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

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

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Research Article

Determination of The Effect of Salicylic Acid Application on Salinity Stress at Germination Stage of Bread Wheat

Melikşah YILMAZ[1], Ferhat KIZILGECI[*2], Nihan TAZEBAY ASAN[3], Ufuk ASAN[4] Asif IQBAL[5], Muhammad Aamir IQBAL[6]

[1]Mardin Artuklu University, Institute of Graduate Education, Department of Field Crops, Mardin, Turkey
[2] Mardin Artuklu University, Kızıltepe Vocational School, Department of Plant and Animal Production, Mardin, Turkey
[3]Şırnak University, Idil Vocational School, Department of Plant and Animal Production, Şırnak, Turkey
[4]Şırnak University, Agriculture Faculty, Department of Field Crops, Şırnak, Turkey
[5]University of Agriculture Faisalabad, Faculty of Agriculture, Department of Agronomy, Faisalabad, Pakistan
[6]University of Poonch Rawalakot, Faculty of Agriculture, Department of Agronomy, Rawalakot, Pakistan

[1]https://orcid.org/0000-0001-8102-2268, [2]https://orcid.org/0000-0002-7884-5463, [3]https://orcid.org/0000-0003-0453-7481
[4]https://orcid.org/0000-0002-7582-7681, [5]https://orcid.org/0000-0001-8884-2648, [6]https://orcid.org/0000-0003-2701-0551

*Corresponding author e-mail: ferhatkizilgeci@artuklu.edu.tr

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Abstract: Under changing climate, abiotic stresses especially salinity have posed serious threats to modern crop production systems of staple crops and chemo-priming with salicylic acid offers a promising remedy. The present study aimed at ameliorating the adverse effects of salt stress through optimization of salicylic acid (SA) for two bread wheat genotypes (DZ17-1 and Empire Plus). The trial was comprised of chemo-priming with different SA levels including 0, 0.5, and 1 mM applied to the seeds of bread wheat genotypes exposed to different salinity levels (0, 50, 100, 150, 200 mM NaCl). The response variables included germination indices, roots length, and weight along with seedling traits. The results revealed that increasing the level of salinity had a negative effect on both genotypes of wheat and all traits studied. The DZ17-7 genotype was found to be more tolerant to salt stress. Among SA concentrations, 1 mM imparted a significant influence on germination, root traits, and seedling parameters. Although SA showed positive effects in salt stress conditions in the study, further studies are needed to clarify the role of SA in providing stress tolerance of plants.

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Ekmeklik Buğdaya Salisilik Asit Uygulamasının Çimlenme Döneminde Tuzluluk Stresine Etkisinin Belirlenmesi

Makale Bilgileri

Geliş: 14.02.2022
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Öz: Değişen iklim koşulları altında, abiyotik stresler, özellikle tuzluluk, temel ürünlerin üretiminde ciddi tehditler oluşturmakta ve salisilik asit bu etkilerin azaltılmasında umut verici bir çözüm sunmaktadır. Bu çalışma, iki ekmeklik buğday genotipine (DZ17-1 ve Empire Plus) salisilik asit (SA) uygulamasının tuz

Anahtar Kelimeler

Abiyotik stres,
Çimlenme,
Kök Uzunluğu

stresinin olumsuz etkilerini iyileştirmesi amaçlamıştır. Araştırmada, farklı tuzluluk seviyelerine (0, 50, 100, 150, 200 mM NaCl) maruz bırakılan ekmeklik buğday genotiplerinin tohumlarına 0, 0.5 ve 1 mM salisilik asit uygulanmıştır. Çalışmada çimlenme indeksleri, kök ve fide özellikleri incelenmiştir. Araştırma sonuçlarına göre, artan tuzluluk seviyesinin hem buğday genotiplerini hem de incelenen tüm özellikleri olumsuz etkilediğini ortaya koymuştur. DZ17-7 genotipinin tuz stresine daha toleranslı olduğu bulunmuştur. SA konsantrasyonları arasında 1 mM, çimlenme, kök özellikleri ve fide parametreleri üzerinde önemli bir etki sağlamıştır. SA, çalışmada tuz stresi koşullarında olumlu etkiler gösterse de bitkilerin stres toleransını sağlamada SA'nın rolünü netleştirmek için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Dipnot: Bu makale Melikşah YILMAZ'ın Yüksek Lisans tezinden üretilmiştir.

1. Introduction

Globally, changing climate and global warming have threatened the food production systems and rapidly increasing human population demands proportionate increase in staple crops production. However, abiotic stresses especially frequent spells of drought and salinity have emerged as daunting challenges posed to multiply crops yield on a per hectare basis (Alghawry et al., 2021; Chowdhury et al., 2021; Iqbal et al., 2021). Wheat (*Triticum aestivum* L.) has been regarded as the most significant cereal crop for ensuring the food security and poverty alleviation strategy of farmers worldwide. It ranks second most important crop after rice and is being cultivated in all continents (Giraldo et al., 2019). It is a staple food for around 36% of the world's population and provides over 20% of daily calories and 55% of total carbohydrates to humans (Siddiqui et al. 2019; Kizilgeci, 2021). Currently, the wheat yield remains low and stagnant, primarily owing to suboptimal growth conditions. (FAO, 2016). Recently, climate change is feared to impart further adverse effects on germination, growth, and yield of wheat crops (Asseng et al., 2015).

Among abiotic stresses, salinity causes numerous biochemical and physiological damages in crop plants and ultimately deteriorates the grain yield and quality (Can et al., 2021). Salinity stress causes osmotic stress and ion toxicity by increasing the assimilation of Na^+ ions and decreasing the Na^+/K^+ ratio due to low osmotic potential in plant roots. Furthermore, this ionic imbalance affects the uptake and transport of other essential ions in target cells and inhibits important plant processes and functions (Arif et al., 2020). In general, among field crops, wheat is more sensitive to salinity, which inhibits plant growth and development. Salinity is the most adverse factor affecting the quality and productivity of wheat by changing its physiological activities as well as its biochemical activities. Soil salinity adversely affects various morphological structures of wheat including seedling growth, plant height, shoot and root length, root, leaf, fresh and dry weight, root/shoot ratio, and chlorophyll content (Iyem et al., 2020). Salinity stress accelerates all phenological stages of wheat (Grieve et al., 1994), reduces the number of tillering (Abbas et al., 2013), the number of spikelets per spike (Frank et al., 1987), grain weight (Abbas et al., 2013), and negatively affect grain yield (Sorour et al., 2019). Ali et al. (2009) reported that wheat exposed to salt stress caused a decrease of up to 45% in yield.

Salicylic acid (SA) or ortho hydroxybenzoic acid [C_6H_4 (OH)CO_2H] is a phenolic type endogenous growth regulator having the potential to ameliorate adverse effects of salt stress under varying pedo-climatic conditions (Javid et al., 2011; Moghaddam et al., 2020). The SA application holds the potential to increase the rate of photosynthesis, stomatal conductivity, and transpiration rate that assist plants to cope with the saline environment (Khan et al., 2003; Arfan et al., 2007). In addition, SA activates antioxidant protection (Xu et al., 2008) along with inhibiting the accumulation of Na^+ and Cl^- in plant cells (Gunes et al., 2007). Furthermore, Abhinandan et al. (2018) reported that SA application remained effective in triggering the vegetative growth of crop plants which reduced the deleterious effects of salinity by virtue of robust growth. In addition, Ma et al. (2017) inferred that SA exogenous application remained effective in improving germination, seedling growth, photosynthesis rate, antioxidant biosynthesis, numerous enzymes activation, stomata opening regulation, and chloroplast development. Moreover, SA was reported to have a critical role in triggering the carotenoids bio-synthesis along with enhancing the rate of de-epoxidation in wheat under saline environment (Moharekar et al., 2003). Previously, it has also been exhibited that SA imparted robustness to the

antioxidant system in Brassica species (Yusuf et al., 2008). The SA application as chemo-priming (pre-sowing seed soaking for 6-72 hrs) might offer one of the biologically viable and promising approaches to cope with adverse effects of salinity on germination and early seedling growth through activation of numerous enzymes and biosynthesis of antioxidants (Choudhary et al. 2021; Zahoor et al., 2021). However, most of the studies have addressed the salinity stress at terminal stages of crop plants while research gaps exist regarding SA application as a seed priming agent in triggering the seed germination and seedling growth traits under saline environments of varying extent.

Thus, it was hypothesized that SA dose optimization for chemo-priming may assist in improving germination rate and seedling growth traits of wheat under varying levels of salinity. To this end, the prime aim of this trial was to assess the impact of different doses of SA on wheat genotypes germination and seedling growth parameters under saline conditions.

2. Material and Methods

The present study was executed in the laboratory of the Field Crops Department of the Faculty of Agriculture, Şırnak University, Turkey in 2020. In the research, material was advanced bread wheat line DZ17-1 (developed by Dicle University, Turkey) and Empire Plus bread wheat genotype. The trial was comprised of different levels of SA (0, 0.5, and 1 mM) applied as a seed priming agent to wheat under varying levels of imposed salinity (0, 50, 100, 150, and 200 mM). The trial was executed as a factorial experiment under a completely randomized design having three replications. The genotypes were subjected to a seed priming process with 10% Sodium Hypochlorite (NaClO) to prevent contamination. The disinfected seeds were then rinsed 3 times with distilled water. The seed priming duration was 12 hours and then dried with drying towels at room temperature. 15 seeds from each genotype were placed on the filter paper in 9 cm plastic petri dishes, which is the germination medium. The trial was carried out in a germination chamber at a constant temperature of 24±1 °C for 8 days, with a day and night length of 18/6 hours. To create salt stress, NaCl solutions were prepared at doses of for each application, and 10 ml were applied to the seeds placed in petri dishes and allowed to germinate. Characteristics examined in the study we are determined according to Yildirim et al. (2015) (coleoptile length, seedling length (mm), root length (cm), shoot dry weight (mg), germination rate (%), germination vigour (%)).

2.1 Statistical analysis

The analysis of variance (ANOVA) of the data obtained from the study was performed using the JMP 10 package program according to the factorial experiment under a completely randomized design. Differences between means were interpreted according to the 5% LSD test.

3. Results and Discussions

3. 1. Coleoptile length

The coleoptile length and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress are given in Table 1.

The highest coleoptile length value was recorded for the DZ17-1 genotype using 1 mM SA + 50 mM NaCl, while the lowest (5.47 mm) was obtained in the DZ17-1 genotype from the application of 0.5 mM SA + 200 mM NaCl. The increase in salt stress caused significant reductions in the coleoptile length of both genotypes. DZ17-1 genotype was found to be more resistant to salt stress conditions than Empire Plus variety. It was determined that salicylic acid application to salt doses had no effect on coleoptile length (Figure 1).

Table 1. Average values of coleoptiles length (mm) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	25.42a-d	24.39abc	22.85c-f	19.33ghi	8.18op
	0.5	24.16a-d	23.32b-e	24.39a-d	13.90lm	5.47p
	1	26.70ab	27.19a	25.83abc	18.42g-j	5.64p
Empire Plus	0	21.60d-g	24.54a-d	20.52e-h	14.75klm	7.13op
	0.5	20.75e-h	18.12h-k	15.50j-m	12.83mn	9.35o
	1	19.65f-i	18.47g-j	16.38i-l	10.04no	7.25op
DZ17-1 mean		25.43A	24.97A	24.35A	17.22C	6.43E
Empire Plus mean		20.67B	20.38B	17.46C	12.54D	7.91E

Differences between means with the same lowercase and uppercase letter are not statistically significant.

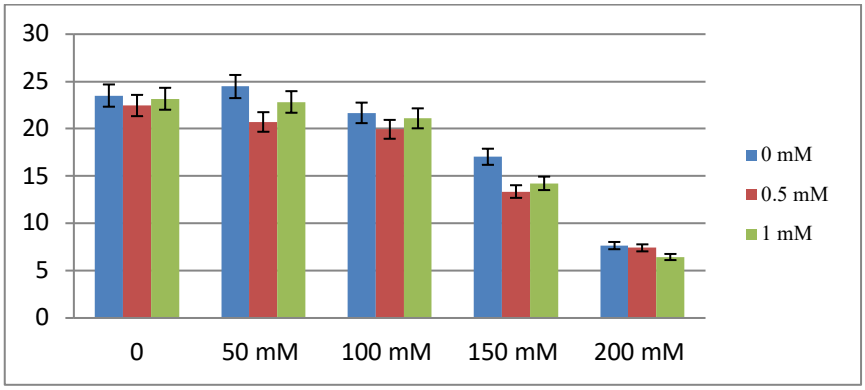

Figure 1. The effects of salicylic acid and NaCl application on the coleoptile length of bread wheat.

Kizilgeci (2021) reported that increasing salt concentrations and decreasing enzyme activities caused a decrease in the coleoptile length of the seeds. Many other researchers have also reported a significant decrease in coleoptile length due to the increase in salt stress (Yildirim et al., 2015; Iyem et al., 2020; Kizilgeci et al., 2020). Responses of genotypes to salicylic acid application under 200 mM salt stress conditions were similar. In our study, it was observed that salicylic acid application was ineffective in reducing the negative effects of salt stress on coleoptile length. Additionally, it was also observed that SA imparted an inhibitory effect in the Empire plus genotype. These findings are in contradiction with those of Canakci and Munzuroğlu (2007) who reported that SA exogenous application in low concentrations increased the coleoptile length under normal conditions, while its high concentrations caused an inhibitory effect on seedling growth and development.

3.2. Root length

The root length values and multiple comparisons of bread wheat genotypes subjected to salicylic acid seed priming under salt stress are given in Table 2.

It was observed that the highest root length (72.28 mm) was obtained in the DZ17-1 genotype by 1 mM SA under non-saline conditions, while the lowest value (6.21 mm) was obtained for Empire Plus genotype by 0.5 mM SA under 200 mM NaCl salt stress. The effects of salicylic acid application on root length properties differed under salt stress conditions (Figure 2). The increase in salt stress resulted in significant reductions in the root length of both genotypes. In accordance with our findings, many researchers have stated that increasing levels of salinity stress resulted in a higher degree of adverse effects on the root length of many field crops (Yildirim et al., 2015; Iyem et al., 2020; Kizilgeci et al., 2020). Additionally, it was also inferred that SA applications up to 100 mM salinity level imparted a positive effect on the root length of the DZ17-1 genotype. Moreover, Empire plus genotype was adversely affected by all doses of SA under varying salinity levels. These findings corroborate with those of Dolatabadian et al. (2009) who opined that SA enhanced the root length of wheat under severe

saline conditions, while Zahra et al. (2011) reported that root elongation was inhibited in parallel with the increase in salicylic acid concentration under salt stress conditions.

Table 2. Average values of root length (mm) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM) 0	50	100	150	200
DZ17-1	0	44.80	37.02	22.74	18.93	9.46
	0.5	49.12	35.72	25.85	13.56	9.81
	1	72.28	41.27	31.72	14.79	9.43
Empire Plus	0	37.42	25.40	19.13	11.34	9.59
	0.5	21.97	21.99	12.60	10.10	6.21
	1	35.50	20.86	15.14	10.99	7.12
DZ17-1 mean		55.40A	38.00B	26.77CD	15.76E	9.57F
Empire Plus mean		31.63C	22.75D	15.62E	10.81EF	7.64F

Differences between means with the same lowercase and uppercase letter are not statistically significant.

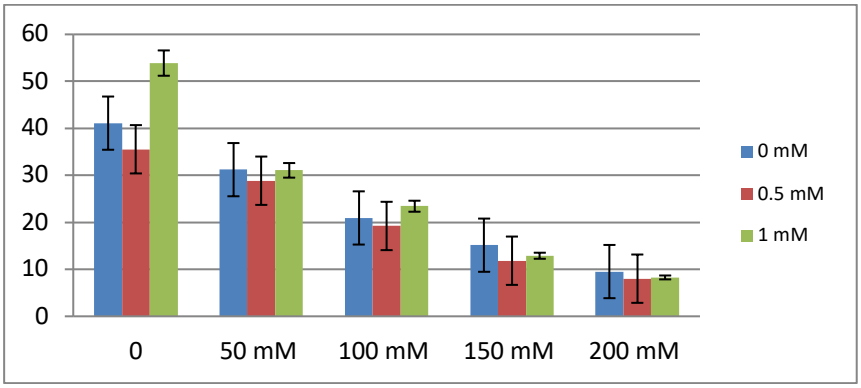

Figure 2. The effects of salicylic acid and NaCl application on the root length of bread wheat.

3.3. Seedling length

The impact of different doses of SA on seedling length of wheat genotypes under varying levels of salinity has been illustrated in Table 3.

Table 3. Average values of seedling length (mm) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM) 0	50	100	150	200
DZ17-1	0	89.92	74.97	52.95	24.54	8.18
	0.5	108.91	88.48	65.28	21.68	5.47
	1	91.86	79.81	60.44	25.68	5.64
Empire Plus	0	74.44	86.33	43.96	18.62	7.13
	0.5	74.87	52.30	40.33	20.63	12.72
	1	78.04	60.41	37.74	13.59	7.25
DZ17-1 mean		96.89A	81.09B	59.56D	23.97F	6.43H
Empire Plus mean		75.78BC	66.35CD	40.68E	17.61FG	9.03GH

Differences between means with the same lowercase and uppercase letter are not statistically significant.

The tallest seedling length (108.91 mm) was obtained for DZ17-1 genotype in 0.5 mM SA + 0 mM NaCl application, while the lowest (5.47 mm) was obtained for DZ17-1 genotype from 0.5 mM SA + 200 mM NaCl application.

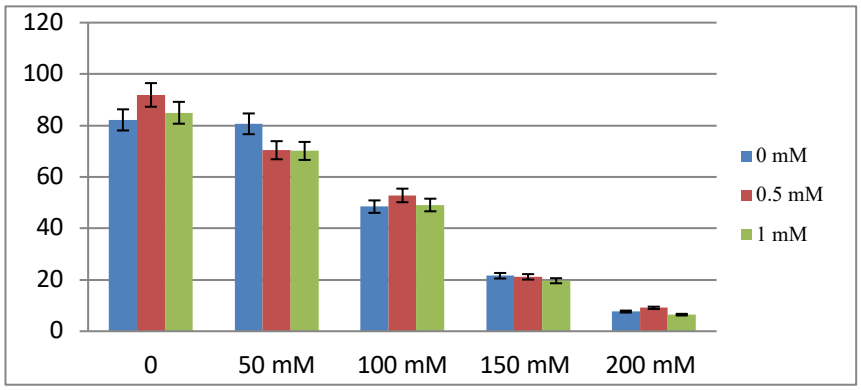

Figure 3. Seedling length in response to different doses of SA under varying levels of salinity.

The increase in salt stress caused significant reductions in the seedling length of both genotypes. However, it was observed that the DZ17-1 genotype was more affected at the highest salt stress compared to the control. It was observed that SA application did not have an effect on increasing seedling length. Likewise, Werner and Finkelstein (1995) reported that high salinity slowed down the plant's water uptake, thus inhibiting root and shoot elongation. Moreover, Shakirova et al. (2003) reported that SA increased the resistance of wheat seedlings to salinity, and 0.05 mM SA application improved plant growth and caused ABA and proline accumulation in wheat.

3.4. Root fresh weight

Table 4 illustrates the root fresh weight of wheat genotypes subjected to chemo-priming with different concentrations of SA under varying levels of induced salinity.

Table 4. Average values of root fresh weight (mg) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	36.7e-k	29.9e-m	37.4e-k	50.5cde	19.7g-m
	0.5	40.3e-i	45.5def	38.8e-j	37.3e-k	35.6e-l
	1	170.8a	73.6bc	84.9b	66.1bcd	42.2e-h
Empire Plus	0	28.9e-m	23.2f-m	23.1f-m	26.4f-m	15.3j-m
	0.5	22.2f-m	12.4lm	24.6f-m	13.8klm	7.2m
	1	43.3d-g	51.4cde	27.7e-m	17.4i-m	19.3h-m
DZ17-1 mean		82.6A	49.7B	53.7B	51.3B	32.5C
Empire Plus mean		31.5C	29.0C	25.1CD	19.2CD	13.9D

Differences between means with the same lowercase and uppercase letter are not statistically significant.

The results revealed that the maximum root fresh weight (170.8 mg) was obtained in the DZ17-1 genotype that was chemo-primed with 1 mM SA and was grown under non-saline conditions, while the lowest value (7.2 mg) was determined in the Empire Plus genotype from the application of 0.5 mM SA + 200 mM NaCl.

The effects of salicylic acid application on root fresh weight properties differed under salt stress conditions (Figure 4). The highest root fresh weight was determined in the combination of 0 mM NaCl +1 mM SA, while the lowest value was determined at 200 mM NaCl + 0 mM SA and 0.5 mM SA.

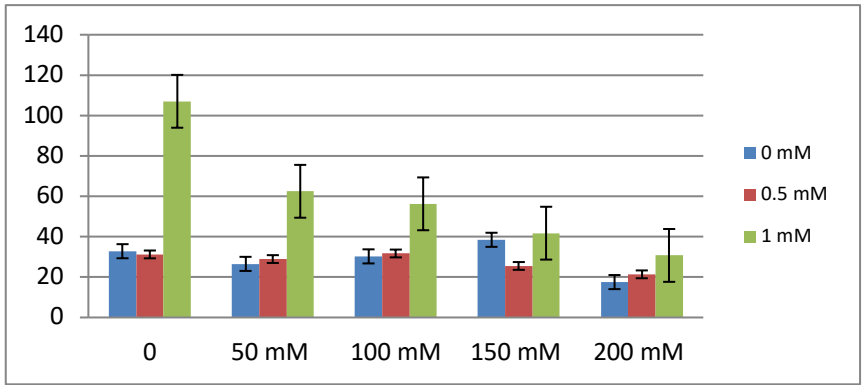

Figure 4. The effects of salicylic acid and NaCl application on the root fresh weight of bread wheat.

The increase in salt stress resulted in significant decreases in root fresh weight of both genotypes. It was observed that the 1 mM SA dose applied in the study had a positive effect on this feature under all stress conditions.

3.5. Seedling fresh weight

Average values of seedling fresh weight and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress are given in Table 5.

Table 5. Average values of seedling fresh weight (mg) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	249.8cd	2404cde	174.9d-j	167.3d-k	30.9no
	0.5	364.7b	294.8bc	196.6c-h	86.3i-o	112.4g-o
	1	505.6a	373.4b	354.6b	153.6d-l	66.5k-o
Empire Plus	0	218.4c-f	213.5c-g	128.1f-n	76.8j-o	26.3no
	0.5	94.3h-o	47.4mno	55.6l-o	50.6l-o	21.6o
	1	145.8e-m	183.5d-i	72.1j-o	33.6no	23.0o
DZ17-1 mean		373.4A	302.8B	242.1C	135.7DE	69.9FG
Empire Plus mean		152.9D	148.1D	85.3EF	53.7FG	23.6G

Differences between means with the same lowercase and uppercase letter are not statistically significant.

As per recorded data, the highest seedling fresh weight (505.6 mg) was obtained in the DZ17-1 genotype that was subjected to chemo-priming with 1 mM SA and grown under optimum growth conditions, while the lowest (21.6 mg) was recorded for Empire Plus genotype having chemo-priming with 0.5 mM SA and was grown under maximum salinity level of 200 mM NaCl.

The increase in salt stress resulted in significant decreases in the seedling fresh weight of both genotypes. Kizilgeci et al. (2020) reported that as the severity of salt stress increased, the seedling's fresh weight decreased. In the present study, it was observed that SA application had an improving effect on seedling fresh weight under stress conditions. Similar to our study, Tepe (2011) reported that SA application has an increasing effect on seedling fresh weight compared to control. Bahrani and Pourreza (2012), in their study investigating the effect of the different salicylic acid applications on seedling fresh weight under salinity stress, obtained the highest seedling fresh weight value in 1.5 mg L^{-1} SA application.

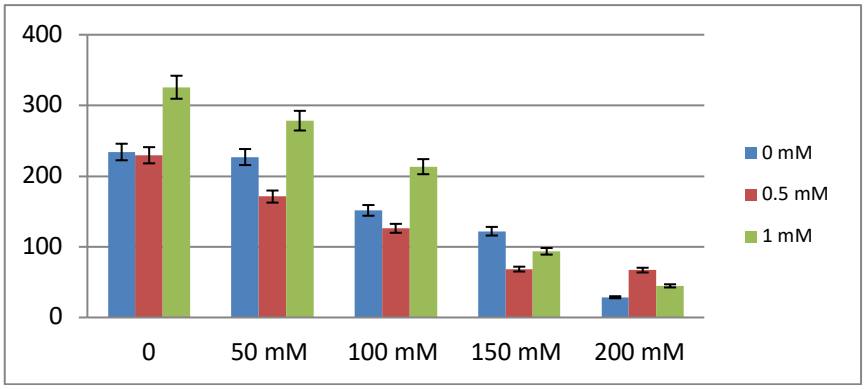

Figure 5. The effects of salicylic acid and NaCl application on the seedling fresh weight of bread wheat.

3.6. Root dry weight

Average values of root dry weight and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress are given in Table 6.

Table 6. Average values of root dry weight (mg) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	23.0	17.2	42.8	31.3	11.6
	0.5	27.5	31.8	25.9	30.5	12.8
	1	40.5	33.1	44.6	41.2	20.1
Empire Plus	0	23.1	15.0	13.2	14.5	8.1
	0.5	11.9	10.5	9.1	12.3	7.2
	1	14.4	28.0	11.8	7.3	6.9
DZ17-1 mean		30.3AB	27.4B	37.8A	34.3AB	14.8CD
Empire Plus mean		16.5C	17.8C	11.4CD	11.3CD	7.4D

Differences between means with the same lowercase and uppercase letter are not statistically significant.

The highest root dry weight value (44.6 mg) was obtained for the DZ17-1 genotype that was chemo-primed with 1 mM SA under 100 mM salinity level, while the lowest value (6.9 mg) was obtained in the Empire Plus genotype chemo-primed with 1 mM SA and exposed to 200 mM salinity level.

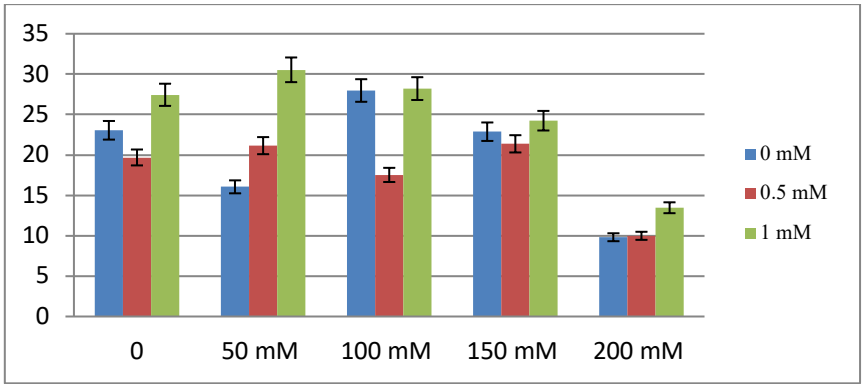

Figure 6. The effects of salicylic acid and NaCl application on the root dry weight of bread wheat.

Salt stress caused a decrease in root dry weight and these results corroborate with those of Yildirim et al. (2015) who also observed a significant decrease in root growth parameters (fresh and dry

weight) in wheat. In addition, Sourour et al. (2014) stated that the toxic effects of a saline environment may be due to the accumulation of salts in cells which disrupted numerous physiological and chemical process, and ultimately root growth was negatively affected, and ultimately significantly lowered root dry weight was recorded. In our study, it was observed that salicylic acid application had an increasing effect on root dry weight. Similarly, Karlidag et al. (2009) obtained the highest root dry weight value of 1 mM SA application in their study.

3.7. Seedling dry weight

The seedling dry weight of bread wheat genotypes as affected by seed priming of salicylic acid under varying levels of salt stress are given in Table 7.

Table 7. Average values of seedling dry weight (mg) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	43.2	38.3	75.7	32.5	9.7
	0.5	55.4	47.7	37.4	21.5	7.7
	1	62.4	50.2	60.3	31.8	36.1
Empire Plus	0	41.8	35.8	22.8	15.3	7.2
	0.5	16.8	17.4	9.9	12.2	7.4
	1	23.1	35.5	13.3	6.1	5.7
DZ17-1 mean		53.6	45.4	57.8	28.6	17.8
Empire Plus mean		27.2	29.5	15.4	11.2	6.7

Differences between means with the same lowercase and uppercase letter are not statistically significant.

The maximum seedling dry weight (62.4 mg) was obtained in the DZ17-1 genotype in the application of 1 mM SA + 0 mM NaCl, according to the general averages of the genotypes, while the lowest seedling dry weight (5.7 mg) was obtained in the Empire Plus genotype by1 mM SA application under salinity level of 200 mM NaCl.

The increasing level of salinity caused a proportionate decrease in the dry weight of the seedlings. These results are in complete agreement with those of Iyem et al. (2020) who reported that salinity significantly enhanced the dry weight of wheat owing to increased biosynthesis of antioxidants and various enzymes which ameliorated the adverse effects of salinity and ultimately seedling growth was promoted which resulted in the maximized seedling weight of wheat. The effects of SA acid application on seedling dry weight under salt stress conditions were similar. El-Tayeb (2005) reported that SA pre-treatment increased the dry weight of barley seedlings under stress conditions and Khodary (2004) reported that SA increased the fresh and dry weight of shoots of maize plants under salt stress.

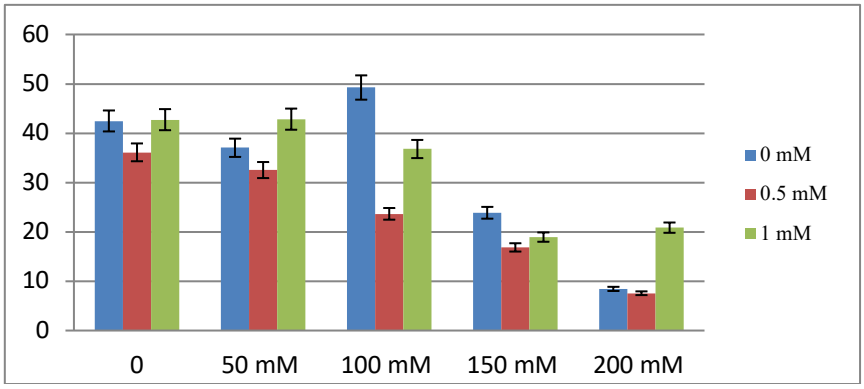

Figure 7. The effects of salicylic acid and NaCl application on the seedling dry weight of bread wheat.

3.8. Germination rate

The impact of chemo-priming with different concentrations of SA on wheat genotypes exposed to different levels salinity have been presented in Table 8.

Table 8. Average values of germination rate (%) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	76.92a	59.62b-e	65.38abc	65.38abc	69.23ab
	0.5	53.85b-f	48.08d-h	55.77b-f	51.92c-g	36.54ghi
	1	59.62b-e	51.92c-g	69.23ab	67.31abc	53.85b-f
Empire Plus	0	57.69b-f	63.46a-d	57.69b-f	57.69b-f	55.77b-f
	0.5	57.69b-f	42.31fgh	46.15e-h	53.85b-f	25.00ij
	1	61.54a-e	65.38abc	42.31fh	32.69hij	19.23j
DZ17-1 mean		63.46A	53.21BC	63.46A	61.54AB	53.21BC
Empire Plus mean		58.97AB	57.05ABC	48.72C	48.08C	33.33D

Differences between means with the same lowercase and uppercase letter are not statistically significant.

The maximum germination rate (76.92%) was obtained in the DZ17-1 genotype in the application of 0 mM SA + 0 mM NaCl, according to the general averages of the genotypes, while the lowest (19.23%) was obtained in the Empire plus genotype from the application of 1 mM SA + 200 mM NaCl.

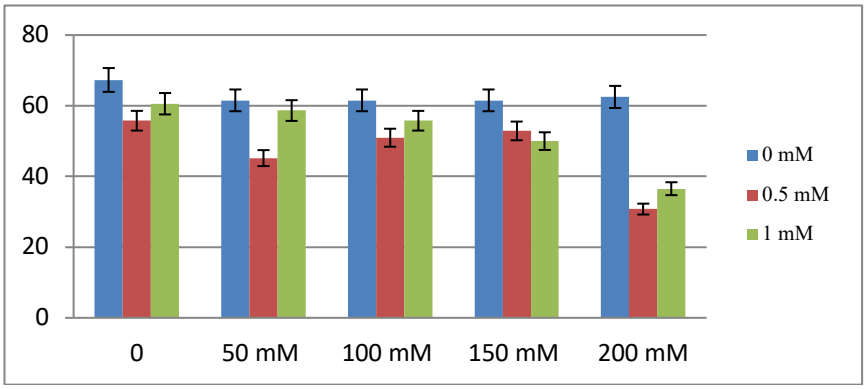

Figure 8. The effects of salicylic acid and NaCl application on the germination rate of bread wheat.

3.9. Germination vigour

Average values of germination vigour and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress are given in Table 9.

The highest germination vigour (92.31%) was obtained in DZ17-1 and Empire Plus genotypes in 0 mM SA + 0 mM NaCl application, while the lowest (36.54%) was obtained in DZ17-1 genotype from 0.5 mM SA + 200 mM NaCl application.

Variety characteristic is an important factor affecting the germination performance of the seed. In previous studies, it was reported that different wheat varieties have different germination rates (Rahman et al., 2008; Moud and Maghsoudi, 2008; Kochak-zadeh et al., 2013). Salt stress had adverse effects on the germination rate of both genotypes. It was observed that the increase in salt stress caused a decrease in the germination rate. Iyem et al. (2020) reported that the increase in salt stress negatively affects germination vigour. Furthermore, El Sabagh (2019) reported that salinity stress delays or reduces germination of wheat probably owing to delayed seed imbibition as saline environment hindered the moisture absorption by wheat seeds.

Table 9. Average values of germination vigour (%) and multiple comparisons of bread wheat genotypes subjected to salicylic acid and salt stress

Genotypes	Salicylic acid (mM)	Salt stress (mM)				
		0	50	100	150	200
DZ17-1	0	92.31	63.46	75.00	67.31	71.15
	0.5	90.38	63.46	57.69	55.77	36.54
	1	88.46	71.15	73.08	65.38	53.85
Empire Plus	0	92.31	71.15	63.46	61.54	55.77
	0.5	84.62	55.77	50.00	53.85	38.46
	1	90.38	73.08	40.38	32.69	36.55
DZ17-1 mean		90.38A	66.03B	68.59B	62.82BC	53.85CD
Empire Plus mean		89.10A	66.67B	51.28DE	49.36DE	43.59E

Differences between means with the same lowercase and uppercase letter are not statistically significant.

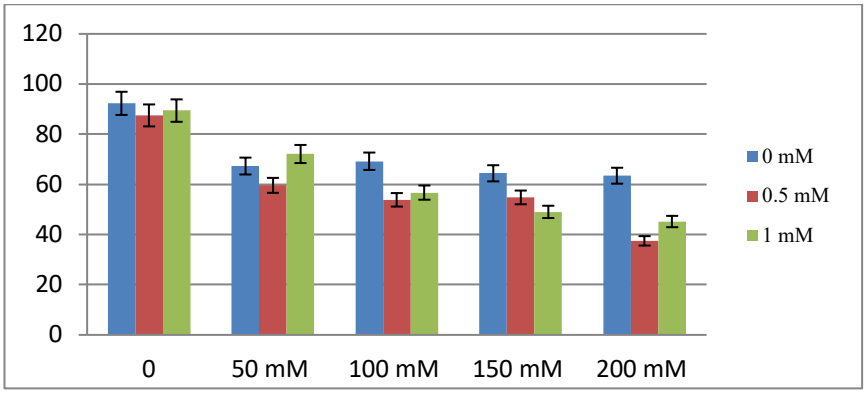

Figure 9. The effects of salicylic acid and NaCl application on germination vigour of bread wheat.

Conclusion

The research findings remained as per postulated hypothesis as varying levels of salinity had adverse effects on seed germination and seedling growth traits, while SA seed priming in varying concentrations remained effective in ameliorating the deleterious effects of saline environment. All levels of salinity had adverse effects on germination and seedling growth of wheat genotypes. It may also be inferred from results that wheat genotype DZ17-1 was salt tolerant compared to Empire Plus genotype, while lower concentration of salicylic acid (1 mM) remained effective in mitigating the adverse effects of salinity as indicated by higher germination, root growth and seedling traits. Although, results of this trial are encouraging in the sense that salt tolerant genotype and most promising SA priming concentration have been determined, but further in-depth trials entailing more genotypes and SA concentrations need field evaluation in order to formulate strategy for general adoption in Turkey and regions having similar climatic conditions.

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Research

The Effect of Humic Acid on Plant Growth, Phytoremediation and Oxidative Stress in Rapeseed (*Brassica napus* L.) Grown Under Heavy Metal Stress

Sibel BOYSAN CANAL[*1], Mehmet Ali BOZKURT[2], Hilal YILMAZ[3]

[1,2]Van YüzüncüYıl University Department of Soil Science and Plant Nutrition,Van,
[3]Kocaeli University, Izmit Vocational School, Department of Plant and Animal Production

[1]https://orcid.org/0000-0001-9027-0458, [2]https://orcid.org/0000-0003-3923-857X, [3]https://orcid.org/0000-0001-9138-3382

*Corresponding author e-mail: sibelboysan@hotmail.com

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Abstract: The aim of this study was to investigate the effects of humic acid (HA) applications on rapeseed (Brassica napus L.) growth, heavy metal uptake, bioconcentration factor (BCF), translocation factor (TF), tolerance index (TI), catalase (CAT), ascorbate peroxidase (APX) enzyme activities and hydrogen peroxide (H2O2) content in polluted soil with lead (Pb), chromium (Cr), cadmium (Cd), and zinc (Zn). Three doses of HA (Control, HA1:500 mg kg-1, HA2:1000 mg kg-1, HA3:2000 mg kg-1) were applied in pots. HA1, HA2, and HA3 applications increased plant growth parameters compared to polluted soil. Compared to the control, HA applications in polluted soil increased the Pb, Cr, Cd, and Zn concentrations in the plant. However, HA applications in polluted soil significantly decreased the heavy metal content in roots and shoots of the plant compared to polluted soil. BCF in both roots and shoots of the plants were greater than 1 for Pb, Cr, Cd, and Zn. However, specifically HA2 application decreased the shoot and root BCF values in polluted soil. TF was smaller than 1 in Pb, Cr, Cd, and Zn in polluted soil. On the other hand, HA applications for Cd increased TF values. Shoot TI decreased 17.37 %, and root TI decreased 9.09% in polluted soil. CAT and APX enzyme activities and H2O2 increased significantly in polluted soil. However, HA applications decreased CAT and APX enzyme activities and H2O2 content in rapeseed. It is concluded that HA application in Pb, Cr, Cd, and Zn polluted soil has a remedial effect on the development of rapeseed by reducing heavy metal content and oxidative stress.

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1. Introduction

The quality of life on earth and the quality of the environment depend on each other. With the increase of urbanization and the development of industry, the use of human-oriented heavy metals has increased. Heavy metals are released into the environment in significant quantities because of industrial activities such as mining, energy and fuel production, and excessive use of pesticides and fertilizer (Okcu et al., 2009). Especially in developed countries, this situation emerges as a more serious problem with each passing day. The most important effect of soil pollution in terms of environmental health is the transfer of pollutants in the soil to the human body due to plants consumed directly or as food for animals that feed on these plants (Singh and Kalamdhad, 2011). Some plants can tolerate heavy metals

that can be toxic to most organisms. Such plants are called "hyperaccumulator plants". It is reported that there are approximately 400 plant species that accumulate metal in their parts. The dominant families with this feature are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae. The Brassicaceae family is the largest family with this feature, with 11 genera and 87 species (Özbek, 2015). Hyperaccumulator plants can absorb one or more heavy metals from the soil. Hyperaccumulator plants can grow easily in exceeding levels of heavy metal contaminated soils and can accumulate metal ions in their different organs (Kürşat, 1999; Rascioa and Navari-Izzo, 2011).

