of identification, i.e. identifying the problem, measurement, analysis, development, validation and control, which are the stages of this methodology, have been evaluated.

In the Toros Tarım company, which was taken as a case study, the factors that caused major losses in the production of the fertilizer and bag factory were investigated using the Six sigma method. In addition, a problem that directly affects the wastage rate and profit margin in production was addressed and analyzed in Minitab program by going down to the root causes, and improvements in production were implemented. With the improvements made, the PE (Polyetlen) film rupture error, which is a common chronic problem of plastic bag manufacturers, has been eliminated and taken under control.

2. Materials and Methods

The commercial success of plastic as a packaging product is the result of its combination of flexibility (from film to rigid applications), durability, light weight, stability, impermeability and easy sterilization. These properties make plastics an ideal packaging material for all kinds of commercial and industrial users. In this study, a woven plastic bag product and plastic (polypropylene and polyethylene) materials were used.

Woven (knitted) plastic bags are bags made of polypropylene plastic material called jute on the outside and polyethylene plastic film on the inside. These bags play a major role in the transportation of fertilizer, which is the lifeblood of agriculture. As with any packaging, sealing and product protection are very important in this type of packaging. The woven plastic production process consists of yarn production line, weaving loom, flexographic printing machine, cutting sewing machine, warp and weft tapes, drafting cylinder, hot cutting, PE (Polyetlen) film line, PE (Polietylen) bag line, winding unit and granulator machine.

In the study, the brainstorming method and six sigma methodology were actively used, which is a general method used to generate a large number of creative and effective ideas on a given topic. In this way, the imagination of the people doing the work can be used to use their ideas generated during their work related to improvement, and their imaginative suggestions can be used for the success of the project as much as possible without any hindrance. Team members were able to develop and suggest improvements and recommendations without any pressure. The six sigma project team included the company's manufacturing engineer, quality engineer, manufacturing manager, quality manager, relevant production line operators, general manager.

The product life for knitted plastic bag is described in Figure 1:

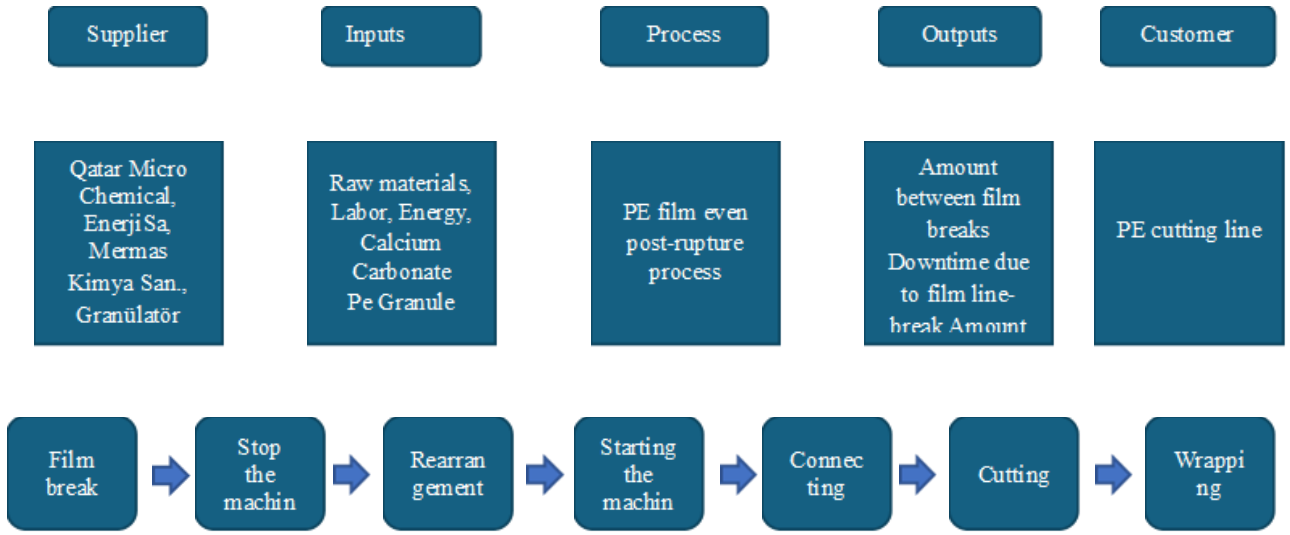

Figure 1. Product Life
Şekil 1. *Ürün Yaşamı*

In order to prevent PE (Polyetlen) film ruptures in the production of woven plastic bags, which are frequently used in fertilizer transportation, the problem was defined correctly in this project, and data were collected from the production lines with root cause analysis and fishbone methods. In the fishbone diagram in Figure 2 all possible causes of the problem are categorized.

According to the following chart, the polyethylene film breaks in the plastic bag are rated according to their importance for the customer and the possible causes of the problem are clearly indicated in the inputs section. The possible causes were prioritized and the number of film breaks, machine downtime, potential impact value and amount of lost product were scored.

When Pareto analysis, in other words prioritization analysis, cooling-related problems, transmission-related malfunctions, electrical fluctuations, resistance temperatures, speed difference between motors are in the top 5. For this reason, all these factors were analyzed one by one.

There are 4 resistances below and 2 resistances above the machine benches in the sampled company. While collecting the data, a large number of measurements were taken by different operators at various times from three shifts. The hours of PE (Polyetlen) film breakage and resistance temperatures were also recorded. The cooling temperature in the machine and the winding motor speeds were measured. Break frequencies were monitored before and after the retrofit, taking into account the total equipment efficiency.

In this study, methods such as Anova, S&S matrix, CTQ (Critical to Quality) quality requirements, voice of the customer were applied in Minitab program before and after process analysis and improvement. With these methods, the problem was accurately defined and a road map for the solution was drawn. Possible causes of the problem with high standard deviations were investigated and the factor that affected the result the most and had the highest deviation was emphasized.

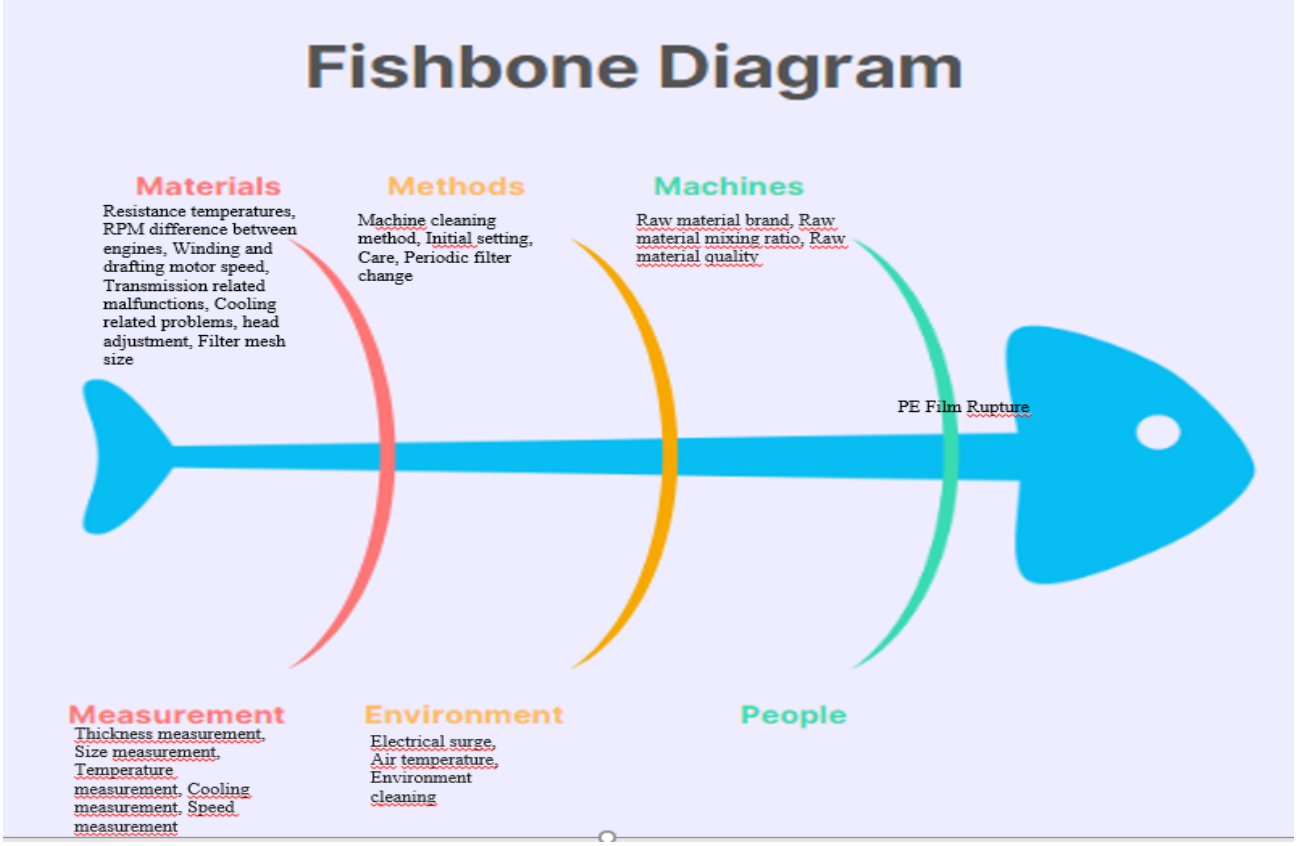

Figure 2. Illustration of possible causes of PE film rupture in a fishbone diagram
Şekil 2. PE film kopmasının olası nedenlerinin balık kılçığı diyagramında gösterimi