Humic acid (HA) is a component of mixed organic matters formed as a by-product of a certain decomposition process of plants, animals, and microbial substances. HA is an organic compound soluble in alkaline medium and insoluble in acidic medium. Humic-based substances consist of functional groups, namely carboxyl, alcoholic and phenolic hydroxyl, carbonyl, and methoxyl groups containing oxygen (Cozzolino et al. 2016). The main heteroatoms in humic substances are oxygen. There are carboxylic (COOH), phenolic-alcoholic (OH), ketonic-quinoid (C=O), and OCH3 (ether and ester) functional groups within humic substances (Kwiatkowska-Malina, 2018). The molecular weight of HA is 50000 g/mol, and the surface area is determined as 2000 m^2/g. HA cation exchange capacity varies between 500 and 1200 me/100g (Alpay, 2013). In the presence of metals, HA has chelating properties. In other words, it can complex well with heavy metals. When HA is decomposed at high pH values, complex reactions and chelation occur between metals and HA molecules. These organic macro molecules also increase the solubility of heavy metals, which significantly affects their biological availability and transportation (Lagier et al., 2000; Alım, 2020). HA interact not only with metals but also with toxic substances in terms of environmental pollution, such as many polluting hydrocarbons, pesticides, and oil. It is asserted that HA forms a strong compound with heavy metals, reducing the stress effect of heavy metals on plant development (Özkay et al., 2016). It has been found that HA increases the intake of nutrients because of the development of the root zone (Çimrin et al., 2001; Gülser and Ayaş, 2016). Rastghalam et al., (2011) investigated the phytoextraction effect of HA in the experiment soils in which various levels of lead (Pb) were applied in their study with rapeseed plants. They reported that HA application was associated with the increase in Pb accumulation in roots and the transport to the shoots in concert with the levels of Pb applications to soils.

The toxic effects of heavy metals exert influence on both physiological and morphological characteristics of plants and cause oxidative stress. The excessive increase of reactive oxygen radicals in plant cells, which is the main cause of oxidative stress, may lead to the deterioration of metabolic functions. However, antioxidative enzymes, which are the secondary defense mechanism in plant cells, may reduce the toxic effects of oxidative stress damage in the plant. (Hamilton et al., 2012).

The present study aimed to determine the effects of applied HA levels on plant growth, heavy metal concentrations, phytoremediation properties, and antioxidative enzyme activity of rapeseed (*Brassica napus* L.) plant grown in soil polluted with Pb, Cr, Cd, and Zn. It was predicted that HA would have a healthy effect on growth and development as well as antioxidant defense mechanisms of rapeseed.

2. Material and Methods

The experiment soil was taken from the study areas of the Faculty of Agriculture of Van Yüzüncü Yıl University. This soil is characterized by low nitrogen, medium calcareous, low organic matter, alkaline pH, and without salt (Table1). For total nitrogen N, according to the Kjeldahl method, the limit values specified are % 0.045-0.090, degree: low nitrogen (Kacar, 1994). For lime ($CaCO_3$), according to the Scheibler calcimeter method, the limit values are % 5-15, degree: medium calcareous (Kacar, 1994). For O.M., according to Walkley- Black wet burning method, the limit values are % 1-2, degree: low organic matter (Müftüoğlu et al., 2014). Salt and pH were measured in 1/2.5 soil-water mixture, with the limit values for pH: >8.5, degree: alkaline, the limit values for salt: <4 dS m^{-1}, degree: without salt (Müftüoğlu et al., 2014). DTPA-Fe, Zn, Mn are at low level DTPA-Cu is at sufficient level (Müftüoğlu et al., 2014). Total Cd, Pb, Zn, and Cr are under toxic levels in the soil. The toxic level for Cd is 1 mg kg^{-1}. Toxic levels for Pb and Cr are100 mg kg^{-1} Toxic level for Zn is 150 mg kg^{-1} (Schachtschabel et al., 1993).

Table1. Experimental soil and humic acid properties

	Experimental Soil	Humic Acid
Texture	Sandy Loam	
pH (1/2.5)	8.15	8-10
Salt, dS m^{-1}	0.35	
Lime, %	6.6	
Organic Material, %	1.02	25
Total N, %	0.056	
Total humic acid+fulvic acid %		65
Extractable with DTPA		
mg kg^{-1}		
Fe	0.90	
Cu	1.40	
Mn	1.24	
Cr	0.06	
Zn	0.60	
Cd	0.08	
Pb	0.30	
Total heavy metal		
mg kg^{-1}		
Zn	45.13	
Cd	0.65	
Pb	9.03	
Cr	95.0	

2.1. Pot experiment

Using the method used by Turan and Esringü (2007) for potting experiments. In the experiment, heavy metals in doses of 100 mg kg^{-1} Cr as chromium nitrate ($Cr(NO_3)_3$), 100 mg kg^{-1} Cd as cadmium sulphate ($CdSO_4.8H_2O$), and 100 mg kg^{-1} Pb as lead nitrate ($Pb(NO_3)_2$ and 250 mg kg^{-1} Zn as zinc sulphate ($ZnSO_4.7H_2O$) were applied to the pots that each was 2.5 kg. The soil polluted with heavy metals added to the potting soil as the liquid was left to incubate for a month. Then, the pot experiment was carried out in the growth chamber. Eight of the rapeseed seeds were planted in each pot. Thinning was done immediately after germination so that three plants were left in each pot. The temperature was adjusted to 20 ± 2°C since rapeseed is a cool weather crop. Chemical fertilizers of 80 mg kg^{-1} phosphorus (P) as triple superphosphate (TSP), 200 mg kg^{-1} nitrogen (N) as ammonium nitrate (NH_4NO_3), and 50 mg kg^{-1} potassium (K) as potassium sulphate (K_2SO_4) were added. A completely randomized experimental design with the three-replication trial was implemented in the trial. Five applications of HA were as follows follows: 1-Control, 2- Polluted soil (PS) with heavy metals, 3- PS+HA_1 (500 mg kg^{-1}), 4- PS +HA_2 (1000 mg kg^{-1}), 5- PS +HA_3 (2000 mg kg^{-1}).

2.2. Chemical and physical analysis of soil

Soil samples were taken from a depth of 0-30 cm. Soil samples were air-dried in a shaded area and passed through a 2 mm sieve. Before filling the pots, the soil was mixed with a liquid-heavy metal mixture. Then the soil was left to incubate for one month. In the soil samples taken before and after the plant harvest, respectively, the soil texture was determined by the Bouyoucos hydrometer method (Bouyoucos, 1951). The pH was measured using in 1:2.5 soil-water mixture (Jackson, 1958). Lime content was determined using Scheibler calcimeter (Hızalan and Ünal, 1966). Soil organic matter was determined using the Walkley Black method (Walkley, 1947). Total N was measured using the Kjeldahl method (Kacar, 1994). The extractable Pb, Cr, Cd and Zn concentrations were determined by the DTPA

method (Lindsay and Norvell, 1978). The total Pb, Cr, Cd, and Zn in the soil were determined according to Khan and Frankland (1983).

2.3. Phytoremediation parameters

Bioconcentration factor (BCF), Translocation factor (TF), and Tolerance Index (TI) parameters were calculated based on studies on phytoremediation. BCF and TF (Esringü et al. 2014) were calculated. TI was calculated (Çifçi, 2020) as follows:

$$BCF = \frac{[\text{(Metal concentration in plant tissue (root or shoot), mg kg}^{-1})]}{[\text{DTPA concentration of soil mg kg}^{-1)}]}$$

$$TF = \frac{[\text{(Metal concentration in the shoots, mg kg}^{-1})]}{[\text{(Metal concentration in the roots, mg kg}^{-1})]}.$$

$$TI\ (\%) = \frac{[\text{(Metal Applied Plant Growth Parameters)}]}{[\text{(Control Plant Growth Parameters)}]} \times 100$$

2.4. Antioxidative enzymes, H_2O_2, and heavy metal analyses in plant

Enzymatic measurements were carried out at 0-4^{0}C. The supernatant was used as a crude enzyme extract for CAT enzyme analyzes. CAT (EC 1.11.1.6) activity was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Çakmak et al., 1993). APX (EC 1.11.1.11) activity was determined following the decrease of ascorbate by measuring the change in absorbance at 290 nm for 1 min (Nakano and Asada, 1981). Leaf sample (0.25 g) was homogenized in 2.5 ml of 1 % TCA. 1 ml, 10 mM KH_2PO_4 (pH=7) phosphate buffer, and 1 ml, 1 M KI (Potassium iodide) were added on 0.5 ml supernatant. The mixture of absorbance was determined at 390 nm (Velikova et al., 2000). Dried plant shoot and root samples were digested with a mixture of HNO_3-$HClO_4$ acids and analyzed for the concentration of Pb, Cd, Cr, and Zn (İbrikçi et al., 1994).

2.5. Statistical analysis

The descriptive statistics were computed for experiment soil and humic acid properties. One-way analysis of variance was performed to compare growth parameters, heavy metal content in the plant, phytoremediation parameters, and antioxidative activity. Significant differences among applications were determined by Duncan's Multiple Range Tests using the 13.0 SPSS software package (Düzgüneş et al., 1987).

3. Results

3.1. Plant growth

The effect of HA applications on growth parameters is given in Table 2 as variance analysis results. The HA applications were statistically significant at all growth parameters ($P < 0.01$). In the study conducted with rapeseed plants, a significant decrease was found in plant height, plant fresh weight, plant dry weight, leaf number, root length, root fresh, and dry weight of rapeseed grown in PS application compared to the control application. However, PS+HA_1, PS+HA_2, and PS+HA_3 caused a greater increase in shoot length, shoot fresh and dry weight, the number of leaves, root length, and root fresh and dry weight compared to the PS application (Table 3).

Table 2. The results of variance analysis on the effects of HA applications on the growth parameters in polluted soil

Variation Source	F(*value*)	P(*significant*)
Shoot length	72.38	0.000
Shoot fresh weight	53.63	0.000
Shoot dry weight	47.76	0.000
Leaf of number	15.38	0.000
Root length	16.37	0.000
Root fresh weight	55.31	0.000
Root dry weight	32.00	0.000

Table 3. The Effect of HA applications on the growth parameters of the rapeseed plants in soil polluted with Pb, Cd, Cr, and Zn

Applications	Shoot **length (cm)**	Shoot fresh weight**(g pot^{-1})**	Shoot dry weight **(g pot^{-1})**	Leaf of number **(per plant^{-1})**	Root **length (cm)**	Root fresh weight **(g pot^{-1})**	Root dry weight **(g pot^{-1})**
Control	22.34 ± 2.51**a***	9.49 ±2.31**a**	0.90 ±0.35**a**	5.61±0.61**a**	10.61±1.9 **a**	0.57±0.16**a**	0.087± 0.04**a**
PS	7.70 ±0.93**c**	1.58 ± 0.15**d**	0.04± 0.01**d**	3.44 ±1.38**b**	4.76±2.06 **d**	0.048± 0.02**c**	0.009± 0.01**c**
PS + HA$_1$	17.70 ± 2.62**b**	4.45±0.58**c**	0.41 ±0.05 **c**	6.17±1.47 **a**	7.22± 3.40**c**	0.19±0.13**b**	0.033± 0.02**b**
PS + HA$_2$	17.19 ±2.18**b**	4.66±1.74**bc**	0.48±0.05**bc**	6.17±1.47**a**	8.76±1.83 **b**	0.21±0.08 **b**	0.032 ±0.02**b**
PS + HA$_3$	18.31±4.18**b**	6.09 ±2.28 **b**	0.61±0.25 **b**	5.78±1.06**a**	8.90± 1.87**b**	0.24±0.10**b**	0.034 ±0.02**b**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn (p<0.05), Polluted Soil:PS.

3.2. Effects of HA applications on heavy metal content in shoot and root of rapeseed (*Brassica napus* L) plant

The effect of HA applications on heavy metal content in shoot and root of rapeseed plants are given in Table 4 as variance analysis results. The HA applications were statistically significant at heavy metal content of shoot and root in the plant (P <0.01). The highest concentrations of Pb, Cr, Cd, and Zn were determined in PS application in both roots and shoot parts of the rapeseed plant. However, PS+HA$_1$, PS+HA$_2$, PS+HA$_3$ applications caused a statistically significant decrease in Pb, Cr, Cd, and Zn contents in both roots and shoots of rapeseed compared to the PS application (Table 5 and Figure 1,2).

Table 4. The results of variance analysis on the effects of HA applications on heavy metal content in polluted soil

Variation Source	F(*value*)	P(*significant*)
Shoot(Pb)	157	0.000
Root(Pb)	784	0.000
Shoot(Cr)	692	0.000
Root(Cr)	200	0.000
Shoot(Cd)	1020	0.000
Root(Cd)	71.41	0.000
Shoot(Zn)	1134	0.000
Root(Zn)	120	0.000

Table 5. The Effect of HA applications on the heavy metal contents in the shoots and the roots of the rapeseed plants in soil polluted with Pb, Cd, Cr, and Zn (mg kg-1)

Applications	Pb		Cr		Cd		Zn	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	3.81± 0.41**c***	27.20±4.78**d**	0.66 ±0.11**d**	10.70± 1.19**d**	0.22±0.04**d**	1.73±0.04**d**	10.43±0.84 e	13.01±0.88**e**
PS	113± 18.69**a**	280 ±8.70**a**	14.57±1.0**a**	140± 8.02**a**	112±3.21**a**	439± 71.64**a**	117± 3.91**a**	263±26.09**a**
PS + HA$_1$	9.38±0.69**b**	174±9.27**b**	3.05± 0.19**b**	74.02±12.58**b**	103±3.01**b**	278 ±40.06**b**	72.51±1.96**b**	236±33.**b**
PS + HA$_2$	8.90± 0.27**b**	141±3.96**c**	1.88 ±0.20**c**	40.57±4.32**c**	73.92±4.43**d**	135 ±17.62**c**	67.59±2.82**c**	146±16.0**d**
PS + HA$_3$	8.93±0.35**b**	150 ±7.67**c**	2.28 ±0.25**c**	69.65±6.67**b**	84.07±3.00**c**	258±48.48**b**	56.29±2.02**d**	205± 4.32**c**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn (p<0.05), Polluted Soil: PS.

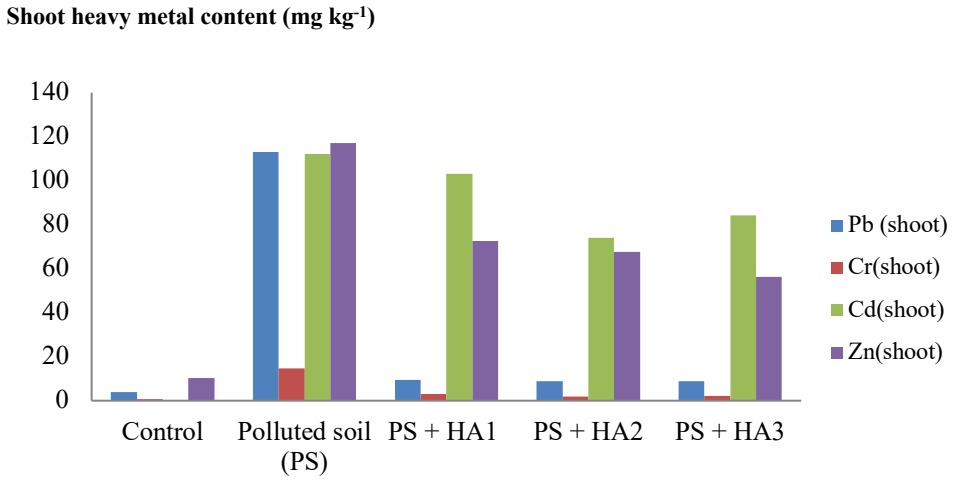

Figure 1. The effect of HA applications on the heavy metal contents in shoots of rapeseed plants in polluted soil.

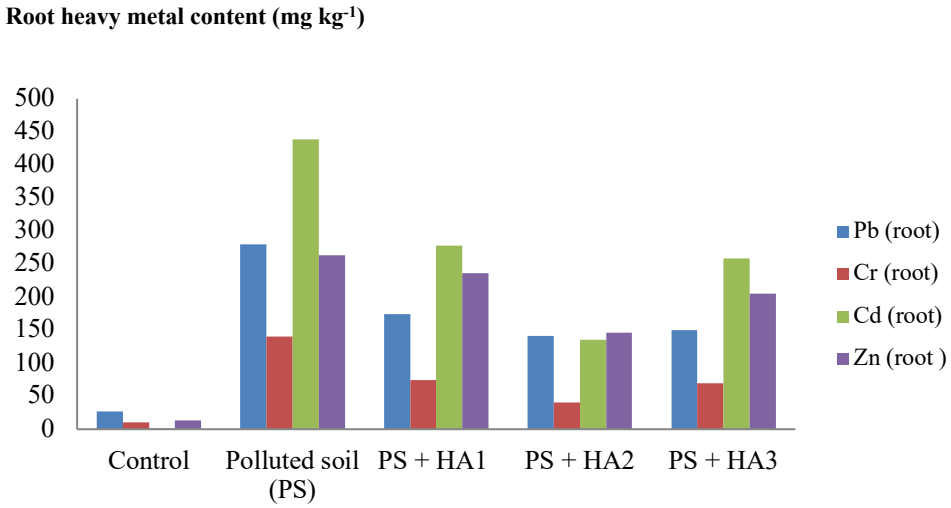

Figure 2. The effect of HA applications on heavy metal content in the root of rapeseed in polluted soil.

3.3. The Effects of HA applications on phytoremediation parameters (BCF, TF, and TI %) of rapeseed (*Brassica napus* L) plant

The effects of HA applications on phytoremediation parameters of rapeseed plants are given in Table 6 as variance analysis results. The HA applications were statistically significant at all phytoremediation parameters (P <0.01**).** In the present study conducted with rapeseed plants, shoot and root BCF values were higher than 1 for Pb, Cr, Cd, and Zn. Nevertheless, PS+HA$_1$, PS+HA$_2$, and

PS+HA_3 applications caused a decrease in the shoot BCF values for Pb, Cr, Cd, and Zn compared to the PS application. PS+HA_1 and PS+HA_2 applications caused a decrease in the root BCF values for Pb, Cr, Cd and Zn compared to the PS application. TF values were lower than 1 for Pb, Cr, Cd, and Zn. However, PS+HA_1, and PS+HA_2 applications for Cd caused an increase in the TF values compared to the PS application (Table 7). In this case, it was determined that both HA applications decreased Pb, Cr, Cd, and Zn accumulation and increased the transport of Cd to the upper organs in the rapeseed plant. While shoot and root TI values were 100 % in the control application, shoot and root TI values were 17.37 % and 9.09 % in PS application. In comparison to PS applications, PS+HA_1, PS+HA_2, and PS+HA_3 applications caused increases in the shoot and root TI values (Table 8).

Table 6. The results of variance analysis on the effects of HA applications on phytoremediation parameters in polluted soil

Variation Source	F(*value*)	P(*significant*)
$BCF_{(shoot)}$ Pb	86.14	0.000
$BCF_{(shoot)}$ Cr	689	0.000
$BCF_{(shoot)}$ Cd	125	0.000
$BCF_{(shoot)}$ Zn	285	0.000
$BCF_{(root)}$ Pb	343	0.000
$BCF_{(root)}$ Cr	56.14	0.000
$BCF_{(root)}$ Cd	24.65	0.000
$BCF_{(root)}$ Zn	23.93	0.000
TLF (Pb)	107	0.000
TLF (Cr)	34.73	0.000
TLF (Cd)	39.03	0.000
TLF (Zn)	21.86	0.000
TI $_{(shoot)}$	43.21	0.000
TI $_{(root)}$	60,626	0.000

Table 7. The Effect of HA applications on the phytoremediation parameters (BCF and TF) of the rapeseed plants in soil polluted with Pb, Cr, Cd, and Zn

	Pb	**Cr**	**Cd**	**Zn**
Applications	**BCF_{shoot}**	**BCF $_{shoot}$**	**BCF $_{shoot}$**	**BCF $_{shoot}$**
Control	0.78± 0.26**b***	7.49±0.33**d**	1.84±0.33**c**	14.95±1.28**a**
PS	3.26±0.59**a**	77.62± 3.81**a**	4.29±0.11**a**	6.66± 0.18**b**
PS + HA_1	0.41±0.01**c**	22.45±0.93**b**	4.22±0.12**a**	4.38± 0.13**c**
PS + HA_2	0.42± 0.01**c**	13.51±1.86**c**	3.25±0.29**b**	4.55±0.41**c**
PS + HA_3	0.76±0.04**b**	21.57± 3.13**b**	4.47±0.15**a**	4.09± 0.04**c**
	Pb	**Cr**	**Cd**	**Zn**
Applications	**BCF(root)**	**BCF (root)**	**BCF (root)**	**BCF (root)**
Control	2.66±0.31**d***	160±17.36**d**	9.39±0.12**b**	18.21±0.67**a**
PS	8.10±0.52**b**	748±96.55**a**	16.75±2.80**a**	15.04±1.55**b**
PS + HA_1	7.61±0.43**b**	547±109**b**	11.42±1.55**bc**	14.30±2.10**b**
PS + HA_2	6.70±0.34**c**	289±17.28**c**	5.92±0.68**d**	9.81±1.37**c**
PS + HA_3	13.63±0.51**a**	659±74.01**a**	13.70±2.55**b**	14.91±0.57**b**
	Pb	**Cr**	**Cd**	**Zn**
Applications	**TF**	**TF**	**TF**	**TF**
Control	0.14±0.02**b***	0.062±0.02**b**	0.13±0.02**c**	0.80±0.08**a**
PS	0.41±0.07**a**	0.104±0.01**a**	0.26±0.05**c**	0.45±0.05**b**
PS + HA_1	0.054±0.001**b**	0.043±0.01**c**	0.37± 0.05**b**	0.18±0.04**c**
PS + HA_2	0.064±0.001**b**	0.047±0.01**c**	0.56±0.08**a**	0.47±0.05**b**
PS + HA_3	0.058±0.001**b**	0.033±0.01**c**	0.34±0.06**bc**	0.28±0.01**c**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn ($p<0.05$), Polluted Soil: PS.

Table 8. The Effects of HA applications on phytoremediation parameter (TI) of rapeseed in soil polluted with Pb, Cr, Cd, and Zn (%)

Applications	TI (shoot)	TI (root)
Control	100±0.001**a***	100±0.001 **a***
PS	17.37±3.73**d**	9.09±4.72 **c**
PS + HA_1	49.21±12.92**c**	37.92±25.21 **b**
PS + HA_2	52.80±25.89**c**	40.58±21.24**b**
PS + HA_3	68.15±32.16**b**	44.19±21.24**b**

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$), TI: Calculated according to the fresh weight of the plant. Polluted Soil: PS

3.4. The Effects of HA applications on antioxidant activity of rapeseed (*Brassica napus* L) plant

The effects of HA applications on the antioxidant activity of rapeseed plants are given in Table 9 as variance analysis results. The HA applications were statistically significant at CAT, APX enzyme activities, and H_2O_2 content ($P<0.01$). In the study conducted with rapeseed plants, the CAT and APX enzyme activities from the antioxidative enzymes and H_2O_2 content in PS application were higher than in the control application. However, PS+HA_1, PS+HA_2, and PS+HA_3 applications caused a decrease in CAT and APX enzyme activities and H_2O_2 content compared to the PS application (Table 10). Accordingly, the application of HA to the heavy metal contaminated soils resulted in a decrease in oxidative stress.

Table 9. The results of variance analysis on the effects of HA applications on antioxidant activity of rapeseed in polluted soil

Variation Source	F(*value*)	P(*significant*)
CAT	90,971	0.000
APX	49,465	0.000
H_2O_2	92,801	0.000

Table 10. The Effects of HA applications on antioxidative activity in rapeseed in soil polluted with Pb, Cr, Cd, and Zn

Applications	CAT(mmolg^{-1}FWMin^{-1})	APX(mmolg^{-1}FW Min^{-1})	H_2O_2(µmol g^{-1} FW)
Control	0.0088±0.001**d***	3.03±0.33**b**	1.58±0.14**c**
PS	0.044±0.001**a**	8.43±1.85**a**	9.49±1.31**a**
PS + HA_1	0.026±0.001**b**	1.97±0.84**c**	3.91±0.47**b**
PS + HA_2	0.031±0.001**b**	2.06±0.21**b**	4.00±0.10**b**
PS + HA_3	0.024±0.001**b**	2.83±0.38**b**	4.15±0.88**b**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn ($p<0.05$), Polluted Soil: PS.

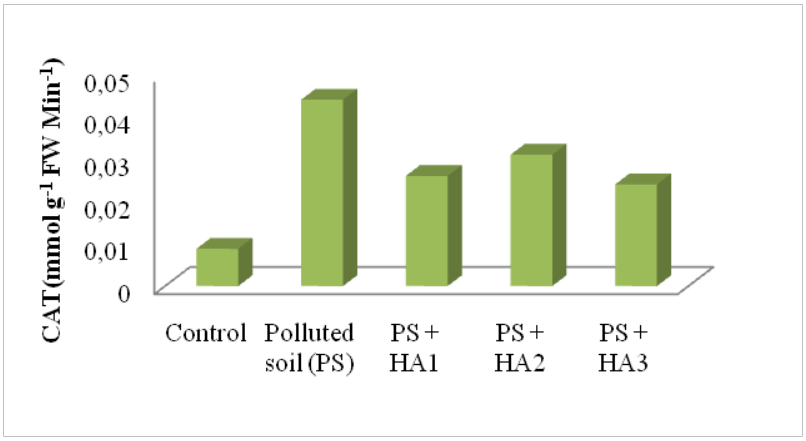

Figure 3. The effects of HA applications on CAT enzyme activities in the rapeseed plants in polluted soil.

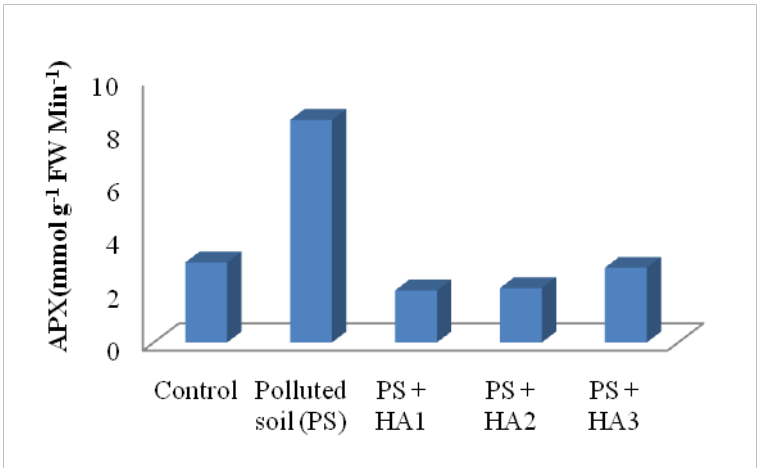

Figure 4. The effects of HA applications on APX enzyme activities in the rapeseed plants in polluted soil.

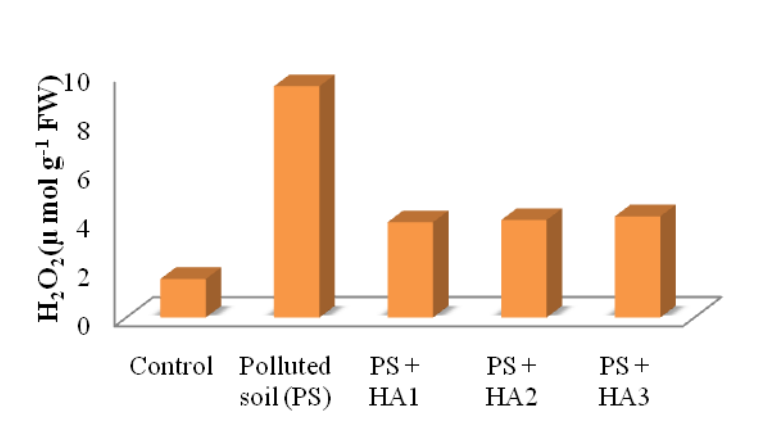

Figure 5. The effects of HA applications on H_2O_2 contents in the rapeseed plants in polluted soil.

According to all these results, HA applications increased some growth characteristics by reducing the inhibitory effects of soil contaminated with Cd, Cr, Pb, and Zn. In addition, HA decreased the effect of oxidative stress due to the decrease of heavy metal concentration in the plant.

4. Discussion

As a part of humus, when HA is added to the soil, it affects the availability of heavy metals in the soil to the plant. This is because it has hydroxyl, carboxylic (COOH), phenolic-alcoholic (OH), ketonic-quinoid (C=O), and OCH3 (ether and ester) functional groups (Nardi et al., 2009). HA affects heavy metal uptake by plants. The addition of HA increases the amount of soil organic matter that changes soil properties, cation exchange capacity, and pH. This change affects the mobility of heavy metals in the soil and affects their uptake by plants (Xu et al., 2018). In the present study carried out with rapeseed plants, humic levels applied to soil contaminated with Pb, Cr, Cd, and Zn caused increases in shoot length**,** shoot fresh and dry weight, leaf number, root length, root fresh and dry weight (Table 3). HA application to soil polluted with Pb, Cr, Cd, and Zn caused decreases in heavy metal content in both root and shoot parts of the rapeseed plants compared to alone polluted soil (Table 5). HA application has been found to be associated with cadmium uptake in tobacco plants when applied to Cd in soil (Evangelou, 2004). Fulvic acid is another component of the humus. Fulvic acid application, either foliar or non-foliar, inhibited Cr uptake in the wheat plant, causing an increase in the plant's biomass and photosynthetic pigments (Ali et al., 2015; Ali et al., 2018).

Shoot and root BCF values in rapeseed plants, which is one of the hyperaccumulator plants, were found as follows: Cr > Zn > Cd>Pb in Pb, Cr, Cd, and Zn polluted soils. Shoot and root BCF values

were greater than 1. Zayed et al. (1998) suggested that metal-accumulating plants should have shoot and root BCF values were greater than 1. However, in the present study, it was determined that HA applications caused a decrease in shoot and root BCF values in rapeseed. TF values were lower than 1 for Pb, Cr, Cd, and Zn. This indicates that rapeseed accumulates heavy metals mostly in the roots, and their transport to the upper organs is low. However, HA applications for Cd caused an increase in the TF values. In a similar study, Pandey (2013) found BCF >1 and TF<1 in the roots of wild castor oil plants. In the present study, the TF values were determined as Zn > Cd > Pb > Cr. In another study, Turan and Esringü (2007) determined TF values in *Brassica Napus* L. as Zn > Cu > Cd > Pb in heavy metal contaminated soils. In our study shoot, TI decreased by 17.37 %, and root TI decreased by 9.09 % in soil polluted with Pb, Cr, Cd, and Zn of the rapeseed plant compared to the control application**.** However, HA application caused an increase in shoot and root TI values (Table 8). In another study conducted with wild castor oil plants in multi-contaminated soil, the TI was lower than the application of EDDS and zeolite to the soil, but the application of nano zeolite caused an increase in TI (Çiftçi, 2020). Similarly, biochar and hydrothermally treated coal gangue (HTCG) applied to rapeseed plant in copper mine tailings resulted in the lowest transfer rate (TR), the lowest root and shoot BCF values, and the lowest TF for Cu, Cd, Cr, Ni, Pb, and Zn compared to the application of (HTCG) together. In the improvement study by rapeseed in the copper mine, it was stated that the combined application of biochar and hydrothermally treated coal gangue reduces the bioavailability of heavy metals (Munir et al., 2020).

In the current study, the CAT and APX activities of the antioxidative enzymes and the H_2O_2 content as a free radical formed as a result of oxidative stress increased significantly in the rapeseed plant compared to the control. However, in comparison to polluted soil with heavy metals, PS+HA_1, PS+HA_2, and PS+HA_3 applications caused a significant decrease in CAT and APX enzyme activities as well as H_2O_2 content (Table 10 and Figures 3,4,5). Antioxidative enzymes are released to reduce the effects of radicals formed in response to oxidative stress. Another building block of humus is fulvic acid. In another study, wastewater application increased the Cr concentration in the wheat plant. On the other hand, fulvic acid application caused a decrease in H_2O_2 levels caused by Cr toxicity in the wheat plant (Ali et al., 2015; Ali et al., 2018). Garcia et al. (2016) stated that HA promotes antioxidative enzyme activity against radicals under heavy metal stress conditions. An aquaculture study conducted by Dobbss et al. (2018) showed that HA application reduces iron uptake under high iron toxicity conditions. However, when HA was treated together with iron, a decrease in antioxidant enzymes was detected compared to the treatments separately.

5. Conclusion

HA has a significant effect on the mobility of heavy metals when applied to soil polluted with heavy metals. This situation significantly affects the heavy metal content of the plant. HA can increase plant development and growth by affecting the soil properties in the growth environment and by influencing plant nutrient availability. We concluded that HA had a positive effect on rapeseed growth, HA has hyperaccumulator properties in soil contaminated with multiple heavy metals thereby reducing the heavy metal uptake and oxidative stress in the plant. HA can increase phytoremediation in polluted soils as it improves plant growth and oxidative stress due to its organic nature.

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Research Article

Morphometric Properties Comparison of Some Turkish and Foreign Apricot Variety Grown at High Altitude

Ferhad MURADOĞLU[*1], Utku KAYAKESER [2], İbrahim BAŞAK[3]

[1]Department of Horticultural, Faculty of Agriculture, Bolu Abant İzzet Baysal University, Turkey
[2]Department of Horticultural, Faculty of Agriculture, Van Yüzüncü Yıl University Turkey
[3]Agriculture and Rural Development Agency, Van Provincial Coordination Unit, Turkey

[1]https://orcid.org/0000-0001-6595-7100, [2]https://orcid.org/0000-0002-1907-673X, [3]https://orcid.org/0000-0003-5160-3229

*Corresponding author e-mail: muradogluf@ibu.edu.tr.

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Correlation matrix,
Fruit quality,
Principal component analysis

Abstract: Apricots are becoming more preferred because of their usage in the fresh, dried, and processing industries and are appreciated by consumers for their pleasant flavor, aroma, and high nutritional value. Apricot cultivation is mostly performed by native varieties in Turkey, and there is insufficient knowledge about the characteristics of foreign varieties. In this study, important Turkish and foreign varieties were evaluated according to their morphological properties using multivariate analyses. The highest fruit weight was detected as 31.90 g (Sakıt-2) in the Turkish varieties and detected 22.36 g (Precoce de Colomer) in the foreign varieties. The highest fruit height, thickness, stone height, and weight were detected in 'Alyanak' and 'Sakıt-2' the Turkish apricot varieties. The 'Soğancı' and 'Sakıt-2' were characterized by the highest stone thickness, pH, fruit height, and weight in Turkish varieties, whereas 'Precoce de Tyrinthe' had the highest total soluble solids in the foreign varieties. The correlation analysis demonstrated significant positive correlations between examined features in Turkish and foreign varieties. In the principal component analysis, the first five components elucidated 93.59% of the total variance. Examined traits were separated into three groups, and 'Sakıt-2', 'Alyanak', 'Hasanbey', and 'Hacihaliloğlu' at the Turkish varieties were placed in the first two groups and characterized by fruit and stone traits, while the foreign varieties formed the other group and were characterize by pH, TSS, and colorimetric traits. The study put forward useful information for the comparison of morphometric traits between Turkish and foreign varieties, and the results can be used in future apricot breeding programs.

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1. Introduction

The market value of fruits is related to their quality characteristics, and pomological traits are basic commercial attention, while they are closely related to the yield of regular high fruit sets, and low drops (Balta et al., 2007; Muradoğlu et al., 2007). Costumers' acceptance is determined by fruit color, visual characteristics, firmness, maturity index (Total Soluble Solids/Titratable Acidity), appealing smell, taste, and eating quality for fresh consumption (Ruiz and Egea, 2008; Krška et al., 2009; Mikulic-

Petkovsek et al., 2016; Suszek et al., 2017). Additionally, the apricot fruit is fantastically approved by consumers due to its richness in nutrients, vitamins, minerals, fibers, sugars, and bioactive phytochemicals (Karabulut, et al., 2007; Leccese et al., 2011; Ali et al., 2011; Kan et al 2014; Fan et al., 2017).

Apricot (*P. armeniaca* L.) kernel is a rich source of protein, fiber, oil, fatty acids, carotenoids, phenolics, antioxidants, and several minerals such as K, Ca, and Mg (Özcan, 2000; Silem et al., 2006; Şeker et al., 2009; Muradoğlu et al. 2011). Because apricot is a climacteric fruit, it cannot be stored for a long time as fresh. While it is mostly consumed fresh, different preservation methods are used to prolong shelf life, such as dried, marmalade, juice, jam, puree, freezing, extrusion products, or packing in a controlled atmosphere (Yıldız, 1994; Haciseferogullari et al., 2007). The main reason for the preference for the apricot fruit is its high flavor, aroma, and sugar content which makes it one of the most popular fruits in the world. The greatest amount of cultivation of apricot is in Mediterranean countries such as Turkey, Spain, Italy, France, and Greece (Greger and Schieberle, 2007). Turkey is the leading country in the world's apricot production, with 833.398 tons. Following Turkey, Uzbekistan, Iran, İtaly, Algeria, and Spain are the main apricot producer countries (FAO, 2020). The apricot tree greatly adapted to Anatolia and many important cultivars such as Kabaaşı, Hasanbey, Hacıhaliloğlu, Soğancı, and Sakıt-2 have been economically cultivated since the centuries (Akın, et al., 2008; Hacıseferoğulları, et al 2007; Karataş and Songül, 2020). Fruit quality is strongly affected by genotypes or cultivars and environmental conditions. Therefore, fruit properties and quality may considerably change among different varieties or the same varieties cultivated in different regions.

Many studies were performed on the characterization of some apricot genotypes or cultivars. But, to our knowledge, there have been no comparative studies on morphometric properties of the Turkish and foreign apricot varieties grown under the same ecological conditions or orchards. With the present study, we aim to compare some Turkish and foreign apricot varieties cultivated in the same orchard based on their fruit, stone, colorimetric, and chemical properties. Additionally, multivariate analyses were used first time in the comparison of Turkish and foreign apricot varieties.

2. Material and Methods

2.1. Plant Material

The ten Apricot varieties which six Turkish varieties ('Alyanak', 'Hacıhaliloğlu', 'Hasanbey', 'Kabaaşı', 'Sakıt-2', and 'Soğancı') and four foreign varieties ('Bebeco', 'Paviot', 'Precoce de Colomer', and 'Precoce de Tyrinthe') were collected from collection orchards of Yuzuncu Yıl University in Van province (Figure 1) at the commercial ripening period and the morphometric traits were evaluated through two consecutive years (2013 and 2014). The cultivars grown at 1730 m altitude are fifteen years old and grafted on 'Zerdali' (wild apricot) rootstock. Orchard was regularly fertigated, and pest and disease control were performed properly. No nutritional deficiency was observed on trees, and all cultural practices were done properly during the study.

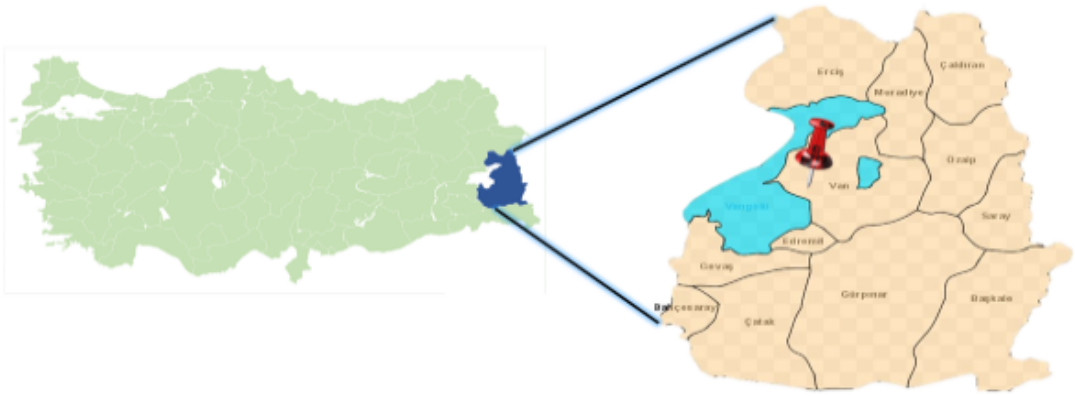

Figure 1: The location of the collection orchard and sampling region.