Table 1. Cause and effect matrix and prioritization
Çizelge 1. *Neden-sonuç matrisi ve önceliklendirme*

	Importance level for the customer		**8**	**9**	**7**	**10**		
	Process Factor	**Outputs** **Inputs**	**Number Of Film Breaks**	**Downtime Due To Rupture**	**OEE Value**	**Amount Of Lost Product**	**Total Value**	**Pareto**
8	Machine	Cooling related problems	9	9	9	9	306	**7%**
7	Machine	Gearbox related malfunctions	5	9	9	9	274	**13%**
17	Enviroment	Electrical surge	9	5	5	9	242	**19%**
4	Machine	Resistance temperatures	9	9	5	5	238	**25%**
5	Machine	Speed difference between engines	9	9	5	5	238	**30%**
6	Machine	Winding and drafting motor speed	9	9	5	5	238	**36%**
15	Method	Maintenance	9	5	5	9	238	**41%**
22	Measurement	Temperature measurement	5	9	9	5	238	**47%**
23	Measurement	Cooling measurement	9	9	5	5	238	**52%**
24	Measurement	Speed measurement	9	9	5	5	238	**58%**
14	Method	Initial setting	5	9	5	5	206	**63%**
16	Method	Periodic filter change	9	5	5	5	202	**67%**
9	Machine	Head setting	5	5	9	5	198	**72%**
10	Material	Raw material brand	5	5	5	5	154	**76%**
13	Method	Machine cleaning method	3	5	5	5	154	**79%**
12	Material	Raw material quality	3	5	5	3	152	83%
20	Measurement	Thickness measurement	3	5	5	3	134	86%
21	Measurement	Size measurement	3	5	3	3	134	89%
18	Enviroment	Air temperature	3	3	3	3	120	92%
11	Material	Raw material mixing ratio	3	1	3	3	102	94%
2	People	Experience	3	3	1	1	68	96%
3	People	Competence	3	1	3	3	68	98%
19	Enviroment	Environment cleaning	1	1	3	3	68	99%
1	People	Education	1	1	1	1	34	100%
Totaly Value for Outputs			**0**	**0**	**0**	**0**	**4298**	

3. Results and Discussion

3.1. Resistance Temperature Values before optimization

Before the retrofitting of the machine tools, the resistance temperature values of the thermocouples were measured periodically. The minimum, maximum and average values of Z1, Z2, Z3, Z4 and D1, D2, D3 resistance temperature values are given in table 1. It was observed that D1, D2 and D3 head resistances dramatically exceeded the upper limit (190°C).

Table 2. Resistance temperature values before ımprovement
Çizelge 2. *İyileştirme öncesi rezistans sıcaklık değerleri*

Resistance	Average Temperature Value (°C)	Min. Measured Value (°C)	Max. Measured Value (°C)
Z1	156,20	145	170
Z2	155,12	132	172
Z3	156,30	140	170
Z4	154,27	140	172
D1	165,96	142	190
D2	167,03	152	190
D3	157,09	145	180

Table 3. Improvement action and responsible table
Çizelge 3. *İyileştirme aksiyon ve sorumluları tablosu*

Actions	Responsable
The problem of overheating (temperature volatility) was discussed with the thermostat supplier company and the recommended thermostat adjustments were made.	Quality control supervisor, production operator
At the end of the recommended settings, the thermostats are deactivated when the thermostats are close to reaching the set temperature (2-3 degrees) and are activated again at the end of the set time and the heating is slowed down and the temperatures are increased to minimum levels above the set values.	Quality control supervisor, production operator
Since the thermocouple (thermocouple, temperature sensor) bearings of the thermostats were deformed, they were renewed to feel the heat more sensitively.	Quality control supervisor, production operator

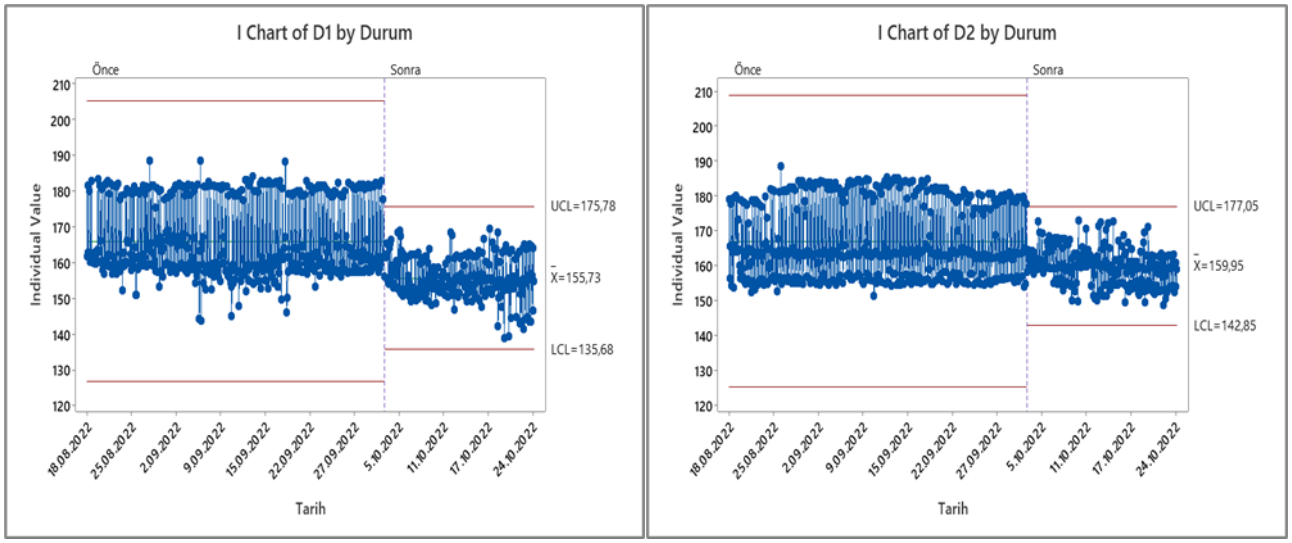

Figure 3. D1-D2 ımprovement before-after distribution between lower and upper limits
Şekil 3. *D1-D2 iyileştirme önce-sonra alt ve üst limitler arasındaki dağılımı*

3.2. Improvement actions

In order to improve this situation, the actions given in Table 2 were implemented by the responsible staff.

3.3. D1-D2 Temperature Values Before-After Improvement

As can be seen in Figure 1, while the upper and lower control limit range of D1 and D2 resistances was wider in the previous case, after the improvements, the variability decreased and the limits narrowed and their averages approached the desired set value of 160 °C.

3.4.Resistance Temperature Values - One Way Anova Table

As can be seen in Figure 2, when the analysis was performed using the Interval Plot module for 7 resistors from the Minitab program, it was seen that before the

improvement, especially the D1-D2 resistance temperatures were higher than the others, while after the improvements made, all resistance temperature values were brought to the set value levels.

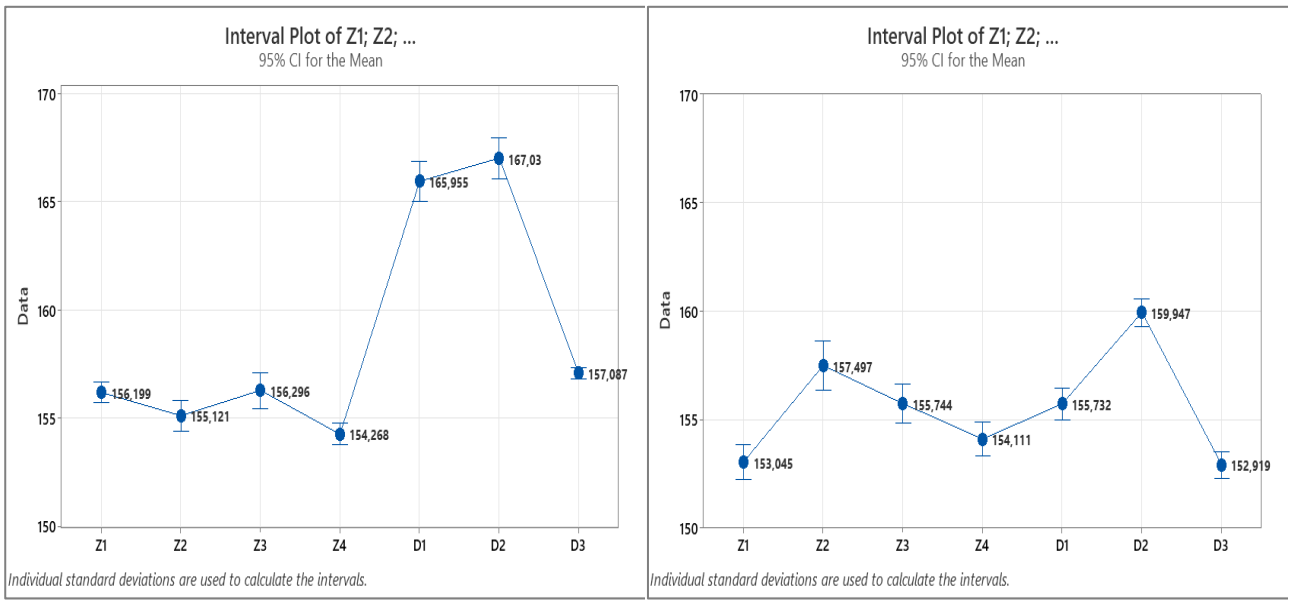

Figure 4. Interval Plot Distribution of Z1, Z2, Z3, Z4 and D1, D2, D3 Resistance Temperatures
Şekil 4. Z1, Z2, Z3, Z4 ve D1, D2, D3 Rezistans Sıcaklıklarının Interval Plot Dağılımı

3.4. Breakage Frequency - Before-After One Way Anova Test Result

As seen in Figures 3 and 4, when the rupture frequency and conditions of PE (Polyetlen) films were analyzed using the I Chart module, Interval Plot and Welch's test from the Minitab program, the monthly rupture conditions and standard deviations before and after the improvement were examined.

The alternative hypothesis was accepted (P-Value: 0,001) and it was accepted that there was a significant difference in the frequency averages after the improvement, i.e. the frequency decreased significantly after the improvement.

The confidence interval for the mean breaking amount is between 70.0-143.7 in the first case and 22.99-50.35 in the second case.

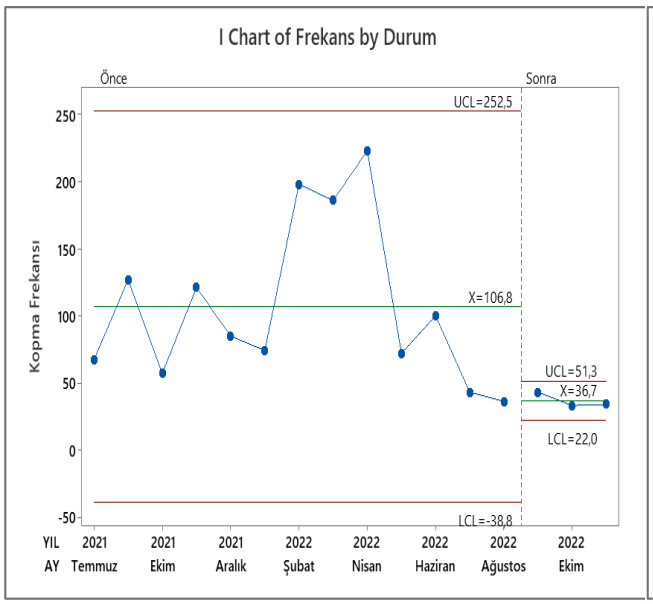

Figure 5. I Chart Distribution of Rupture Frequencies on a Monthly Basis
Şekil 5. *Aylar Bazında Kopma Frekanslarının I Chart Dağılımı*

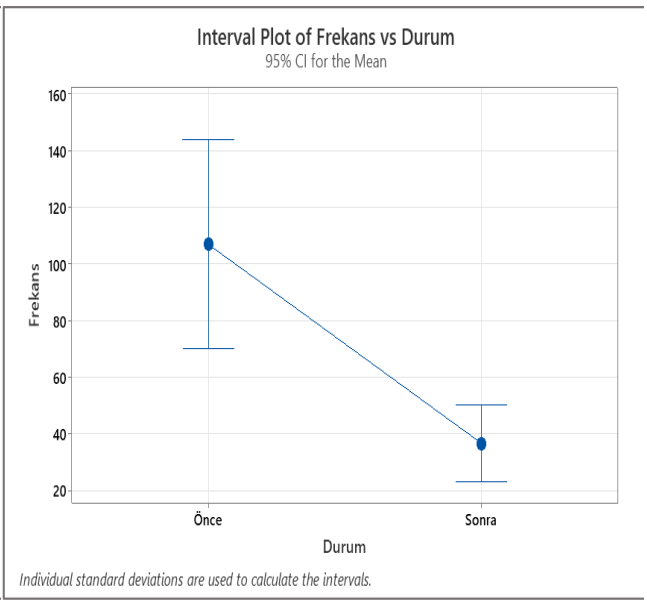

Figure 6. Interval Plot Distributions of Rupture Frequencies
Şekil 6. *Kopma Frekanslarının Interval Plot Dağılımları*

Means

Durum	N	Mean	StDev	95% CI
Önce	13	106,8	61,0	(70,0; 143,7)
Sonra	3	36,67	5,51	(22,99; 50,35)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are eq
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Durum	1	12,7671	16,62	0,001

4. Conclusion and Discussion

The Six Sigma kaizen approach is a customer-oriented methodology based on numerical data and statistical analysis that aims to achieve consistent improvement. The success of the Six Sigma project is ensured by the progress of the work flow, the appropriate response to buyer expectations, the reduction of inappropriate issues and deficiencies in the product or service, the creation of processes and services to best meet business needs, the implementation of the infrastructure and the appropriate team and leadership system to keep the gain and progress continuous.

This was the case in the production of plastic bags. Periodically, these methods were applied every week as non-conforming products increased in the bag production machines and various analyzes were made in the Minitab program, different operators were made to work on the machines at different time intervals and different personnel took measurements from the products. The distribution of the inter-break quantities (mt) in PE (Polyetlen) films was analyzed month by month, and the resistance temperature values before and after the remediation, the monthly breakage rates and their standard deviations were analyzed. The causes of film breakage were analyzed as human, machine, dimension, material and environmental by using fishbone diagram and 5 cause analysis methods and the root cause was tried to be found.

In the chronic problem of the enterprise, statistical methods and Minitab tools where the most effective results are obtained; S&S matrix, pareto diagram, cause-effect diagrams, ANOVA, Interval plot, Box Plot and I Chart analyzes come to the fore.

In order to prevent PE (Polyetlen) film breaks in the production of woven plastic bags, which are frequently used in fertilizer transportation, the distribution of cooler temperatures, which is one of the prominent factors in PE (Polyetlen) film breaks as a result of pareto analysis, was examined. It was observed that there was not a very large variability. Similarly, engine and transmission speeds were analyzed. Again, it was observed that the deviation in the number of revolutions was not high. There are 4 resistances below and 3 resistances above the machine benches. It was observed that the deviations in the resistance temperatures were high and the temperature values exceeded the lower and upper limits. In this case, new optimal lower and upper limits were determined. In each shift, the resistance temperatures in the lines were recorded and accordingly the set values in the resistance were determined. The upper limit was set for the first resistance to stop as Z1 approaches 165. In this case, the other resistance was allowed to increase, and then when the temperature reached the upper limit, it was again switched to the other resistance. It was found that the resistances could not take accurate readings due to dirt getting into the resistances and the resistances were replaced with thermocouples. As a result of the improvements, a radical reduction in the amount of PE (Polyetlen) film breakage in the bag was observed.