2.2. Method

About 40 similar fruits were harvested uniformly from five different trees. The fruit quality traits: fruit width (FWD), fruit thickness (FT), fruit height (FH), stone width (SWD), stone thickness (ST), and stone height (SH) were measured using a caliper with an accuracy of 0.1 mm. Fruit weight (FWT) and stone weight (SW) were measured with an electronic scale that has a 0.01 g precision. The color parameters (L*, a*, b* Chrome*, and Hue^{o}) were determined by using a hand-type colorimeter (Minolta Co., model CR-400, Tokyo, Japan). pH was determined by a table-type pH meter (Thermo Science, Orion star A 111). Total soluble solids (TSS) were determined using a hand refractometer (ATC, NTRM01). TA was measured by titration method by adding 0.1 N NaOH to fruit juice (5 ml) until the final pH reaches 8.1. (Ruiz et al., 2008; Muradoğlu et al., 2011)

2.3. Climate Description of Research Area

Climatic data belonging to the research area, as shown in Figure 2, obtained from the Turkish State Meteorological Service are presented as the monthly average of two years (2013 and 2014). The range of monthly minimum, maximum, and average temperatures were 7.6-14.7 °C, 1.9-28.0 °C, and -3.4 °C - 22.2 °C, respectively. The minimum, maximum, and average temperatures during the vegetative period (from March to November) ranged between -2.7-5.7 °C, 6.6-17.1 °C, and 1.4-10.7°C, respectively. January and February were characterized by the lowest temperatures, whereas July and August had the highest temperatures. The monthly precipitation varied between 3.4 and 56.0 kg/m^2. The highest precipitation occurred in the winter months, and the summer months were the lowest in the research area.

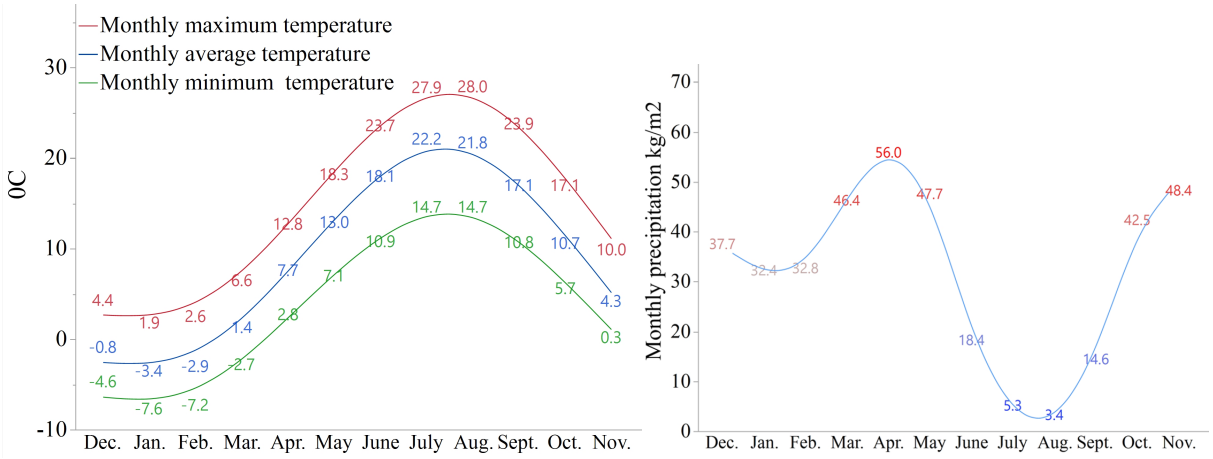

Figure 2. Climatic data belong to the research area.

2.4 Statistical Analysis

The average data from two years (2013-2014) were subjected to analysis of variance using SPSS 23.0 statistical software. Data were presented as the mean and standard error. One-way ANOVA and Duncan multiple range tests were used to compare the varieties, and the statistical significance level was taken as 5% in the calculations. Moreover, the correlations matrix was determined by Pearson's correlation analysis using R Studio software with the package 'Corrplot' (Wei et al., 2017) and the cluster dendrogram was created according to Ward's method. The principal component analysis (PCA) was performed to clarify the relationships of features with each other and varieties using R Studio software with the package of 'ggplot2' (Wickham, 2016).

3. Results and Discussions

The sixteen morphological characters that average for two years (2013-2014) belong to Turkish and foreign apricot varieties were determined. Morphological characters include fruit (width, thickness, height, and weight), stone (width, thickness, height, and weight), color (lightness, green/redness,

blueness/yellowness, chroma, lightness's angle), total soluble solids, pH, and titratable acidity were evaluated.

3.1. Morphometric Traits of Turkish and Foreign Varieties

The apricot fruit is also consumed fresh, and consumers primarily prefer the fruit in terms of visual appearance, and minorly defected or low-quality fruits could be used for processing. Therefore, fruit dimensions are an important description factor of fruit quality and are one of the crucial criteria for consumers' preferences. The fruit dimensions, FWD, FT, FH, and FWT, were detected between 27.29-39.42 mm, 31.72-39.45 mm, 24.34-38.42 mm, and 10.68-31.9 g, respectively in the Turkish varieties, while they ranged from 29.06 to 35.95 mm, 34.01 to 35.28 mm, 30.68 to 33.9 mm, and 19.42 to 22.36 g, respectively in the foreign varieties.

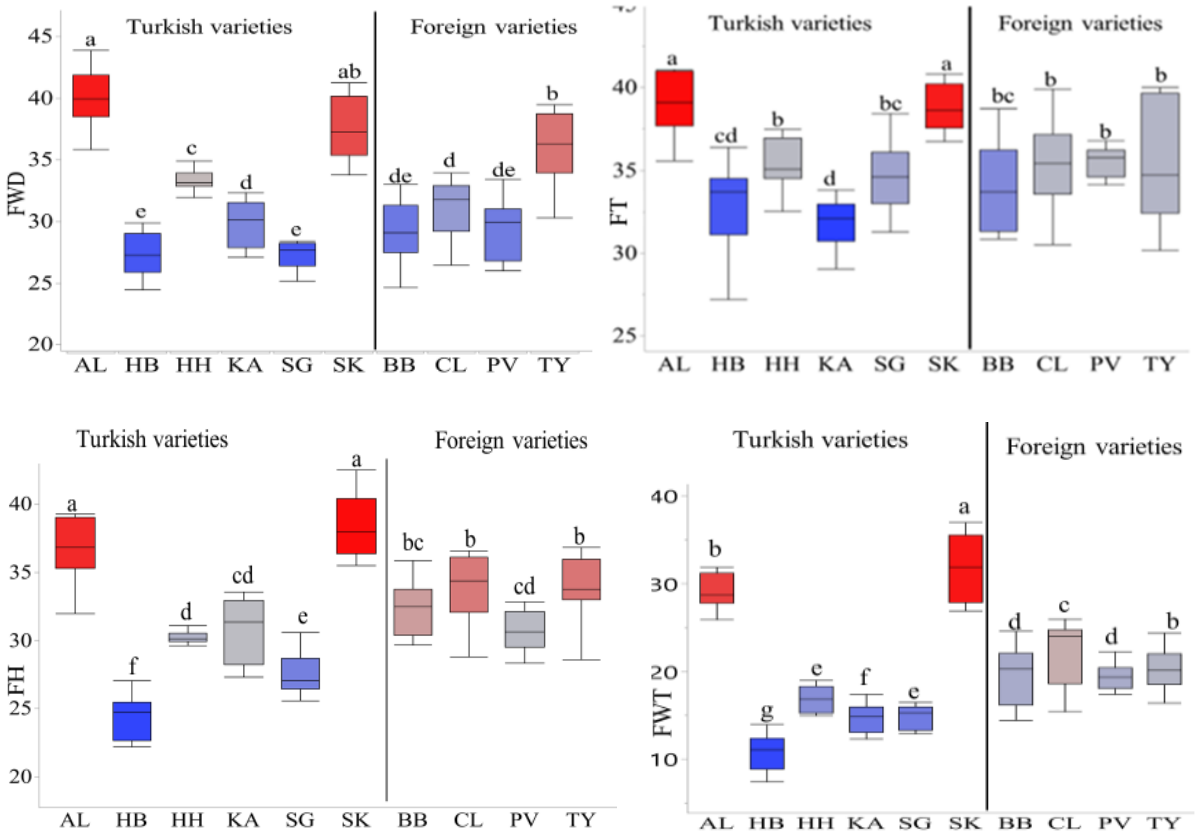

Figure 3. Fruit dimension features of Turkish and foreign varieties. AL; Alyanak, HH; Hacıhaliloğlu, HB; Hasanbey, KA; Kabaaşı, SK; Sakıt-2, SG; Soğancı, BB; Bebeco, PV; Paviot, CL; Precoce de Colomer, TY; Precoce de Tyrinthe.

The fruit dimensions were highest in Turkish varieties 'Alyanak' and 'Sakıt-2' compared to foreign varieties (Figure 3). Krichen et al. (2014) reported that fruit dimensions of 112 Tunisian apricot accessions ranged from 2.0 to 10 g for fruit weight and from 1.0 to 1.8 g for stone weight. Studies on fruit height, width, thickness, and 100 kernel weight were previously reported to vary from 14.0 to 19.17 mm, from 9.99 to 10.20 mm, from 3.3 to 6.27 mm, and from 28.7 to 65.1 g, respectively (Gezer et al., 2003; Vursavuş and Özgüven, 2004). Velardo-Micharet, et al. (2021) declared fruit height, diameter, and weight as varying from 46.6 to 53.9 mm, 48.4 to 57.5 mm, and 58.0 to 97.4 g, respectively. Another study was carried out in the Iğdır region on six apricot cultivars, fruit and stone weights were recorded as 27.88-52.9 g and 1.96-2.94 g, respectively (Muradoğlu et al., 2011). The fruit pomological data were in line with the previous findings; nevertheless, the differences are related to cultivar, yield (high and low fruit set up), maturity, soil types, nutritional status, and climatic conditions. The fruit set and pomological characters are seriously affected by climatological events. As shown in Figure 2. the research area where Located in eastern Turkey at a high altitude is show suitable climate conditions for

apricot growing. Besides in some years, extreme temperature fluctuations including late spring frost that occur in late April-early May can be causing an important threat to fruit sets and the yield of apricot varieties. Because apricot is a sensitive species and is seriously affected by climatological conditions.

Apricot stones (kernel) are generally by products in the fruit processing industry and thinking as discarded material. Apricot, a very important commercial fruit, is in the world and numerous apricot stones are exposed in food processing. Recent studies showed that apricot stones are rich in unsaturated fatty acids (Özkal, 2004), minerals such as K, Ca, and Mg (Muradoğlu et al. 2011), contains dietary fiber (Dwivedi and Ram, 2006), and several biological activities by its antioxidant, anticarcinogenic and, antimicrobial properties (Mandalari et al., 2010; Yiğit et al., 2009). For these reasons, apricot stones have been used in many foods and industrial areas in recent years.

Significant statistical differences were observed in terms of stone dimensions between the Turkish and the foreign apricot varieties (Figure 4). The SWD, ST, SH, and SWT of Turkish varieties changed from 9.31 to 17.04 mm, from 21.05 to 25.24 mm, from 10.15 to 18.81 mm, and from 1.23 to 2.13 g, respectively. These values changed to 8.40-9.68 mm, 19.93-22.60 mm, 14.14-16.51 mm, and 1.36-1.35 g, respectively for foreign varieties. Turkish varieties had higher stone dimensions than foreign varieties. Mratinic et al., (2011) reported that the stone weight of twenty Macedonia wild apricot ranged from 1.81 to 4.85 g. The stone length and width were previously reported by Velardo-Micharet, et al., (2021) as from 26.4 to 28.2 mm and between 21.6 and 23.4 mm from apricot cultivars, respectively. Our results were in a close relationship with previous studies and minor differences are due to varieties or high altitudes that are affected by climatic factors and fruit characters.

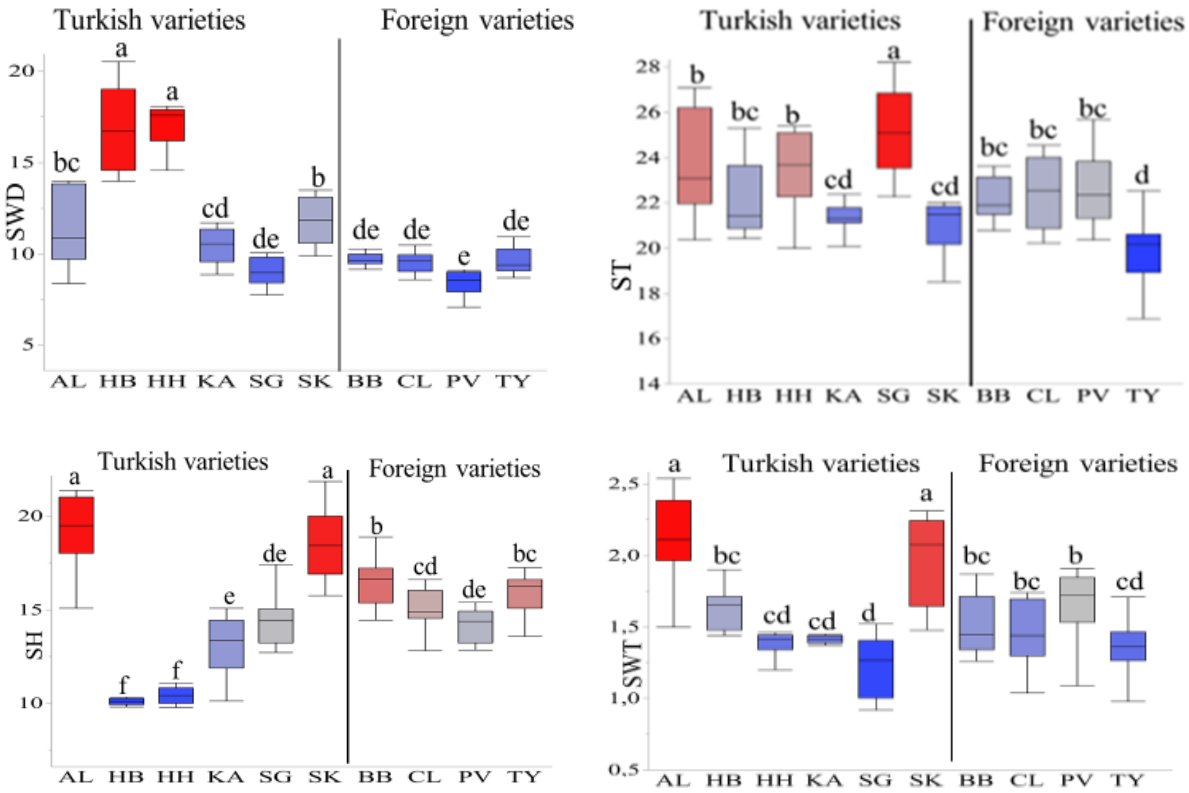

Figure 4. Stone dimension features of Turkish and foreign varieties. AL; Alyanak, HH; Hacıhaliloğlu, HB; Hasanbey, KA; Kabaaşı, SK; Sakıt-2, SG; Soğancı, BB; Bebeco, PV; Paviot, CL; Precoce de Colomer, TY; Precoce de Tyrinthe.

Apricot fruit color is very important commercially and customer prefers acceptable fruit color should be skin color ranging from yellow to deep orange, with a distinct red blush in many modern cultivars. According to Callahan (1995) and Moreau-Rio (2006), shiny, yellow, and orange apricot genotypes with a high red-blush ratio are very desirable to customers. Therefore, chromatic parameters such as l*, a*, b*, C*, and hue^{o}* are crucial and have been widely used to describe fruit quality. In

addition, the color variables have been connected to the cultivars and presence of phenolic compounds, anthocyanins, and antioxidant capacity in fruits (Akin et al., 2008; Christiensen, 2000; Cömert et al., 2020). Therefore, numerous researches were performed on chromatic parameters by Akın et al., (2008); Fan et al., (2017) and Velardo-Micharet, et al (2021) to determine the fruit quality of apricot. In this study, the fruit color of Turkish apricot varieties was detected 50.39-55.75 for L*, 15.77-30.02 for a*, 33.9-42.34 for b*, 27.75-49.06 for C*, and 32.15-66.39 for hue°. In foreign verities, fruit color was observed as 52.39-62.14 for L*, 17.98-27.66 for a*, 37.99-48.40 for b*, 45.84-55.67 for C*, and 55.66-62.34 for hue° respectively. The color of apricot varieties was higher in foreign varieties except for a* and hue° than Turkish varieties (Figure 5). Moreover, color is the most important maturity indicator for many fruit species and chromatic traits are affected by a sequence of agents such as cultivar, lighting, cultivation management, and maturity stage.

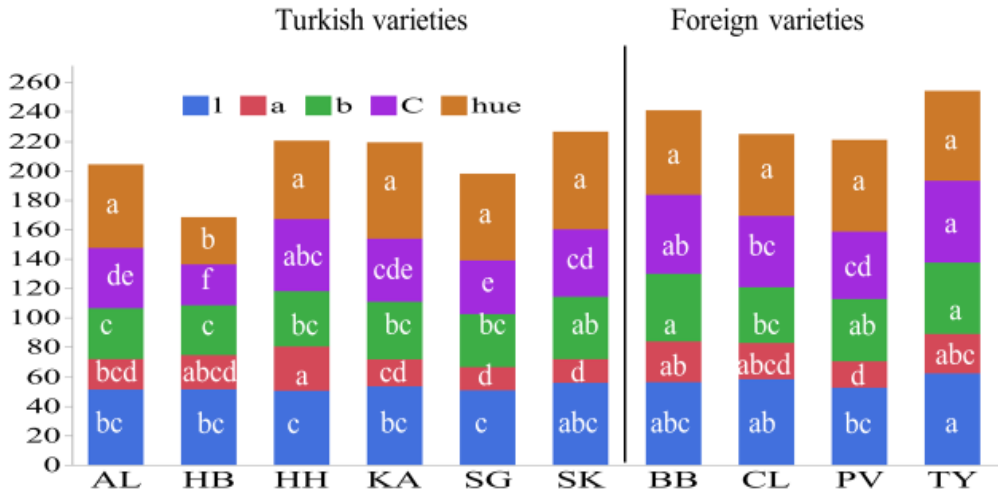

Figure 5. Chromatic features of Turkish and foreign varieties. AL; Alyanak, HH; Hacıhaliloğlu, HB; Hasanbey, KA; Kabaaşı, SK; Sakıt-2, SG; Soğancı, BB; Bebeco, PV; Paviot, CL; Precoce de Colomer, TY; Precoce de Tyrinthe,

Apricot quality is generally characterized by parameters of soluble solids content, sugars, titratable acidity, flesh firmness, and peel color (Génard et al., 1994). Fruit taste contributing to fruit quality was determined by sweetness and acidity, which is related to the content of soluble sugars and types of organic acids (Mikulic-Petkovsek et al., 2016). TSS and pH for Turkish varieties ranged from 3.05-4.20 % to 3.66-5.42 and for foreign varieties ranged from 3.55-5.00% to 3.95-5.14 respectively. TA varied from 0.37 to 3.18% in Turkish varieties and 0.60-2.39 % in foreign varieties (Figure 6). In a study conducted on 13 Turkish apricot cultivars, pH and titratable acids varied between 3.68-5.04 and 0.22-1.40% respectively (Karataş and Şengül, 2020). In another study, the pH and total acid values were reported as 3.83–6.61 and 0.08% and 0.28% as malic acid (Akin et al., 2008). In the studies conducted on some apricot cultivars, total acidity, pH, and soluble solid contents were found 0.14-0.68%, 4.81-5.45 and 12.50-24.00% by Muradoğlu et al., (2011) and 0.96-1.89%, 3.90-4.70, and 11.70-14.40 Brix, respectively by Mratinic, et al., (2011). According to the researchers, soluble solids contents of over 10% and titratable acidity of 0.7–1.0% are critical for consumer acceptance. Our titratable acidity amounts were in the range of acceptance threshold, but soluble solids contents were below. The main reasons were variety differentiation, environmental conditions, and harvest date such as early harvest.

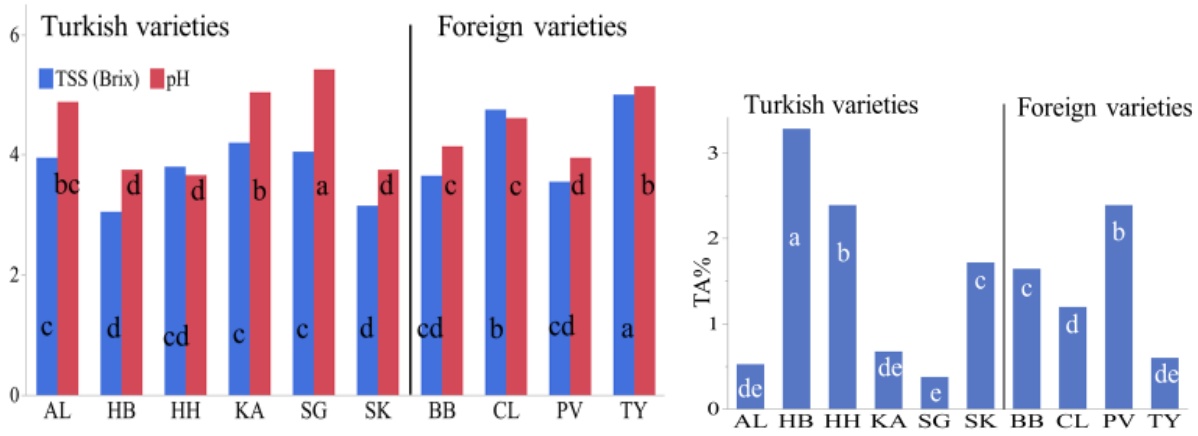

Figure 6. Chromatic features of Turkish and foreign varieties. AL; Alyanak, HH; Hacıhaliloğlu, HB; Hasanbey, KA; Kabaaşı, SK; Sakıt-2, SG; Soğancı, BB; Bebeco, PV; Paviot, CL; Precoce de Colomer, TY; Precoce de Tyrinthe.

3.2. Morphological Distribution in Turkish Apricot Varieties

The studied morphological characters in Turkish varieties were grouped into four categories depending on the correlation matrix and the clustering analyses (Figure 7A). These analyses were successfully used in the previous studies by Liu et al., (2021) in the comparison of relationships.

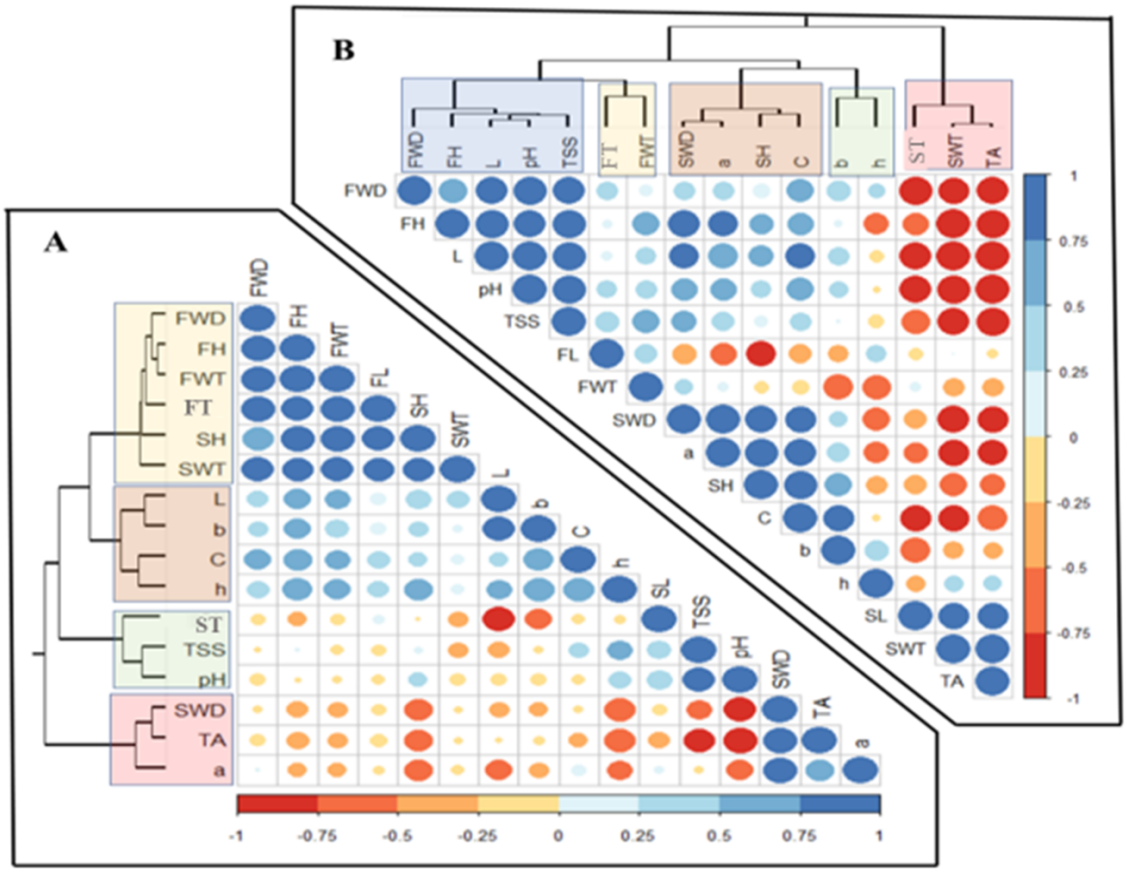

Figure 7. Correlation matrix and cluster dendrogram of morphological characters in Turkish (A) and foreign (B) varieties. FWD: fruit width, FT: fruit thickness, FH: fruit height, FWT: fruit weight, SWD: stone width, ST: stone thickness, SH: stone height, SWT: stone weight, L: lightness, a: green/redness, b: blueness/yellowness, C: chroma, hue°: lightness's angle, TSS: total soluble solids, and TA: titratable acidity.

Six morphological characters (Fruit width, fruit thickness, fruit height, fruit weight, stone height, and stone weight) were grouped in the first category (Fig 7A). The highest FWD, FT, SH, and SWT were determined in the 'Alyanak' variety, while the highest FH and FWT were determined in the 'Sakıt-2' variety (Figure 3)

Some calorimetric parameters such as L, b, C, and hue^{o} were grouped in the second category (Figure 7A). The highest L*, b*, and hue^{o} were detected in the 'Sakıt-2' variety, while the highest C* was detected in the 'Hacıhaliloğlu' variety (Figure 5).

Morphological characters in the third group were separated into two clusters. Maximum ST and pH were detected in 'Sakıt-2' and the highest TSS was detected in the 'Kabaaşı variety (Figures 4 and 6)

SWD, TA, and a* were grouped into four categories: The maximum SWD and a* were observed in 'Hacıhaliloğlu', while the maximum TA was observed in 'Hasanbey' (Figures 4, 5, and 6).

3.3. Morphological Distribution in Foreign Apricot Varieties

The studied morphological characters in foreign varieties were grouped into five categories depending on the correlation matrix and clustering analyses (Figure 7B).

FWD; FH, L*, pH, and TSS parameters were formalized in the first group. In this cluster, all examined morphological characters were the highest in 'Precoce de Tyrinthe' (Figures 3, 5, and 6)

Two characters, FT and FWT, were placed in the second group. The highest FL was determined in 'Paviot', while the maximum FWT was in 'Precoce de Colomer' (Figure 3).

The third group consisted of four characters SWD, a*, SH, and C*. The maximum a*, and SH were determined in 'Bebeco', while maximum SWT and C* were determined in 'Paviot' and 'Precoce de Tyrinthe' (Figures 4 and 5).

From the colorimetric parameter, b* and hue^{o} were composed of the fourth group. The highest b* was detected in the 'Precoce de Tyrinthe', while the highest hue^{o} was in 'Paviot' (Figure 5).

ST, SWT, and TA parameters were grouped in the fifth cluster. The maximum ST, SWT, and TA were detected in 'Paviot' (Figures 4 and 6).

3.4. Differences in Morphological Characters Between Turkish and Foreign Apricot Varieties

Relationships between morphological characters of Turkish and foreign varieties were detected by creating a correlation matrix. Significant positive correlations were observed among fruit and stone characters in the Turkish varieties. On the contrary, TA with pH and a* with SWD had a significant negative correlation in both Turkish and foreign varieties (Figure 7). These results agree with previous findings by Krichen et al., (2014) and Cömert et al., (2020). PCA was used to determine critical characters and to minimize the number of effective factors. Therefore, PCA has been used intensely by researchers to define critical characteristics of fruit species for the last decade (Mratinic, et al., 2011; Güler et al., 2021; Muradoğlu et al., 2021). In this study, PCA was conducted to characterize morphological characters of Turkish and foreign varieties. The PCA showed that 93.59% of the observed total variability was described by the first five components. PC1 and PC2 identified 40.1%, and 23.2% of total variations, respectively, and the first two components described 63.32% of the total variability. Turkish and foreign varieties were separated into three groups. The first group was associated with the 'Alyanak', and 'Sakıt-2' variety that was related to FWD, FH, FWT, FT, SH, SWT, and hue^{o}*. The second group was formed mostly of foreign varieties as 'Precoce de Colomer', 'Bebeco', 'Paviot', 'Precoce de Tyrinthe', 'Soğancı', and 'Kabaaşı' linked with colorimetric data such as L*, b*, c*, TSS, and pH characters. 'Hasanbey' and 'Hacıhaliloğlu' varieties formed the third group, and these varieties were characterized by ST, SWD, TA, and a*. Relationships among varieties were shown on a biplot (Figure 8). In a similar study on local apricot genotypes, Mratinic, et al., (2011) reported that the first three components represented 70.85% of the total variance.

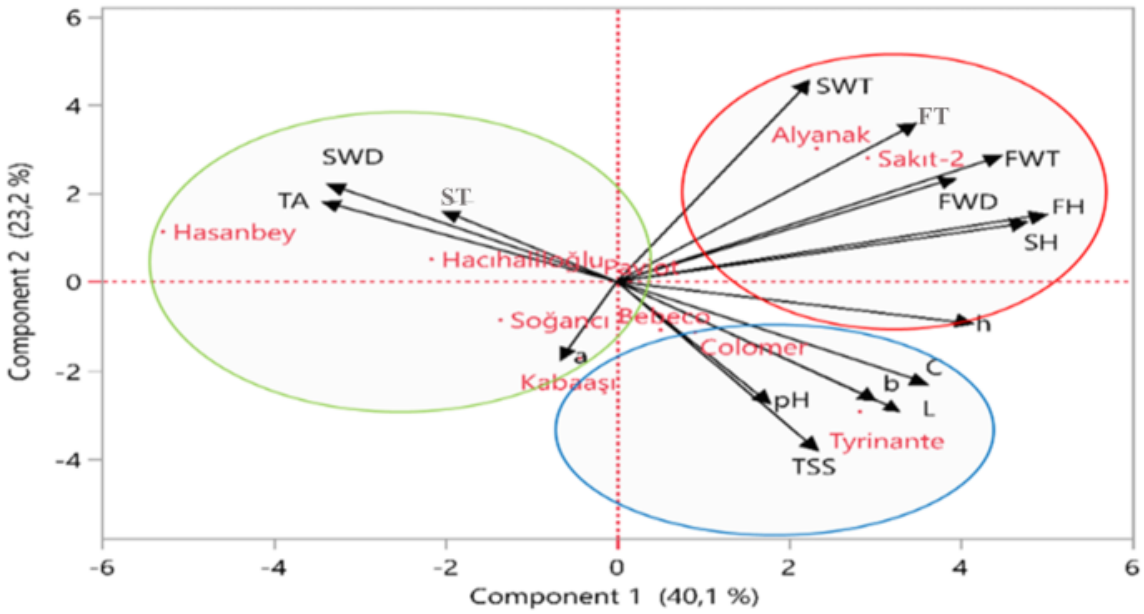

Figure 8. The principal component analysis (PCA) shows the interrelation of Turkish and foreign apricot variety and morphological characters.

4. Conclusions

The morphological properties of varieties are major indicators in determining fruit quality. That's why it is important to investigate fruit quality related to consumer preferences. In the present study, significant differences were observed in Turkish and foreign varieties according to their morphometric properties.

The fruit dimensions (FWD, FT, FH, and FWT) of varieties were the highest in 'Alyanak' and 'Sakıt-2' in the Turkish varieties. The highest values regarding stone width were recorded from the 'Hasanbey' and 'Hacıhaliloğlu' varieties, length from the 'Soğancı' variety, while 'Alyanak' and 'Sakıt-2' varieties have the biggest fruit height and weight. Fruit stone features were the highest in Turkish varieties compared to foreign varieties. The color was higher in foreign varieties except for a* and hue°. The highest soluble solid contents were recorded from the 'Precoce de Tyrinthe' in foreign varieties. Contrarily, pH and total titratable acids were higher recorded from 'Soğancı' and 'Hasanbey' in Turkish varieties.

The results showed that Turkish varieties have higher morphometric characters and these varieties had better performance in the ecological conditions of the study.

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Research Article

Effect of "Avigen" Immunomodulator on Beta-lysine Activity in Broilers

Rumen KARAKOLEV[*1], Preslava PETROVA-TSENIN[2], Reneta PETROVA[3], Tsvetoslav KOYNARSKI[4]

[1,2,3]National Diagnostic Science and Research Veterinary Medical Institute, 1606 Sofia, Bulgaria
[4]Trakia University, Department of Animal Husbandry, Faculty of Veterinary Medicine, 6000 Stara Zagora, Bulgaria

[1]https://orcid.org/0000-0001-7018-398X, [2]https://orcid.org/0000-0002-4931-9587, [3]https://orcid.org/0000-0003-2454-5411
[4]https://orcid.org/0000-0003-1876-2372

*Corresponding author e-mail:rumenkarakolev@abv.bg

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Natural immunity

Abstract: The beta-lysine activity of blood serum and egg white in broilers and broiler breeders from the ROSS 308 hybrid reared in industrial conditions was studied using a photometric method. The birds from the experimental groups were treated with polybacterial immunomodulator "AVIGEN", the anti-stress preparation "ASPIVIT C" and the preparation "BIOXAN", which helps to increase the resorption surface of the intestinal mucosa.We found increased activity of beta-lysine in the blood serum in immunomodulator-treated broilers. No significant differences were found in the activity of beta-lysine in the blood serum of the experimental and control parent herds until the onset of lay. On the onset of lay, we observed an increase in beta-lysine activity in the blood serum, especially pronounced in the experimental herd. We also registered an increase in this indicator in the egg white of the birds from the experimental flock from the 2nd to the 7th day after laying. Elevated serum beta-lysine concentrations during the onset of lay, legitimizes beta-lysine as a stress-induced factor of non-specific immunity. The higher levels of beta-lysine in the blood serum and egg white of the experimental herds are probably due to the stimulation on the mucous membranes with the polybacterial immunomodulator AVIGEN.

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1. Introduction

Beta-lysine is a non-specific immune defense factor in birds that has been little studied. "Beta-lysine" means the thermostable bactericidal substances in the serum, active against bacilli, which do not need the presence of complement to perform their function (Weinert, 2013). The beta-lytic activity of the serum provides information about the state of the body in animals and humans in different physiological and pathological processes of different etiology, so it is important in clinical trials in veterinary and human medicine (Sarukhanov et al., 2016). Henriksen et al. (2012), Wu, (2013), Xie et al. (2015), Ouidir et al. (2015) performed experiments on lysine synthesis with the help of other microorganisms – *Saccharomycescerevisiae, Mycobacterium tuberculosis, Pseudomonas aeruginosa.* Ayasan and Okan (2010) investigate the effects of containing lysine on the fattening performance of broiler chickens. Beta-lysine is also known to be a precursor to the biosynthesis of antibiotics, neurotransmitters, and several polymers with high molecular weight (Hungetal., 2013). Our previous

studies (Karakolev et al., 2014; Karakolev, 2015; Karakolev and Nikolov, 2015) followed the physiological fluctuations in the beta-lysine activity of the blood serum in broilers and hen embryos from two hybrids of laying hens, and in the egg white of commodity eggs, depending on the age and hybrid affiliation of the laying hens. In a detailed study of the role of *Schizochytrium limacinum* in the diet of broilers on their natural immunity, Sotirov et al.(2021) also found a positive effect of the beta-lysine index in the experimental group. Investigating the possibilities for influencing the humoral immune response in laying hens, Bozakova et al., (2018) also draw attention to the changes in the activity of beta lysine under the influence of the immunomodulator «Immunobeta». According to Zemskov et al.(2018) beta lysine is involved in the mechanisms of immune homeostasis, in induction, regulation, and possibly in the correction of the immune response. The protective effects of other bacteriolysins, such as lysozyme and the complement system, have been studied in sufficient depth.However, literature data on beta lysine are extremely scarce, with no data on the beta-lysine activity of blood serum and egg white in broiler breeders, as well as the factors potentially influencing this activity.

With the present studies, we aimed to determine the beta-lysine activity of the blood serum of broilers and in the blood serum and egg white in broiler breeders treated with the immunomodulator "AVIGEN", as one of the main factors of natural immunity in the studied birds.

2. Material and Methods

2.1. Polybacterial immunomodulator "AVIGEN"

The product is a concentrated form of lipopolysaccharide components of the thermostable endotoxin extracted from Gram-negative bacteria from the Enterobacteriaceae family.

2.1.1. Method of treatment

The immunomodulator was administered in liquid form, containing 3,000 doses (ten days) in 1000 ml.

For broilers - orally, through drinking water from the 1st to the 10th day. The experimental and control flocks of broilers numbered 12,000 birds each. From the birds treated with the immunomodulator in the experimental flock, as well as from the control one, 45 birds were randomly selected, from which blood was taken for testing.

For broiler breeders - during the growing up period, orally, through drinking water from the 1st to the 10th day and from the 120th to the 130th day of life. The experimental and control flocks of broilers breeders numbered 12,500 birds each. From them, we took 45 samples of blood serum for the tests.

The field experiments were conducted in a poultry farm of Planeta 98 Ltd.

The subject of the study were broiler breeders and broilers from the hybrid ROSS 308, grown on the floor under industrial conditions. The birds from the experimental flocks received with the water and the preparations “ASPIVIT C” and “BIOXAN” in a dosage of 5 ml per 10 l of water. Both preparations have an ancillary effect. The first has an anti-stress effect on birds, and the second improves the permeability of mucous surfaces.

2.2. Sampling

At certain intervals (6-, 12- 18-, 24-, 36-week-old), we took blood from the axillary vein. The separated blood serum was stored at 4^{o}C. We collected blood samples from broilers at 10, 18, and 28 days of age. Serum testing was performed no later than 24 hours after sampling. We collected 45 eggs in a day (2, 7, 10, and 14) from control and experimental parent flock in the first two weeks after laying. The egg white test was performed no later than 6 hours after sampling.

2.3. Determination of beta-lysine activity

The beta-lysine activity of blood serum and egg white was determined by a spectrophotometric method describedby Bucharin et al. (1977), modified by Karakolev and Nikolov (2015). The research was performed in flat-bottomed plates. We used a pre-prepared spore suspension of Bacillus subtilis ATCC 6633. We added the controls with an automatic pipette - 80 µl of saline + 80 µl of spore

suspension in each of the first 4 wells. We then instilled the experimental sera with an automatic pipette - 80 µl serum + 80 µl spore suspension in each of the following wells, according to the number of samples tested. We homogenized by a plate shaker. Optical density measurements were performed using a BioTek L80 spectrophotometer at a wavelength of 630 nm, before incubation. We incubated the plate in a plate incubator with a timer (37ºC for 2 hours). Immediately after incubation, we again measured the optical densities at the same wavelength. Since the optical densities of the controls did not change for 2 hours in the incubator, we performed the calculations by taking the changes in the optical densities of the samples for each well separately, according to the formula:

$$\% \text{ of lysis} = OD1 - OD2 / OD1 \times 100, \quad (1)$$

where OD1 is the optical density of the sample before incubation and OD2 is the optical density of the sample after incubation.

2.4. Statistical analysis

The optical density results were calculated for the control wells and experimental sera expressed as a percentage of the test culture lysis. Data were processed by independent t test with the fixe defect model using Data analysis tool pack, Microsoft Excel 2016, Microsoft Corporation Ltd. at a level of significance $P < 0.05$.

3. Results

3.1. Values for beta-lysine activity in the blood serum of broiler breeders

Measured values for beta-lysine activity in the blood serum of broiler breeders are presented in Table 1. Monitoring of changes in the indicator began at 6 weeks of age and continued until 36 weeks of age. Significant differences were found in beta-lysine activity at 18 weeks of age, coinciding with the laying of birds and the strong physiological stress that accompanies this process. Not coincidentally, at this stage, there is a slight increase in serum beta-lytic activity in the control group. The changes are physiologically conditioned.