As a result of the analyses, necessary changes were made in the quality control plan and related forms used to check the conformity of the products coming out of production, and trap samples were sent to production, and validation activities were carried out by adding regular measurements (break frequency, resistance temperatures, etc.) to the forms. In addition, 24,012 TL/year savings were achieved by reducing downtime due to film breaks in the PE (Polyetlen) Film line, and 13,938 TL/year savings were achieved financially by reducing the amount of waste.

Awareness and practical trainings were provided to the personnel. In six sigma projects, it has been determined once again that the most important issue, as important as making the change, is the adaptation and support of this change by the entire staff of the company.

In the future, it is thought that by making use of this article, improvements can be made in production processes or machines in plastic bags or in another sector by using 6 sigma methodology and statistical analysis methods applied in the thesis.

Conflıct of Interest

The article is a research study within the scope of PhD and was carried out under the supervision and supervision of the advisor Prof. Dr. Recai GÜRHAN and there is no conflict of interest in terms of name priority.

Author Contrıbutıon

Burcu ER was responsible for the preparation of the article and the conduct of the research as the corresponding author. Prof. Dr. Recai GÜRHAN was the supervisor of the article and the research.

Thank You

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Two new invasive species for Tokat province: *Zaprionus indianus* Gupta, 1970 and *Zaprionus tuberculatus* Malloch, 1932 (Diptera: Drosophilidae)

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Abstract: This study was carried out in peach orchards of Akyamaç, Kemalpaşa and Kömeç villages of Tokat centre in 2023. As a result of the observations made in the orchards of the mentioned villages, damaged peach fruits were cultured in the laboratory. *Zaprionus tuberculatus* Malloch, 1932 and *Zaprionus indianus* (Gupta, 1970) (Diptera: Drosophilidae), which are invasive species, were detected as a result of the culture processes. Especially *Z. indianus* was observed to be intensively reared from the cultures. These species are the first records for the insect fauna of Tokat province.

Keywords: Invasive species, *Zaprionus indianus*, *Zaprionus tuberculatus*, Drosophilidae, Tokat, Türkiye

Tokat ili için iki yeni istilacı tür: *Zaprionus indianus* (Gupta, 1970) ve *Zaprionus tuberculatus* Malloch, 1932 (Diptera: Drosophilidae)

Öz: Bu çalışma, Tokat merkeze bağlı Akyamaç, Kemalpaşa ve Kömeç köylerinin şeftali alanlarında 2023 yılında gerçekleştirilmiştir. Adı geçen köylere ait şeftali bahçelerinde yapılan gözlemler neticesinde zarar görmüş şeftali meyveleri laboratuvarda kültüre alınmış ve kültür işlemleri neticesinde istilacı türlerden olan *Zaprionus tuberculatus* Malloch, 1932 ve *Zaprionus indianus* (Gupta, 1970) (Diptera: Drosophilidae) tespit edilmiştir. Özellikle *Z. indianus*' un kültürlerden yoğun olarak çıktığı görülmüştür. Tespit edilen bu türler Tokat ili böcek faunası için ilk kayıt niteliğindedir.

Anahtar kelimeler: İstilacı türler, *Zaprionus indianus*, *Zaprionus tuberculatus*, Drosophilidae, Tokat, Türkiye

1. Introduction

Drosophilidae is an important family with 4700 species belonging to 77 genera worldwide (Bächli, 2023). The Palaearctic fauna currently consists of 482 species from 27 genera (Brake & Bächli, 2008). The drosophilid fauna of Türkiye, is poorly known with 36 species from six genera (Koçak & Kemal, 2013). The genus *Zaprionus* Coquillett, 1902 is recognised by the longitudinal white stripes on the frons and mesonotum and totally have 59 species, of which 48 are known from the Afrotropical region (Yassin & David, 2010). Among these species, the invasive species that are widely distributed in the African continent are known as *Zaprionus indianus*, *Z. tuberculatus* and *Z. ghesquierei* (Chassagnard & Kraaijeveld, 1991).

Zaprionus indianus is a polyphagous species that has been observed infesting the fruits of over 70 plant species in its native Africa (Raspi et al., 2014; Joshi et al., 2014). This species is currently globally distributed and is accepted as cosmopolitan. It is found in temperate and tropical regions (Commar et al., 2012). It has been reported from different hosts such as fig, loquat, guava, oranges, palm, longan, cashew, pomegranate, grape, apricot, and cherries (Vilela, 2001; Steck, 2005; van der Linde et al., 2006; Al-Jboory & Katbeh-Bader, 2012; Joshi et al., 2014). Although *Z. indianus* is not considered to be a pest in Africa, it spread rapidly in Brazil and caused the loss of more than 40% of the fig crop in one region in 1999 (Stein et al., 2003). This species was initially documented in Türkiye in 2017 within the Eastern Mediterranean Region, and found infesting various fruits such as persimmon, blackberry, peach, mulberry, fig, cherry, peach, and plum (Çatal et al., 2019).

Another invasive species Z. *tuberculatus* originates from the Afrotropical region and the islands of the Indian Ocean and is an invasive species for the European continent (Chireceanu et al., 2015). This invasive species was determined for the first time in Türkiye by Patlar et al. (2012) in Adana. The same researchers suggested that this species may be a potential agricultural pest for fig crops like *Z. indianus*.

In addition, Çatal et al. (2021) reported that this species is widespread in the Mediterranean region of Türkiye.

Over the past few years, *Z. indianus* has escalated to a point where it has the potential to detrimentally impact fruit production and international trade in numerous countries worldwide. Researchers across various nations are actively conducting studies to identify this pest and devise effective solutions. This study was carried out to reveal the presence of *Z. indianus* and *Z. tuberculatus* in peach orchards in Tokat province.

2. Material and methods

Surveys were carried out in peach orchards in Akyamaç, Kemalpaşa and Kömeç villages of Tokat (Türkiye) in 2023. The infected peach fruits were gathered and transferred to 5-liter plastic jars (containing 4-5 cm of sterile soil) with ventilation holes and stored at 25±2 ℃ and 60±5% relative humidity conditions in the Entomology Laboratory of the Middle Black Sea Transitional Zone Agricultural Research Institute, Tokat, Türkiye for the emergence of flies. Adult emergence was monitored by checking every 24 hours and the adults obtained were placed in 70% alcohol. To prepare the female genitalia, the last part of the abdomen was removed, boiled in 10% KOH solution and cleaned from the other parts (Tschorsnig, 1985). The genitalia were then preserved in glycerine. Species identification was made according to van der Linde (2010) and Yassin and David (2010) and confirmed by Dr. Asime Filiz ÇALIŞKAN KEÇE (Çukurova University, Department of Plant Protection, Faculty of Agriculture, Balcalı, Adana, Türkiye). Flies were photographed using a Leica MC170 digital camera that was attached to a Leica M205 C stereomicroscope and Leica DM 2000 microscope. The drosophilid specimens are deposited in the Plant Protection Museum in Tokat Gaziosmanpaşa University, Agricultural Faculty, Tokat, Türkiye.

3. Results

Zaprionus indianus and *Z. tuberculatus* adults were obtained from infested peach fruits in 2023. Both species are new records for the insect fauna of Tokat province.

3.1. *Zaprionus indianus* Gupta, 1970

Diagnosis: White stripe surrounded by black from the head to the end of the scutellum**.** The black stripes are of equal width everywhere (Figure 1a,b). There are a row of composite spines on the fore femur. These spines are directly on the leg, not on any tubercles (Figure 1c). Abdominal tergal bristles with dark spots at the base (Figure 1d) (van der Linde, 2010). Female of ovipositor and spermatheca as Figure 2a,b.

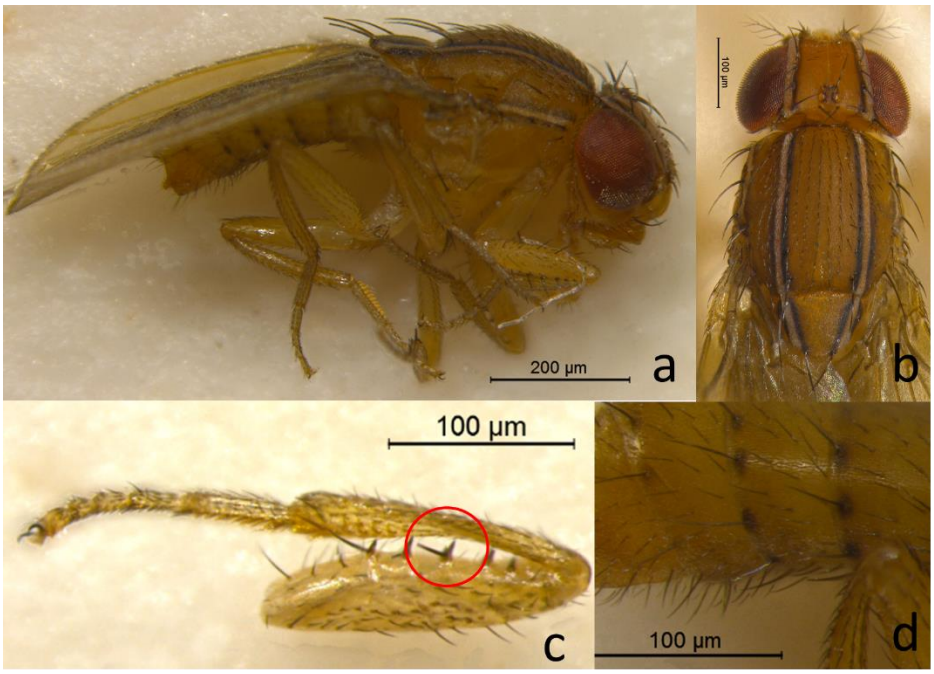

Figure 1. *Zaprionus indianus* adult-female. **a)** General view, **b)** Head and thorax (dorsal view) **c)** A row of composite spines on the fore femur, **d)** Abdominal tergal bristles

Şekil 1. *Zaprionus indianus ergin-dişi. a) Genel görünüm, b) Baş ve toraks (dorsal görünüm) c) Ön femur üzerinde bir sıra kompozit dikenler, d) Abdominal tergal setalar*

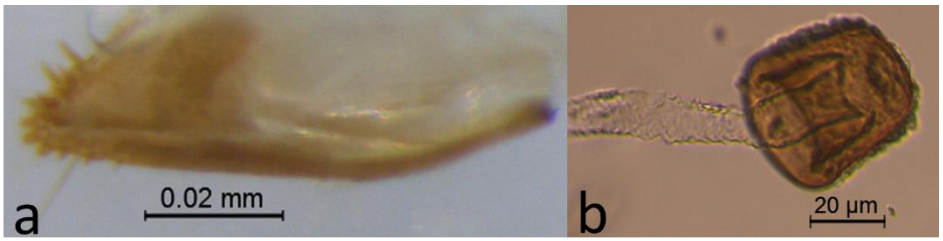

Figure 2. *Zaprionus indianus*-female. **a)** Ovipositor, **b)** Spermatheca
Şekil 2. *Zaprionus indianus-dişi. a) Ovipozitör, b) Spermateka*

Material examined: Tokat (Centre, Akyamaç), N40°21'17" E36°29'34", 627 m, 34♂, 47♀; 23 August 2023. Host plant: peach.

Distribution in Türkiye: Adana, Hatay, Mersin, Osmaniye (Çatal et al., 2019; 2021).

Distribution in the World: Avrupa, Africa, Asia, Central, North and South America (EPPO, 2020b, c).

3.2. *Zaprionus tuberculatus* Malloch, 1932

Diagnosis: The frons has a white median stripe (Figure 3b). There is a protruding tubercule bearing a bristle on the fore femur (Figure 3a,c) (Yassin & David, 2010). Female of ovipositor and spermatheca as Figure 4a,b.

Material examined: Tokat (Centre), Akyamaç, N40°20'45" E36°29' 36", 593 m, 13♂, 7♀; N40°20' 53" E36°29'18", 593 m, 12♂, 5♀; Kemalpaşa, N40°20'45" E36°31'11", 611 m, 3♂, 5♀; Kömeç, N40°20' 56" E36° 27' 23", 610 m, 5♂, 4♀; N 40°21'1" E36°27' 15", 621 m, 3♂, 2♀; 19 September 2023. Host plant: peach.

Distribution in Türkiye: Adana (Patlar et al., 2012; Çatal et al., 2021), Aydın (Başpınar et al., 2022), Uşak (Zengin, 2020), Hatay, Kahramanmaraş, Mersin, Osmaniye (Çatal et al., 2021).

Distribution in the World: Africa, Cyprus, Greece, Israel, Italy, Malta, Spain, Romania (EPPO, 2020a).

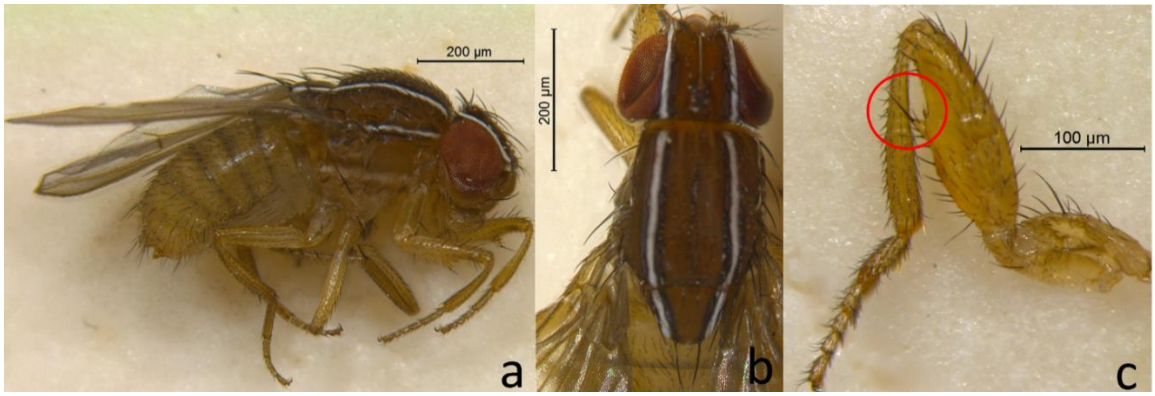

Figure 3. *Zaprionus tuberculatus* adult. **a)** General view (female), **b)** Head (frons with a median white stripe) and thorax (dorsal view-female), **c)** Protruding tubercule bearing a bristle on the fore femur (male)
Şekil 3. *Zaprionus tuberculatus ergin, a) Genel görünüm (dişi), b) Baş (bir beyaz şeritli frons) ve toraks (dorsal görünüm-dişi), c) Ön femurda, üzerinde bir seta bulunan tüberkül (erkek)*

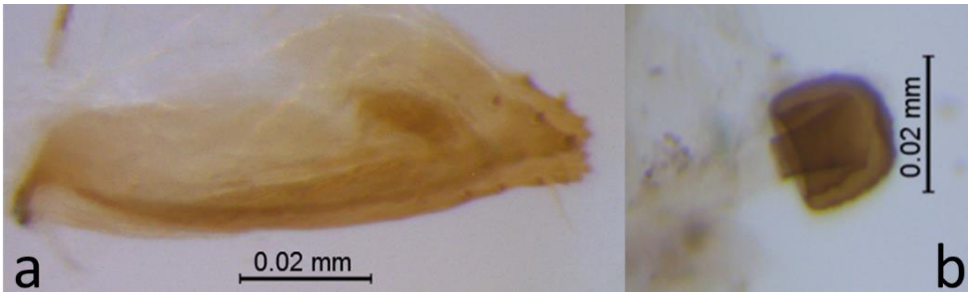

Figure 4. *Zaprionus tuberculatus* female. **a)** Ovipositor, **b)** Spermatheca
Şekil 4. *Zaprionus tuberculatus dişi. a) Ovipozitör, b) Spermateka*

Discussion

Most drosophilid species are saprophagous and feed on decaying plant material (Schmitz et al., 2007). However, differently, some species cause significant economic losses by damaging unripe fruit and are also invasive species. The most important of these is *Drosophila suzukii* (Matsumura), which is widespread throughout the world and causes economic damage to many fruit species. It is known that up to 80% of the strawberries in Europe have suffered crop losses due to this pest (Lee et al., 2011). Another invasive species, *Z. indianus*, is recognised as the primary pest causing significant economic losses especially in figs in many countries of the world. (Commar et al., 2012). In 1999, *Z. indianus* caused a 40% crop loss of figs in the state of São Paulo (Stein et al., 2003). In addition, Bernardi et al. (2017), reported that this species prefers strawberry fruits mechanically injured by various factors or damaged by *D. suzukii* females. The pest status of the other species *Z. tuberculatus* and its potential risk for agriculture is not clear. However, the fact that this species was obtained from 49 fruit species suggests that it may pose a risk in agricultural production in the future. In addition, the similar feeding patterns and ecological requirements of this species with *Z. indianus* also reveal its potential to pose a risk in the future.