Table 1. Beta-lysine activity (%) of blood serum in broiler breeders ROSS hybrid, n=45

Age, in weeks	Experimental flock X̄±SD	Control flock X̄±SD	P value
6	18.26±0.23	12.34±0.46	P<0.00001
12	21.58±0.42	14.05±0.63	P<0.00001
18	47.50±4.95	32.29±1.55	P<0.00001
24	29.86±1.05	15.27±0.95	P<0.00001
36	20.41±0.61	15.70±0.35	P<0.00001

The increase in beta-lysine activity in the blood serum of laying birds from the experimental flock is significantly more pronounced - 47.50% vs. 32.29% in controls. As can be seen from the table, the values of the studied indicator are higher during the whole observed period in the birds treated with the immunomodulator, while in the controls, the activity of beta-lysine is close to the physiological norm with a small increase during the laying period. The obtained data show an increase in beta-lysine activity in the blood serum of laying birds (18th week), significantly higher in the experimental flock. After the onset of lay, beta-lysine levels again decreased at 24 and 36 weeks, and in birds receiving the immunomodulator, it remained higher compared to controls.

3.2. Values for beta-lysine activity of egg white in broiler breeders

In research of egg white, we found higher beta-lysine activity in the experimental parent flock compared to the control. These differences are particularly pronounced on the 2nd and 7th days of laying. The results obtained by us show that beta-lysine is one of the earliest factors of non-specific

protection of the chicken embryo, which is active at the beginning of prenatal development. In the control herd, these values fluctuate physiologically between 28% and 15% activity. In birds treated with a polybacterial immunomodulator, beta-lysine activity in egg white was almost twice as high. This is an indicator directly related to the non-specific, humoral immune defense and the development of the embryo, as well as to the hatchability of the breeding eggs.

Table 2. Beta-lysine activity (%) of egg white in broiler breeders ROSS hybrid, n=45

Days of laying	Experimental flock $\bar{x} + SD$	Control flock $\bar{x} + SD$	P value
2	42.06±1.27	28.45±0.24	P<0.001
7	40.20±1.44	22.19±0.82	P<0.001
10	28.45±0.56	22.63±0.85	P<0.001
14	20.82±0.55	15.50±0.57	P<0.001

3.3. Values for beta-lysine activity in the blood serum of broilers

In research of blood sera from broiler chickens, we found that the activity of beta lysine generally had higher values in immunomodulator-treated birds. Already on the 10th day of the life of broilers, the studied indicator has an average value of 24.59, or 37 % more than the controls. On day 28, beta-lysine activity increased again to 37.35%, while in untreated birds, it remained at 16.54 % activity.

Table 3. Beta-lysine activity (%) in the blood serum of broilers ROSS hybrid, n=45.

Age in days	Experimental flock $\bar{x} + SD$	Control flock $\bar{x} + SD$	P value
10	24.59±0.69	15.53±0.52	P<0.001
18	25.14±0.36	17.44±0.27	P<0.001
28	37.35±0.86	16.54±0.69	P<0.001

4. Discussion

In all our blood serum and egg white tests, we found higher beta-lysine activity in birds from the experimental flocks receiving an immunomodulator. Probably, it is positively influenced by stimulation of the mucous membranes with the polybacterial immunomodulator, as well as by the action of the preparations "ASPIVIT C" and "BIOXAN", which help to increase the resorption surface of the intestinal mucosa. In addition, the application of the anti-stress preparation "ASPIVIT C" provides additional equalization in the conditions of the experiments that were conducted under production conditions. This makes it possible to exclude the influence of some technological stressors and to believe with a high degree of confidence that the increase in beta-lysine activity in experimental birds is due to the effect of the immunomodulator "AVIGEN". Our results also show that age is not a determinant of beta-lysine expression. Birds respond well regardless of age and category, and breeders treated with immunomodulator, lay eggs containing more beta-lysine, especially in the first 7 days after the onset of laying. Several studies on germ-free animals demonstrate that symbiotic bacteria and/or bacterial molecules (for instance, lipopolysaccharide, β-glucan, and peptidoglycan) can fully trigger adaptive immunity (Gensollen et al., 2016; Ganalvonarburg et al., 2016, Macpherson et al., 2017). The intestinal mucosa plays an important role in the initial triggering of the immune response and the following regulation of its maturation (Hrnciv et al., 2008).

Polybacterial immunomodulators are a powerful inducer of lysozyme and complement (Karakolev et al., 2014). The induction of beta lysine in egg white and blood serum is studied for the first time under the influence of the preparations we use – "AVIGEN", "ASPIVIT C" and "BIOXAN". The present experiments also establish their influence on the activity of beta-lysine, which is part of the non-specific immune factors in the blood serum. Obviously, beta-lysine is one of the earliest factors of innate immunity, as it is already present in the avian embryo, and its additional activity can be induced by lipopolysaccharides from enterobacteria included in the drinking water of birds, as our experiments

show. We evaluate the effect of the other two preparations only as supportive - anti-stress in “ASPIVIT C” and increasing the resorptive capacity of the mucous membranes in “BIOXAN”.

In our previous studies, the physiological values of beta-lysine in the blood serum in broiler breeders and broilers were monitored. Unlike broilers, in parent flocks, there is a strong stress factor associated with the onset of laying and accompanying hormonal and immune rearrangement in the body (Karakolev, 2015; Karakolev and Nikolov, 2015). Nevertheless, the concentration of beta-lysine may be influenced by some factors that have a beneficial effect on the activity of complement, lysozyme, and other indicators of the natural (innate) immune response. A number of authors (Ganalvonarburg et. al., 2016; Zemskov et al., 2018; Bozakova et al., 2018) consider the factors that may influence the mechanisms of non-specific, innate resistance and emphasize that polysaccharide substances are one of the most effective for this purpose. Hung et al. (2013) consider that AblAs from methanoarchaea are lysine 2,3-aminomutases that may function as potential biocatalysts for the synthesis of β-lysine *in vivo* and *in vitro*. Okanishi et al.(2013), and Zhang, et al.(2013), Weinert et al.(2013), also developed genetic methods for the biosynthesis of lysine for biotechnological purposes.

From the present experiments, it is clear that lipopolysaccharides from enterobacteria contained in concentrated form in the immunomodulator "AVIGEN" have a positive effect on the activity of beta-lysine fractions in the blood serum of broilers and especially in the parental forms during laying and about two weeks thereafter. The increased activity of beta- lysine is an important part of the non-specific immune defense and the possibilities for its activation in stressful periods of breeding are of particular importance for the health status of birds. Similar data in broilers was reported by Sotirov et al.(2021), which compared the effect of the application of *Schizochytrium limacinum* on some indicators of natural immunity in broilers. The authors found an increase in the activity of beta-lysine in the blood serum in experimental group III, accompanied by a slight decrease in the values of lysozyme and complement. These data also confirm the results of our previous studies on the inverse correlation between lysozyme and complement values on the one hand and beta-lysine activity on the other. Comparison of the results regarding the effect of *Schizochytrium limacinum* in the diet of broiler chickens and the measured activity of beta lysine of 11.49% show that beta-lysine is less affected than as a result of exposure to lipopolysaccharides from enterobacteria. Bozakova et al.(2018) investigated the effect of the immunomodulator “Immunobeta” on innate humoral immunity in laying hens and also found some effect on beta lysine activity. In the conditions of temperature stress, Bozakova et al.(2018) tracks the effect of the immunomodulator "Immunobeta" on innate humoral immunity in turkeys and laying hens, which supports our data on the special role of beta-lysine in stressful situations for the body.

After administration of the immunomodulator "AVIGEN", the activity of beta-lysine in the blood serum of broilers from the ROSS 308 hybrid reached 37.35%, which is significantly above the physiological limit observed in the control flock. The increase in the activity of beta-lysine in the blood serum in broiler breeders who took the immunomodulator in our experiments is especially significant and long-lasting. The discovery of almost twice the activity of beta-lysine in egg white shows the importance of beta-lysine as one of the earliest protective factors of innate immunity in birds and the importance of immunomodulators for their health status.

Conclusion

Based on the results obtained, it can be concluded that the immunomodulator "AVIGEN", containing lipopolysaccharides from enterobacteria and taken with drinking water in an appropriate dosage, causes an increase in beta-lysine activity in the blood serum of broilers and broiler breeders. The effect of beta-lysine activity does not depend on the age and category of the birds.

For the first time, it is established that breeding eggs laid by broiler breeders receiving the immunomodulator "AVIGEN" have higher levels of beta-lysine, especially in the first weeks after laying.

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Research Article

The Effects of Some Dormancy Breaking Treatments and Temperature on Seed Vigor of Gum Tragacanth (*Astragalus gummifer* Labill.)

Gülüm GÜREL[1], Bilal KESKİN[*2], Süleyman TEMEL[3]

[1] Department of Field Crops, Postgraduate Education Institute, Igdir University, Igdir, Turkey
[2,3] Department of Field Crops, Faculty of Agricultural, Igdir University, Igdir, Turkey

[1]https://orcid.org/0000-0003-0120-3902, [2]https://orcid.org/0000-0001-6826-9768, [3]https://orcid.org/0000-0001-9334-8601

*Corresponding author e-mail: bilalkeskin66@yahoo.com

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Abstract: This research was carried out to determine the effects of germination temperature and 12 dormancy breaking applications on the germination of the seeds of the gum tragacanth (*Astragalus gummifer* Labill.) bush. The research was carried out in the Field Crops Department laboratory, Iğdır University Faculty of Agriculture, in 2019. Gum tragacanth seeds were germinated for 28 days in the dark at constant temperatures of 10, 15, 20, and 25 °C and variable temperatures of 20/10 °C, 20/15 °C, 25/10 °C, and 25/15 °C. As a result of the research, the highest total germination rate was determined at 10.7% at 25/10 °C and 25/15 °C temperatures. It was determined that there was 89.3% dormancy in gum tragacanth seeds. Then, 12 dormancy breaking methods (matrix priming, hydro priming, gibberellic acid (GA_3, potassium nitrate, cold, moist stratification, warm moist stratification, warm+cold moist stratification, cold+warm moist stratification, cold water, hot water, mechanical scarification, and chemical scarification) were applied. After dormancy breaking applications were made, the seeds were germinated again at 25/15 °C. At the end of the study, it was revealed that the highest total germination percentage with 50.7% was obtained from the application of hot water for 2 minutes. On the other hand, it was determined that matric priming, hydro priming, gibberellic acid, potassium nitrate, cold, moist stratification, warm moist stratification, cold+warm moist stratification, mechanical scarification, and chemical scarification applications did not have any effect on removing the dormancy status of gum tragacanth seeds.

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Footnote: This study was produced from Gülüm GÜREL's Master's thesis.

1. Introduction

The genus *Astragalus*, which includes annual and perennial herbaceous and shrubby plants and belongs to the leguminous family, includes 3000 species. *Astragalus* species are spread over a wide area such as Europe, Asia, and North America, with 463 species and 41% (210 species) of these species endemic in Turkey (Dinç et al., 2013; Erkul and Aytaç, 2013). *Astragalus* species are widely used as a feed source for animals, as a raw material for medicine, dye, textile industry, as a source of nectar for bees, and also in the control of erosion areas (Demir and Keskin, 2016; Keskin and Temel, 2019; Bagheri

et al., 2015; Budge et al., 2012; Lee et al., 2007; Abd Kadir et al., 2013). *Astragalus brachycalyx*, *Astragalus gummifer*, *Astragalus kurdicus,* and *Astragalus microcephalus* species from which gum called tragacanth is extracted are widely found in Turkey, Iran, Caucasus, and Afghanistan regions, and this gum obtained is used in pharmacy, paint and weaving industries (Khan and Abourashed, 2010).

Gum tragacanth (*Astragalus gummifer* Labill.) is a perennial herb that can grow up to 60 cm. It is piled rooted, and its stem and shoots are thorny. Its flowers are white and pollinated by bees. When their stems are cracked and scratched, they ooze a gum called tragacanta (tragacanth gum). It is resistant to sandy, acidic, and arid areas (Keskin and Temel, 2019). Gum tragacanth spends the winter period in a dormant state and forms new shoots and leaves with the onset of temperatures, and the newly formed fresh shoots and leaves maintain their greenery throughout the summer. Flowering continues between May and October, and fruit formation continues for a long period, from June to November (Keskin and Temel, 2019).

Gum tragacanth, which is used in many areas, grows in natural areas, and these plants are not cultivated and grown in the field. Therefore, these plants cannot be controlled, and their generation may be extinct. The germination rate is expected to be high in cultivated seeds. On the other hand, non-cultured plants are more likely to have hard seeds and dormancy. It is also difficult to obtain seeds in these plants. Knowing the optimum germination temperatures of the seeds and the level of dormancy characteristics, if any, and which methods and methods are effective on breaking dormancy in seeds in order to spread the plants of high importance to wider areas will contribute to the dissemination of this plant to wider areas. Dormancy is defined as the inability of seeds to germinate due to internal (water and gas impermeability of the seed coat, chemical substances in the seeds) and external (temperature, oxygen, light) factors even though the environmental conditions are suitable. The practices required to break dormancy in seeds vary from species to species and even between seeds of the same species from different origins. In order to break dormancy, stratification, soaking in water, using growth regulators, washing, drying, heat and light treatment, mechanical scarification, chemical scarification, and combinations of one or more of these are used (Obalı, 2009; Agrawal and Dadlani, 1995; Erken and Kaleci, 2010; Moradi et al., 2016; Lagha et al., 2001; Tuncer, 2019; Maesaroh and Demirbağ, 2020).

In the literature review on the germination status and dormancy breaking methods of the seeds of gum tragacanth, it was seen that no study was conducted. Therefore, this study was planned to determine the temperature at which gum tragacanth showed high germination and which methods methods would be appropriate for breaking the dormancy in seeds.

2. Materials and Methods

2.1. Plant material

The study was carried out in the laboratory of the Department of Field Crops, Faculty of Agriculture, Iğdır University, in 2019. The seeds of gum tragacanth were cut in October-November and dried in the shade after being brought to the laboratory. The dried shoots were crushed with a plastic board on the bottom of the sieve, and the seeds were removed from the shoots. Damaged and broken seeds in the seeds were removed. The 1000 grain weight of the seeds was determined as 3.7 g. Seeds were stored in airtight packages at 5 °C until used. Seed viability and dormancy applications were carried out according to ISTA rules (ISTA, 2017).

2.2. Germination test

The seeds were germinated in the dark at constant temperatures of 10, 15, 20, and 25 °C and variable temperatures of 20/10 °C, 20/15 °C, 25/10 °C, and 25/15 °C. 3x25 seeds were used in each application. The seeds to be germinated were kept in 5% sodium hypochlorite for 60 seconds and then left to dry for 30 seconds on germination papers for half an hour. After the seeds were placed on the germination paper in a 120x20 mm glass petri dish, they were left on the germination papers with 25 seeds in each glass petri dish and then covered with a second germination paper. Germination papers were wetted with water prepared by adding 0.2 g of pomarsol to 1 liter of distilled water as starting water to prevent fungus growth. After the establishment of the experiment, daily counts were made for 28 days, and rootlets germinating above 2 mm were accepted as germinated (ISTA, 2017). At the end of the 28th day, normal, abnormal, and dead seeds were counted.

2.3. Dormancy breaking applications

12 dormancy breaking methods were applied to gum tragacanth milkvetch based on ISTA (2017) rules. After the dormancy breaking methods were applied, the seeds that showed the highest total and normal germination at the end of the germination tests were subjected to germination tests for 28 days at 25/10 °C and 25/15°C. Since the highest total and normal germination in all dormancy breaking applications occurred at 25/15 °C, statistical analyzes were made based on data at only 25/15 °C temperature.

2.3.1. Matrix priming

Seed: vermiculite: water; The seeds and vermiculite were placed in gauze nets in a 2: 1: 3 ratio and placed in opaque containers with water. The dishes were kept in the dark for 24, 36, and 48 hours at 15 °C and then dried to their initial weight at 25 °C.

2.3.2. Hydro priming

The seeds to be used in hydro priming application were kept in water at 20 °C for 5 hours, and then surface drying was applied to the seeds. After the surface drying process, the seeds in the tulle pouch were left on the wire tray in the aging pots filled with water so that they do not come into contact with water. The mouth of the aging containers was covered with a cling film in an airtight manner, and the lids of the containers were tightly closed. Seeds kept in aging containers at 20 °C for 60, 72, and 96 hours were dried at room temperature until they reached their initial weight.

2.3.3. Gibberellic acid

The seeds were kept in the dark for 24 hours in 250, 500, and 1.000 ppm GA_3 solutions so that they were completely submerged, and then surface drying was applied to the seeds.

2.3.4. Potassium nitrate

The seeds were kept in the dark for 6 hours in 2% and 4% KNO_3 solutions so that they were completely submerged, and then surface drying was applied to the seeds.

2.3.5. Cold moist stratification

After the seeds were placed between completely saturated coarse filter papers, they were placed in tulle pouch, and the applications were kept at 5 °C for 3 and 4 weeks.

2.3.6. Warm moist stratification

The seeds were kept for 1 and 2 weeks at 20 °C between completely saturated coarse filter papers and surface drying was applied to the seeds.

2.3.7. Warm+cold moist stratification

The seeds were kept between completely saturated coarse filter papers at 20 °C for 1 and 2 weeks, then kept at room temperature for 24 hours and then kept at 5 °C for 3 and 4 weeks. Afterwards, surface drying was applied to the seeds.

2.3.8. Cold+warm moist stratification

Seeds were kept between completely saturated coarse filter papers at 5 °C for 3 and 4 weeks, then 24 hours at room temperature, and then at 20 °C for 1 and 2 weeks. Then, surface drying was done on the seeds.

2.3.9. Coldwater

The seeds were kept in tulle pouch for 1, 2, and 4 weeks at 5 °C, completely submerged in water, and then surface drying was performed on the seeds.

2.3.10. Hot water

The seeds were kept in tulle pouch for 2 and 4 minutes in boiling (100 °C) water so that they were completely submerged in the water. Then the surface drying process was carried out.

2.3.11. Mechanical scarification (Sanding)

Seeds were abraded in a shaking device for 5, 10, and 15 minutes in size 10 sandpaper.

2.3.12. Chemical scarification (Sulfuric acid)

Seeds were kept in 96% H_2SO_4 for 10, 20, and 30 seconds. After the application, the seeds were washed with pure water and left to dry on blotting paper.

2.4. Comparison of dormancy breaking applications

In the germination tests, the germination rate control data obtained at 25/15 °C temperature were accepted, and a comparison was made with the dormancy breaking application, which obtained the highest germination rate after each dormancy breaking method was applied.

2.5. Statistical analysis

The analysis of variance was carried out according to the randomized plots experimental design according to the JMP 5.0.1 package program of the research data. The mean of the important factors is grouped according to the least significance difference (LSD).

3. Results and Discussion

3.1. Germination rates of *Astragalus gummifer* at different temperatures

There were significant changes in the total and normal germination rates of gum tragacanth milkvetch seeds germinated at different temperatures. Total germination rates were 2.7%, 8.0%, 8.0%, 8.0%, 5.3%, 8.0%, 10.7% and 10.7%. at 10, 15, 20, 25, 20/10, 20/15, 25/10 and 25/15 °C temperatures, respectively. On the other hand, normal germination rates were 0.0%, 4.0%, 5.3%, 8.0%, 5.3%, 8.0%, 9.3% and 9.3%, respectively. The highest total and normal germination rates were found at 25/10 °C and 25/15 °C temperature values. There was no significant change in abnormal germination rates at different temperatures. Although the germination rate of *Astragalus gummifer* seeds increased slightly due to the increase in temperature, it was observed that the seeds showed dormancy at a significant rate (89.3%). It is an expected situation to show dormancy in uncultured plants. *Astragalus gummifer* seeds were not found to germinate normally at 10 °C, the lowest temperature used in the study, while there was some germination due to the increase in temperature, but this germination rate remained at 9.3% (Figure 1).

It was determined that there was no significant change in the germination rate between different temperature values without pretreatment, and the highest germination rate was 10% in *Astragalus adsurgens*, 3.17% in *Astragalus maritimus*, 4% in *Astragalus arpilobus* and *Astragalus bibullatus*, and the species showed significant dormancy. (Kondo and Takeuchi, 2004; Bacchetta et al., 2011; Albrecht and Penagos, 2012; Long et al., 2012). On the other hand, it was determined that *Astragalus adsurgens* germinated at different temperature values at 30 °C, *Astragalus gines-lopezii* at 15/25°C and *Astragalus membranaceus* at 10 °C (Jaganathan et al., 2019; Zhou et al. al., 2012; Schnadelbach et al., 2016). Generally, *Astragalus* seeds appear to show high dormancy. *Salsola kali* subsp. *ruthenica* seeds at 10 and 25 °C (Obalı, 2009), *Alhagi pseudodalhagi* seeds at 25 °C (Moradi et al., 2016). As can be seen in previous research, the germination temperatures of the seeds vary according to the plant genus and species.

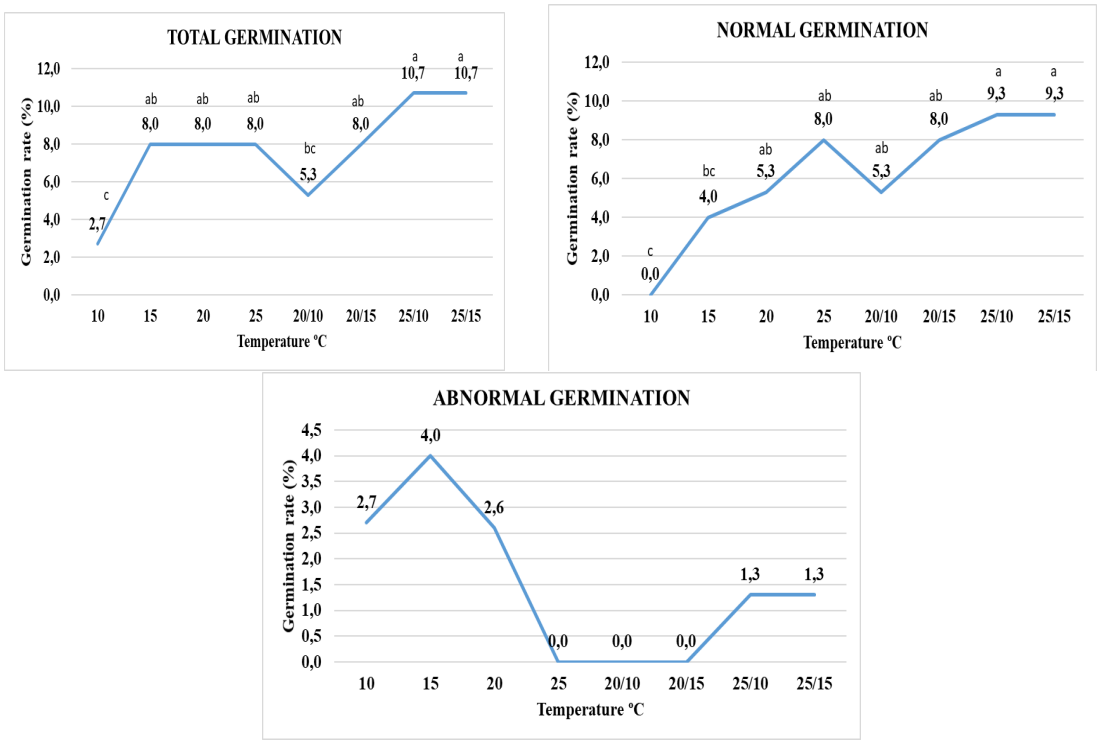

Figure 1. Total, normal and abnormal germination rates of *Astragalus gummifer* seeds at different temperatures (Total germination LSDs: 3.0**, Normal germination LSDs: 4.7**, Abnormal germination LSDs: 4.7ns), **P < 0.01 are significant within the probability limits, ns is insignificant.

3.2. Germination rates of *Astragalus gummifer* in dormancy breaking applications

3.2.1. Matrix priming

While matrix priming application at different times had a significant effect on total seed germination and normal seed germination rates of gum tragacanth milkvetch, it was observed that it did not have a significant effect on abnormal seed germination rate. When matrix priming was applied for 24, 36, 48 hours, total seed germination rates in gum tragacanth milkvetch seeds were 8.0%, 22.7%, and 8.0%, respectively. The highest total seed germination rate was 22.7% in the matrix priming application for 36 hours. The lowest total germination rate of 8.0% was observed in the seeds applied 24 and 48 hours of matrix priming. Normal germination rates were determined as 20.0% in gum tragacanth milkvetch seeds, which were applied matrix priming for 36 hours (Table 1).

In the literature studies, it was seen that matrix priming application was not tested in *Astragalus* species. However, studies on different plant species (*Allium cepa*, *Abelmoschus esculentus* and *Allium ampeloprasum*) determined that matrix priming increased the seed germination rate compared to control and was a successful method in breaking dormancy (Özden et al., 2018a; Pandita et al., 2010; Ozden et al., 2018b). In the present study, it was found that matrix priming application increased the germination rate in *Astragalus gummifer* seeds and revealed that it was an effective method in breaking dormancy, which also supports previous studies.

3.2.2. Hydro priming

The effect of different hydro priming applications on seed germination rates of gum tragacanth milkvetch was not significant. With the application of hydro priming for 48, 72, and 96 hours, total seed germination rates of gum tragacanth milkvetch were 10.7%, 9.3%, and 2.7%, while normal germination rates were 10.7%, 6.7%, and 2.7%, and abnormal germination rates were 0.0%, 2.7%, and 0.0%, respectively,

In previous studies, no hydro priming application was found to break the dormancy in the seeds of *Astragalus* species. However, they determined hydro priming of *Nigella sativa* (Tajbakhsh et al.,

2014), *Allium cepa* (Özden et al., 2018a), and *Lactuca sativa* (Rao et al., 1987) seeds increased the germination rate compared to the control and was a successful method in breaking dormancy.

3.2.3. Gibberellic acid

Gibberellic acid application at different concentrations had a significant effect on the total germination and abnormal germination rate of gum tragacanth, but the effect on the normal germination rate was not significant (Table 1).

Table 1. The effects of some dormancy breaking practices on seed viability of *Astragalus gummifer*

Aplications	Application levels	Total germination (%)	Normal germination (%)	Abnormal germination (%)
Matrix Priming	24 hour	8.0 b	8.0 b	0.0
	36 hour	22.7 a	20.0 a	2.7
	48 hour	8.0 b	8.0 b	0.0
LSD value and significant		6.05**	0.00**	6.05 ns
Hydro priming	48 hour	10.7	10.7	0.0
	72 hour	9.3	6.7	2.7
	96 hour	2.7	2.7	0.0
LSD value and significant		ns	ns	ns
Gibberellic acid	250 ppm	6.7 b	6.7	0.0 b
	500 ppm	10.7 a	6.7	4.0 a
	1000 ppm	10.7 a	9.3	1.3 ab
LSD value and significant		3.83**	ns	3.02*
Potassium nitrate	%2	10.7	9.3	1.3
	%4	10.7	9.3	1.3
LSD value and significant		ns	ns	ns
Cold moist stratification	3 week	24.0 a	21.3 a	2.7
	4 week	4.0 b	2.7 b	1.3
LSD value and significant		9.94**	5.74**	ns
Warm moist stratification	1 week	9.3	8.0	1.3
	2 week	14.7	12.0	2.7
LSD value and significant		ns	ns	ns
Warm + cold moist stratification	1 week+3 week	14.7 b	12.0 bc	2.7
	1 week+4 week	24.0 a	17.3 a	6.7
	2 week+3 week	12.0 b	9.3 c	2.7
	2 week+4 week	16.0 ab	16.0 ab	0.0
LSD value and significant		9.23**	4.80*	ns
Cold + warm moist stratification	3 week+1 week	10.7	10.7	0.0
	3 week+2 week	14.7	12.0	2.7
	4 week+1 week	14.7	13.3	1.3
	4 week+2 week	9.3	6.7	2.7
LSD value and significant		ns	ns	ns
Cold water	1 week	13.3 b	8.0	5.3
	2 week	20.0 a	13.3	6.7
	4 week	14.7 b	12.0	2.7
LSD value and significant		3.70**	ns	ns
Hot water	2 minute	50.7	38.7	12.0 b
	4 minute	46.7	20.0	26.7 a
LSD value and significant		ns	ns	5.74**
Mechanical scarification	5 minute	20.0 a	10.7	9.3 a
	10 minute	9.3 b	9.3	0.0 b
	15 minute	9.3 b	9.3	0.0 b
LSD value and significant		6.05**	ns	3.02**
Chemical scarification	10 second	6.7 b	6.7	0.0
	20 second	10.7 a	10.7	0.0
	30 second	9.3 ab	8.0	1.3
LSD value and significant		3.02*	ns	ns

*P < 0.05 significant at probability limits, **P < 0.01 significant at probability limits, ns not significant.

Total germination rates of gum tragacanth seeds were 6.7%, 10.7%, and 10.7% in 250, 500, and 1000 ppm applications of gibberellic acid. While the highest total germination rate was obtained in 500 and 1000 ppm gibberellic acid application, the lowest total germination rate was found in 250 ppm

application. Gibberellic acid application at different concentrations did not have an effect on increasing the germination rates obtained without any dormancy breaking the application. Although the application of gibberellic acid in breaking dormancy is a common application, the application of gibberellic acid in gum tragacanth seeds did not make a significant contribution to the increase in germination rate. In studies on some *Astragalus* species, it has been revealed that gibberellic acid application is not effective in breaking dormancy (İkram et al., 2014). On the other hand, it is an effective method in breaking dormancy (Zhou et al., 2012; Keshtkar et al., 2008). On the other hand, it was determined that the application of gibberellic acid to *Centaurea tchihatcheffii*, *Capparis ovata*, *Arbutus andrachne*, *Thlaspi lilacinum*, *Draba brunifolia,* and *Vitis vinifera* seeds increased the germination rate of seeds and was an effective method in breaking dormancy (Okay and Günöz, 2009; Gökçöl and Duman, 2018). ; Onursal and Gözlekçi, 2007; Kırmızı, 2017; Akkurt et al., 2013). However, in studies conducted on *Achillea gypsicola*, *Saponaria halophila,* and *Chamaecytisus pygmaeus* species, it was determined that gibberellic acid application was not effective in increasing the seed germination rate (Çolak, 2011; Erken et al., 2014; Açıkgöz and Kara, 2019).

3.2.4. Potassium nitrate

There was no significant difference between 2% and 4% potassium nitrate application on total germination, normal germination, and abnormal germination rate of gum tragacanth. Total germination, normal germination, and abnormal germination rates were 10.7%, 9.3%, and 1.3%, respectively, in both potassium nitrate applications (Table 1). In previous studies, it was determined that potassium nitrate application was not effective in increasing the germination rate of some *Astragalus* seeds (İkram et al., 2014), while it increased the germination rate in some species (Zhou et al., 2012). In the current study, it was determined that potassium nitrate application was not effective in increasing the germination rate of gum tragacanth seeds.

3.2.5. Cold moist stratification

Changes in total germination and normal germination rates were observed in gum tragacanth seeds, which were subjected to cold moist stratification at different times. The total germination rates in the seeds germinated after the 3rd week and 4th week of cold moist stratification application were 24.0% and 4.0%, respectively, while the normal germination rates were 21.3% and 2.7% (Table 1). Considering these values, total germination and normal germination rates were higher in seeds that were cold moist stratification for 3 weeks. It has been determined that there will be significant decreases in germination rates of gum tragacanth seeds in case of longer cold stratification (4 weeks).

In a study conducted by Cavieres and Almedia (2018), although cold stratification increased the germination of *Astragalus looseri* seeds by 9% compared to the control, this increase was found to be statistically insignificant. Germination rates were determined as 7.7% in control application and 16.7% in cold stratification. Long et al. (2012) applied *Astragalus arpilobus* seeds in wet sand at 4 °C for 4, 8, 12, and 16 weeks. Cold stratification did not increase the germination rate of seeds, and they determined that it was not an effective method for breaking dormancy. Jones et al. (2016) applied cold stratification to *Astragalus filipes* seeds for 3 weeks at 4 °C. Cold stratification increased the germination rate of seeds, and it was determined to be an effective method for breaking dormancy. Isavand et al. (2005) applied cold stratification to *Astragalus siliquosus* seeds. Cold stratification increased the germination rate of seeds, and it was determined to be an effective method for breaking dormancy.

Studies have shown that cold folding has a positive effect on breaking dormancy. In the current study, it has been seen that cold stratification is an effective method in breaking dormancy and encourages germination, which supports previous studies.

3.2.6. Warm moist stratification

There was no significant difference in seed germination rates between 1 and 2 weeks of warm stratification of seeds of gum tragacanth. The total germination rates of seeds, which were germinated after 1 and 2 weeks of warm stratification, varied between 9.3% and 14.7%, normal germination rates between 8.0% and 12.0%, and abnormal germination rates between 1.3% and 2.7%, respectively (Table 1). In a study, It was determined that the application of warm stratification to *Salsola kali* subsp. *ruthenica*. Seeds decreased the germination rate compared to the control and had no effect on the breaking of dormancy (Obalı, 2009).

3.2.7. Warm+cold moist stratification

It was observed that the effect of warm+cold stratification at different times on gum tragacanth seeds on total germination rate and the normal germination rate was significant, but the effect on abnormal germination was insignificant (Table 1). In 1 week warm+3 weeks cold, 1 week warm+4 weeks cold, 2 weeks warm+3 weeks cold, and 2 week warm+4 week cold stratification, total germination rates were 14.7%, 24.0%, 12.0% and 16.0%, normal germination rates were determined as 12.0%, 17.3%, 9.3%, and 16.0%, respectively. The highest total germination and normal germination rates were obtained in 1 week warm+4 weeks cold stratification (Table 1). Previously, no study has been found in which warm+cold stratification has been tested in seeds of *Astragalus* species. However, it was determined that 3 week warm stratification + 12 week cold stratification application of *Fraxinus ornus* seeds had a significant effect on the germination rate (Tilki, 2005). On the other hand, it was determined that the application of warm+cold stratification to *Flueggea anatolica* seeds had no effect on breaking dormancy (Avşar and Ok 2009).

3.2.8. Cold+warm moist stratification

It was observed that cold+warm stratification application of gum tragacanth seeds at different times did not have a significant effect on total, normal and abnormal seed germination rates (Table 1). In the current study, as a result of 3 weeks cold+1 week warm, 3 weeks cold+2 weeks warm, 4 weeks cold+1 week warm, and 4 weeks cold+2 weeks warm stratification, total seed germination rates were 10.7%, 14.7%, 14.7%, and 9.3%, normal seed germination rates were 10.7%, 12.0%, 13.3%, and 6.7%, respectively.

3.2.9. Cold water

While significant differences were observed in the total germination rate of gum tragacanth seeds by soaking in cold water for 1, 2, and 4 weeks, no significant differences were observed in normal and abnormal germination rates. Total germination rates were found as 13.3%, 20.0%, and 14.7% in cold water soaking for 1, 2, and 4 weeks, respectively. In applications, the highest total germination rate was obtained with 20.0% in cold water soaking for 2 weeks (Table 1). In a study conducted on the subject, it was determined that keeping *Astragalus adscendens* and *Astragalus podolobus* seeds in cold water at 4 °C for 10 days increased the germination rate of seeds and was an effective method in breaking dormancy (Tavili et al., 2014). In another study, when *Salsola kali* subsp. *ruthenica* seeds were washed in running water for 24, 48 and 72 hours, germination rates of 87.33%, 86.67%, and 84.67% were obtained, respectively. In the control, a germination of 84% was achieved. Therefore, it was determined that the germination rate decreased with the increase in the soaking time of the seeds (Obalı, 2009).

3.2.10. Hot water

There were significant changes in the total, normal and abnormal seed germination rates of gum tragacanth seeds in different times (2 and 4 minutes) soaking in boiling water. Total germination rates of gum tragacanth seeds kept for 2 and 4 minutes in hot water were 50.7% and 46.7%, normal germination rates were 38.7% and 20.0%, and abnormal germination rates were 12.0% and 26.7%, respectively (Table 1). On the other hand, significant differences were observed in abnormal germination rates in 2 different periods of soaking in hot water. The highest total and normal germination rates were obtained in the application of soaking gum tragacanth seeds in boiling water for 2 minutes. It was determined that there was a decrease in the total germination and normal germination rates of the seeds that were kept in boiling water for a longer time and an increase in the abnormal germination rate at the same time. It is estimated that hot water application has a significant effect on the permeability of the seed coat and contributes to the increase in germination rate.

In many studies, the seeds of *Astragalus* species were soaked in hot water. *Astragalus maritimus* and *Astragalus verrucosus* seeds in 100 °C hot water (Bacchetta et al., 2011), *Astragalus adscendens* and *Astragalus podolobus* seeds in hot water for 5 minutes (Tavili et al., 2014), *Astragalus hamosus* seeds in 60, 70, 80, 90 and 100 °C hot water for 5, 10, 15 and 20 minutes (Patane and Gresta, 2006), *Astragalus cyclophyllon* seeds in 60, 80 and 100 °C hot water for 5 and 10 minutes (Keshtkar et al., 2008), *Astragalus arpilobus* seeds in 70, 80, 90 and 100 °C hot water (Long et al., 2012) and *Astragalus podolobus* seeds in boiling water for 1 minute (Agh et al., 2017) applied soaking and determined that soaking in hot water is an effective method for breaking dormancy. On the other hand, it has been

determined that soaking in hot water for seeds of *Astragalus filipes*, *Astragalus gines-lopezii,* and *Astragalus cicer* is not an effective method for breaking dormancy (Kildisheva et al., 2018; Schnadelbach et al., 2016; Statwick, 2016).

3.2.11. Mechanical scarification

Mechanical scarification of gum tragacanth seeds for 5, 10, and 15 minutes caused significant changes in total germination and abnormal germination rates. On the other hand, it was determined that mechanical scarification application for different durations did not cause significant changes in the normal germination rate (Table 1). As a result of mechanical scarification application of gum tragacanth seeds for 5, 10, and 15 minutes, total germination rates were 20.0%, 9.3%, and 9.3%, while abnormal germination rates were determined as 9.3%, 0.0%, and 0.0%, respectively. It was observed that mechanical scarification caused an increase in abnormal germination rate in the application for 5 minutes, but there was no significant change in normal germination.

Mechanical scarification has been applied to seeds of many *Astragalus* species. Mechanical scarification increased the germination rate of the seeds compared to the control in *Astragalus peckii*, *Astragalus penduliflorus*, *Astragalus filipes*, *Astragalus siliquosus*, *Astragalus hamosus*, *Astragalus cicer*, *Astragalus fridae*, *Astragalus tribuloides*, *Astragalus bibullatus,* and *Astragalus contortuplicatus* seeds and determined that mechanical scarification is an effective method for breaking dormancy (Miklas et al., 1987; Isavand et al., 2005; Eisvand et al., 2006; Fateh et al., 2006; Patane and Gresta, 2006; Arbabian et al., 2009; Albrecht et al., 2012; Molnár et al., 2015; Pearson, 2015; Jones et al., 2016; Schnadelbach et al., 2016; Siles et al., 2016; Statwick, 2016; Kildisheva et al., 2018; Dziurka et al., 2019).

3.2.12. Chemical scarification (Sulfuric acid)

It was observed that keeping gum tragacanth seeds in 96% sulfuric acid for 10, 20, and 30 seconds caused a difference in total germination rates, while it did not cause a significant change in normal germination and abnormal germination rates. The total germination rates in seeds kept for 10, 20 and 30 seconds in chemical scarification were found to be 6.7%, 10.7%, and 9.3%, respectively. The highest total germination rate was observed in the seeds that applied chemical scarification for 20 seconds (Table 1). When the seeds of gum tragacanth were kept in sulfuric acid for 10 and 20 seconds, no abnormal germination was observed. However, abnormal seed germination was observed at the rate of 1.3% when kept for 30 seconds.

As chemical scarification, sulfuric acid applications at different concentrations and durations have been applied to many *Astragalus* species. It was determined that the application of sulfuric acid to *Astragalus cicer* and *Astragalus hamosus* seeds did not increase the germination rate of the seeds compared to the control, and it was not an effective method for breaking dormancy (Siles et al., 2016; Statwick, 2016). On the other hand, the application of sulfuric acid to the seeds of *Astragalus vulnerariae*, *Astragalus adsurgens*, *Astragalus penduliflorus*, *Astragalus maritimus*, *Astragalus verrucosus*, *Astragalus adscendens*, *Astragalus podolobus*, *Astragalus siliquosus*, *Astragalus hamosus*, *Astragalus lehmannianus*, *Astragalus cyclophyllon*, *Astragalus armatus*, *Astragalus sinicus*, *Astragalus cicer,* and *Astragalus arpilobus* determined that it is an effective method in breaking dormancy (Miklas et al., 1987; Kondo and Takeuchi, 2004; Isavand et al., 2005; Eisvand et al., 2006; Patane and Gresta, 2006; Keshtkar et al., 2008; Kim et al. al., 2008; Bacchetta et al., 2011; Long et al., 2012; Abudurehman et al., 2014; Tavili et al., 2014; Dilaver et al., 2017; Kheloufi et al., 2018; Dziurka et al., 2019).

3.3. Comparison of dormancy breaking applications

Each dormancy breaking application with the highest total germination rate was compared with the germination rates obtained in the control (25/15°C) application. Seed germination rates of gum tragacanth are given in Figure 2 according to the statistical analysis made to compare dormancy breaking practices with each other.

It was determined that the differences between different dormancy breaking treatments on total germination rate, normal germination rate, and abnormal germination rate of gum tragacanth were statistically significant. The highest total germination and normal germination rates of 50.7% and 38.7%, respectively, were obtained from gum tragacanth seeds kept in hot water for 2 minutes. Gum tragacanth

seeds kept in hot water for 2 minutes had 40% more total germination and 29.4% more normal germination than the control treatment.

Compared with the control application, it was determined that hydro priming, gibberellic acid, potassium nitrate, warm moist stratification, cold + warm moist stratification, and chemical scarification applications did not cause a significant change in the total germination rates of gum tragacanth seeds. On the other hand, it was determined that matrix priming increased the total germination rate of seeds by 12%, cold moist stratification 13.3%, warm + cold moist stratification 13.3%, cold water soaking 9.3%, and mechanical abrasion 9.3%, compared to the control application.

Compared with the control application, it was determined that hydro priming, gibberellic acid, potassium nitrate, warm moist stratification, cold+warm moist stratification, cold water soaking, mechanical scarification, and chemical scarification did not cause any increase in the normal germination rate of gum tragacanth seeds. On the other hand, matrix priming, which increased the total germination rate of gum tragacanth seeds slightly compared to the control application, caused an increase in normal germination rate as 10.7%, cold moist stratification 12.0%, and warm + cold moist stratification 8.0%.

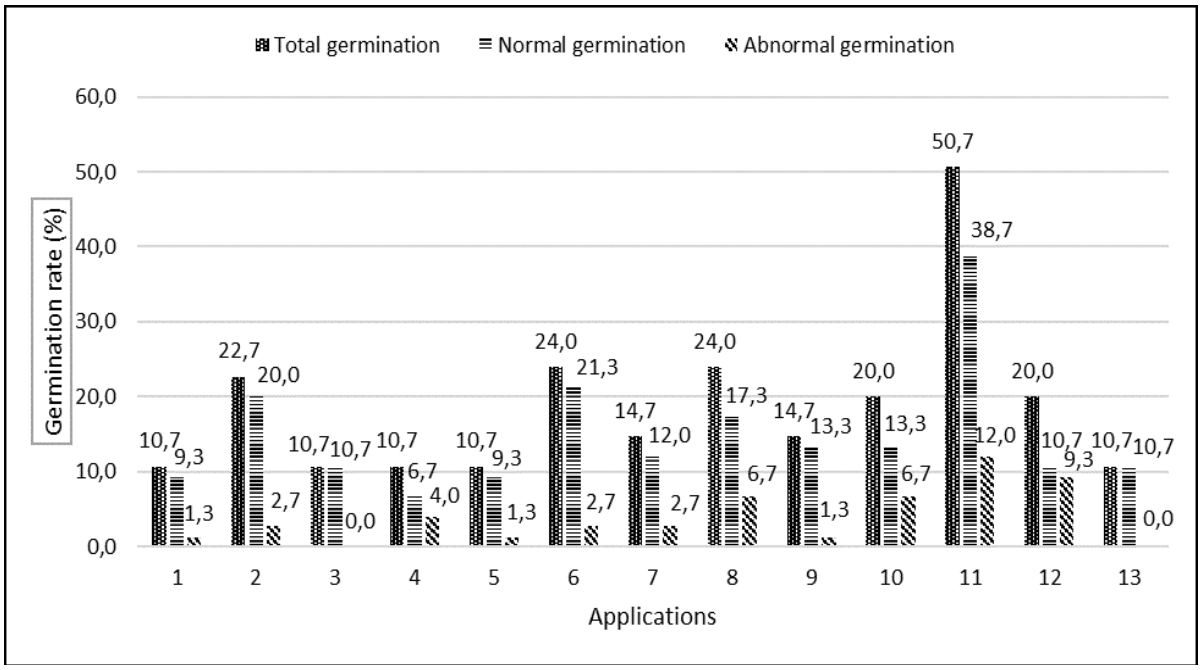

Figure 2. Effects of different dormancy breaking treatments on germination of *Astragalus gummifer* seeds (at 25/15°C temperature degrees). Treatment= 1: Control, 2: Matrix priming (36 hours), 3: Hydro priming (48 hours), 4: Gibberellic acid (500 ppm), 5: Potassium nitrate (%2), 6: Cold moist stratification (3 weeks), 7: Warm moist stratification (2 weeks), 8: Warm + cold moist stratification (1 week+4 week), 9: Cold + warm moist stratification (4 week+1 weeks), 10: Cold water (2 weeks), 11: Hot water (2 minutes), 12: Mechanical scarification (5 minutes), 13: Chemical scarification (20 seconds). (Total germination LSDs: 7.1**, Normal germination LSDs: 6.0**, Abnormal germination LSDs: 4.6**), **P < 0.01 is significant in the range of probability.

Compared with the control application, matrix priming, hydro priming, gibberellic acid, potassium nitrate, cold moist stratification, warm moist stratification, cold+warm moist stratification, and chemical scarification applications did not affect the abnormal germination rate of gum tragacanth seeds. Compared to the control application, it was determined that there was 5.4% more abnormal germination rate in the warm+cold moist stratification application, 5.4% in the cold water soaking, and 10.7% in the hot water soaking.

Conclusion

In the current study, the most suitable germination temperatures were found at 25/10°C and 25/15°C values for the seeds of the gum tragacanth bush. The highest dormancy breaking application was observed in gum tragacanth seeds kept in hot water for 2 minutes. According to the control application, matrix priming, cold moist stratification, warm moist stratification, warm+cold moist

stratification, cold+warm moist stratification, cold water soaking, hot water soaking, and mechanical scarification applications caused a slight increase in germination rates by breaking the dormancy state of the gum tragacanth seeds. On the other hand, it was determined that hydro priming, gibberellic acid, potassium nitrate, and chemical scarification applications did not have significant effects on breaking the dormancy of gum tragacanth seeds.

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Research Article

Determination of Sex-Reversal Rate and Growth Performance in Diallel Hybrids of Nile Tilapia (*Oreochromis niloticus*) and Blue Tilapia (*Oreochromis aureus*)

Francis Oster NWACHI[1], Arnold Ebuka IRABOR [*2]

[1,2]Delta State University, Agriculture Science Faculty, Fisheries and Aquaculture, Department 12345, Abraka, Nigeria

[1]https://orcid.org/0000-0001-9828-2330, [2]https://orcid.org/0000-0002-2276-6897

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

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Abstract: A total of 200 fish samples (100 *Oreochromis niloticus* (Nile tilapia) and 100 *Oreochromis aureus* (Blue tilapia)) were used for this research aimed to produce a population of tilapia species that is skewed toward the male population. Three mating periods were carried out in total. The males were removed from the hapa after each round of egg production to allow repercussion. Fries from the experimental units were collected, counted, and stocked in a tank with a dimension 3 x 1 x 4 m^2 and were fed to satiation while maintaining the basic water quality. The sex ratio data were subjected to student t-test analysis, while that of growth parameters were examined by ANOVA on Spss version 25. Results obtained showed a true hybridization between Nile tilapia and Blue tilapia that produced offspring that skewed toward the male population. A male skewed population was produced through interspecific crossing of two related strains of tilapia. This pattern repeated itself throughout the times when mating pairing was initiated. At the end of the trials, the hybrids and their reciprocals produced a higher number of male fish compared to the female. Hybrid 1 produced 93% male, while hybrid 2 produced 71% male. Furthermore, better performance was recorded for the hybrids compared to their pure strain despite the lower feed intake. Conclusively, a male skewed population is best produced through interspecific crossing of two related strains of hybrid tilapia.

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1. Introduction

Tilapia is regarded as an aquatic chicken because of its prolific nature (Menaga and Fitzsimmons, 2017). They are the third most important aquaculture product, just behind salmon and carp (Naylor et al., 2021). With the genus Oreochromis consisting of *O. niloticus*, *O. aureus,* and *O. mossambicus,* they remain one of the most widely cultured. Tilapia culture is popular because of its ability to thrive in most aquaculture systems (Nwachi and Esa, 2016). Khaw et al. (2016) infer that tilapia has almost all the traits that are associated with a fish with excellent culturable traits. Despite the fact that tilapia has the potential to do well in culture, its production in Africa remains at a minimal level (Goni et al., 2020; Ovharhe et al., 2020).

Tilapia could be cultured in different environments because of its ability to adapt to different environments. It is of note that strains of tilapia could be found in every body of water ranging from fresh water to marine environments. They are easy to culture, while their early maturation makes them it a choice for stock improvement. Although the aforementioned made it clear that tilapia is a fish that is farmed because of its desirable characteristics, drawbacks do exist while culturing this fish (Ng and Romano, 2013). Tilapia is known to quickly reproduce and populate the water body that it is introduced to (Oladimeji et al., 2015). This gives rise to a population of mini fish that are of little or no economic value.

Proliferation implies that there isn't enough food to go around for the army of mini fish that are produced. At a certain time in Southeast Asia, tilapia is regarded as weed fish (fish to be eliminated) before stocking the folk's fish as a result of the fish not reaching market size in culture (Nwachi et al., 2020). However, the ability of the male fish to reach market size on time made it possible for breeders to focus on raising monosex tilapia with a focus on the male fish because tilapia exhibit sexual dimorphism (Novelo et al., 2020).

A study by Nwachi and Esa (2016) infers that hybridization is a process of combining different strains with the purpose of producing a hybrid. It also involves mating fish with traits of interest, especially if they could be inherited. Strain or line crossing could be initiated depending on the need. Production of monosex tilapia has been a success because fertilization is external, making it possible to produce fish with a different number of chromosomes (Lago et al., 2016). Haque et al. (2016); Lozano et al. (2014) reported the use of interspecific breeding to produce hybrids of interest, which is in line with the way in which tilapia can easily accept related strains. A number of crosses have been used to produce male skewed sex individuals (Lahav and Lahav 1990; Rosenstein and Hulata 1994; Wohlfarth 1994; Verdegem el al., 1997; El-Hawarry 2012; Felix et al., 2019).

Despite some success recorded by the crossing of interspecific strains to achieve a population that is skewed toward males, proper documentation on the use of reciprocal mating and the growth rate achieved has not been fully exploited. Hence, there is a need to carry out diallel pairing of pure strains of *O. niloticus* to *O. aureus* to produce a high male sex ratio and a male of *O. aureus* to *O. niloticus to* produce a reciprocal.

The general objective was to produce a population of fish skewed towards a male population, with the specific objective of the pairing of male *O. aureus* (Blue tilapia) to female *O. niloticus* and the reciprocal. Also, evaluated the growth rate and colour variation of both the pure strain and the hybrid.

2. Material and Methods

The work was carried out at the research center of the Department of Aquaculture and Fisheries Delta State University Abraka, Nigeria. The candidate fish was collected from notable tilapia cage culture farms in River Benue and Ase creek. A total of 100 of each strain of *Oreochromis niloticus* (Nile tilapia) and *Oreochromis aureus* (Blue tilapia) were collected, respectively. The weight and length of the 12 months old female broodstocks range from 48.70 g to 168.50 g at 13.6 cm – 21.40 cm, while the 14 months old male range from 50.60 g to 190.60 g at 14.40 cm – 23.50 cm.

A pure strain of this stock was required; hence, prior to purchase for the research, they were subjected to further identification to species level with the aid of microsatellite by a group of experts at the African Bioscience Laboratory Ibadan Nigeria and Inqaba, West Africa after which a total of 36 females and 12 males that were identified as the pure strain was selected. A man-made lake of 100 sq.m was chosen for the work. Hapa of $1x1x1m^2$ was made from micro mesh size net that could retain fry but allow free flow of water. Water quality parameters such as temperature, pH, and dissolved oxygen were constantly monitored to ensure they were within the acceptable levels 27 °C, 7.5, and 6.9 mg/L^{-1}, respectively, as reported by Mohammadi et al. (2021).

Paring was at a ratio of 1:3 for male and female broodstocks. Mouth clipping was carried out on the male fish to reduce the incidence of cannibalism. The female fish is stocked for five days in the hapa before the introduction of the male fish. The male fish is crossed with the female fish at random to produce half-sib so that phenotypic and heritability correlation could be carried out. Mating of male to female *O. niloticus* (pure strain 1) and male to female *O. aureus* (pure strain 2) made up treatments 1 and 2, while male *O. aureus* to female *O. niloticus* (hybrid 1) and male *O. niloticus* to female *O. aureus* (hybrid 2) made up treatments 3 and 4. All treatments were in triplicates.

2.1. Spawning and fry collection

The paired broodstocks were fed to satiation twice a day at 07:00 and 04:00 GMT with commercial feed (Stretches) of size 3 mm at 25% crude protein. Free swimming fries were scoped out of the water. The mouths of the brooders were also examined for fries and fertilized eggs that were collected and transferred to the hatchery for completion of the hatching process. A total of three mating periods were carried out. The male fish was removed from the hapa after each round of egg production to allow repercussion.

2.2. Fry rearing and sexing

Fries from the experimental units were counted and stocked in a tank of $3x1x4m^2$. They were fed to satiation while maintaining the basic water quality as described by Boyd and Lichtkpper (2002). The fries were fed with artemia and green algae from the pond in a progressive manner and were subsequently fed with various sizes of commercial feed to satiation. Three different culture tanks were used in raising each set that was produced at the same time to reduce the incidence of cannibalism. After a period of twelve weeks, methylene blue and hand lenses were used in separating the sexes. The male fish has only one opening through which both milt and urine pass, while the females have different openings for eggs, and urine exists.

2.3. Data analysis

The sex ratio was calculated with the aid of the Independent Groups t-test, while growth analysis was done by Anova on IBM SPSS v25 while Duncan Multiple Range was used to differentiate the means (Irabor et al., 2021).

3. Results

In Table 1, the result of interspecific breeding infers that a true hybridization between Nile tilapia and Blue tilapia produces a population that is skewed toward the male population. The mating of the pure strain produced both populations; mating between male and female pure 1 produced 126 males and 174 females in the first trial. At the end of the last trial, 470 males and 592 females were produced from a total of 1062. Pure 2 produced 414 males from a total of 1103 after the third trial. Similarly, the trial produces a 44% male population for pure 1 and a 37% for pure 2. Hybrid 1 produces a total of 925 males and 70 females from a total of 995. This makes it 93%, while the hybrid 2 produces 71% from a total of 993, making it 663 males and 270 females.

Table 1. Number of male fish from the trials

Trails	Pure strain 1	Pure strain 2	Hybrid 1	Hybrid 2
1	126(300)	115(318)	206(220)	198(225)
2	146(362)	143(355)	339(355)	215(310)
3	198(400)	156(430)	380(420)	250(398)
Average	470(1062)	414(1103)	925(995)	663(933)
Percentage	44	37	93	71

Values without superscripts are the same.

The mating of Nile tilapia to blue tilapia gives strains at a different number of days. It takes a mean of 24.16 days for the first trail to produce fries for pure 1 and 24.67 days for pure 2. The average number of days for pure 1 and pure 2 was 15.97 and 16.13, respectively. In the first mating, the hybrid recorded success after 28.21 and 28.47 days, respectively, for the hybrids 1 and 2. However, the average number of days for success to be recorded was 19.21 for pure 1 and 19.6 for pure 2. Although the numbers of successful days for the two pure strains were the same at the first mating, they were significantly different from the hybrids (Table 2). This trend repeated itself throughout the times that pairing for mating was initiated (Table 3).

Table 2. Mean number of fries produced at each crossing

Mean (Days)	Pure strain 1	Pure strain 2	Hybrid 1	Hybrid 2
1	24.16 ± 0.33^{a}	24.67 ± 0.63^{a}	28.21 ± 0.54^{b}	28.47 ± 0.34^{b}
2	13.44 ± 0.14^{a}	13.87 ± 0.76^{a}	16.31 ± 0.65^{b}	16.63 ± 0.5^{b}
3	10.32 ± 0.23^{a}	9.85 ± 0.85^{a}	13.12 ± 0.11^{b}	13.71 ± 0.41^{b}
Average	15.97	16.13	19.21	19.6

The superscripts were used to show different averages, and values with the same superscripts are the same.

Table 3. Growth Performance of Interspecific hybridization

Mean (Days)	Pure strain 1	Pure strain 2	Hybrid 1	Hybrid 2
Initial weight (g)	48.01 ± 0.67	48.00 ± 0.75	48.02 ± 0.32	48.00 ± 0.41
Final live weight (g)	82.23 ± 0.23^{a}	77.19 ± 0.11^{a}	91.62 ± 1.54	89.29 ± 1.23
MWG (g)	34.22 ± 0.77^{a}	29.19 ± 0.65^{a}	43.60 ± 0.71^{b}	41.29 ± 0.12^{b}
MDWG (g/day)	0.36 ± 1.23^{a}	0.31 ± 1.41^{a}	0.46 ± 0.33^{b}	0.44 ± 0.51^{b}
PWG (%)	66.58 ± 0.71^{a}	57.73 ± 0.22^{a}	86.52 ± 0.62^{b}	82.51 ± 0.31^{b}
TFI (g)	114.72 ± 1.43^{a}	114.02 ± 1.67^{a}	117.19 ± 1.56^{b}	116.42 ± 1.89^{b}

The superscripts were used to show different averages, and values with the same superscripts are the same; MWG is the mean weight gain; MDWG is the mean weight gain per day; TFI is the total feed intake.

4. Discussion and Conclusion

The reports by Assis et al. (2017); Lago et al. (2016); Nwachi and Esa (2016) inferred that tilapia exhibits sexual dimorphism, making the male fish much more valuable than the female, and the practice of all-male fish, culture is an option to adapt to if the goal of the culturist is to produce food sources for human. A number of ways that could lead to the exclusive production of a population that is skewed toward the male proportions were evaluated.

However, in this study, the use of interspecific breeding by pairing Nile tilapia with Blue tilapia was examined. Pure strains of each of the base parents were crossed, and the resulting fries were examined. Nwachi et al. (2020) reported the sexual difference that was shown at the mating of reddish coloured fish to their wild counterparts, with the conclusion that difficulties (delayed) in producing fries take place because of differences in their colours, despite the fact that they are from the same strain. In this study, more variation was observed in the hybridization matches (produced from pairing the interspecific and pure strains) when compared to that of the pure strain.

It is of note that the interspecific strain produced a population with more males compared to the mating of the pure strains with each other. In Table 1, the mating of the hybrids produced 93 and 71% male proportion, while the crossing of the pure strain produced 37 and 44%. Eknath and Hulata (2009)**;** El-Zaeem et al., (2012) opined that a pure strain of Nile tilapia crossed with Blue tilapia produces a population that is skewed towards the male gender which is in agreement with the results of this study. Similarly, the time at which swim-up appears in the hatching hapa, which is an indication that fries have been produced for the pure strain, is shorter compared to the hybrids. In Table 2, after the three trials, the average length of time for spawning to take place is 15.97 and 16.13 days, respectfully, for the pure strains, while it takes the hybrid 19.21 and 19.60 days, respectively. This assertion was in line with the work of Nwachi et al. (2020) on the effect of colour on the diallel mating of wild tilapia to UPM red tilapia.

Similarly, the hybrid gave a better growth rate throughout the study, as shown in Table 3. The mean growth rate of the hybrid was higher compared to the pure strain despite lower total feed intake, and this was attributed to the genotype of the hybrid. Lugert et al. (2019); De Verdal et al. (2018) were of the opinion that hybrids performed better than their parents, even with a lower feed intake. The mean daily growth rate of 43 and 41g is higher than the 36 and 31g recorded in their parents.

Conclusively, a male skewed population was produced through the interspecific crossing of two related strains of tilapia. The hybrids and their reciprocals produced a higher number of male fish compared to the females. Similarly, better performance was recorded by the hybrids when compared with their pure strains despite the lower feed intake. This means that the production of the hybrid for commercial purposes will give more income.

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Research Article

Effects of Traditional Tillage, Conserved Tillage and No Tillage Methods and Some Allelopathic Practices on Weed Growth in Organic Vineyards

Koray KAÇAN[*1], Fadime ATEŞ[2], Engin ÇAKIR[3], İkbal AYGÜN[4]

[1]Muğla Sıtkı Koçman University, Research and Application Center, 48000, Muğla, Turkey
[2] Viticulture Research Institute, Atatürk Neighbourhood, Horozköy, 45125, Manisa, Turkey
[3,4]Ege University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, 35040, İzmir, Turkey

[1]https://orcid.org/0000-0003-3316-9286, [2]https://orcid.org/0000-0003-4466-4573, [3]https://orcid.org/0000-0003-4573-4991
[4]https://orcid.org/0000-0003-1144-913X

*Corresponding author's e-mail: koray099@hotmail.com

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Abstract: This study was carried out to determine the effects of some tillage methods; it included conventional tillage and conservation tillage with some weed control applications on weed manifestation in organic vineyards. The organic vineyard experiment area was designed as main and sub-plots. The effects of some methods of conventional tillage, no-tillage, and conservation tillage on weed coverage, densities, fresh weight, and dry weight were determined in the organic vineyard experiment area. These tillage methods were applied in the main plots. A chisel and heavy-duty disk harrow were used for conservation tillage methods. The plough and disc harrow were also applied as conventional methods. Other allelopathic methods (olive mill wastewater, radish (*Raphanus sativus* L.), and broccoli (*Brassica oleracea* L.) were applied as sub-plots in the experiment area. As a result of the statistical analysis of the values obtained in the study, the most effective method, the application of the plough and disc harrow, was determined for weed coverage and fresh and dry weight weeds in the main plots. The olive mill wastewater was also determined as the most effective application in the sub-plots. In terms of grape yield, the most effective method in the main plots was the plough + disc harrow application (6.8068 kg vinestock^{-1}). The planting of broccoli (6.4485 kg vinestock^{-1}) was determined as the most effective sub-plot application for grape yield.

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1. Introduction

Equivalent to the increasing use of pesticides in agricultural areas, it is inevitable that the practice of sustainable agriculture will be disturbed as a result of improper practices. In addition, the negative effects of intensive pesticide use on the environment and human health cause increasing health concerns. Therefore, the conscious use of agricultural land to meet the adequate nutritional needs of the world's population (İlter et al., 1998, Aksoy and Altındişli 1999) and alternative methods to conventional agriculture and their integrated management should be investigated. The loss of crops caused by diseases, pests, and weeds is approximately 67.15% in some important crops such as wheat, corn, paddy,

cotton, and soybeans in the world. This loss is caused by 13.78% from disease, 21.75% from pests, and 32% from weeds (Oerke and Dehne 2004).

In Turkey, depending on the type and density of weeds, the average yield loss varies between 10%–50% (Tepe, 1998); however, the yield loss is known to occur in even larger amounts depending on crops. The amount of pesticides used to eliminate these losses is increasing. Besides the many damaging effects of weeds, the most important damage is that they cause a decrease in crops; this decrease amounts to an average loss of 10% in worldwide production (Oerke, 2006). Pesticide use is increasing the cost of production exponentially and causes the irreversible destruction of agricultural systems, as resistance to harmful organisms is caused by intensive pesticide use. According to FAO (Food and Agriculture Organization), the worlds' pesticide use has reached 4 122 334 tons (FAOSTAT, 2018), meaning 41.5% of the pesticide market is used by herbicides, plant growth regulators, and growth inhibitors, 27.1% is used by insecticides, 21.5% by fungicides and 9.9% by other chemicals (FAOSTAT 2017). Using the amount of pesticides is 60 020 tons in Turkey. This amount consists of 20 450 tons of fungicide, 12450 tons of insecticides, herbicides 10025 tons, rodenticides 259 tons, and 6 835 tons of other pesticides (FAOSTAT, 2018). It is known that 0.015%–6% of pesticides used in agriculture reach target organisms. The remaining use of the 94.0%–99.9% of pesticides is mixed with the ecosystem (Yıldız et al., 2005).

Pesticide residue damages the soil, flora, and fauna, which play an important role in the soil. They also pass from the soil to the crops and from there to humans and animals causing harmful effects on the food chain. Pesticides enter groundwater and the atmosphere through evaporation, adversely affecting the reproductive ability of fish and bird populations, along with many other organisms, thereby causing the destruction of organisms (Kortekamp, 2011). However, in organic agriculture, which alternates conventional agriculture with chemicals, one of the most important problems in organic production is weed growth (Reddiex et al., 2001).

Climate change is also emerging as a serious growing threat, largely due to its negative impacts on agricultural production and global food security. (FAO, 2009, Wheeler and Von Braun, 2013). In the last 50-100 years, an increase of approximately 0.5 °C in annual average surface temperature has been observed in different parts of the world (IPCC, 2014). Furthermore, climate change affects the ability of ecosystems to effectively capture carbon and maintain balance in the nitrogen cycle (Fu et al., 2020, Succarrie et al., 2020). There is evidence that conservation tillage (reduced tillage and no tillage) increases yields (Naab et al., 2017) and reduces soil degradation in conserved tillage practices, as well as the addition of plant waste to soil is one mechanism supporting the positive effects of these practices on soil health, soil organic carbon, and yield. The high nitrogen and phosphorus content in (cutting cover crops and incorporating them into the soil before planting the target crop) has been explained as the reason behind their ability to improve yields and soil health (Nziguheba et al., 2000).

Three long-term experiments were conducted with a combination of reduced and conventional plow tillage and stubble tillage to determine weed infestation levels in organic farming. As a result of the treatment, tillage by chisel plough resulted in significantly highest annual weed density compared to all other treatments. The natural *C. arvense* infestation showed the highest shoot density in the plough/chisel treatment (Gruber and Claupein 2009). In another study; showed that zero tillage application increased grain yield by 49% and 18%, gross yield by 43% and 14%, and reduced the total amount of weeds for four years in tillage applications (Sasode et al., 2020). Compared to conventional tillage with zero tillage systems, some It has been observed that perennial weeds have decreased. (Thomas et al., 2004). Therefore, a tillage reduction can be expected in Long-term organic crop systems. Using cover crop mulches for weed control can also lead to a change in the weed community. It can cause an increase in perennial weeds (Ryan et al., 2009). Fewer weeds and lower weed biomass in reduced tillage plots were also observed compared with tilled plots and no-till plots (Vaisman et al., 2011). Besides the amount of cover crop residue, other factors such as field history, cultural practices, and the weed seed bank can also determine the weed type. Species present in the year following the cover crop should b. evaluated in the context of weed competition.

Turkey has the most favorable conditions for grape production and is one of the country. Thus, it ranks sixth in the world in terms of grape production. Furthermore, Turkey has the highest rate of organic grape exports. According to statistics in 2017, 4 200 000 tons of grapes are produced in an area of 416.907 ha (FAOSTAT, 2017). These production figures include 50.2% fresh grapes, 38.1% drying

grapes, and 11.6% wine grapes (TUIK, 2019). The yield for grape production in Turkey is 10 074 kg ha^{-1}. Moreover, organic grapes are produced in 116.283 tons on 403,047 ha^{-1}.

In organic farming applications, the highest costs refer to the expenditures related to the control of weeds (Uygur and Lanini 2006). Therefore, in order to produce successful organic production, weeds must be effectively controlled. The 'organic agriculture' system refers to controlled and certified agricultural production at every stage, from production to consumption, without using chemical input. It is recommended to apply the appropriate soil tillage methods in the control of weeds. However, excessive tillage applications that may cause soil erosion are not allowed (TMOARA, 2005). The most important reasons for tillage are the elimination of crop competition with weeds and supporting the early growth of the crop (Triplett and Dick 2008).

The success of weed control can be measured by crop yield. Chemical weed control is widely used in conventional agriculture. Production costs decrease according to the intensity of the weeds' pressure. Herbicide application rates can be reduced if weeds remain below the economic damage threshold in the crop. Therefore, alternative weed control methods are important for sustainable agriculture. However, perennial weeds with longer life spans and deep root systems cannot be reduced by herbicides. The seed reserve in the soil and weed infestation tends to increase under conservation tillage and no-tillage (Légère et al., 2011).

Weeds can obstruct the early development of the crop both by reducing the nutrient content of the soil and by reducing the soil temperature in conservation tillage and no-tillage applications (Triplett and Dick, 2008; Boomsma et al., 2010). At the same time, they can also significantly reduce crop yield (Davis et al. 2005). Generally, the presence of weeds depends on soil conditions, cultivation treatments, thermal conditions, and weed seed reserves in the soil (Shahzad et al., 2016, Skuodiene et al., 2018).

Thanks to the ploughing system, more than 33% of weeds on the surface of the soil were carried to the deeper layers of the soil as a result of ploughing, which significantly decreases the emergence of weeds (Woźniak, 2007). On the other hand, opinions on crop infestation with weeds in the ploughing and no-till systems are inexplicit. Therefore, our primary objectives were to determine the control efficiency of different tillage systems and organic practices on weeds.

2. Materials and Methods

2.1. Experiment area

This study was conducted at the Viticulture Research Institute between 2015 and 2018 in Manisa 38° 38' 0,9.40" N, 27° 23' 59.43" E). The variety was seedless Royal which is planted at a range of 3 m - 2 m in the experiment area. Some organic weed control practices consisted of radish, broccoli, and mill olive waste water.

2.2. General features of the Manisa province

Manisa province is only 41 kilometers away from the Aegean Sea. It is located between 27 08' and 29 05' east longitudes and 38 04' and 39 58' north latitudes, with an area of 13,810 km2 (Figure 1). The prevailing climate in Manisa is also referred to as the Mediterranean land climate type. Temperatures rise in the summer, while rainfall intensifies in the winter. The months of summer are very hot, as the characteristics of the continental Mediterranean climate prevail in Manisa. The average annual temperature is 16.3ºC. The coldest months are January and February. The Western Anatolia region has the precipitation characteristics of the Mediterranean climate type (MMMID, 2019).

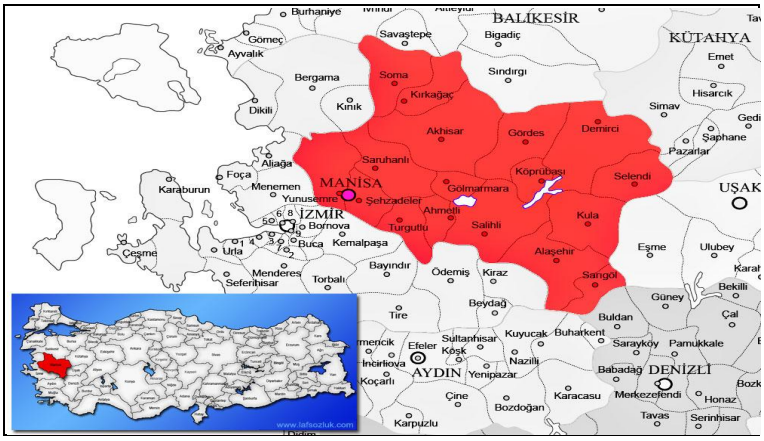

Figure 1. Map of the province of Manisa, Turkey.

2.3. Soil analysis

The soil samples were taken from 0–30 cm depth, and some physical and chemical analyses were performed to determine the initial soil properties. Cation exchange capacity (CEC) was determined by using sodium acetate (buffered at pH 8.2) and ammonium acetate (Sumner and Miller 1996). The Kjeldahl method was used to determine organic N (Bremner 1996), while plant-available P was determined by using the sodium bicarbonate method (Olsen et al., 1954).

Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (Rhoadesö 1996). Soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to McLean (McLean, 1982). Soil organic matter was determined using the Smith-Weldon method (Nelson and Sommers, 1982). Ammonium acetate was buffered at pH 7 (Thomas, 1982). was used to determine exchangeable cations. After extraction, the P, K, Ca, Mg, and Na contents were determined using an inductively coupled plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484- 4794, USA). The analysis results for soil physical and chemical properties are given in Table 1.

Table 1. Chemical and physical characteristics of the soil

Evaluated Characters	**Values**	**Evaluated Characters**	**Values**
pH (1 mol KCL dm^{-3})	**7.50**	K (mg kg^{-1})	593.3
Salt (dS m^{-1})	**0.015**	Ca (mg kg^{-1})	620
Lime (%)	**5.62**	Mg (mg kg^{-1})	463.5
Organic matter (%)	**1.41**	Fe (mg kg^{-1})	5.15
N (%)	**0.18**	Cu (mg kg^{-1})	2.84
P (mg kg^{-1})	**2.68**	Zn (mg kg^{-1})	0.78
Soil type	**Sandy-loamy**	Mn (mg kg^{-1})	6.58

2.4. Climate data

The climate values measured between 2015-1018 in the experimental area are given in Table 2-3.

Table 2. Weather data at the experimental station between 2015 and 2018

	Annual	January	February	March	April	May	June	July	August	September	October	November	December
°C	16.1	6	8	10	15	20	24	27	26	23	17	12	8
Average High Temperature													
°C	22.0	10	8	15	21	26	31	34	33	30	23	17	12
Average Low Temperature													
°C	10.2	2	0	5	9	13	17	20	19	16	11	7	4
Average Precipitation													
mm	740	129	109	88	59	24	9	-	-	26	49	90	160
Average Length of Day													
Hours	12.7	10.3	11.2	12.4	13.7	14.8	15.3	15.1	14.1	12.9	11.6	10.5	10

Table 3. Precipitation data at the experimental station between 2015 and 2018

Date	**Liquid Precipitation (mm)**			**Number of Days**		
Elem	**> PRCP**	**EMXP**		**DP01**	**DP10**	**DP1X**
Year	**Total Liquid Content**	**Extrem Max Precip.**	**Max. Precip. Date of occurence**	**Precip >= 0.01**	**Precip >=0.10**	**Precip >= 1.00**
2015	725.4	50.8	Oct-25	77	50	9
2016	669.3	86.1	Jan-18	69	37	6
2017	693.7	74.9	Mar-08	82	49	8
2018	564.9	26.9	Dec-10	86	46	2

The number of days above 20 ºC in 2017 was 68. It was 68 days in 2018. It was 41 ºC in July 2017, 39 ºC on the 19th of August 2018. 2017 average was 23.5 ºC, 2018 average was 24.1 ºC. (NOAA, 2021. NOAA's National Centers for Environmental Information).

2.4. Experiment details

Experiments were conducted in 2015 and 2018 in the experiment vineyards of the Viticulture Research Institute. The experiment contained four replicates and made use of a randomized complete block split-split plot design. A row of the vineyard was left as a space buffer between each main plot. Each main plot was divided into three subplots with the four vines in each subplot, and the two vines maintained as buffers. The first treatment (the main plots) split-plot used the conventional conservation and no-tillage methods. The conservation tillage methods included the use of a chisel and heavy duty disc harrow. Broccoli, radish, and olive mill wastewater were investigated as Organic allelopathic weed control applications. Experiments were undertaken with four replicates applications consisting of 12 vines per plot.

2.5. Soil tillage Applications

Conventional Tillage (CT): In this method, it was carried out as an intensive cultivation applied by farmers in the region. The conventional tillage with the use of a disc harrow and a plough were carried out twice a year. 1. Plough applications in Autumn with two passes of disc harrow application. 2. Weed count and plough application with two passes of disc harrow application in April.

Conservation Tillage (CST1): This method was applied in ways using a chisel. Conservation Tillage : 1. Soil tillage applications with chisel in Autumn 2. Weed count and chisel application in April

Conservation Tillage (CST2): This method was applied in ways using a heavy disk harrow. 1. Soil tillage applications with heavy duty disc harrow in Autumn. 2. Weed count and heavy duty disc harrow application in April.

No Tillage (NT): Grape production was carried out without soil tillage in the experiment plots. Weeds were count in April. (Figure 2).

Control (C): Weedy plots control.without any weed control.

Control application

Chisel application

Plough application

Disc harrow application

No tillage application

Figure 2. The soil tillage applications

2.6. Organic allelopathic weed control applications

Olive mill wastewater, radish, and broccoli were used to determine the effectiveness of weed control in organic viticulture. These plants were selected because of their allelopathic properties. In order to compensate for the missing nutrients in the vineyard, stable manure (1.5 tons da^{-1}) and green fertilizer (barley + vetch + fababean 2.5 + 3.5 + 7.5 kg da^{-1}) were applied.

2.7. Identification of weeds and measurements

While counting, the broadleaf weeds were counted by the whole plant in the plots, while the narrow-leaf weeds were counted by the shoot number. The recognized weed species were recorded, and the unrecognized species were numbered and brought to the laboratory. Afterwards, their diagnosis was made as a result of the comparison of plants in the flora of Turkey (Davis, 1965; Davis, 1988). In order to determine the effect of the applications on weeds, the type and number of weeds were determined by throwing a 50 x 50 cm^{-2} frame on each plot 28 and 56 days after the application (Figure 3). The effects of the applications were determined as a percentage by applying the following Abbott formula to weed coverage and weed density (Abbott, 1925).

$$\%\ EFFECT = \left(\frac{Weed\ Coverage{-}Density\ in\ Unapplied\ Control\ Parcels{-}Weed\ Coverage{-}Density\ in\ Applications\ Parcels}{Weed\ Coverage{-}Density\ in\ Unapplied\ Cotrol\ Parcels}\right) \times 100$$

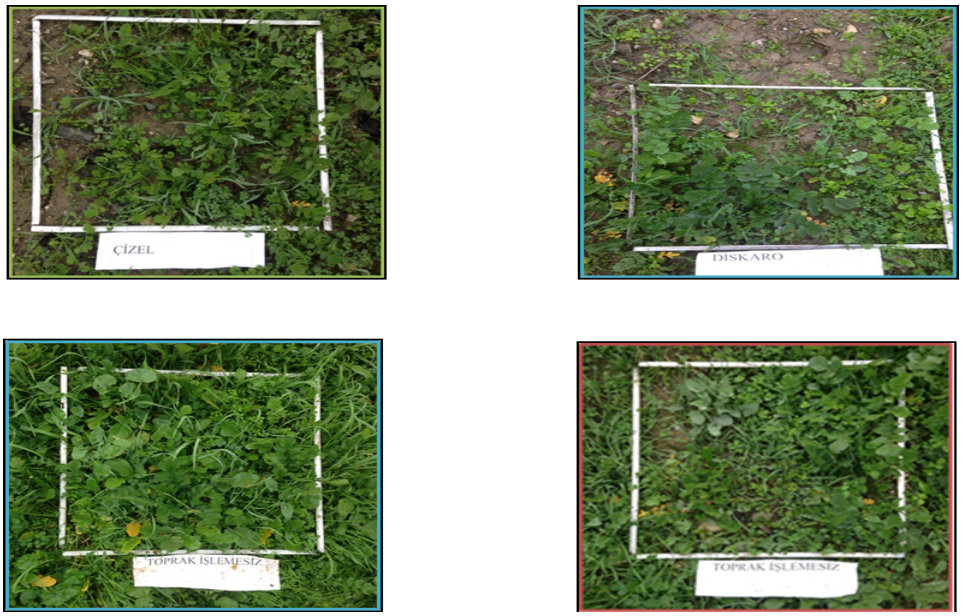

Figure 3. Images of weed counts from the experiment area.

Effects of the applications on grape yield: The number of grapes obtained from the vines in the application plots were weighed, and the yields were determined as grape kg vine^{-1}. Statistical Analysis: The values obtained in the counts performed in the plots in the experiment area were applied using one-way variance (ANOVA) analysis and the IBM SPSS v22 package program; the differences between the averages obtained from the measurements were subjected to variance analysis by Duncan Multiple Comparison.

3. Results

3.1. Effects of different soil tillage methods on weed coverage

The results of the Duncan Multiple Comparison Test on the different tillage methods between 2015 and 2018 for weed coverage are shown in Table 4. As a result of the statistical analysis of weed coverage, differences between the main plot and sub-plot applications and the weed coverage obtained between the years were found to be statistically significant for $p \leq 0.05$. At the same time, applications–years and the interaction of the main plot applications between the sub-plot applications are statistically significant for $p \leq 0.05$.