These species, which are generally adapted to warm regions, can spread to colder regions with global climate change. Türkiye, as a country with different climates and elevations, is a suitable country for the spread of species belonging to the family Drosophilidae. Until now, species belonging to this family were not considered as a threat to Turkish agriculture. However, interest in this family has increased after it was discovered that invasive species such as *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* (Gupta) caused major economic losses in fruit production (Orhan et al., 2016; Çatal et al., 2019).

In this study conducted in the centre of Tokat, *Zaprionus indianus* and Z. *tuberculatus* adults were obtained from ripe peach fruits cultivated in August and September. In particular, *Z. indianus* was much more intensively were reared from cultures. Çatal et al. (2021), reported that both species were detected in peach fields in the Mediterranean region. Also, Çatal et al. (2019), stated that *Z. indianus* caused significant damage to some fruits, including peaches, in the Eastern Mediterranean region. Santos et al. (2003) reported that *Z. indianus* caused damage to ripe peaches in Brazil.

Tokat province has a transition climate between the Black Sea climate and the steppe climate in Central Anatolia. In general, the summer season is hot in low areas. The climatic characteristics and agricultural production pattern of the province provide an advantage for the spread and population increase of the invasive species *Z. indianus* and *Z. tuberculatus*. In this study, the presence of *Z. indianus* and *Z. tuberculatus* in peach fields in Akyamaç, Kemalpaşa and Kömeç villages of Tokat province where peach production is important was revealed. It is thought that especially *Z. indianus* may pose a threat to peach cultivation in the future. For this reason, it is important to carry out studies to determine the detection, prevalence, density and damage status of these invasive species both in peach areas and in other fruits which are the hosts of these species.

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Molecular Characterisation of 28S Region of *Xiphinema index* (Dorylaimida: Longidoridae) from Türkiye

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Abstract: *Xiphinema index* (Dorylaimida: Longidoridae) is a virus vector nematode and is highly distributed worldwide. In this study, the DNA of the *Xiphinema index* nematode was subject to Polymerase chain reaction (PCR) using D2A and D3B primers to analyse the 28S region. The *X. index* population in the study was recovered from a fig (*Ficus carica* L.) plantation in Tekirdağ, Türkiye. At first, the nematodes were identified morphologically and then molecularly. After PCR, amplified DNA was sequenced for molecular purposes, and data from the D2-D3 region of the 28S rRNA molecule of *X. index* were subjected to GenBank sequence comparison using BLAST (Basic Local Alignment Search Tool). The local sequence submitted to GenBank with accession number PQ165044 showed identity (98.26-99.81%) with several *Xiphinema index* hits. Local *X. index* sequence clustered close with Spanish (HM921406, HM921400, HM921399, HM921398, HM921363, HM921349, HM921347 and HM921364) and Crete/Greece (KJ802882) sequences in Neighbor-joining and Maximum likelihood analysis comparing the local sequence, 16 different *X. index* accessions and 14 accessions from various species.

Keywords: Dagger nematode, 28S rRNA, Ficus carica L., Türkiye

Türkiye'de *Xiphinema index* (Dorylaimida: Longidoridae)'in 28S Bölgesinin Moleküler Karakterizasyonu

Öz: *Xiphinema index* (Dorylaimida: Longidoridae) virüs vektörü nematod türü olup dünya genelinde oldukça yaygındır. Bu çalışmada, *Xiphinema index* nematodunun DNA'sı ile, 28S bölgesini analiz etmek için D2A ve D3B primerleri kullanılarak Polimeraz Zincir Reaksiyonu (PCR) yapılmıştır. Çalışmada *X. index* popülasyonu, Türkiye, Tekirdağ'daki bir incir plantasyonundan (*Ficus carica* L.) toplanmış, ve türün filogenetik analizini gerçekleştirmek için DNA'sı D2A ve D3B primerleri ile amplifiye edilmiştir. Nematodlar önce morfolojik olarak, daha sonra moleküler olarak tanımlanmıştır. PCR sonrası amplifike yapılan nematod DNA'sı sekansa tabi tutulmuştur ve *X. index*'in 28S rRNA molekülünün D2-D3 bölgesinden gelen veriler BLAST (Basic Local Alignment Search Tool) kullanılarak GenBank dizi karşılaştırmasına tabi tutulmuştur.Yerel sekans PQ165044 aksesyon numarası ile GenBank'a kaydedilmiş ve diğer *Xiphinema index* türleri ile (% 98.26 – 99.81) benzerlik göstermiştir. Yerel *X. index* dizisi, 16 farklı *X. index* aksesyonu ve farklı *Xihpinema* türlerine ait 14 aksesyon ile yapılan Neighbor-joining ve Maximum likelihood analizlerinde yerel sekans, İspanya (HM921406, HM921400, HM921399, HM921398, HM921363, HM921349, HM921347 ve HM921364 ve Girit/Yunanistan sekanslarıyla aynı dalda kümelenmiştir.

Anahtar kelimeler: *Kamalı nematod, 28S rRNA, Ficus carica L., Türkiye*

1. Introduction

The dagger nematode, *Xiphinema index* (Dorylaimida: Longidoridae), is one of 260 ectoparasitic nematodes of the genus *Xiphinema* belonging to family Longidoridae (Decraemer, 2007). This species was first described by Thorne and Allen in 1950 from the root zone soils of the fig tree (Ficus carica). Since then, it has been reported from various locations worldwide (Jawhar, 2006; Leopold et al., 2007). Severe damage caused by this nematode has been documented in Mediterranean countries, where dense populations result in root swelling, necrosis, wilting of above-ground plant parts, and general stunting (Van Zyl et al., 2011).

X. index plays a primary role in the spread of virus diseases, which are among the most dangerous plant

pathogens worldwide. More than 2.000 viruses infect plants, animals, and other microorganisms, with 1.200 of them parasitising plants (Bernardo et al., 2018). Unlike bacterial and fungal pathogens that can be controlled with pesticides, virus diseases pose the greatest threat to agricultural production due to the lack of effective control methods. The disease potential of viruses varies depending on the virulence of the isolate and the susceptibility of the cultivar (De Klerk & Loubser, 1988). *X. index* was initially identified as the vector of grapevine fanleaf virus (GFLV) (Hewitt et al., 1958), one of the most harmful nematode-borne virus diseases affecting vineyards. This virus is widely distributed across several countries, causing disruptions in carbohydrate metabolism and hormonal balances, significant decreases in photosynthesis, and increased grapevine respiration rates (Basso et al., 2017). Severe infections can lead to irregularly maturing clusters with non-uniform, poorly developed berries, resulting in yield reductions of up to 80%. The damage inflicted on infected plants varies based on the virus isolate, infection severity, and grape variety susceptibility (Martelli and Savino, 1990; Andret-Link, 2004). Nematodes can acquire virus particles within 15 minutes of feeding on young root tips and can simultaneously transmit these particles to healthy plants. Nematodes that carry virus particles can maintain their ability to transmit the virus for up to 9 months, complicating efforts to eradicate viruses when nematode populations persist (Taylor and Raski, 1964). Moreover, nematodes can survive in the soil for several months even after the host plant is removed, posing ongoing risks for future infections.

Morphology and morphometric observation have been mostly preferred to identify *Xiphinema* species (Kumari and Liskova, 2009). However, many species have very similar characteristics, which make them difficult to distinguish under mixed populations (Sırca 2007). Besides, this kind of identification is only possible using adult nematodes and can only be performed by experts. (Kumari et al., 2010). Because of these limitations, several researchers developed molecular techniques for rapid and reliable species determination. Again, genetic similarities between populations of different countries and species can be easily revealed with molecular techniques (Vrain et al., 1992; Wang et al., 2003). The D2-D3 segments of the 28S region can be successfully applied to separate different *Xiphinema* species. (Blaxter et al., 1998; Powers, 2004; Daramola et al., 2019; Fayaz et al., 2022). This method identifies nematodes in every developmental stage (Hübschen et al., 2004a; Hübschen et al., 2004b).

The nematode species *Xiphinema index*, has been detected by several researchers in our country, particularly in vineyards. It has been found in the several provinces including Şanlıurfa, İzmir and Manisa, (Yıldız & Elekcioğlu, 2011; Mistanoğlu et al, 2015; Öztürk et al., 2017; Kasapoğlu et al., 2018).

This study aimed to molecularly characterize the *Xiphinema index* by amplifying the D2-D3 region of 28S rRNA with general primers and to determine the closeness using local sequence data and other GenBank records.