Statistical analysis was carried out by taking the average of the weed covering values obtained at the time of both counts of tillage applications. The weed coverage obtained at the second count time shows similar information to the data in the first count time but was increased by 8%–30% with the development of weeds (Table 4). Compared to the control plots (C), the plow and disc harrow (CT) were the most effective application by reducing the coverage of weeds by 70.39%. The least effective application was the NT method, with 33.34%.

The main plot applications on weed fresh weights were found to be statistically as significan in terms of the applications and interactions of the main applications with years in organic experiment plots. The CT applications were the most effective application by reducing the fresh weight of weeds by 68.31%. Other applications were found to be effective at rates of CST1 32.47%, CST2 29.64%, NT 16.84%, respectively. The most effective application was the plough and disc harrow method CT (53.37%) in terms of dry weights of weeds. In plots of no-tillage (7.42%) was the least effective application. Other applications had an efficiency of 28% to 31.07%.

Table 4. Effects of main plot applications on weeds (2015–2018)

Treatment	CT	CST1	CST2	NT	C	SE	ANOVA
Weed coverage (%)	24.97d	51.94c	54.44bc	56.22b	84.34a	5,357	***
Fresh Weed Weight (g m^{-2})	225.14d	479.72c	499.84c	590.73b	710.40a	12.846	***
Dry Weed Weight (g m^{-2})	57.58c	88.95b	85.57b	114.92a	124.14a	3.642	***

ns, not significant. ANOVA: ***P <0.001, **P <0.01, *P <0.05. The Standart Error = SE, CT: Conventional, CST1: Conservational, CST2: Conservational, NT: No-Tillage, C: Control.

3.2. The effects of Organic allelopathic weed control applications on weed coverage in the experiment

All the Organic allelopathic weed control applications were reduced to between 40.12% and 50.34% of the weed coverage compared to the control plots. According to the control plots, the olive mill wastewater application (50.34%) was the most effective application, while broccoli (44.94%), radish (42.20%), and the control plots were listed (Table 5).

The effects of the Organic allelopathic weed control applications on fresh weed weights and year interactions were found to be statistically important ($p \leq 0.05$). Olive mill wastewater, the most effective application, had an efficiency rate of 48.57% for weed fresh weights. The least impact was obtained from the application of radish (%35.20).

As for the weed dry weight, the effects on weed dry weights were found to be important in the sub-plot's applications. Nevertheless, the weight differences in their interactions between the sub-plots *year and sub-plots* main plot applications were insignificant. The olive mill wastewater had the highest effective rate (46.87%) for the dry weight of weeds. The least impact was obtained from the application of radish (30.10%).

Table 5. Effects of Organic allelopathic weed control applicationss on weeds (2015–2018)

Treatment	OW	B	R	SC	C	SE	ANOVA
Weed coverage (%)	41.88d	46.44c	48.75b	50.50b	84.34a	1.668	***
Fresh Weed Weight (g m^{-2})	365.34c	425.12b	460.27b	544.94a	710.40a	16.349	***
Dry Weed Weight (g m^{-2})	65.95d	85.13c	85.66c	110.28b	124.14a	5.460	**

ns, not significant. ANOVA: ***P <0.001, **P <0.01, *P <0.05. The Standart Error = SE OW: Olive mill wastewater, B: Broccoli, R: Radish, SC: Sub-plots control, C: Control.

The effects of the applications in the weed coverage in 2015 were 20.39% higher than the effects in 2017, which were the least impactful. The effects in other years have followed to 2015 effect (Table 6). According to the statistical analysis of fresh weed weight, differences in the fresh weight of weeds obtained from applications over the years were found to be important. In 2017 and 2018, with the decrease in the amount of precipitation, the increase in the average temperature, especially the hot months of July and August, had a negative effect on the fresh and dry weight of weeds. Accordingly, the lowest weed fresh weight was determined in 2017, while the highest weed fresh weight was attained in 2016. Moreover, differences in the distribution of dry weed weights were obtained from applications between the years and were found to be important. Accordingly, the lowest weed dry weight was achieved in 2017, while the highest dry weights were achieved in 2016 (Table 6).

Table 6. Effects of the applications on weeds according to years (2015–2018)

Years	2015	2016	2017	2018	ANOVA
Weed coverage (%)	48.00c	54.15b	60.30a	55.07b	*
Fresh Weed Weight (g m^{-2})	752.07b	831.03a	159.79d	261.76c	***
Dry Weed Weight (g m^{-2})	82.07b	124.75a	78.45b	86.71b	**

ns, not significant. ANOVA: ***P <0.001, **P <0.01, *P < 0.05.

3.3. Effects of different tillage methods on grape yield

Statistically, compared to the effects of grape yields of the applications, the obtained yield differences were statistically important in the tillage methods. The results of the yield were important in the interactions between the year and the applications. The yields obtained from the conventional plots were about 2.65 times the yield of the control plots. The no-till application had nearly 2.62 times the yield of the control plots. While the third efficiency was obtained from the disc harrow application (CST2), the lower yield was obtained from the chisel application (CST1). (Table 7).

Table 7. Effects of the applications on grape yield (kg Vinestock^{-1}) (2015–2018)

Treatments	CT	CST1	CST2	NT	C	SE	ANOVA
Yield	6.80d	4.40c	5.29b	6.71d	2.56a	0.213	***

ns, not significant. ANOVA: ***P <0.001, **P <0.01, *P <0.05. The Standart Error = SE, CT: Conventional, CST1: Conservational, CST2: Conservational, NT: No-Tillage, C: Control.

The grape yield differences obtained from the sub-plots were found to be statistically important. Except for the control plots, all other sub-plot applications were included in the same statistical group (b). While the highest efficiency was obtained from broccoli, olive mill wastewater and Radish applications were also followed this application (Table 8).

Table 8. Effects of the sub-plot applications on grape yield (kg Vinestock^{-1}) (2015–2018)

Treatments	OW	B	R	SC	C	SE	ANOVA
Yield	6.17b	6.45b	6.13b	3.51a	2.54a	0.347	***

ns, not significant. ANOVA: ***P <0.001, **P <0.01, *P <0.05, The Standart Error = SE, OW: Olive mill wastewater, B: Broccoli, R: Radish, SC: Sub-plots control, C: Control.

4. Discussion

In general, weed seeds tend to come to the surface in no-tillage soil while also preventing the proliferation of weeds in well-tillaged soil. The seeds of weeds remained in the first 10 cm layer of soil before soil tillage, while the seeds were spread across 20 cm of the soils' surface by the tillage of the soil (Buhler et al., 2001). More than 50% of the total weed seeds are located at a depth of 0 to 5 cm, and this percentage decreases as soil depth increases (Buhler et al. 1997, Chauhan et al., 2006). Moreover, it is stated that the plough buries weeds in the soil and prevents them from germinating, and destroys their existing shoots (Boström, 1999). Plough tillage ensures that germination conditions are limited by spreading weed seeds deeper into the soil so that seed dormancy lasts longer (Børresen and Njos, 1994). However, weeds are effectively controlled by conventional tillage in the early season (Steckel et al., 2007); therefore, weed infestations may occur with this tillage system in the late season.

For many years, it is inevitable that the weeds adapt to conventional tillage systems in the same way for many years. Thus, new alternative systems are needed to compensate for crop losses (Harker and Clayton, 2004).

Conservation tillage systems not only improve the physical properties of soil, but they also enhance soil water availability (Unger 1994, Drury et al., 1999). At the same time, it may facilitate root growth (Martino and Shaykewich, 1994). Conservation tillage may be more productive than conventional tillage because it improves the soil quality and water use efficiency of crops (Samarajeewa et al., 2006).

Zero tillage (ZT) is one of the effective practices of conservation tillage, which reduces costs for land preparation, fuel consumption, equipment use, labor cost and increases crop yield by protecting soil and water (Farooq et al., 2011, Jabran and Mehmood 2015). Nevertheless, it also restricts the growth of the main root axis in the early stages of plant development. It also restricts the growth of the main root axis in the early stages of plant development (Lampurlanes et al., 2001). Many researchers draw attention to the change in weed flora after the application of the conservation tillage system. Moreover, while perennial weeds can be controlled in conventional tillage systems, they can become a major problem in conservation tillage applications (Nyagumbo, 2008; Mashingaidze et al., 2012). Therefore,

conservation tillage contains high weed densities as opposed to conventional systems during the initial years of adoption (Cardina et al., 2002, Sosnoskie et al., 2006). The conservation tillage system also encourages weed seed banks and germination of higher weed emergence (Barberi and Lo Cascio, 2001). Therefore, this is needed to control new weed practices, which help control or reduce weed populations in conservation tillage methods. Allelopathy is a viable tool for weed management in conservation tillage (Jabran and Farooq, 2013, Jabran et al., 2015). Alternatively, allelopathy can also be used due to resource competition and non-chemicals in the weed control system.

According to the statistical consequences of the effects of the applications on weed fresh weights, the plough and disc harrow method (225.15 g m^{-2}) was found to at least the fresh weight of weed, and it also had 68.30% effectiveness when compared to the control plots in the organic vineyard. This application has been followed by chisel, disc harrow and no-tillage applications. As for the effectiveness of sub-plot applications, in terms of weed fresh weight, the effective applications of the olive mill wastewater, broccoli and radish applications are listed respectively.

The most effective application for the dry weights applications in the experiment area was the plough and disc harrow method (53.62%). In the sub-plot applications, olive mill wastewater (46.87%) was the most effective application. Studies with olive mill wastewater investigated the possibility of solid and liquid forms as fertilizers and herbicides. The effectiveness of different doses was evaluated in olive mill wastewater in wheat fields, while the solid form of olive mill wastewater was tested in the fields of sunflower and maize (Boz et al., 2003). According to the results obtained, it was determined that the olive mill wastewater was 90% effective against little hogweeds (Portulaca oleracea) in the wheat fields. Some doses of olive mill wastewater prevented the total weed density at rates ranging from 39%–100%. In another study for the control of weeds in fig nurseries, olive mill wastewater was applied before planting of the fig seedlings and was successful in the control of annual weeds, especially Portulaca oleracea, etc. This effectiveness also continued for three months (Öğüt, 2007).

In this study, the plough and disc harrow method showed the highest effect (70.39%) in terms of weed coverage in the experiment field. This application was followed by the chisel (38.41%) and no-tillage (33.34%) methods. Tillage has been shown to reduce weed populations in perennial agro-ecosystems such as vineyards (Kazakou et al., 2016, Hall et al., 2020). In different studies, the most effective application was the plough tillage for weed coverage in winter wheat fields (Kende et al., 2017). The plough and rotavator disc harrow tillage method as a similar example, as well as the wheat mulch application, were the most effective for weed biomass in maize fields (Din et al. 2013).

It has been determined that the application of no-tillage and clipped weeds increased crop yields. Other cover crops also increased yield by increasing the water content and the amount of carbon in the soil (Kaçan and Boz, 2014, Hashimi et al., 2019). In this study, the highest yield per vinestock was obtained from the plough and disc harrow method (6.80 kg $Vinestock^{-1}$) in the main plots. Moreover, the highest yield was harvested from broccoli (6.45 kg $Vinestock^{-1}$) in the sub-plots. In the same way, the applied mulch textile without soil tillage was determined as the most effective application in terms of grape yield in organic vineyards (Kaçan and Boz, 2014).

Conclusion

The plough and disc harrow method had the most effective application in terms of weed control in the main plots. Olive mill wastewater was determined as the most effective of the applications as subjects in sub-plots for the 2015–2018 period in the organic vineyard. The plough and disc harrow method also achieved the highest yield, while the yields of no-tillage application were very close to this yield. Accordingly, there was no tillage application evaluated in terms of workforce and energy, and it was revealed that allelopathic plants and organic waste should be included in the weed management system for sustainable agriculture.

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Research Article

Validity Control of Markers Used in Molecular Marker Assisted Selection in Tomato

Ceylan Pınar UÇAR[*1], Suat ŞENSOY[2]

[1,2]Van Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri ABD, Van Türkiye
[2]Van Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi Bahçe Bitkileri Bölümü, Van Türkiye

[1]https://orcid.org/0000-0001-9056-9353, [2]https://orcid.org/0000-0001-7129-6185

*Corresponding author e-mail: ceylanucar2@gmail.com

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SCAR,
Solanum lycopersicum L.

Abstract: This study aimed to check the validity of the SCAR and CAPS markers developed for certain diseases and pests on some tomato cultivars and genotypes in molecular marker-assisted selection. For this purpose, developed molecular markers for resistance were tested for tomato wilt virus (TSWV), Fusarium wilt (FOL), Tomato leaf curl virus (TYLCV), and root-knot nematode (RKN). SCAR Scr-001 markers for TSWV, TAO1 CAPS marker, and P743DF1-P743DR1, P743DF3- SCAR P6-25 markers for FOL; Scar P6-25 marker which TYLCV; SCAR Mi-23 and PMI, of RKN, CAPS APS and C8B markers for P743DR3, P743DF1-P743DR1, At2F- ToMV were selected. These selected markers were screened in 24 tomato genotypes, 9 of which were commercial and 12 local genotypes as well as the control group, Mountain Merit, NCICELBR, and NCI123S. SCAR Scr-001 marker for TSWV; TAO1 CAPS marker and P743DF3-P743DR3, P743DF1-P743DR1, At2-F-At2-R SCAR markers for FOL; P6-25 SCAR marker for TYLCV; and SCAR Mi-23 and PMI markers for RKN gave results. In this context, it was concluded that the mentioned markers could be efficiently used in marker-assisted selection studies in tomatoes.

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Domateste Moleküler Markör Yardımlı Seleksiyonda Kullanılan Markörlerin Geçerliliğinin Kontrolü

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Anahtar kelimeler

CAPS,
Domates,
Moleküler markör yardımlı seleksiyon,
SCAR,
Solanum lycopersicum L.

Öz: Bu çalışmada, domateste bazı hastalık ve zararlılar için geliştirilen SCAR ve CAPS markörlerinin moleküler markör destekli seleksiyon çalışmalarında geçerliliğini kontrol etmek amaçlanmıştır. Bu amaçla Domates lekeli solgunluk virüsü (TSWV), Fusarium solgunluğu (FOL), Domates yaprak kıvırcıklığı virüsü (TYLCV), kök-ur nematodu (RKN), gibi hastalık ve zararlılara dayanıklılık için geliştirilmiş olan moleküler markörler test edilmiştir. Bu markörlerden, TSWV için SCAR Scr-001 markörü, FOL için CAPS TAO1 markörü ve P743DF3-P743DR3, P743DF1-P743DR1, At2F- At2R SCAR markörleri; TYLCV için SCAR P6-25 markörü, RKN için SCAR Mi-23 ve PMİ markörleri kullanılmıştır. Bu markörlerin kontrolü, 9'u ticari 12'si yerli çeşit ve kontrol grubu olarak da Mountain Merit, NCICELBR ve NCI123S genotipleri olmak üzere 24 domates genotipinde PCR yöntemiyle yapılmıştır. TSWV için SCAR Scr-001 markörü; FOL için TAO1 CAPS markörü ve P743DF3-P743DR3, P743DF1-P743DR1, At2-F- At2-R SCAR markörleri; TYLCV için P6-25 SCAR markörü ve RKN için

SCAR Mi-23 ve PMİ markörlerinden sonuç alınabilmiştir. Bu bağlamda sonuç alınan SCAR ve CAPS markörlerinin domateste moleküler yardımlı seleksiyon çalışmalarında etkin bir şekilde kullanılabileceği sonucuna varılmıştır.

1. Giriş

Personatae takımının Solanaceae familyasından yer alan domates türleri Yeni Dünya'dan diğer kıtalara yayılmıştır. Türkiye'ye domatesin Adana'dan girdiği bilinmektedir. Ülkemizin iklim şartlarının uygun olması nedeni ile domates yetiştiriciliği hızla yayılmış ve bu sebzeyi işleyecek sanayi 1970'li yıllardan itibaren kurulmaya başlamıştır. Türkiye domates üretiminde dünya ülkeleri içinde alt sıralardan hızla üst sıralara yükselmeyi başarmıştır (Şalk ve ark., 2008).

Domates lekeli solgunluk virüsü, nematod, fusarium, domates sarı yaprak kıvırcıklığı virüsü, hastalıkları ve zararlıları dünyada olduğu gibi Türkiye'de de büyük verim kayıplarına sebep olmaktadır. Domates Lekeli Solgunluk Virüsü (TSWV), 1915'de Avustralya'da tespit edilmiştir. Virüsün başlıca vektörü olan *Frankliniella occidentalis* Perg. tripsinin kıtaya yayılması ile diğer ülkelerde de hastalık saptanmaya başlamıştır (Cho ve ark., 1989). Türkiye'de Tekinel ve ark. (1969), Mersin ili çevresinde yetiştiriciliği yapılan çeşitli sebzelerde bu virüsün belirtilerine rastlamıştır. Çanakkale'de tütün yetiştirilen bölgelerde görülmüş, bunun arkasından Balıkesir, Manisa, Uşak ve Samsun illerinde de tespiti yapılmıştır (Azeri, 1981). 1997 yılında Şanlıurfa'nın domates yetiştirilen alanlarında Güldür (1997) tarafından TSWV etmeninin olduğu ilk kez raporlanmıştır. Domates kök-ur nematodu Meloidogyne spp. (RKN), tamamen bitkinin kök kısımlarına yapışarak beslenen bir parazittir. Domateste kök ur nematodlarının varlığı, bitki köklerinde oluşturduğu yumrular ile belli olur ve nematodlar bu oluşan urlar içerisinde kendine yaşam alanı sağlamaktadır. Bu urlar, bitkideki su dolaşım sistemini bozar ve kökün işlevlerini engeller (Thorne, 1962). Domates bitkisinde gelişme yavaşlar ve durur, bodurlaşma meydana gelir. Domatesin yaprak kısımlarında sararma, çiçek ve meyve dökülmeleri görülür. Enfeksiyon ağır bir pozisyona gelirse bitki tamamen kuruyabilir (Trudgill ve Blok, 2001). Fusarium solgunluk etmeninin (Fusarium spp) tropik ve subtropik iklim şartlarında ve kumlu topraklarda daha zararlı olduğu görülmektedir. Etmen dayanıklı sporları olan klamidiospor formunda olumsuz şartları geçirebilir. Tohum ve toprak kökenli bir hastalık etmenidir (Dilmaç ve ark., 2020). Domates Sarı Yaprak Kıvırcıklık Virüsü (TYLCV), domates yetiştiriciliğini etkileyen en önemli hastalıklardan birisi olup, geminivirüs (çift parçacık halinde) grubundandır ve beyazsinek ile taşınır. TYLCV'nin konukçu dizisi oldukça geniştir. Konukçularının bazılarında belirti bulunmayabilirken, hastalık o bitkinin içinde bulunabilir ve ergin beyazsineklerle hastalık etmeni bulunmayan sağlıklı bitkilere taşınabilir (Çolak Ateş ve ark., 2017).

Moleküler markör destekli seleksiyon (MAS) kullanılmasının avantajları, çevre etkisi nedeniyle örtülen genlerin tespit edilmesinde kolaylık sağlamaktadır: MAS ile resesif genler de güvenli bir şekilde belirlenebilmekte; Analizi yapılacak olan genotip sayısı büyük ölçüde azalmakta; Araştırmanın başında aktarılmak istenilen geni bulundurmayan genotiplerin çoğu elemine edileceği için daha efektif bir ıslah programı oluşturulabilmekte; Geriye melezleme ile dayanıklılığın aktarılmasını kolaylaşmakta; fenotipik olarak taranması zor olan özelliklerin belirlenmesinde kolaylık sağlanmakta; erken dönemde genotip bilgisi elde edilebilmekte; ve ebeveynlerin belirlenip melezlemelere erken dönemde başlanması gibi birçok avantaj sağlamaktadır (Güleç ve ark., 2010). Ayrıca, karantina kapsamındaki bazı hastalıklara dayanıklılık için testlemeye gereksinimin olmaması ve az sayıda bitkiyle yapılan çalışmalara olanak sağlaması iş gücü ve maliyeti düşürmede katkı sunmaktadır.

Markör tipi, moleküler markörlerin ıslahta ve genetik çeşitliliğin belirlenmesinde kullanılmasında büyük öneme sahiptir (Sensoy ve ark. 2007; Güleç ve ark., 2010; Ertuş ve ark., 2014; Taş ve ark., 2021). Kodominant markörlerin heterozigot bireyleri ayırabilme özelliği yüzünden, MAS'ta özellikle kodominant markörler kullanılmaktadır. MAS programları dominant özelliklerin yanında genellikle kodominant SCAR (Sequence Characterized Amplified Regions) ve CAPS (Cleaved Amplified Polymorphic Sequence) markör sistemleri üzerine yoğunlaşmıştır (Collard ve Mackill, 2008). SCAR markörleri genetik haritada belirli bir gen ya da özellikle bağlantılı olarak tespit edilen RAPD-AFLP gibi bantlardan yola çıkılarak geliştirilebilmektedir. PCR-RFLP olarak da bilinen CAPS (Cleaved Amplified Polymorphic Sequence), uygun primerler kullanılarak PCR ile çoğaltılmış DNA bölgelerinin restriksiyon enzimleriyle (endonükleaz) parçalanmasına dayanan ve sonucunda DNA parçacık uzunluk polimorfizminin elde edildiği bir tekniktir (Filiz ve Koç, 2011).

Moleküler markörler, farklı özellikleri ve karakterleri DNA düzeyinde ölçen ve araştırılan genotiplerde istenen bir geni ya da özelliği izlemek için kullanılabilen araçlardır. Modern ıslah programlarında birden fazla dayanıklılık geninin ıslah hat veya çeşitlerine aktarılması hedeflenmektedir. Moleküler markörler 1980'den bu yana çoğu bitkide yoğun bir şekilde kullanılmaktadır. Özellikle domateste hastalıklara dayanıklılık genleriyle bağlantılı markörler geliştirilerek ıslah programlarında kullanılmaktadır (Grube ve ark., 2000). Moleküler markörlerin geliştirilmesi, kantitatif karakterlerle çalışmayı daha kolay hale getirdiğinden dolayı büyük ilgiyle karşılaşılmıştır. Mevcut çalışmada, domateste farklı araştırmacılar tarafından geliştirilen markörlerin ülkemizdeki bazı domates çeşitleri ve genotiplerinde çalışıp çalışmadığının kontrolü amaçlanmıştır.

2. Materyal ve Yöntem

Bu çalışmada 24 domates genotipi kullanılmıştır. Domates genotiplerinin 9'unu F1 hibrit çeşitler, 15'ini farklı bölgelerden temin edilmiş olan yerel çeşitler (Erzurum sırık, Van sırık, Siirt yerli1, Siirt yerli 2, Gönen, Sencan sırık, Köylüm, Armut, Yayla, Sırık kapıdağ, Oturak geleneksel ve Patika) oluşturmuştur. Ayrıca, North Caroline Üniversitesi'nden temin edilmiş olan ve hastalık ve zararlılara dayanıklılık durumları bilinen 3 genotip (Mountain Merit, NCICELBR ve NC123S) kontrol genotipleri olarak çalışmada yer almıştır. Yedisi SCAR ve 1 CAPS markörü olmak üzere 8 adet markör çifti çalışmada kullanılmıştır (Çizelge 1). Genotiplere ait tohumların viyollere ekimi Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü iklim odasında yapılmıştır. Yaklaşık 15-20 gün sonra bitkiler 2-3 gerçek yaprak dönemindeyken, genç yapraklardan örnekler alınmıştır. DNA izolasyonu, Doyle ve Doyle (1990)'a göre geliştirilmiş olan CTAB metodunun modifiye edilmesi ile yapılmıştır. Elde edilen DNA örneklerini elektroforezde görüntülemek için % 2 hazırlanmış agaroz jel kullanılmıştır. Baz büyüklüklerinin belirlenmesi amacıyla da 100 bp DNA ladder kullanılmıştır. Yirmi dört domates genotipi üzerinde SCAR ve CAPS markör yöntemleri kullanılmıştır. CAPS markör tekniğinde gen spesifik markör çiftleriyle PCR ortamında çoğaltılan amplifikasyon ürünleri kesme enzimleri ile kesilmiştir (Jiang ve ark., 1997). Çizelge 1'de belirtilen TAO-1 CAPS markörü için RsaI kesme enzimi kullanılmıştır. SCAR markör yöntemiyle Sw-5 genine ait scr-001 markörü, I-3 genine ait P43DF3-P43DR3 ve P43DF1-P43DR1 markörleri, I-1 genine ait At2-F-At2R markörü, Ty3 genine ait P6-25 markörü, Mi-1 genine ait Pmi ve Mi-23 markörleri PCR ortamında çoğaltılmıştır. Scr-001, At2F-At2R, TAO-1 markörlerine ait amplifikasyon ürünleri %2 elektroforez jel ortamında yürütülmüştür. P43DF3-P43DR3 ve P43DF1-P743DR1, P6-25, Mi-23, Pmi markörleri ise kapillar elektroforez ile görüntülenmiştir.

Çizelge 1. Çalışmada kullanılan moleküler markörlere ait primer dizilimleri

Hastalık/ Zararlı	Gen	Markör	Markör tipi	Markör ID	Markör dizilimi Kaynak
TSWV	Sw-5	SCAR	Dominant	Scr-001	F=CTGGGTGAGTCTTGACAT Dianese ve ark., 2010 R=CTGGGTGAGTACATCAGATT
FOL	I-3	SCAR	Ko-dominant	P7-43DF3 P7-43DR3	F=CACGGGATATGTTRTTGATAAGCATGT Barillas ve ark., 2008 R=GTCTTTACCACAGGAACTTTATCACC
FOL	I-3	SCAR	Ko-dominant	P7-43DF1 P7-43DR1	F=GGTAAAGAGATGCGATGATTATGTGGAG Barillas ve ark., 2008 R=GTCTTTACCACAGGAACTTTATCACC
FOL	I-1	SCAR	Dominant	At2-F At2-R	F=CGAATCTGTATATATTACATCCGTCGT Arens ve ark., 2010 R=GGTGAATACCGATCATAGTCGAG
FOL	I-2	CAPS	Ko-dominant	TAO1	F=GGGCTCCTAATCCGTGCTTCA Staniaszek, 2007 R=GGTGGAGGATCGGGTTTGTTTC
TYLCV	Ty-3	SCAR	Ko-dominant	P6-25	F=GGTAGTGGAAATGATGCTGCTC Ji ve ark., 2008 R=GCTCTGCCTATTGTCCCATATATAACC
RKN	Mi-1	SCAR	Ko-dominant	Pmi	F=GGTATGAGCATGCTTAATCAGAGCTCTC Arens ve ark., 2010 R=CCTACAAGAAATTATTGTGCGTGTGAATG
RKN	Mi-1	SCAR	Ko-dominant	Mi-23	F=TGGAAAAATGTTGAATTTCTTTTG Seah ve ark., 2007 R=GCATACTATATGGCTTGTTTACCC

3. Bulgular

Domateste domates lekeli solgunluk virüsüne dayanıklılık sağlayan ve *L. peruvianum*'dan aktarılan sw-5 genine ile ilişkili markörler dizayn edilmiş ve ıslah çalışmalarında kullanılmıştır (Shi ve ark., 2011). Bu çalışmada sw-5 genine dayalı olarak Dianese ve ark., (2010) tarafından geliştirilen Scr-001F/R markör çifti 24 domates genotipi üzerinde taranmıştır (Şekil 1 ve Çizelge 2). Bu dominant markörün dayanıklılık bandı 400bç'de görüntü vermektedir. Dokuz F_1 ticari çeşidin 3'ünde 400bç'lik dayanıklı bant elde edilmiş ve 6'sında herhangi bir bant elde edilememiştir. Yerli çeşitlerin 12 tanesinde herhangi bir dayanıklılık DNA bandı elde edilememiştir. Kontrol grubunda ise Mountain Merit ve NC123S homozigot dayanıklılık DNA bandı elde edilmiştir.

Fusarium oxysporum f.sp. *lycopersici* ırkı-3'e bağlı olarak geliştirilmiş olan SCAR 43DF3-43DR3 markörü 24 domates genotipinde analiz edilmiştir (Şekil 2 ve Çizelge 2). F_1 ticari çeşitlerin 4'ünde 650bç duyarlı bant elde edilmiştir. Yerli çeşitlerin de 4'ünde duyarlı DNA bant profili elde edilmiştir. Kontrol grubunda ise Mountain merit 650 bç ve 875 bç'de DNA bant profili vermiş ve bu çeşidin heterozigot olduğu tespit edilmiştir. NCICELBR çeşidi 650 bç'de DNA bandı vermiş ve duyarlı bir genotip olduğu görülmüştür. NC123S genotipi ise 875bç baz uzunluğu vererek dayanıklılık özelliği göstermiştir.

Fusarium oxysporum f.sp. lycopersici ırkı-3'e bağlı olarak geliştirilmiş olan 43DF1-43DR1 markörü de 24 domates genotipi üzerinde test edilmiştir (Şekil 3 ve Çizelge 2). Dokuz F_1 ticari çeşidin 1'inde 1060 bç baz uzunluğu veren duyarlı bir bant elde edilmiştir. Yerli çeşitlerin 6'sı da duyarlı bant vermiştir. Kontrol grubunda NC123S genotipinde 1270 bç dayanıklılıkla ilişkili bant elde edilmiştir. Bunun yanıda Mountain Merit'te hiç bant elde edilmezken, NCICELBR'de DNA duyarlılık bandı elde edilmiştir.

Fusarium oxysporum f.sp. lycopersici ırkı-1'e bağlı olarak geliştirilmiş olan At-2F/R markörünün de 24 domates genotipi üzerinde taraması yapılmıştır (Şekil 4 ve Çizelge 2). Dokuz ticari çeşidin 8'inde 130 bç büyüklüğünde dayanıklılık ile ilişkili bir bant elde edilirken, sadece bir çeşitte ilişkili DNA bant profili belirlenememiştir. Yerli çeşitlerin 7'sinde dayanıklılığı gösteren DNA bant profili elde edilmiştir. Kontrol grubunda NCICELBR ve NC123S genotipleri DNA dayanıklılık bandını oluştururken, Mountain Merit genotinde herhangi bir DNA bandı elde edilememiştir.

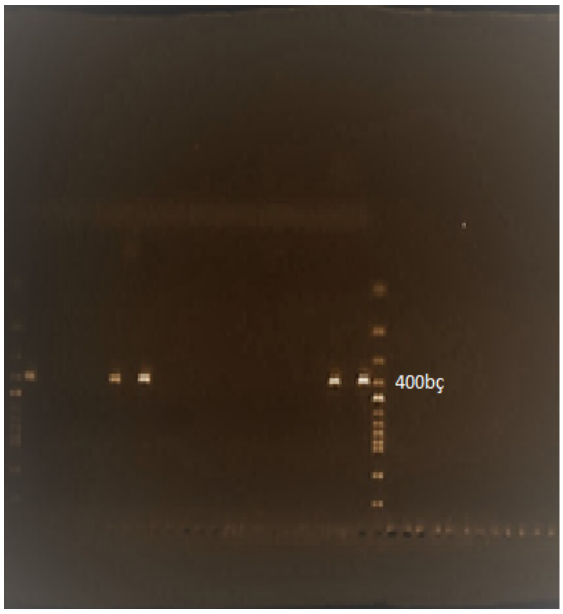
Fusarium oxysporum f.sp. *lycopersici* ırkı-2'ye bağlı olarak geliştirilmiş olan CAPS TAO1 kodominant markörünün de 24 domates genotipi üzerinde taraması yapılmıştır (Şekil 5 ve Çizelge 2). Bu markör ile elde edilen PCR ürününün RsaI kesme enzimiyle kesilmesi sonucu DNA bant profili elde edilmiştir. Ticari F_1 çeşitlerin 6'sında 250 bç ve 500 bç büyüklüğünde olmak üzere her iki baz uzunluğu tespit edilmiş ve bu çeşitlerdeki dayanıklılığın heterozigot formda olduğu anlaşılmıştır. Yerel çeşitlerin 9'unda sadece 250bç'de DNA bandı tespit edilmiş ve genotipler homozigot duyarlı olduğu belirlenmiştir. Kontrol grubunda NC123S çeşidinde her iki büyüklükte DNA bant profili elde edilerek bu genotipin heterozigot özellik gösterdiği gözlemlenirken, Mountain Merit ve NCICELBR genotiplerinde DNA bant profili elde edilememiştir.

Domates sarı yaprak kıvırcıklığı virüsüne dayanıklılık sağlayan ty-3 genine yönelik tasarlanmış olan P6-25 kodominant markörü de 24 domates genotipi üzerinde taranmıştır (Şekil 6 ve Çizelge 2.). Dokuz ticari F_1 hibrit çeşitten 5 tanesinde hem 450 bç duyarlılık ile ilişkili DNA bandı hem de 650 bç dayanıklılık ile ilişkili DNA bandı elde edilerek heterozigot durumun varlığı tespit edilmiştir. Bu F_1 çeşitlerin 4 adedinde ise sadece 450 bç uzunluğundaki duyarlılık ile ilişkili DNA bant profili elde edilmiştir. Yerli çeşitlerin 10 tanesinde DNA duyarlılık ile ilişkili bant elde edilirken, 2'sinde herhangi bir DNA bant profili elde edilememiştir. Kontrol grubunda yer alan Mountain Merit, NCICELBR, NC123S 450 bç'de duyarlı bant olduğu tespit edilmiştir.

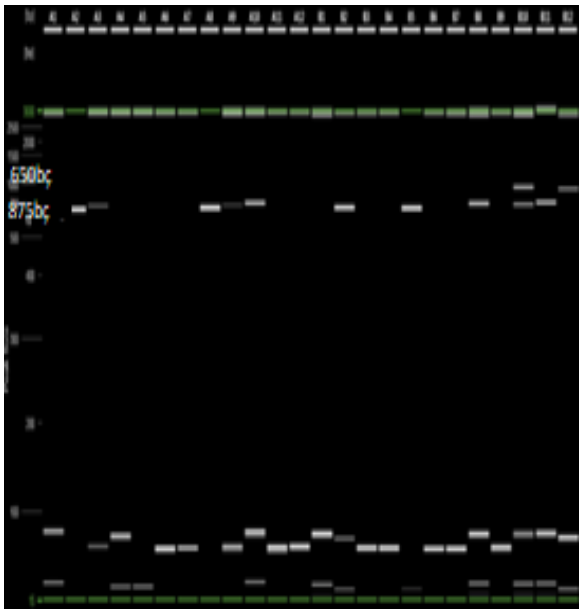
S. peruvianum'dan aktarılan Mi lokusundaki Mi 1-2 dayanıklılık geni için geliştirilmiş markörlerden bir diğeri olan PMİ markörünün 24 domates genotipi üzerinde analizi yapılmıştır (Şekil 7 ve Çizelge 2). Dokuz ticari çeşidin ikisinde 350 bç uzunluğunda duyarlılık ile ilişkili bir DNA bandı elde edilmiş; birinde 550 bç dayanıklılık ile ilişkili DNA bandı elde edilirken, birinde ise her iki DNA bant profili görülmüş ve bu genotipin heterozigot yapıda olduğu tespit edilmiştir. Yerli çeşitlerin ise sadece 2'sinde DNA duyarlılık bandı elde edilmiştir. Kontrol grubunda yer alan NCICELBR'de DNA duyarlılık bandı elde edilirken, diğer 2 çeşitte herhangi bir DNA bant profili elde edilememiştir.

Solanum habrochaites'den aktarılan Mi-1 genine dayalı olarak geliştirilmiş olan SCAR ko-dominant Mi-23 markörünün 24 domates genotipi üzerinde analizi yapılmıştır (Şekil 8 ve Çizelge 2).

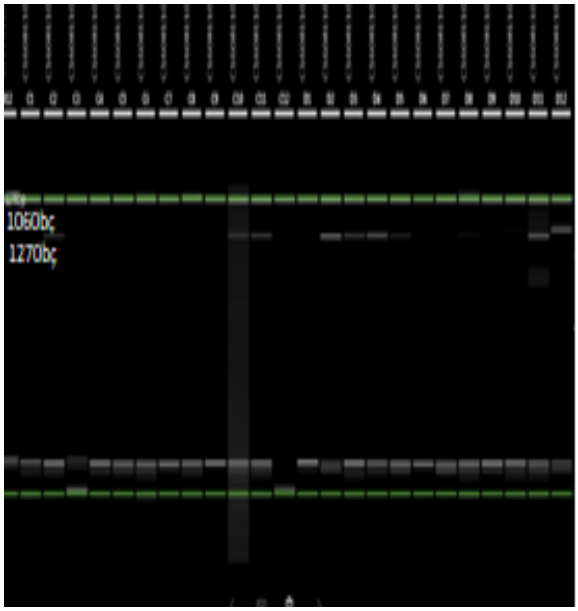
Bunlardan 9 ticari F_1 çeşidinin 5'inde 430 bç uzunluğunda DNA dayanıklılık bandı elde edilmiştir. Bir ticari çeşit ise 380 bç'de duyarlı ve 430 bç'de DNA dayanıklılık bandı oluşturarak heterozigot özellik göstermiştir. Yerli çeşitlerin 9'unda DNA dayanıklılık bandı elde edilmiştir. Kontrol grubundan Mountain Merit genotipinde heterozigot (H), NCICELBR duyarlı (S), NCI123S (R) DNA dayanıklı bant profili elde edilmiştir.



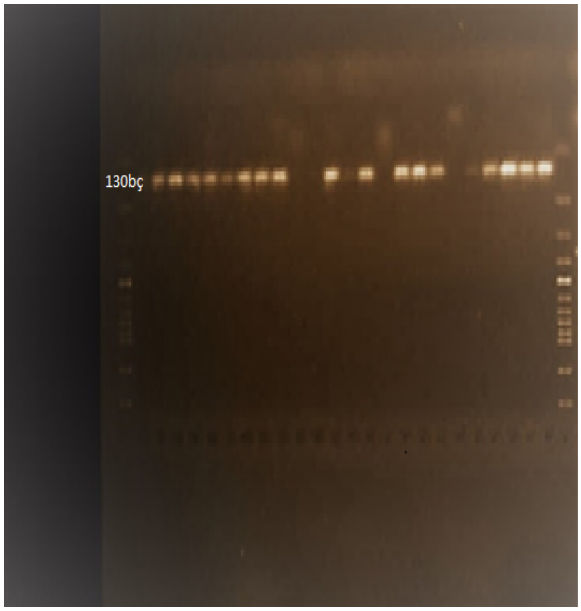
Şekil 1. Scr-001 dominant markörü ile elde edilen 400bç DNA bandı.



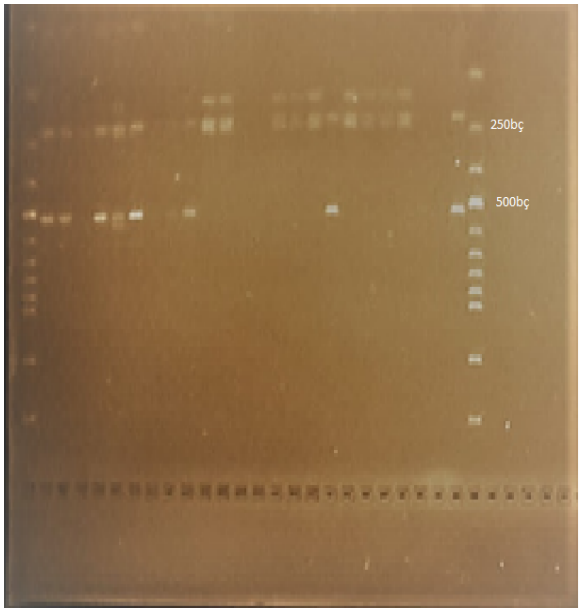
Şekil 2. 43DF3 markörü PCR ürünlerinin Kapillar elektroforez görüntüsü 100bç ladder kullanılmıştır (R:875bç, S: 650bç).



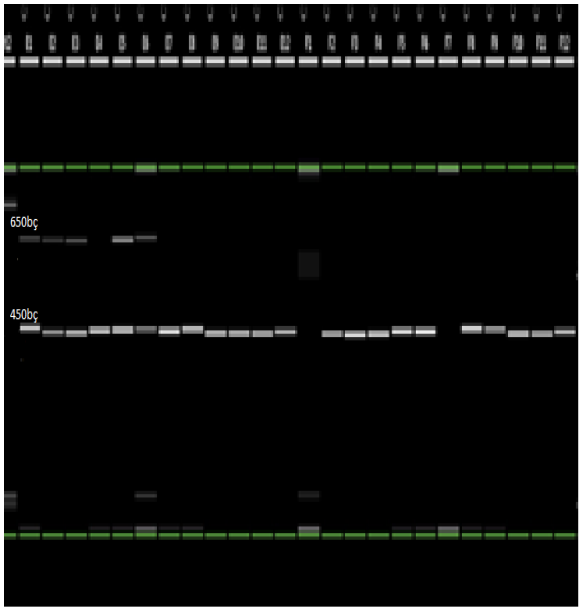
Şekil 3. 43DF1-43DR1 markörü PCR ürünlerinin Kapillar elektroforez görüntüsü (R:1060 bç, S: 1270bç).



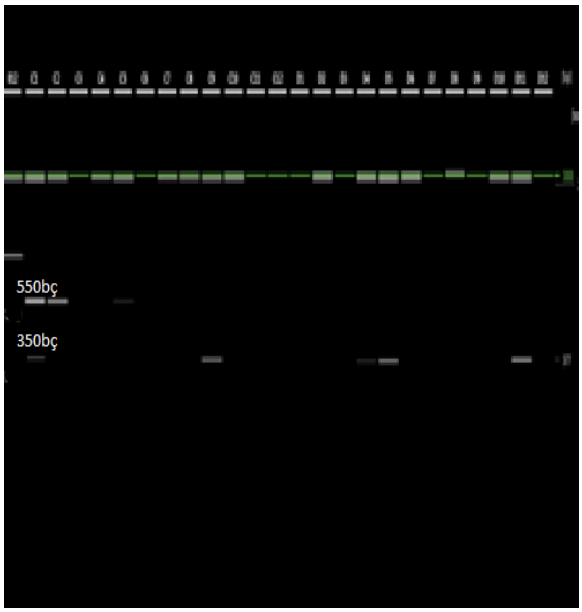
Şekil 4. At-2 markörüne ait (dominant) elde edilen 130bç bant.



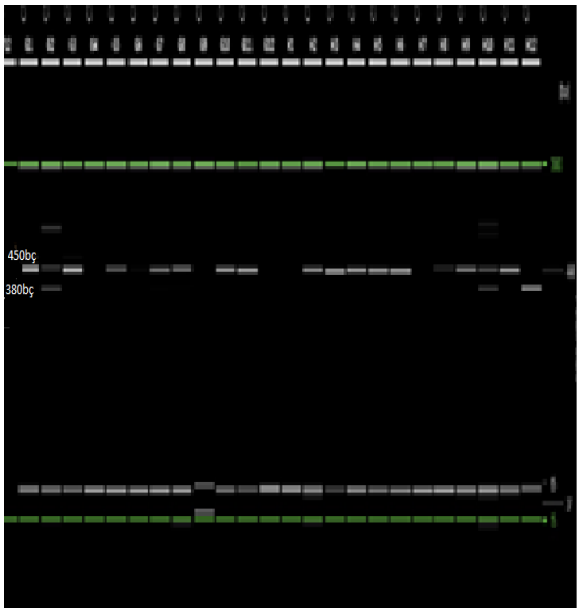
Şekil 5. TAO1 markörüne ait elde edilen S:250bç R:500 bant.



Şekil 6. P6-25 markörü PCR ürünlerinin Kapillar elektroforez görüntüsü (R:650bç, S: 450bç).



Şekil 7. PMİ markörü PCR ürünlerinin Kapillar elektroforez görüntüsü (R:550bç, S: 350bç).



Şekil 8. Mİ-23 markörü PCR ürünlerinin Kapillar elektroforez görüntüsü (R: 430 bç S: 380 bç).

Çizelge 2. Domates popülasyonuna ait genotiplerin bazı hastalık ve zararlılara karşı dayanıklılığı sağlayan genlerle ilişkili olarak geliştirilen moleküler markörlerle taranmasından elde edilen sonuçlar

Hastalık/ Zararlı	TSWV			FOL												TYLCV			RKN							
Gen	SW-5			I-1			I-2			I-3						TY-3			Mİ-1							
Markör	SCR-001			At-2			TAO-1			P7-43DF1			P7-43DF3			P6-25			Mi-23			Pmi			
	F1	Y.Ç.	K.G.	F1.	Y.Ç.	K.G.	F1	Y.Ç.	K.G.	F1	Y.Ç.	K.G.	F1	Y.Ç.	K.G	F1	Y.Ç.	K.G.	F1	Y.Ç	K.G	F1	Y.Ç.	K.G.		
Hm Duyarlı	-	-	-	-	-	-	-	9	-	1	6	1			1	4	10	3	-		1	2	2	1		
Hm Dayanıklı	3	-	2	-	-	-	-	-	-			1	4	4	1				5	9	1	1				
Heterozigot				-	-	-	6		1						1	5			1		1	1				
Bant Mevcudiyeti	-			8	7	2																				

*Hm: Homozigot, F1: Hibrit çeşit, K.G: Konrol grubu, Y.Ç: Yerli çeşit.

4. Tartışma ve Sonuç

Moleküler markörler domateste tarımsal açıdan birçok önemli özellik için genlerin ve QTL'lerin belirlenmesinde veya haritalanmasında moleküler yardımlı seleksiyon yöntemiyle kullanışlı ve faydalı olabilmektedir. Bununla birlikte, literatürde belirtilen tüm markörler domates ıslah programlarında uygulanamamaktadır. Markörleri yeniden tanımlayabilmek, belirli ıslah hatlarında faydalılık, çoğaltılabilirlik, yeni polimorfik markörleri tanımlamak ve geliştirmek için genellikle ek çabalar gereklidir. Bu çalışmada, domates lekeli solgunluk virüsü, fusarium solgunluğu, domates yaprak kıvırcıklığı virüsü, kök-ur nematodu, hastalık ve zararlılar için literatürde belirtilen geliştirilmiş olan markörlerden seçilmiştir. Seçilen CAPS ve SCAR markörleri 24 domates genotipi üzerinde test edilmiştir.

Domates lekeli solgunluk virüsü için test edilen SCAR Scr-001 markörü için çalışılan domates genotiplerinde sonuç elde edilmiştir. Çalışmada elde edilen sonuçlar, Roselló ve ark. (2001)'nın yaptığı çalışmada Huallanca, Ancash, Peru'da toplanan *Lycopersicon peruvianum*'un PE-18 genotipinden geliştirilen UPV 1 ıslah hattında Sw-5 alleliği kontrol eden ve hibrit yetiştiriciliğinde dayanıklılığı arttırabilmek için yaptıkları çalışmada Sw-5 ile bağlantılı SCAR Scr001F/R dominant markör çifti kullanılmıştır. Analizlerin görüntülenmesi sonucunda 400 bç DNA büyüklüğündeki bant sonuç vermiştir. Sw-5'te çalışan SCAR markırı UPV-1'de benzer şekilde çalışmış ve ticari hibrit çeşitlerinin geliştirilmesinde UPV-1'in önemli olduğunu göstermişlerdir (Roselló ve ark., 2001). Elde edilen sonuçlar bu çalışmada elde edilen sonuçlar ile paralellik göstermiştir ve bu markörün klasik testlemeye alternatif olabileceği belirtilmiştir.

Fusarium solgunluğu ile ilişkili TAO1 CAPS markörü de bu çalışmadaki 24 genotip üzerinde denenmiş ve genotiplerde heterozigot ve homozigot DNA bant profilleri elde edilmiştir. Ülkemizde yürütülen başka bir çalışmada TAO1 CAPS markörü ile test edilen 92 adet domates hattından 20 adet dayanıklı, 25 adet duyarlı ve 47 adedinde hem duyarlı hem hassas (heterozigot) DNA bant profili elde edilmiş ve TAO1 CAPS markörünün FOL'a karşı klasik testlemeye göre dayanıklı hat ve çeşit geliştirmede kolaylık sağlayabileceği belirtilmiştir (Pınar ve ark., 2013a). P743DF3-P743DR3 marköründe de çalışmamızda yapılan sonuçlara göre kontrol gurubu homozigot DNA bant profili vermesi paralellik göstermiştir. Barillas ve ark. (2008), Fusarium solgunluğu I-3 dayanıklılık genine bağlı olarak geliştirilmiş olan SCAR 43DF3/R3 markörünü farklı domates genotipleri üzerinde test etmişler ve duyarlı bant 650 bç'de ve dayanıklı bant 875 bç'de elde edilmiştir. Bunun sonucunda I-3 geniyle SCAR 43DF3/R3 ko-dominant markörünün bağlantılı olduğu tespit edilmiştir (Barillas ve ark. 2008). Barillas ve ark. (2008)'nın yaptığı çalışmada ayrıca Irk-3'e bağlı olarak geliştirilmiş olan P743DF1-P743DR1 ve 43DF1-43DR1 markörleri de domates genotipleri üzerinde test edilmiş ve SCAR 43DF1\R1 markör çifti duyarlı genotiplerde 1060 bç'de ve dayanıklı genotiplerde ise 1270 bç'de bant elde edilmiş; denemenin sonucunda I-3 geniyle SCAR 43DF1/R1 ko-dominant markörünün

bağlantılı olduğu değerlendirilmiştir ve domates hatlarında markör destekli seleksiyon kullanılabileceği belirtilmiştir. Arens ve ark., 2010'da yaptığı çalışmada domates çeşitlerinde I-1 genine bağlı dayanımı test etmek için At-2 markörünü kullanmışlar ve bu markörün I-1 gen lokusunun varlığını tespit etmek için kullanılabileceğini ifade etmişlerdir. Ülkemizde yürütülen başka bir çalışmada I-1 genine bağlı olarak geliştirilmiş olan At-2F/R markörü ile yapılan bir moleküler tarama sonucunda 92 adet F2 popülasyonu genotipinin 66'sı130 bç büyüklüğünde DNA bant profili vermiş ve çalışma sonucunda *Fusarium oxysporum f.sp*'ye dayanıklılığın belirlenmesinde bu markörlerin yol gösterici olabileceği belirtilmiştir (Pınar ve ark., 2013b).

Domates yaprak kıvırcıklığı virüsü için literatürde geliştirilen SCAR P6-25 markörü, 24 domates genotipi üzerinde denenmiş ve olumlu sonuç alınmıştır. Pınar ve ark. (2013a) yaptığı çalışmada Ty-3 dayanıklılık geni için P6-25 markörünün test edilmesinden sonra 92 adet domates genotipin 24'ünde homozigot dayanıklı bant ve 18'inde duyarlı bant elde edilirken, 50'sinde her iki bant da elde edilmiştir. Bu çalışmanın sonucunda belirtilen hastalığa karşı moleküler belirteçler klasik testlemeye göre dayanıklı hat ve çeşit geliştirmede kolaylık sağlayabileceğini belirtmişlerdir (Pınar ve ark., 2013a). Prasanna ve ark., (2015) çalışmalarında Domates yaprak kıvırcıklığı virüsü için geliştirilen moleküler markörleri Hindistan'da ıslah çalışmalarında etkin bir şekilde kullanılabileceğini göstermişlerdir.

Kök-ur nematoduna ait Mi-1 dayanıklılık genine ait literatürde geliştirilen SCAR ko-dominant PMİ markörünün de bu çalışmada 24 domates genotipi üzerinde taraması yapılmıştır. PMİ markörüne ait markör sonuçların El Mehrach ve ark. (2005)'nın yaptığı çalışmayla paralellik gösterdiği tespit edilmiştir. El Mehrach ve ark. (2005)'nın yaptığı çalışmada Mi1-2 bölgesi için dört markör belirlenmiştir. Bunlardan biri olan Pmi markörü PCR'a dayalı olarak anonim olarak seçilen domates genotiplerinde denendikten sonra, duyarlılık için 350 bç ve dayanıklılık için 550 bç'de DNA bant profili vermiştir. Bunun sonucunda Mi-1 dayanıklılık geni için PMİ markörünün kullanılabileceği ifade edilmiştir (El Mehrach ve ark., 2005).

Kök-ur nematodu ile ilişkili olarak literatürde Mi-1 dayanıklılık genine yönelik geliştirilen kodominant SCAR Mi-23 ve PMİ markörlerinin de bu çalışmada 24 domates genotipi üzerinde taraması yapılmış ve bu markörlerden olumlu sonuç alınmıştır. Özarslandan ve ark. (2010)'nın yaptığı çalışmada domates bitkileri, *Meloidogyne javanica* ile inoküle edilmiş ve genotiplerin dayanıklılık özellikleri klasik yöntemle taranmış ve bu domates genotipleri REX-F1-REX-R2 ve Mi23F-Mi23R'ye özgü primerler kullanılarak da duyarlı, dayanıklı veya heterozigot yapıda bantlar taşıyıp taşımadıkları belirlenmiş ve klasik tarama ile DNA markörlerinin kullanımı arasında açık bir ilişki olduğunu saptanmıştır. Bu markörlerin *M. javanica* dayanıklılık ıslahı için markör destekli seleksiyonda kullanılabileceği sonucuna varılmış ve yürüttüğümüz bu çalışmayla da paralellik gösterdiği tespit edilmiştir.

Sonuç olarak, literatürde domates ıslah programlarında moleküler markör yardımlı seleksiyon amaçlı geliştirilmiş bazı markörlerin ülkemizde de etkin bir şekilde kullanılabileceği anlaşılmıştır. Ayrıca yabani kaynaklı domates türleri dayanıklılık genlerinin ticari hibrit çeşitlerine aktarılmasından dolayı etkin bir şekilde kullanıldığı görülmekle birlikte yerel kaynaklarda bu markörler mevcut olmadığı da teyit edilmiştir. Bununla birlikte, entansif tarım uygulamaları yüzünden hastalık ve zararlı etkenlerinin sürekli kendilerini değiştirme kapasitesine sahip olduğu unutulmamalıdır. Bu yüzden hastalık ve zararlılara dayanıklılık sağlayan yeni dayanıklılık genlerinin bulunması ve bunlarla ilişkili güvenilir ve etkin markörlerin ıslah programlarına gelecekte yoğun bir şekilde kazandırılması önem arz etmektedir.

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Research Article

Determination Effects of Active Dry Yeast on Morphological and Chemical Components of Maize Plants Grown in Alkaline Soils for Silage Purposes

Mohammad Gazy ALOBAIDY[1], Zübeyir AĞIRAĞAÇ[2], Şeyda ZORER ÇELEBİ[*3]

[1]Van Yuzuncu Yil University, Institute of Natural and Applied Science PhD, 65000, Van, Turkey
[2,3]Van Yuzuncu Yil University, Agriculture Faculty, Field Crops Department, 65080, Van, Turkey

[1]https:/orcid.org/0000-0002-9894-9673, [2]https:/orcid.org/0000-0003-1414-1472, [3]https:/orcid.org/0000-0003-1278-1994

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

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Silage

Abstract: In agriculture, the use of environmentally friendly materials both in place of and alongside chemical ones is continuously increasing. Two field experiments were conducted during the 2019 and 2020 seasons to investigate the effects of foliar application of three doses (5.0, 7.5, and 10 g L^{-1}) of active dry yeast were sprayed on maize (*Zea mays* L. Cv. Tuano) at two intervals, the first 54 days after planting (DAP), and the second 15 days later. Plants were grown for silage purposes under alkaline soil conditions at the Experimental and Research Station of the Field Crops Dep., Van Yuzuncu Yıl University-(VYYU), Turkey. Measurements were taken three times at the vegetative stage 64, 74, and 84-DAP, and one times at the dough stage 117-123 DAP. Morphologically, the results showed that the foliar application of different doses of yeast increased the plant height (cm), stem, leaves, and cobs weight per plant and total plant weight (g), number of cobs $plant^{-1}$ (piece), and green and dry herbage yield (ton da^{-1}) at 117-123 DAP during the two seasons. In contrast, there was no significant increase in plant height (cm), chlorophyll as SPAD (The Soil Plant Analysis Development) value, and the number of leaves per plant at 64 DAP. Chemically, the spraying of the yeast improved the P, K, Ca, Mg, Fe, Cu, Se, and Zn concentrations at 117-123 DAP as well as chlorophyll content at 74 and 84 DAP compared to the control. From this study, it could be concluded that the highest values of the studied parameters were recorded when active dry yeast was used at a dose of 10 g L^{-1} on maize under high pH soil.

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1. Introduction

Maize or corn is one of the most important cereal crops in the world. It is grown under a wide range of agronomic and environmental circumstances (Khan et al., 2014; Rouf Shah et al., 2016). It is the main source of energy and food source for humans and animals in addition to its content of natural phytochemical compounds such as pigments, substantial fats, and starch (Rouf Shah et al., 2016; Kaul et al., 2019). Moreover, over the past few decades, the silage of corn has become the main fodder ingredient in the dairy cows' rations (Khan et al., 2014). The cultivation area of corn produced for silage in Turkey is approximately 4.7 million decares. Total silage corn production is 23.2 million tons, and the silage yield is 4915 kg da^{-1} on average, although it may vary by region (Acar et al., 2020). 73% of

corn production in the world and 90% in developed countries is used for animal feeding. In Turkey, 70% of corn production, which ranks third after wheat and barley, is used for animal nutrition (Öz et al., 2017).

Nutrient materials solubility and availability, in addition to soil microorganism activity, mainly depend on soil reaction (pH). Most micronutrients, for instance, are less available in neutral-alkaline soils compared to acid soils, which are considered the preferred conditions to plant growth (Lončarić et al., 2008). In general, under alkaline soil conditions, the availability of most macronutrients is increased. In contrast, the availability of micronutrients and some macronutrients such as phosphorus is decreased. This decrease leads to a negative impact on plant growth and yield (Jiang et al., 2017). According to the salinity and alkalinity criteria used in the Turkey Improved Soil Map studies, there is a problem with salinity and alkalinity in an area of 1.518.722 ha in Turkey. Where 0.5% is alkaline, 8% is slightly salty alkali, and 17.5% is salty alkali. The barren lands are equivalent to 5.48% of the total cultivated agricultural lands (Sönmez and Beyazgül, 2008).

The use of bio/organic fertilizers is increasing all over the world for environmental and human health safeguard purposes, which has become endangered as a result of the excessive use of chemical fertilizers and pesticides (Agamy et al., 2013). Bio-stimulants influence plant metabolism if it used in low concentrations through prompting of natural plant growth regulators driving and vigor, reinforcement of nutrients absorption, enhancement of root growth, and increasing resistance to stress as abnormal conditions. In addition, these materials could be used as systemic agents when they are applied as a foliar application (Hassan et al., 2020).

Abbas (2013) and Alsaady et al. (2020) concluded that the application of bio-stimulants such as yeast in small quantities affected several metabolic processes and promoted plant growth, productivity, and development through increased photosynthesis, natural hormones, ion uptake, and protein synthesis as well as a relative increase in the availability of the micronutrients in the soil. It may also increase antioxidants and enhance metabolism and water holding capacity.

Yeast *Saccharomyces cerevisiae* extract, which is plant nutritious as well as an, environmentally friendly, and convenient to use, has a feature over common plant growth regulators and soil conditioners (Xi et al., 2019) and includes natural plant hormones i.e., free and associated IAA, free GA_3 and associated GA_3 (Twfiq, 2010) and vitamins, amino acids, necessary macro and micronutrients for plant growth, and growth stimuli such as Cytokinins (Medani and Taha, 2015) and it is counted as a natural source of biostimulants that motivate some enzymes, cell expansion and division, synthesis of pigments, and nucleic acid and protein formation (Wanas, 2006), and also it releases CO_2 which leads to improvement of photosynthesis (Kurtzman and Fell, 2005). Al-Shaheen et al. (2019) stated that the application of active dry yeast had positive effects on corn plants. All sprayed maize plants with active dry yeast extract produced the highest yield rate of all the studied characters.

This research aimed to study the effect of spraying bio-fertilizers (active dry bread yeast) in improving the growth of maize crops for silage purposes under alkaline soil conditions.

2. Material and Methods

Two field experiments were performed to evaluate the effects of active dry yeast on the growth, morphological changes, and chemical constituents of maize (*Zea mays* L. Cv. Tuano) for silage purposes. The experiments were carried out in the experimental field of VYYU-Campus, Fac. Agric., Field Crops Department as well as in the Science Application and Research Center labs, Van, Turkey, during the two successive summer seasons, 2019 and 2020. Some climatic data of the province of Van during the years of the experiment are given in Table 1. The average temperature in Van city in the 2019 and 2020 seasons were higher than the average temperature for the long years. In contrast, total precipitations in the experimental seasons (2019 and 2020) were lower than the long-term ones. The same trend was recorded for the average humidity in Van city during the 2019 and 2020 seasons were lower compared to long years average, as shown in Table 1.

Table 1. Some climate values of the experimental area for the years 2019 and 2020 and the long-term average*

Month	Average Temperature (°C)			Total Precipitation (mm)			Average Humidity (%)		
	2019	2020	Long Years	2019	2020	Long Years	2019	2020	Long Years
April	7.2	8.6	8.4	36.2	50.9	57.4	66.1	65.4	59.3
May	15.4	14.5	13.4	15.3	27.8	45.3	51.9	54.0	55.1
June	21.4	19.3	18.8	7.2	13.4	16.4	45.4	44.4	47.1
July	23.0	23.0	22.7	0.4	17.9	6.9	39.0	46.4	42.3
August	23.7	21.6	22.9	0.9	10.0	5.3	40.2	44.5	40.5
September	18.8	20.1	18.3	0.8	5.6	20.4	43.9	41.3	43.9
October	13.4	13.3	12.0	24.1	1.8	48.2	52.9	47.2	57.3
Mean/Total	17.6	17.2	16.6	85	127	199	48.4	49.0	49.3

* Van Meteorology Regional Directorate records.

Soil samples were randomly taken each year before cultivation at the two depths of 0-20 and 20-40 cm, and were subjected to some physical and chemical analysis (VYYU, Fac. Agric., Dep. Soil Science and Plant Nutrition) were illustrated in Tables 2. Experiments soil pH ranged between 7.94 and 8.16 in the 0-20 cm and 20-40 cm, respectively. The experiment's soil was sandy-clay in VYYU-Campus, as shown in Table 2.

Table 2. Some physical and chemical analysis of the soil experimental field

Deep	pH Sat.	Clay (%)	Silt (%)	Sand (%)	Lime (%)	Ca+Mg (me $100g^{-1}$)	CEC (me $100g^{-1}$)	Organic Matter (%)
0-20	7.94	35.08	20.95	43.97	21.06	13.30	16.00	1.87
20-40	8.16	31.76	18.88	49.39	21.41	14.25	17.00	1.69

Maize seeds were obtained from the Van Directorate of Provincial Agriculture and Forestry. Seeds were sown on 15th May in both seasons, and all experimental units were 5.0×3.5 m, containing 6 rows (70 cm between rows) and 12 cm between plants. The distance between plots has been arranged as 2 m and between blocks as 3 m. With the planting, fertilizer will be applied to all plots at the rate of 8 kg N da^{-1} at the first batch and 8 kg P_2O_5 da^{-1}. When corn is 50-60 cm tall, second dose of nitrogen fertilizer will be applied at the rate 10 kg N da^{-1} (Sabancı, 2015). Hoeing has been done two times to cover the bottom of the plant stem, aerate the soil, and fight weeds. Plants were irrigated with a sprinkler irrigation system. The active dry yeast was prepared from bread yeast (*Saccharomyces cerevisiae*), by solving dry yeast in hot water, then added sugar in a ratio of 1:1 and kept in a warm place for 24 hours, according to Morsi et al. (2008). Three different doses of active dry bread yeast (5.0, 7.5, and 10.0 g L^{-1}), as well as control plots treated with tab water, were sprayed on maize plants under alkali soil stress two times started at 54 DAP and repeated after 15 days.

2.1. Data Recorded

In both experiments, three samples on five plants per plot, i.e., at 64, 74, and 84-DAP, were read from each treatment 10 days after each application. In which each one was represented by three replicates for the morphological studies (plant height (cm), number of leaves per plant), and total chlorophyll SPAD. At the dough stage (117-123 DAP), a sample was taken to record the following: plant height (cm), number of leaves per plant, number of cobs per plant (piece), stem, leaves, cobs weight per plant (g), plant weight (g), stem diameter (mm), and green and dry herbage yield (ton da^{-1}). Macronutrients (P, K, Ca, Mg) and micronutrients (Fe, Cu, Mn, Mo, Se, Zn) concentrations were determined using by wet digestion inductively coupled plasma-optical emission spectrometer (ICP-OES) and atomic absorption spectrophotometer (AAS) in VYYU, Science Application and Research Center labs according to Association of official analytical chemists (1995, chap. 50).

2.2. Experimental design and statistical analysis

The field experiments were arranged in the Randomized Complete Block Design (RCBD) with three replications. Three different doses of active dry bread yeast (*Saccharomyces cerevisiae*), as well

as control, were sprayed on maize plants. All data recorded were subjected to normal statistical analysis (One-Sample Kolmogorov-Smirnov Test), and the test distribution is normal. One-way analysis of variance (ANOVA) and Duncan multiple comparison test were used to evaluate the treatment effects. Then the combined analysis of the two seasons was done according to Steel and Torrie (1960). The differences among treatment mean values were compared using Duncan multiple range test, and a p-value of 5% was used for statistical significance as reported by Gomez and Gomez (1984). All statistical analysis was performed by using the analysis of variance (ANOVA) technique of SAS Software package Stat-view-9.0.1 (SAS Institute, 2002).

3. Results and Discussions

3.1. Vegetative Characters

The effects of active dry yeast applications on plant height (cm), number of leaves per plant, and leaf chlorophyll content (SPAD value) are given in Table 3. Progressive significant height of maize plant, number of leaves per plant, and SPAD measurements were achieved with the foliar application of active dry yeast. When 10 g of yeast was sprayed on the plant above soil, the plant height and number of leaves of maize reached the highest records of the second and third vegetative stages than the yeast-free treatment, control, while the differences between effects of yeast concentrations at 64 DAP were insignificant.

Table 3. Effect of dry yeast foliar application on vegetative characters of maize grown under high pH soil conditions at 64, 74, and 84 DAP during 2019 and 2020 seasons

Treatments / **Spray**	Plant height (cm)	Number of leaves per plant	SPAD Value
	64 Days After Planting		
Control	*137.83±26.84 ^{NS}	10.20±0.872 ^{NS}	41.07±5.06 ^{NS}
M1: 5.0 g L^{-1}	137.03±9.99	10.30±0.755	38.82±2.62
M2: 7.5 g L^{-1}	146.50±19.02	10.80±0.361	43.14±3.80
M3: 10.0 g L^{-1}	131.97±17.60	10.90±0.265	42.28±2.13
Mean	138.33	10.55	41.33
***P*-Value**	0.4758	0.2774	0.5168
S.E.D.**	9.500	0.505	2.930
	74 Days After Planting		
Control	163.15±10.79 ^{c}	11.84±0.703 ^{NS}	36.11±3.47 ^{c}
M1: 5.0 g L^{-1}	196.36±12.48 ^{b}	12.03±0.701	42.19±0.68 ^{b}
M2: 7.5 g L^{-1}	204.23±17.62 ^{ab}	12.33±0.351	43.87±1.99 ^{b}
M3: 10.0 g L^{-1}	216.47±13.83 ^{a}	12.73±0.252	48.45±1.33 ^{a}
Mean	195.05	12.23	42.66
***P*-Value**	0.0003	0.0967	0.0001
S.E.D.	11.360	0.442	1.740
	84 Days After Planting		
Control	196.31±11.18 ^{d}	13.33±0.635 ^{NS}	38.55±1.53 ^{c}
M1: 5.0 g L^{-1}	220.76±11.14 ^{c}	13.43±0.751	45.03±1.14 ^{b}
M2: 7.5 g L^{-1}	233.13±11.33 ^{b}	13.55±0.253	47.25±0.49 ^{b}
M3: 10.0 g L^{-1}	247.67±14.02 ^{a}	14.29±0.278	51.08±0.70 ^{a}
Mean	224.47	13.65	45.48
***P*-Value**	0.0001	0.0781	0.0001
S.E.D.	9.780	0.430	0.855

The means within each column within main effects and interactions followed by the same letter are not significant at $P \leq 5\%$.
*Mean±Standard deviation. **S.E.D.: Standard errors of differences of means, NS: insignificant difference.

The illustrated parameters (Table 3) are in agreement with the findings presented by (Abu Khouder et al., 2019; Al-Shaheen et al., 2019; Al-madhagi., 2019); they pointed out that the application of yeast increased the plant height, number of leaves per plant and rate of chlorophyll in the plant leaves. In this context, the highly impactful CO_2 release was due to the use of active dry yeast, which subsequently leads to an increase the net plant photosynthesis (Ferguson et al., 1995; Vas and Papanas,

2019). The increase of the concentration of chlorophyll when the foliar application of yeast is because it contains the natural hormones that motivate the chlorophyll formation. Yeast includes Cytokinin, which is vital for chloroplast up growth and is the backbone of chlorophyll synthesis EzzEL-Din and Hendawy (2010). Moreover, activating of plant vegetative growth using dry yeast could be due to its effect on the transmission of the nutritional signal that constitutes growth regulators (El-Ghadban et al., 2003).

3.2. Morphological Characters at the Dough Stage

The impact of spraying active dry yeast on morphological characters of maize at the dough stage for the mean of the two seasons (2019 and 2020) is indicated in Tables 4 and 5. Using the 5.0, 7.5, and 10.0 g L^{-1}doses of dry yeast have a positive effect on the maize plant growth for silage purposes in comparison with the control treatment (without yeast spraying). Plant height, stem weight per plant, and plant leaf weight in the dough stage differed significantly according to doses of yeast and control applications ($p \leq 0.05$). The greatest value was detected in corn treated with active dry yeast (10 g L^{-1}), followed by (7.5 g L^{-1}), then by (5 g L^{-1}), and the smallest values were in control non-sprayed plants. While no significant difference was detected between the M2: 7.5 g $L^{-1}$and M3: 10.0 g $L^{-1}$of dry yeast on the plant leaf weight (Table 4).

Table 4. Effect of dry yeast foliar application on morphological characters of maize grown under high pH soil conditions at the dough stage during the 2019 and 2020 seasons

Treatments / **Spray**	Plant height (cm)	Number of leaves per plant	Stem diameter (mm)	Stem weight plant^{-1} (g)	Leaves weight plant^{-1} (g)
Control	*220.40±26.84 ^{d}	13.93±0.25 ^{NS}	24.56±1.27 ^{NS}	220.53±53.1 ^{d}	103.03±14.99 ^{c}
M1: 5.0 g L^{-1}	246.97±9.99 ^{c}	14.24±0.90	23.22±0.99	289.87±58.3 ^{c}	129.53±12.45 ^{b}
M2: 7.5 g L^{-1}	266.03±19.02 ^{b}	14.68±0.11	22.65±3.38	316.73±58.9 ^{b}	141.60±6.77 ^{ab}
M3: 10.0 g L^{-1}	282.91±17.6 ^{a}	14.68±0.01	24.75±1.19	396.73±73.2 ^{a}	158.00±3.50 ^{a}
Mean	254.08	14.38	23.79	305.97	133.04
***P*-Value**	0.0001	0.1103	0.5259	0.0001	0.0009
S.E.D.**	15.770	0.387	1.592	50.100	8.540

The means within each column within main effects and interactions followed by the same letter are not significant at $P \leq 5\%$.
*Mean±Standard deviation. **S.E.D.: Standard errors of differences of means, NS: insignificant difference.

On the other hand, there was not a significant difference between maize plants treated with all dry yeast concentrations and control on the number of leaves per plant and stem diameter (mm) of maize plants grown under alkaline soil conditions at the dough stage during 2019 and 2020 seasons as Table 4 shown. This may explain that these traits are associated with the genetic cultivar of corn. It is well known that the actual performance of any cultivar depends on its genetic parameters interacting with all surrounded environmental conditions (Saleh et al., 2018). For the cobs number per plant, the data presented in Table 5 indicated that because of the applying active dry yeast to maize, the highest value of number of cobs per plant (1.57) was recorded at 10 g L^{-1}, while the lowest value of number of cobs per plant (1.07) was obtained as a result of the non-application of dry yeast 0 g L^{-1} was obtained in the control plots. On the other hand, it is seen that there are no statistically significant differences between the M1: 5.0 g L^{-1} and M2: 7.5 g L^{-1} of dry yeast doses on the number of cobs per plant and the untreated plants (control). As well as between M2: 7.5 g L^{-1} and M3: 10.0 g L^{-1}.

The results in Table 5 showed that the foliar application of active dry yeast had significantly augmented cobs weight plant^{-1} and plant weight of the aerial parts indicators compared with control (spraying with tap water). The maximum mean values have been recorded by applying 10 g L^{-1} of dry yeast in the average of both seasons 2019 and 2020, while the minimum values in the untreated control. Increasing the active dry yeast concentration from 0 to 10 g L^{-1} increased the cobs weight per plant and plant weight of the aerial parts. It is clear that the level of 10 g L^{-1} of dry yeast gave the highest record of studied plant weight of the aerial parts parameter 940.03 g, with an increase of 102.69% compared to the untreated plants as a control (recorded 463.77 g). Green and dry herbage yield (ton da^{-1}) characteristics recorded significant responses when maize plants were treated by the different levels of active dry yeast in comparison to not sprayed plants in the average of the two seasons, 2019 and 2020,

as shown in Table 5. Although there is a significantly green herbage yield (ton da^{-1}) difference between control and plants sprayed by yeast, there are slight differences (not significant) between the concentrations of active dry yeast themselves. For dry herbage yield (ton da^{-1}), generally, the differences between the concentrations of active dry yeast themselves and between yeast doses and control were significant in the dough stage during the 2019 and 2020 seasons.

In this context, there are many studies on the application of yeast in different plants such as maize, sunflower, and broad bean. There are results that plant growth is positively affected the growth characteristics as well as yield and its components reported by some researchers (Seadh et al., 2015; Altunlu et al., 2019; Al-Shaheen et al., 2019; Abed and Zeboon, 2020; Al-Ani et al., 2020). The results mentioned in Table 4 are in the same trend. The increase may be due to the effect of the yeast spray because it can produce phytohormones such as Auxins, Gibberellic acid, Cytokinins, and some vitamins (EL-Kholy et al., 2007), which by their physiological action are stimulated the elongation and cell division. Besides, they contain amino acids and some mineral elements such as nitrogen and others that enhance plant growth, and this result is consistent with what (Ahmed et al., 2011). In this context, the yeast catalyzes the release of CO_2 from fermentation, which will eventually contribute to photosynthesis and increase the products resulting from the process (Khalil and Ismael, 2010).

Table 5. Effect of dry yeast foliar application on morphological characters of maize grown under high pH soil conditions at the dough stage during the 2019 and 2020 seasons

Treatments / **Spray**	Number of cobs $plant^{-1}$ (g)	Cobs weight $plant^{-1}$ (g)	Plant weight (g)	Green herbage yield (ton da^{-1})	Dry herbage yield (ton da^{-1})
Control	*1.07±0.116 ^{b}	140.20±16.56 ^{d}	463.77±80.5 ^{d}	7.50±0.98 ^{b}	1.63±0.18 ^{d}
M1: 5.0 g/L	1.23±0.322 ^{b}	280.40±40.6 ^{c}	699.80±101.9 ^{c}	8.79±0.57 ^{a}	2.23±0.14 ^{c}
M2: 7.5 g/L	1.30±0.100 ^{ab}	314.40±24.3 ^{b}	772.73±85.5 ^{b}	8.90±0.28 ^{a}	2.63±0.32 ^{b}
M3: 10.0 g/L	1.57±0.058 ^{a}	385.30±44.4 ^{a}	940.03±87.2 ^{a}	9.30±0.33 ^{a}	3.18±0.35 ^{a}
Mean	1.29	280.07	719.08	8.62	2.41
***P*-Value**	0.0175	0.0001	0.0001	0.0001	0.0017
S.E.D.**	0.1472	27.320	72.800	0.4945	0.2135

The means within each column within main effects and interactions followed by the same letter are not significant at $P \leq 5\%$.
*Mean±Standard deviation. **S.E.D.: Standard errors of differences of means.

3.3. Chemical Characters at the Dough Stage

It is seen in Table 6 that the phosphorus, potassium, calcium, magnesium, and iron concentrations have increased significantly as a result of the applied different dry yeast levels. The smallest contents of previous minerals were in the control plants, followed by the records obtained from applications of active dry yeast, which the highest values recorded with M3: 10 g L^{-1} yeast. M3 active yeast dose recorded increment by 47.05% in P, 61.73% in K, 92.67% in Ca, 51.42% in Mg, and 173.68% in Fe concentrations in comparison with non-treated plants as control. In this regard, Duru (2020) reported that calcium, sulfur, and potassium are essential mineral elements in human and animal nutrition. Here, it is known that P is a vital part of nucleic acids, phospholipids, adenosine triphosphate (ATP), and several coenzymes. Also, K affects osmosis and the operation of stomata by its role as a cofactor involved in protein formation, enzymes activation, and main solute functioning in water balance. Fast-growing animals seem to have higher potassium requirements, and an increased protein level increases the demands. Potassium is the main cation in intracellular fluid and actions in acidic medium, organization of osmotic pressure, conduction of the nerve impulse, muscle contraction, especially the heart muscle, cell membrane function, and Na^{+}/K^{+}-ATPase. Potassium is also required during glycogen synthesis. Like that, Ca is important in the formation and cell wall constancy and in the maintenance of membrane composition and permeability, the stimulation of certain enzymes coordinates many responses of cells to stimuli (Soetan et al., 2010). For animals, calcium acts as a component of bones and teeth and nerve and muscle function regulation. In blood coagulation, Ca activates the conversion of prothrombin to thrombin and also participates in the coagulation of milk. Calcium activates many enzymes such as ATPase, succinic dehydrogenase, lipase, etc. (Malhotra, 1998; Murray et al., 1999; Soetan et al., 2010).

In this regard, Mg is part of the chlorophyll particles; it activates many enzymes and an active substance of several enzyme systems in which thymine pyrophosphate is a cofactor. It also stimulates pyruvic acid carboxylase, pyruvic acid oxidase, and the condensing enzyme for the reactions in the Krebs cycle. It is also a component of bones and teeth. Fe is a component of cytochromes, electron transport, stimulates some enzymes, and plays a role in the formation of chlorophyll. Iron acts like hemoglobin in oxygen transportation. In cellular respiration, Fe acts as a major constituent of enzymes that contribute to biological oxidation, such as cytochrome C, C1, A1, etc. (Malhotra, 1998; Murray et al., 1999; Soetan et al., 2010). While the solubility of Iron, Fe^{2+}, and Fe^{3+} ions decrease with an increase in pH being extremely low in calcareous soils, where noticeable Fe deficiency in plants not adapted to these conditions occurs (Römheld and Nikolic, 2006).

Table 6. Effect of dry yeast foliar application on P, K, Ca, Mg, and Fe concentration (%) of maize grown under high pH soil conditions at the dough stage during the 2019 and 2020 seasons

Treatments / **Spray**	P	K	Ca	Mg	Fe
Control	*0.170±0.024 ^{d}	0.784±0.056 ^{d}	0.628±0.054 ^{d}	2.112±1.713 ^{c}	0.076±0.005 ^{d}
M1: 5.0 g L^{-1}	0.214±0.012 ^{c}	1.083±0.054 ^{c}	0.897±0.028 ^{c}	2.767±2.332 ^{b}	0.139±0.017 ^{c}
M2: 7.5 g L^{-1}	0.221±0.016 ^{b}	1.156±0.083 ^{b}	1.076±0.064 ^{b}	3.191±2.711 ^{a}	0.171±0.019 ^{b}
M3: 10.0 g L^{-1}	0.250±0.026 ^{a}	1.268±0.050 ^{a}	1.210±0.031 ^{a}	3.198±2.786 ^{a}	0.208±0.015 ^{a}
Mean	0.214	1.073	0.953	2.817	0.149
***P*-Value**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
S.E.D.**	0.001333	0.00971	0.02393	0.0539	0.00317

The means within each column within main effects and interactions followed by the same letter are not significant at $P \leq 5\%$.
*Mean±Standard deviation. **S.E.D.: Standard errors of differences of means.