2. Material and method

2.1. Collection of soil samples, extraction, and identification of *Xiphinema index*

In our previous studies, the *Xiphinema index* has been detected in various plants in Thrace, whereas in our survey in PhD thesis, it was found in high populations at depths of 30-60 cm in fig orchards. In this study, soil samples were collected in March 2017 from a fig orchard where the *Xiphinema index* was previously identified. A molecular study was conducted with nematodes isolated from these soil samples. Female nematodes were extracted from a 200 gr subsample using Cobb's sieving and centrifugal flotation method (Jenkins, 1964; Brown & Boag, 1988). To confirm the *X. index*, slides were prepared from heat-killed, fixed (in a double strengthen formalin-triethanolamine (7 ml formaldehyde+2 ml triethanolamine+ 71 ml distilled water) solution, and on a slide-mounted female (Thorne and Allen, 1950; Seinhorst, 1959; Hooper, 1961). Species were identified by comparing morphometric and morphologic parameters of female nematodes with descriptions of Thorne & Allen and keys of Loof and Luc, 1990, 1950 and other researchers. The images of the females were taken with a Celestron microscope. The females, confirmed to be *X. index,* were picked and transferred to PCR tubes for DNA extraction and further molecular studies.

manufacturer's procedure was followed in the extraction process. Individuals were hand-picked under the microscope to extract DNA and placed into 0,2 ml microcentrifuge tubes containing 2.5 µl tissue preparation and 10 µl extraction solutions. Following

2.2. 28S Phylogenetic analysis with *Xiphinema index*

To isolate the *Xiphinema index,* The Sigma Aldrich Extract-N-Amp™ Tissue PCR Kit was used, and the

processing at 55°C for 10 minutes and 95°C for 3 minutes, a neutralisation solution was added to the extract, and the DNA concentration (A260/280 A260/230) was measured using a spectrophotometer. The extracted DNA was stored at -20 °C until molecular studies.

Amplification of nematode DNA was performed with D2A (5′ ACAAGTACCGTGAGGGAAAGT 3′) and D3B (5′ TCGGAAGGAACCAGCTACTA 3′) primer pair (Nunn, 1992). The PCR reaction was carried out with the following program: 95 °C for 3 min, followed by 39 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min, ending with one cycle at 72 °C for 5 min. The Amplification product was separated on a 1.5 % agarose gel in 1×TAE (Tris-acetate-EDTA) buffer.

The PCR product was sequenced following UV visualisation. The sequencing was performed in the Central Research Laboratory at Namık Kemal University using the Beckman Coulter GenomeLab GeXP Genetic Analysis System. Sequence data from the D2-D3 region of the 28S rRNA molecule was edited with Bioedit 7.2.5 (Biological sequence alignment editor) software and subjected to Blast (Basic Local Alignment Search Tool) sequence comparison in NCBI (National Center for Biotechnology Information). Neighbour-joining (1000 Bootstrap) and maximum likelihood were performed on Mega 7 software, comparing the *X. index* (16 accessions) and other *Xiphinema* sequences (14 species/accessions) from 10 countries (Table 1).

Table 1. The list of NCBI GenBank accessions used in the phylogenetic analysis
Çizelge 1. *Filogenetik analizlerde kullanılan NCBI GenBank aksesyonları*

ACCESSIONS	SPECIES	LOCALITY
HM921406; HM921400; HM921399; HM921398; HM921363; HM921349; HM921347; HM921348; HM921364;	*Xiphinema index*	Spain
KX244910	*Xiphinema index*	Spain
HG969307	*Xiphinema index*	Hungary
AY601628	*Xiphinema index*	Argentina
KM283422; MF996703	*Xiphinema index*	Iran
KJ802882; KJ802881	*Xiphinema index*	Crete, Greece
JQ780362	*Xiphinema diversicaudatum*	Czech Republic
KJ802879	*Xiphinema cretense*	Crete, Greece
MT271611	*Xiphinema hyrcaniense*	Iran
KX244900	*Xiphinema cadavalense*	Spain
KX244915	*Xiphinema pseudocoxi*	Spain
KY623487	*Xiphinema tica*	Costa Rika
GU549474	*Xiphinema globosum*	Spain
KY131240	*Xiphinema japonicum*	Spain
KU052864	*Xiphinema bakeri*	Japan
AY601629	*Xiphinema basiri*	Cuba
KC567170	*Xiphinema belmontense*	Spain
KC567166	*Xiphinema baetica*	Spain
KX244887	*Xiphinema andalusiense*	Spain
KT308868	*Longidorus intermedius*	Spain

3. Results

The *Xiphinema index* specimens used in the molecular studies had elongated bodies [3±0.06 (2.97-3,11)] mm] that formed an open spiral shape when at rest or deceased, with the lip region continuous with the neck contour. The a=60.4±2.91 (56-66), the c=78.52±1.52 (76.2-80.8), and the c′=0.83±0.01 (0.82-0.84). The oesophagus had a narrow anterior part that widened towards the posterior. The posterior part of the oesophagus was cylindroid. The oesophagus-intestinal valve was small and conoid-rounded, and the stylet was long. A conical-shaped cardia was located at the junction of the oesophagus and intestine. Stylet 203.4±5.6 (195-213) µm long with odontophore 79.1±2.16 (76,6-80,0) µm and odontostylet 123.4±3.6 (116.4-127.9) µm. Guiding ring 120.4±1.28 (118.0-122.3) µm from anterior end. Vulva was a depressed transverse slit located anterior to mid-body. Reproductive system amphidelphic, with reflexed ovary. The vulva appeared as a depressed transverse slit situated anterior to the mid-body [38.6±2.4 (36-40.6)]. The reproductive system was amphidelphic, with a reflexed ovary. The tail was convex-conoid, with greater curvature dorsally and a small peg called mucro. The tail was 0.87±0.22 times as long as the anal body width (Thorne and Allen, 1950). Mucro length was 8.11±002 (7.8-8.30) (Figure 1).

The morphometric values of individuals were similar

to those of the Spanish, Chilean, Serbian and Lebanese populations (Body length 2.7-3.65 mm; Stylet=193-217 µm; Tail=32-48.6µ; V=36-43.9%) (Barsi and Lamberti, 2000; Jawhar et al, 2006; Gutierrez et al. 2011; Meza et al., 2011).

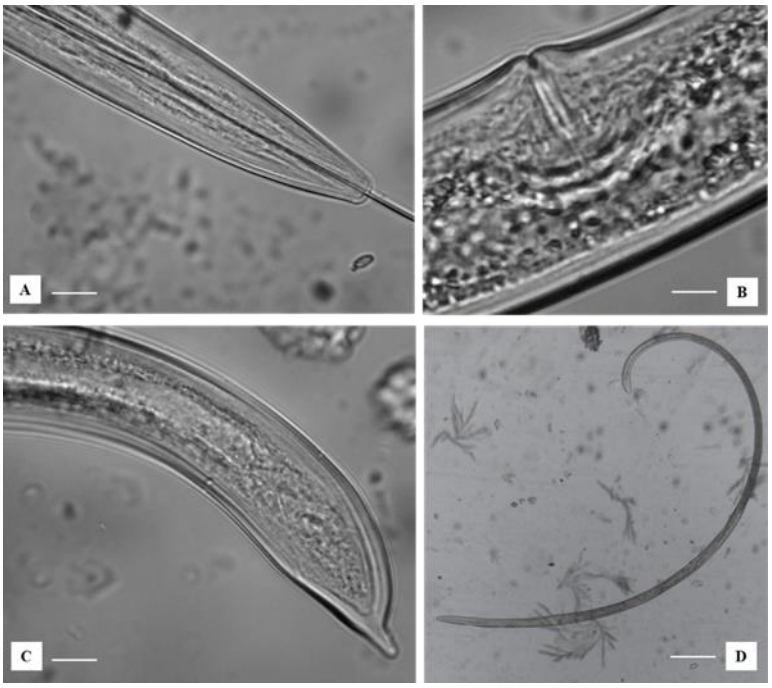

Figure 1. *Xiphinema index* female A. Head with odontostylet B. Vulval region C. Tail and mucro (Scale bar: 20 µm) D. Female entire body
Şekil 1. *Xiphinema index dişi A. Baş bölgesi ve odontostilet B. Vulva bölgesi C. Kuyruk ve mucro D. Dişi vücut genel görünüm*

The partial sequence of the *X. index* from Tekirdağ (PQ165044) was compared with records from other countries, revealing over 98% identity with 16 hits of the 28S ribosomal RNA gene partial sequence of the *X. index* in the NCBI GenBank. According to Blast (Basic Local Alignment Search Tool), the sequence showed 99.81% identity (519-520 nt/1 gap) with sequences from Greece (KJ802882) and 99.41% identity (560-563 nt/1 gap) with sequences from Spain (HM921364). Spanish accessions HM921406, HM921400, HM921399, HM921398, HM921363, HM921349, HM921347, and HM921364 exhibited a 99.3% identity score. The lowest identity was observed with the Iranian accession KM283422 (98.78%; 567-574 nt/2 gaps). Nucleotide differences ranged from 1 to 7 in closely related sequences, with gaps between all sequences ranging from 1% to 3%.

The closest match was *X. diversicaudatum* MG994934 (92.08%) when comparing identity with other *Xiphinema* species. Other close matches include *X. pyrenaicum* AY601626 (97.52%), *X. cretense* KJ802880 (91.89%), *X. cadavalense* KX244900 (91.73%), *X. hyricaniense* MT271611 (91.73%), *X. pseudocoxi* KX244915 (91.70%), *X. tica* KY623487 (91.57%), *X. japonicum* KY131240 (91.36%), *X. bakeri* KU052864 (91.36%), *X. belmontense* KC567170 (91.35%), *X. basiri* AY601629 (91.35%), *X. coxi* AY601631 (91.30%), *X. baetica* KC567166 (91.23%), *X. andalusiense* KX244887 (91.21%), *X. abrantinum* AY601625 (90.83%), and *X. turdetanense* KX244920 (91.21%).

Figures 2 and 3 represent the Neighbor-joining and Maximum likelihood trees generated by comparing the Tekirdağ sequence and 31 different *Xiphinema* accessions from NCBI. In both trees, the local *X. index* clustered in the nearest clade with sequences from Spain, Hungary, and Greece. All *X. index* species were found in the same clade as other non-*X. index* species were grouped in other clades.

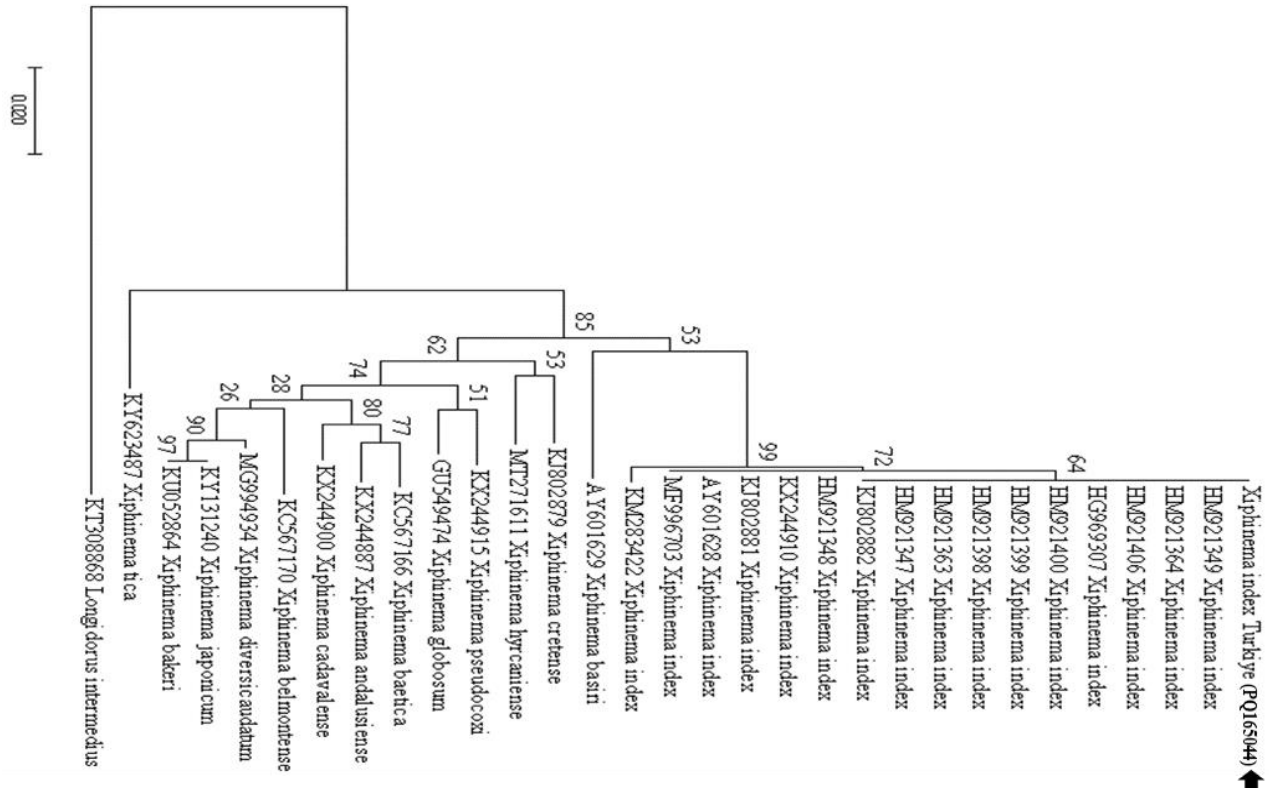

Figure 2. Neighbor-joining tree generated by comparing 28S rRNA gene D2-D3 regions.
Şekil 2. *28S rRNA geni D2-D3 bölgelerinin karşılaştırılmasıyla oluşturulmuş Neighbor joining ağacı*

Figure 3. A maximum likelihood (Kimura-2 parameter model) tree generated by comparing 28S rRNA gene D2-D3 regions.
Şekil 3*. 28S rRNA geni D2-D3 bölgelerinin karşılaştırılmasıyla oluşturulmuş Maximum Likelihood (Kimura-2 Parametre modeli) ağacı*

4. Discussion

In previous studies conducted in Thrace, *Xiphinema index* nematode has been vineyard areas (Ozturk et al., 2017). Although this nematode species can parasitize many cultivated plants, it is reported to reproduce best on fig trees, acting as its primary host. Research has determined that *X. index* has a lifespan of 60 to 64 weeks on *Ficus carica*, with a total reproductive capacity ranging from 140 to 160 progeny (Brown & Coiro, 1985). Fig trees, which grow along roadsides and various other locations, are commonly found between rows of vines or along vineyard edges, especially in districts like Şarköy in Thrace. The presence of these host plants, particularly in vineyards where viral infections are prevalent, can facilitate the proliferation of vector nematodes, thereby exacerbating the spread of epidemics. To prevent the spread of *grapevine fanleaf virus* (GFLV), one of the most significant viruses affecting vineyards, it is essential for new vineyard sites to be free of nematode infestations. Soil samples from these areas are analyzed to assess the presence of vector nematode infestations.

Identifying *X. index* mostly relies on observing morphologic and morphometric characteristics under a microscope. *X. index* shows identical morphological features with many species, such as *X. vuittenezi* and *X. italiae* (Van Zyl, 2011). Its closest relative is *X. diversicaudatum* Micoletzky, 1927, and *X. index* is distinguished by the more anterior position of the vulva, four pairs of caudal pores compared to six pairs in *X. diversicaudatum*, and the smaller body structure. Morphological identification is difficult, and misidentification is sometimes possible. For this reason, researchers are using molecular methods for diagnosis. Many species-specific primers were designed based on the sequence divergence of the DNA region of species and closely related species (Esmenjaud & Bouquet, 2009). Several studies have previously achieved molecular confirmation of cyst nematodes, lesion nematodes, stunt nematodes, and root-knot nematodes. The internal transcribed spacer (ITS), region of ribosomal DNA, cytochrome c oxidase subunit I (COI) and some other genetic markers were generated and successfully applied in identifications. (Blaxter et al., 1998; Powers, 2004). (Vrain et al., 1992; Wang et al. 2003; Hübschen et al., 2004). Ribosomal RNA is divided into two subgroups in eukaryotic organisms such as nematodes: the large subunit covering the 60S, 5S, 5.8S, and 28S regions and the small ribosomal unit containing the 18S region (Lafontaine and Tollervey, 2001). The D2 and D3 segments of 28S rRNA are frequently used in phylogenetic studies because it is easy to design diagnostic primers from this region, and the gene encoding 28S rRNA is more variable than 18S (Subbotin et al., 2008).

In this study, the 28S region was used in the molecular characterisation and phylogenetic studies of the *Xiphinema index*. The results revealed that the local population was highly identical to those in Spain, Crete, and Greece. Furthermore, Blast analysis showed a 98% or more identity with Iranian and Hungarian accessions. These significant findings contribute to *the* existing body of knowledge on the phylogeny of the *X. index* in different countries.

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Author contributions

Lerzan ÖZTÜRK carried out molecular studies, and İbrahim Halil ELEKÇİOĞLU approved the nematode identification, reviewed the paper, and approved.

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