For micronutrients concentrations, it appeared from the results mentioned in Table 7 that active dry yeast applications increased the copper, selenium, and zinc contents of the samples statistically when compared to control plants. The highest Cu and Se amounts of the samples obtained from these applications were determined as 49.342 and 1.825 ppm for M3, respectively. In contrast, the highest Zn concentration resulted in M1 and M2, with non-significant changes in the copper concentration between them observed. Furthermore, the lowest concentrations detected by control with 27.190, 0.331, and 76.618 ppm for Cu, Se, and Zn, respectively. It was determined that there was an insignificant difference in the concentrations of Manganese elements between the yeast doses and the control (untreated plants).

Copper is a component of vital redox and lignin-biosynthetic enzymes (Soetan et al., 2010), and it plays a role in iron intake (Chandra, 1990). Moreover, it is significant for growth and bone synthesis (Malhotra, 1998; Murray et al., 1999). Additionally, copper is required for the synthesis of collagen and elastin fibers that provide structure and elasticity to connective tissue and blood vessels (Larson, 2005).

Selenium is integrated originally into plants by sulfur substitution in the cysteine and methionine (Soetan et al., 2010). Selenium is a mineral that is a component of a complete defense system that shelters the lively organism from the hurtful effect of reactive oxygen species (ROS) (Murray et al., 1999).

Here Malhotra (1998) and Soetan et al. (2010) indicated that zinc is effective in the construction of chlorophyll, invigorates some enzymes, and functions as a part in the creation of auxin, chloroplasts, and starch. Moreover, the absorbed zinc that enters the liver was exported to peripheral tissue in plasma, bound to albumin after being united into zinc metalloenzymes. These results may be reflected that increasing the zinc concentration in the maize plants sprinkled with active dry yeast could lead to good results on animals' health and production.

On the contrary, the use of dry yeast on corn has recorded a negative effect on molybdenum concentrations, where the control was the highest and gradually decreased with increasing the yeast doses from 5.0 to 10.0 g L^{-1}. The Mo concentration was relatively high in corn silage and other feeds linked with the lowest Cu concentrations resulting in undesirable ratios between Cu and Mo (Miltimore and Mason, 1971). This may refer to the presence of an antithesis association between Cu and Mo concentrations in maize plants.

Table 7. Effect of dry yeast foliar application on Cu, Mn, Mo, Se, and Zn concentration (ppm) of maize grown under high pH soil conditions at the dough stage during the 2019 and 2020 seasons.

Treatments / **Spray**	Cu	Mn	Mo	Se	Zn
Control	*27.190±2.73 ^{d}	116.048±27.99 ^{NS}	0.754±0.06 ^{a}	0.331±0.27 ^{d}	76.618±4.30 ^{c}
M1: 5.0 g L^{-1}	33.533±5.67 ^{c}	116.359±3.04	0.394±0.07 ^{c}	0.802±0.09 ^{c}	84.573±20.74 ^{a}
M2: 7.5 g L^{-1}	46.207±7.18 ^{b}	115.032±12.41	0.538±0.27 ^{b}	1.208±0.26 ^{b}	83.675±7.90 ^{a}
M3: 10.0 g L^{-1}	49.342±9.25 ^{a}	115.750±21.33	0.401±0.06 ^{c}	1.825±0.70 ^{a}	79.816±16.36 ^{b}
Mean	39.07	115.80	0.52	1.04	81.17
***P*-Value**	< 0.001	0.908	< 0.001	< 0.001	< 0.001
S.E.D.**	0.3470	1.9010	0.002404	0.00765	0.5190

The means within each column within main effects and interactions followed by the same letter are not significant at $P \leq 5\%$.
*Mean±Standard deviation. **S.E.D.: Standard errors of differences of means, NS: insignificant difference.

Similar results were in harmony with those of Abbas (2013) on beans, and Nasser et al. (2019) on lentils, they reported that chemical composition such as mineral elements improved under applying of yeast. On the other hand, Abbas (2013) revealed that the role of yeast could also be attributed to its effect on enzymatic activity, the production of some natural plant hormones, improving the ability to absorb nutrients, diversion of phosphorus from insoluble state to soluble one, and increasing its absorbability by plants, all of these increase the mineral content in plants.

4. Conclusion

In the present study, foliar dry yeast applications to maize had a positive effect on the morphological and chemical properties, and as a result of these applications, the highest properties, including the amount of the studied properties, were obtained at a dose of 10 g L^{-1}. The findings obtained from this study, when considered overall, it could be concluded that foliar applications of active dry yeast during the beginning vegetative stages might be recommended to increase vegetative growth, yield, and quality of maize for silage purposes in the soils that have high pH under Van-Turkey conditions. Furthermore, the fact that yeast is a natural and safe substance for the plants and the environment and economically feasible.

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Research Article

Performance of Some Standard Quince Cultivars under Ecological Conditions of Bafra, Samsun

Ahmet OZTURK[*1], Zaki Ahmad FAIZI[2], Tahsin KURT[3]

[1] Ondokuz Mayis University, Agriculture Faculty, Horticulture Department, 55200, Atakum, Samsun
[2,3] Ondokuz Mayis University, Post-graduate Institute, Horticulture Department, 55200, Atakum, Samsun

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

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

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Abstract: This study was carried out to determine the phenological, morphological, pomological, and yield characteristics of some standard quince cultivars. The earliest flowering occurred in 'Limon', and the earliest harvest was recorded in 'Gördes' cultivar. There were statistical differences between cultivars and years in terms of the examined characteristics. The highest rootstock diameter was observed in 'Limon', and the highest stem diameter was recorded in 'Gördes' quince cultivar. In the study, fruit weight varied between 334.91-377.93 g, geometric diameter varied between 86.02-87.26 mm, flesh firmness varied between 11.33-11.71 kg cm^{-2}, TSS content varied between 11.88-12.70 %, pH varied between 3.31-3.62, titratable acidity varied between 0.51-0.62 %. Fruit number per tree, yield per tree, yield per stem cross-section area, and yield per crown volume were higher in 'Limon' than in other cultivars. Among the cultivars, fruit number ranged from 33.07 to 51.62 tree $^{-1}$, yield ranged from 9.82 to 15.41 kg tree $^{-1}$, yield efficiency ranged from 0.61 to 0.95 kg cm^{-2}, and yield per crown volume ranged from 8.78 to 12.01 kg m^{-3}. Differences between cultivars in terms of L*, a*, and chroma were observed. While, no differences were determined between cultivars in terms of b* and hue^{o}. Among the cultivars, L* value varied from 62.58 to 76.83, the chroma varied from 33.10 to 45.11, and the hue^{o} varied from 111.98 to 115.06. As a result of the study, it can be said that the fruit yield and quality characteristics of 'Limon' cultivar were higher than the other cultivars.

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1. Introduction

The origin of quince (*Cydonia oblonga* Mill.) is Northern Iran, the Caspian Sea region, South Caucasus, and Anatolia. Also, Crimea, Northern Greece, Turkestan, Southern regions of Europe, and extending to North Africa, are said to be centers of origin as can be found in the wild form of in those areas (Ozcagiran et al., 2005). Quince, which is a pome fruit, has been more limited both in terms of distribution as well as a production area and amount compared to other pome fruits such as apple and pear (Ozcagiran et al. 2005; Bolat and Ikinci, 2015). According to FAO's 2019 data, 666 589 tons of quince is produced on a 93 699 ha area in 37 different countries, and Türkiye has a share of 27.1% in the world quince production with a production of 180 542 tons. China (125 480 tons, 18.8%), Uzbekistan (84 937 tons, 12.7%), and Iran (81 594 tons, 12.2%) followed Türkiye in terms of production amount.

Türkiye, which supplies 41.4% of the world's quince with an amount of 15 698 tons, is in the first place in quince export among 55 countries (FAOSTAT, 2021).

The use of vegetative propagation methods such as cuttings and adventive shoots in the quince, as it is a self-fertile fruit type that is very productive, easy to harvest and store, and without pollinator problems, has caused the number of varieties to be limited (Ozbek, 1978; Ercan and Ozkarakas, 2005; Ozcagiran et al., 2005). Quince varieties such as 'Bardak', 'Demir', 'Limon', 'Bencikli', 'Tekkeş', 'Midilli', 'Ege 22', and 'Eşme' are among the quince cultivars commercially are grown in Türkiye. Quince, which is less likely to be damaged by late spring frosts due to late flowering, can be grown in home gardens as a mix or in orchards in almost every region of Türkiye (Ozcagiran et al., 2005).

Quince fruits, whose fruit structure and ripening are similar to apples and pears, never soften excessively. In addition to its fresh consumption can be processed as jam, marmalade, fruit juice, canned food, etc. Quince, is also used as a dwarf rootstock for pear in moist soils (Ozbek, 1978; Ercan et al., 1992; Ozcagiran et al., 2005). Adaptation studies on quince are so less compared to apples and pears, and even it is pome fruit with the highest production after apples and pears.

Quince, which is a native plant of Anatolia, can be grown in almost all regions up to an altitude of 1 000 m (Ozcagiran et al., 2005). Quince production in Türkiye follows an increasing tendency every year, except for the years when extreme climatic conditions are experienced. Türkiye quince production amount was 189 251 tons in an area of 7 737 hectares in 2020, which Sakarya (103 238 tons, 54.6%) was the first in quince production, followed by Bursa (15 616 tons, 8.3%) and Denizli (7 312 tons, 3.9%) (TSI, 2021). The Black Sea Region has a share of 4.2% in the production area of pome fruits with 4.4% production amount in Türkiye, while it has a share of 3.1% in the quince production area and 5.4% in the production amount. In the Black Sea Region, which ranks 4th in quince production in Türkiye, Samsun ranks 3rd (12.0%) in Black Sea Region quince production after Amasya (36.1%) and Corum (13.2%). In the quince production of Türkiye, Samsun ranks 17th with 1 232 tons quince production (Ozturk and Serttas, 2021). In the province of Samsun, the Bafra district ranks 3rd with a total of 12 270 quince trees, of which 6 150 fruitings and 6 120 non-fruiting, with the production of 160 tons in 0.9 hectares (TSI, 2021). It is essential to determine the cultivation potential of new quince cultivars in the district and to reveal their performance in the production of quince, since the ecological conditions of the district are suitable and it has brought good income to the producer in recent years.

The objective of this study was to determine some phenological, morphological, pomological, and chemical properties of 'Gördes', 'Ekmek', and 'Limon' quince cultivars under ecological conditions of the Bafra district of Samsun.

2. Material and Methods

2.1. Materials

This study was carried out at the Bafra Agricultural Research Center of Ondokuz Mayis University (41°33'50" N, 35°52'23" E, and 20 m altitude) in 2018. Orchard was established with 1-year-old saplings at 3.5x3.5 m distances. In the study, 'Gördes', 'Ekmek', and 'Limon' quince cultivars grafted on BA-29 quince clone rootstock were used. The plants were irrigated with drip irrigation between 15 May to 15 September. Fertilization was done with 15-30-15+ME fertilizer at the beginning of summer and 20-20-20 NPK-containing fertilizer in autumn with drip irrigation. Weed control was carried out by mulching the black ground on the row and regularly breaking the weeds with a rotovator between the rows. The properties of experimental area soil were recorded as 2.73-10% clay (low), 13.21-20% silt (moderate), 6.5-20% sand (moderate), pH 7.5 (slightly alkaline), 0.2-0.3 dS m^{-1} salt (no salt), 0.3-0.5 organic matter (low), 3-6% lime ($CaCO_3$) (less), 0.03-0.06% N (less), 5-10 ppm P (medium) level and the soil depth was more than 1 meter.

2.2. Methods

2.2.1. Phenological observations

Phenological observations such as flower bud burst, first flowering, full flowering, and fruit set and harvest date in the examined quince cultivars were carried out according to Yarılgac (2001) and Ercan and Ozkarakas (2005). In addition, the number of days from full flowering to harvest was determined according to these phenological dates.

2.2.2. Morphological investigations

Rootstock diameter (mm) by measuring 10 cm below the grafting union with a digital caliper (Mitutoyo CD-20CPX) sensitive to 0.01 mm at the end of the growing season of all trees in each replication in each cultivar, trunk diameter by measuring the trunk from approximately 20 cm above the soil level (mm) was determined. Plant height (cm) was determined by measuring the distance between the soil level and the top of the shoot with a tape measure. In addition, crown width (cm), crown length (cm), and crown height (cm) were measured to determine the crown volume (m^3) and the trunk cross-sectional area (cm^2) using the trunk diameter (Ozturk and Ozturk, 2014; Kucuker and Aglar, 2021).

2.2.3. Pomological investigations

In the examined quince cultivars, 30 fruits were randomly harvested from each replication when the lint on the peel surface could be easily wiped off by hand and when the color of the fruit skin turned yellow. Fruit weight of the harvested fruits was determined by a digital scale (Weightlab WL-3002L) sensitive to 0.01 g, fruit width (mm), fruit length (mm), and fruit height (mm) were determined with a digital caliper sensitive to 0.01 mm, and the geometric diameter of the fruit was calculated (Ozturk et al., 2015). Flesh firmness (kg cm^{-2}) was determined using a hand penetrometer (Bicasa, Italy) with an 8 mm tip from two areas along the equatorial region of the fruit where the peel was removed. In the juice obtained from fruits, the amount of total soluble solids content (TSS, %) was determined by using a digital hand refractometer (Atago PAL-1, Japan), pH was determined by a digital pH meter (PHSJ-4A, China), and titratable acidity (%) was determined by the titration method (% malic acid) with 0.1 N NaOH (Kılıc et al. 1991). L*, a*, b*, chroma, and hue^o values in the fruit peel from both sides of the equatorial part of the fruit were determined with a color measuring device (Konica Minolta CR 300, Japanese).

In the examined cultivars, the number of fruits per tree (piece) by counting the fruits on each tree before harvest, the yield per tree (kg) by weighing the harvested fruit, and yield per cross-sectional area (kg cm^{-2}) was calculated as yield per tree divided by the trunk cross-sectional area. Also, yield per crown volume yield (kg m^{-3}) was calculated as yield per tree by dividing the crown volume (Bolat et al., 2019).

2.2.4. Statistical analysis

The research was established in a randomized block design with 3 replications and 5 plants in each replication. The obtained data were analyzed in the IBM SPSS 21.0 statistical package program, and the differences between the averages were determined by the 'Duncan Multiple Comparison Test' at $p<0.05$ level.

3. Results and Discussion

The earliest flower bud burst in quince cultivars grown in Bafra ecological conditions occurred on 10 April in 2020 and 13 March in 2021. In both experimental years, the first flowering and full flowering occurred in Limon at the earliest and in 'Ekmek' quince cultivars at the latest. Fruit set was observed in 'Gördes' at the earliest and in Limon quince at the latest during the experiment. In the research, the earliest fruit harvest in 2020 was observed in 'Gördes' variety on 29 September, Limon on 10 October at the latest while 'Gördes' on 5 October at the latest in 2021, followed by Limon quince on 18 October. The cultivar with the lowest number of days from full bloom to harvest in both experimental years was 'Gördes' (160 days and 150 days, respectively), and the cultivar with the highest number of days (174 days and 167 days, respectively) was recorded Limon quince (Table 1). In the research, it was determined that the phonological properties differ according to the cultivars and years. In the quince, Tekintas et al. (1991) cited that in local quince cultivars grown in the Van district, bud bursting was 4-5 May, full flowering was 24-28 May, and fruit harvest was 5-18 October; Koyuncu et al. (1999) reported that bud bursting was 08-14 May, first flowering was 14-20 May, full flowering was 22-25 May, end of flowering was 24-27 May, harvest date was 17-18 October, the number of days from full flowering to harvest was 146 - 148 days in Ekmek cultivar grown in Van district. Ercisli et al. (1999) reported that the fruit ripening date in quince varies according to varieties and years, and they reported that in some quince cultivars grown in Oltu (Erzurum) district, the earliest harvest occurred between 7-10 October in Ekmek cultivar and 23-27 October in Anzavdere genotypes at the latest. In quinces grown

in Gevaş (Van) district, flower bud burst on May 2-5, full bloom on May 15-20, fruit harvest on October 6-16 were occurred, and the number of days from full bloom to harvest varied between 143-151 days (Yarılgac, 2001). Ercan and Ozkarakas (2005) determined that the first flowering of 31 quince varieties and types selected from the Aegean Region was between 15 March to 10 April, full flowering between 23 March to 30 April and fruit harvest between 4 October to 28 November. The first flowering on 10-28 April, full bloom on 15 April - 2 May, the end of flowering on 22 April - 07 May, and the fruit harvested on 30 Sep - 5 Oct from Eşme and Limon quince cultivars in Tokat ecological conditions (Gercekcioglu et al., 2014). Eşme cultivar at ecological conditions of Şanlıurfa showed, bud burst on 19-26 March, the first flowering on 22-30 April, the full flowering on 27 April-7 May, harvest on 24 Oct. - 03 Nov., and the number of days from full flowering to harvest reported 180 days (Bolat and Ikinci, 2015). It can be said that the results of the phenological observations determined in our research are compatible with studies of others. It can be mentioned that the difference in phenological characteristics is due to the cultivar and the district where Quince is grown (Koyuncu et al., 1999; Ozcagiran et al., 2005). In the research, flowering and fruit set occurred shortly after the bud burst in 2020 (approximately 25 days), while in 2021, this period lasted for about 50-55 days. It can be said that this situation is due to the temperature difference between years. As a matter of fact, Ozbek (1978) stated that the flowering time varies according to the climatic conditions of the year, latitude, altitude and he also noted that the flowering period of a tree changes according to the weather conditions, as all the flowers on the tree open in a short time in hot and dry weather, unlike the flowering in the same tree could take longer in cool and rainy weather.

The effect of cultivars and production years on rootstock and stem diameter were significant. Average rootstock diameter varied between 57.35 to 62.63 mm in cultivars and 44.86 to 74.05 mm in years average. 'Limon' quince cultivar had the highest rootstock diameter (62.63 mm). The stem diameter varied between 45.54 to 50.49 mm in cultivar averages and 34.87 to 62.14 mm in terms of years average. The highest stem diameter (50.49 mm) was observed in the 'Gördes' quince (Table 2). Tatari et al., (2020) reported that rootstock diameter ranged from 8.80 mm to 11.06 mm in 3-year-old promising hybrid quince genotypes in Isfahan (Iran) ecological conditions. Bolat and Ikinci (2015) noted that the stem diameter ranged between 5.22 cm and 12.30 cm of 'Eşme' quince cultivar grown in Şanlıurfa ecological conditions during the 5-12 years of the experiment. Rootstock diameter obtained value was higher in the study than Tatari et al. (2020), and the trunk diameter was similar to the results of Bolat and Ikinci (2015). It can be said that the differences in results are due to the genetic structure and ecological differences of the growing region.

Table 1. Phenological features of some quince cultivars under Bafra ecological conditions

Cultivars	Flower bud burst date	First flowering date	Full flowering date	Fruit set date	Harvest date	Number of days from full flowering to harvest
				2020		
Gördes	12 April	16 April	23 April	1 May	29 September	160
Limon	10 April	14 April	20 April	5 May	10 October	174
Ekmek	12 April	18 April	24 April	3 May	1 October	171
				2021		
Gördes	15 March	3 May	7 May	10 May	5 October	150
Limon	13 March	1 May	5 May	15 May	18 October	167
Ekmek	15 March	5 May	8 May	13 May	10 October	161

The effect of the cultivars on the tree height in the examined quince cultivars was insignificant, but the research years were significant. The tree height was observed between 244.30 to 259.07 cm (Table 2).

Table 2. Morphological features of some quince cultivars under Bafra ecological conditions

Years	Cultivars	Rootstock diameter (mm)	Stem diameter (mm)	Tree height (cm)	Trunk cross section area (cm^2)	Crown volume (m^3)
2020	**Gördes**	47.92±2.9 c*	36.13±0.9 c	236.33±4.6 bc	10.26±0.5 c	0.52±0.3 c
	Limon	44.80±1.7 cd	35.30±1.1 c	234.05±5.3 bc	9.80±0.5 c	0.60±0.1 c
	Ekmek	41.86±0.9 d	33.19±0.4 c	230.10±8.7 c	8.65±0.2 c	0.54 ±0.1c
2021	**Gördes**	68.84±1.1 b	64.85±3.1 a	281.80±5.4 a	33.16±3.1 a	4.12±0.3 a
	Limon	80.46±1.6 a	55.77±0.9 b	273.13±8.7 a	24.67±3.4 b	4.41±0.1 a
	Ekmek	72.85±0.2 b	65.80±1.1 a	258.50± 8.5 ab	33.99±0.5 a	3.66±0.1 b
Factor Means						
Cultivar	**Gördes**	58.38±9.8 b	50.49±6.1 a	259.07±6.1 a	21.71±1.3 a	2.32±1.9 ab
	Limon	62.63±9.6 a	45.54±6.9 b	253.59±2.8 a	17.24±0.9 b	2.50±2.1 a
	Ekmek	57.35±7.5 b	49.49±7.1 ab	244.30±3.9 a	21.32±1.3 ab	2.10±1.7 b
Year	**2020**	44.86±4.1 b	34.87±1.8 b	233.49±6.9 b	9.57±1.1 b	0.55±0.2 b
	2021	74.05±5.4 a	62.14±6.5 a	271.14±7.1 a	30.61±6.0 a	4.06±0.4 a
Probability						
Year		0.001	0.001	0.001	0.001	0.001
Cultivar		0.019	0.001	0.214	0.043	0.050
Year x Cultivar		0.003	0.043	0.052	0.046	0.099

*: values within the same columns followed by different letters were significantly different ($p < 0.05$) for each parameter.

The tree height was observed 2.34-2.76 m in the 3-year-old 'Eşme' quince cultivar grafted on the quince seedling and 2.49-2.80 m in the Limon quince cultivar at the ecological conditions in Tokat (Gercekcioglu et al., 2014). The same researchers stated that the difference between the production years, especially the results of the following years compared to the first year, was due to the differences in the age and growth vigor of the trees. The effects of research years and varieties on trunk cross-sectional area and crown volume were significant. The highest trunk cross-sectional area was in 'Gördes' (21.71 cm^2) and the lowest (17.24 cm^2) in 'Limon' cultivar. The highest crown volume was in the 'Limon' (2.50 m^3) and the lowest (2.10 m^3) in the 'Ekmek' cultivar. According to the research years, the trunk cross-sectional area varied between 9.57-30.61 $cm^{2,}$ and the crown volume varied between 2.10-2.50 m^3 (Table 2). Bolat and Ikinci (2015) stated that, the trunk cross-sectional area changes according to the years, found that the trunk cross-sectional area of 'Eşme' quince cultivar was 21.41 cm^2 in 5-year-old plants and 118.83 cm^2 in 12-year-old plants in Şanlıurfa ecological conditions. Gercekcioglu et al., (2014) stated that the crown volume varies according to years and cultivars, and they reported that the crown volume was 1.75-2.16 m^3 in 'Esme' and 1.93-2.16 m^3 in Limon quince cultivars in Tokat ecological conditions. Researchers have pointed out that the difference in cultivars is due to genetic structure and growing conditions. In contrast, the difference in terms of years is due to the age of the trees and ecological conditions. Although the examined cultivars in the study were three years old, slightly higher stem cross-sectional area and crown volume were determined compared to studies including similar cultivars of the same age in different ecological conditions. We can attribute this situation to the fact that the research area has the ideal sandy-loam soil structure and climatic characteristics desired by the quince and the regular annual maintenance operations such as irrigation, fertilization, and weed removal.

The cultivars and research years had significant effects on fruit weight in the study. The fruit weight varied between 334.91 g (Limon) - 377.93 g (Gördes) in terms of cultivar averages and 276.34 g - 431.34 g in terms of research years average (Table 3). The fruit weight of quince cultivars observed 209.4-272.0 g in quinces from Van district by Tekintas et al. (1991); 205.3 g (Limon) - 435.0 g (Midilli) in Aegean Region by Ercan et al. (1992); 255.56 g-530.0 g in Oltu district by Ercisli et al. (1999); 168.9-203.1 g in the Van district by Koyuncu et al. (1999); 121.84-350.96 g in Gevaş (Van) district by Yarılgac (2001); 198.3-452.8 g in Aegean Region by Ercan and Ozkarakas (2005); 257.4-510.4 g in Marmara Region by Buyukyilmaz and Yalcınkaya (2007); 269.4-409.6 g in Kalecik clones by Dumanoglu et al. (2009); 196.93-461.62 g in Çukurova conditions by Kuden et al. (2009); 194.01-297.86 g in Spain by Rodriguez-Guisado et al. (2009); 265.4-415.9 g in some quince clones by Legua et al. (2013); 330.08 g (Eşme) - 352.86 g (Limon) by Gercekcioglu et al. (2014) in Tokat ecology; 349.26 g in Şanlıurfa

conditions by Bolat and Ikinci (2015); 175.12-329.44 g by Erçisli et al. (2015); 88.0-573.0 g by Pinar et al. (2016) in quinces in Egirdir conditions; 135.63-530.74 g by Koc and Keles (2018) in Yozgat conditions. Fruit weight seems to be consistent with previous studies.

Table 3. Pomological features of some quince cultivars under Bafra ecological conditions

Years	Cultivars	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Geometric diameter (mm)
2020	**Gördes**	432.30±9.1 a*	91.21±2.1 a	103.73±5.8 a	94.31±2.5 a
	Limon	403.51±3.2 b	92.01±2.1 a	91.42±2.7 ab	90.14±2.1 ab
	Ekmek	458.20±9.4 a	94.18±1.4 a	98.22±2.1 ab	93.90±0.7 a
2021	**Gördes**	323.55±5.4 a	76.00±3.6 b	86.33±7.3 b	80.22±5.4 b
	Limon	266.31±4.9 b	78.33±1.4 b	90.33±1.9 ab	81.91±0.9 b
	Ekmek	239.17 ±9.5 b	80.67±5.7 b	92.00±4.9 ab	85.20±5.6 ab
Factor Means					
Cultivar	**Gördes**	377.93±6.1 a	83.61±5.3 a	95.03±4.1 a	87.26±6.4 a
	Limon	334.91±7.5 b	85.17±5.6 a	90.88±3.6 a	86.02±5.1 a
	Ekmek	348.68±6.3 b	87.43±5.4 a	95.11±4.7 a	89.55±7.7 a
Year	**2020**	431.34±2.8 a	92.47±3.1 a	97.79±7.9 a	92.78±3.4 a
	2021	276.34 ±3.8 b	78.33±6.3 b	89.56±8.2 a	82.44±7.4 b
Probability					
Year		0.001	0.001	0.049	0.001
Cultivar		0.001	0.495	0.589	0.600
Year x Cultivar		0.001	0.957	0.234	0.040

*: values within the same columns followed by different letters were significantly different ($p < 0.05$) for each parameter.

There was an insignificant effect of cultivars on fruit width, fruit length, and geometric diameter in this study. The fruit width varied between 83.61 - 87.43 mm among the cultivars, fruit length 90.88 - 95.11 mm, and geometric diameter between 86.02 - 89.55 mm (Table 3). Ercisli et al. (1999) in the quinces of Oltu district reported, fruit width 78.98-102.37 mm, fruit length 72.58-121.24 mm; Koyuncuoglu et al. (1999) in Ekmek quince cultivar observed, fruit width 7.38-7.57 cm, fruit length 8.35 cm; Yarılgac (2001) said that, fruit width was 5.83-8.19 cm, fruit length was 5.64-9.81 cm in Gevas district quinces; Dumanoglu et al. (2009) in Kalecik quince clones in Ankara ecological conditions expressed which, fruit width was 77.3-88.3 mm, fruit length was 92.9-112.6 mm; Rodriguez-Guisado et al. (2009) recorded that, in quince clones originating from Spain fruit width was 74.53-86.07 mm, fruit length was 76.01-85.62 mm; Gercekcioglu et al. (2014) in Eşme and Limon quinces observed that, fruit width was 81.16-90.89 mm, fruit length was 93.63-111.38 mm; according to Bolat and Ikinci (2015) Esme quince cultivar had a fruit width of 87.62 mm, a fruit length of 98.64 mm, and a fruit volume of 429.32 cm^3; Pinar et al. (2016) examined fruit width of 63.0 mm, fruit length of 50.0 mm; Koc and Keles (2018) said that fruit width was 6.32-9.36 cm, fruit length was 5.32-10.84 cm; Uzun et al. (2020) reported that fruit width was between 44.81-79.25 mm and fruit length was between 55.62-94.03 mm in quince genotypes collected from Kayseri district. Emphasizing that the variety has a significant effect on fruit sizes, Ercisli et al. (2015) found that fruit width was 68.56-90.53 mm, fruit length was 75.32-91.68 mm, the geometric diameter was 70.72-90.98 mm, and fruit volume was 185.39-391.98 cm^3 in the quince cultivars they examined. It can be said that the results about fruit sizes obtained from the research are compatible with similar previous studies.

There was no significant effect of cultivars and research years on the fruit firmness of the examined quince cultivars. The fruit flesh firmness ranged between 11.33-11.71 kg cm^{-2} in the study. The cultivars and research years had significant effects on TSS, pH, and acidity. In terms of cultivar averages, TSS content varied between 12.70-11.88%, pH 3.31-3.62, acidity 0.51-0.62%. The highest TSS (12.70%) in 'Ekmek', pH (3.62), and acidity (0.62%) in 'Limon' were determined (Table 4).

Table 4. Chemical features of some quince cultivars grown under Bafra ecological conditions

Years	Cultivars	Flesh firmness (kg cm^{-2})	Total Soluble Solid (%)	pH	Titratable acidity (%)
	Gördes	11.58±0.3 a*	12.67±0.8 b	3.15±0.1 d	0.47±0.1 d
2020	**Limon**	11.50±0.3 a	12.93±0.8 ab	3.56±0.1 bc	0.49±0.2 d
	Ekmek	11.67±0.2 a	13.20±0.2 a	3.18±0.1 d	0.48±0.1 d
	Gördes	11.17±0.8 a	11.53±0.1 d	3.96±0.1 a	0.61±0.2 b
2021	**Limon**	11.92±0.4 a	10.83±0.1 e	3.68±0.1 b	0.75±0.1 a
	Ekmek	11.00±0.1 a	12.20±0.2 c	3.44±0.1 c	0.55±0.1 c
Factor Means					
	Gördes	11.38±0.9 a	12.10±0.6 b	3.56±0.4 a	0.54±0.1 b
Cultivar	**Limon**	11.71±0.6 a	11.88±1.1 b	3.62±0.1 a	0.62±0.1 a
	Ekmek	11.33±0.4 a	12.70±0.6 a	3.31±0.4 b	0.51±0.1 b
Year	**2020**	11.58±0.4 a	12.93±0.3 a	3.30±0.2 b	0.48±0.1 b
	2021	11.36±09 a	11.52±0.6 b	3.69±0.2 a	0.63±0.1 a
Probability					
Year		0.523	0.001	0.001	0.001
Cultivar		0.622	0.001	0.001	0.001
Year x Cultivar		0.474	0.001	0.001	0.001

*: values within the same columns followed by different letters were significantly different ($p < 0.05$) for each parameter.

Ercisli et al. (1999) reported that the flesh firmness was 1.21-3.86 kg (with a 5 mm tip), TSS was 11.80-16.00%, the pH was 3.53-4.06, the acidity was 0.51-2.06% in quinces from Oltu district; Koyuncu et al. (1999) noted that the flesh firmness was 7.79-8.74 kg cm^{-2}, the TSS was 12.16-14.20%, the pH was 3.11-3.39, the acidity was 1.45-1.70% in the Ekmek cultivar grown in Van district; Yarılgac (2001) cited that, flesh firmness was 9.01-10.74 lb (with 11.1 mm tip), The TSS was 9.95-17.80%, pH was 3.11-6.65, acidity was 0.59-1.41% in the Gevaş district; Ercan et al. (2005) reported that, the flesh firmness was 6.25-14.50 lb cm^{-2} (with 11.1 mm tip), TSS was 11.75-17.10% in quinces collected from the Aegean Region; Buyukyilmaz and Yalcınkaya (2007) cited that, the fruit firmness was 4.80-6.88 kg, the TSS was 14.7-15.9%, the acidity was 1.01-1.85 g 100 ml^{-1}; Dumanoglu et al. (2009) noted that, the flesh firmness was 64.7-80.2 N, the TSS was 12.8-16.5%, the acidity was 0.9-1.5% in Kalecik quince clones under Ankara ecological conditions; Kuden et al. (2009) determined that, the TSS was 12.85-17.28%, acidity was 0.71-1.22% in quinces under Pozanti (Adana) ecological conditions; Rodriguez-Guisado et al. (2009) cited that, TSS was 15.0-17.20%, pH was 3.96-4.09, acidity was 4.03-5.46 g L^{-1} in quince clones originating from Spain; Legua et al. (2013) cited that flesh firmness was 4.73-9.85 kg cm^{-2} (with 8 mm tip), TSS was 13.40-18.63%, acidity was 5.28-9.54 g malic acid L^{-1}; Gercekcioglu et al. (2014) reported that the flesh firmness of Eşme and Limon quince cultivars was 36.30-39.21 lb, the TSS was 13.37-13.93%, pH was 2.71-3.26, acidity was 8.38-12.91 g L^{-1} In the ecological conditions of Tokat; Szychowski et al. (2014), who reported that fruit firmness was 5.08-11.60 kg, TSS was 11.3-15.5%, pH was 2.82-3.05, acidity was 0.99-1.56% in Spanish quince clones; Bolat and Ikinci (2015) reported that the flesh firmness was 7.73 kg cm^{-2} (with 8 mm tip), TSS was 15.60%, pH was 3.49, acidity was 0.63% of Eşme cultivar in Şanlıurfa condition; Pinar et al. (2016) cited that fruit firmness was 5.08-11.60 kg, TSS was 11.3-15.5%, pH was 2.82-3.05, acidity was 0.99-1.56% in some important quince cultivars; Uzun et al. (2020) reported that the TSS varied between 9.00-18.00% and acidity between 0.61-2.40% in quinces collected from Kayseri district. It can be said that the results obtained from the research are compatible with the results of similar studies used in the research, some of which are also used, and the differences that arise are caused by the genetic structure, ecology, rootstock, and care conditions.

Except for yield on crown volume, cultivars had a significant effect on the number of fruits per tree, yield per tree, and the yield on trunk cross-sectional area in this study. The effects of the research years on the number of fruits per tree, yield per tree, the yield on the trunk cross-sectional area, and the yield on the crown volume were significant (Table 5).

Table 5. Yield performance of some quince cultivars grown under Bafra ecological conditions

Years	Cultivars	Fruit number (pieces tree $^{-1}$)	Yield (kg tree $^{-1}$)	Yield per trunk cross sectional area (kg cm^{-2})	Yield per crown volume (kg m^{-3})
2020	**Gördes**	15.76±0.2 c*	6.82±0.3 e	0.67±0.1 bc	13.21±0.4 a
	Limon	24.62±1.5 c	9.94±0.7 cd	1.03±0.1 a	19.27±3.1 a
	Ekmek	17.43±1.1 c	7.96±0.2 de	0.92±0.1 a	15.18±2.2 a
2021	**Gördes**	54.87±1.5 b	17.77±0.8 b	0.55±0.1 cd	4.36±0.4 b
	Limon	78.62±6.1 a	20.88±1.3 a	0.87±0.2 ab	4.74±0.3 b
	Ekmek	48.70±1.2 b	11.67±0.7 c	0.34±0.1 d	3.18±0.1 b
Factor Means					
Cultivar	**Gördes**	35.31±2.1 b	12.29±6.1 b	0.61±0.1 b	8.78±1.9 a
	Limon	51.62±3.1 a	15.41±6.2 a	0.95±0.2 a	12.01±2.1 a
	Ekmek	33.07±1.7 b	9.82±2.2 c	0.63±0.3 b	9.18±1.7 a
Year	**2020**	19.27±4.3 b	8.24±1.5 b	0.87±0.2 a	15.89±0.2 a
	2021	60.73±4.7 a	16.77±4.3 a	0.59±0.2 b	4.09±0.4 b
Probability					
Year		0.001	0.001	0.001	0.001
Cultivar		0.001	0.001	0.003	0.341
Year x Cultivar		0.004	0.001	0.013	0.484

*: values within the same columns followed by different letters were significantly different ($p < 0.05$) for each parameter.

The number of fruits varied between 33.07-51.67 tree $^{-1}$ and the yield per tree varied between 9.82-15.41 kg, the yield on trunk cross-sectional area varied between 0.61-0.95 kg cm^{-2}, and the yield on the crown volume varied between 8.78-12.01 kg m^{-3} in the examined cultivars. The highest number of fruits per tree, yield per tree, the yield on trunk cross-section area, and the yield on crown volume were determined in Limon (51.62 tree $^{-1}$, 15.41 kg tree $^{-1}$, 0.95 kg cm^{-2}, and 12.01 kg m^{-3}, respectively) (Table 5). Ercan et al. (2005) reported that yield per tree ranged between 31.61-178.50 kg in the unknown rootstock and age of quince cultivars; Buyukyilmaz and Yalcınkaya (2007) noted that yield in trunk cross-sectional area was 0.33-4.61 kg for promising quince cultivars for the Marmara Region; Gercekcioglu et al. (2014) cited that number of fruits per tree was 7.22-23.44 and the yield per tree was 2.5-6.33 kg in Eşme and Limon quince cultivars in the ecological conditions of Tokat; Bolat and Ikinci (2015) reported that the yield per plant varies between 5.1-47.6 kg and the yield per trunk cross-sectional area varies between 219.46-400.52 g cm^{-2} due to the research years in Eşme cultivar in Sanliurfa district. The number of fruit per tree and the yield were generally higher in the second year than in the study's first year. It is stated that the number of fruits per tree and the yield increase as progress the age of trees (Gercekcioglu et al., 2014; Bolat and Ikinci, 2015). It was determined that the yield on trunk cross-sectional area and crown volume was higher in the first year of the study than in the second year. In this case, it is thought that the trunk cross-sectional area and crown volume increased faster (approximately 4 times increase) in the second year compared to the first year of the study. According to this fast increase, the fact that the yield did not increase in the same way was effective in lowering these values compared to the first year.

The effect of cultivars on L*, a*, and chroma of fruit skin color characteristics of quince cultivars examined in the study was significant, but the effect on b* and hue^{o} was insignificant. The L* value, which expresses the brightness of the fruit skin in quince varieties, was the highest in the Limon (76.83) and the lowest in the 'Ekmek' (62.58). The a* value was the highest (-14.66) in the 'Gördes' and the lowest (-17.69) in the 'Limon'. The b* value, which represents the yellowness of the bark, ranged from 28.50 to 46.77. The chroma value, which is the saturation of the color, was the highest (45.11) in the 'Gördes' and the lowest (33.10) in the 'Ekmek'. The hue^{o} value varied from 111.98 to 115.06 (Table 6).

Table 6. Fruit skin color values of some quince cultivars under Bafra ecological conditions

Years	Cultivar	L*	a*	b*	Chroma	Hue^o
2020	**Gördes**	50.49±0.9 d*	-13.29±0.2 a	26.72±0.2 a	31.93±0.7 d	118.08±.04 a
	Limon	68.59±1.5 b	-18.53±0.2 c	35.18±1.1 a	38.94±1.1 bc	118.11±0.1 a
	Ekmek	58.38±0.1 c	-16.34±0.1 b	31.45±0.2 a	31.81±0.8 d	115.84±0.5 ab
2021	**Gördes**	84.01±2.8 a	-16.02±0.6 b	30.27±2.2 a	58.28±3.0 a	105.88±0.2 c
	Limon	85.07±1.2 a	-16.85±0.1 b	58.37±1.4 a	40.29±0.1 b	112.02±0.5 b
	Ekmek	66.77±2.4 b	-18.69±0.7 c	36.97±0.6 a	34.40±1.3 cd	113.53±3.7 b
Factor Means						
Cultivar	**Gördes**	67.25±1.8 b	-14.66±1.6 a	28.50±2.4 a	45.11±1.4 a	111.98±6.7 a
	Limon	76.83±0.9 a	-17.69±0.9 b	46.77±2.1 a	39.61±1.4 b	115.06±3.4 a
	Ekmek	62.58±0.5 c	-17.51±1.5 b	34.21±2.8 a	33.10±2.2 c	114.68±3.7 a
Year	**2020**	59.15±0.9 b	-16.05±2.2 a	31.12±3.8 a	34.22±3.7 a	117.34±1.2 a
	2021	78.62±0.5 a	-17.18±1.4 b	41.87±6.5 a	44.32±4.4 b	110.48±4.5 b
Probability						
Year		0.001	0.007	0.254	0.001	0.001
Cultivar		0.001	0.001	0.273	0.001	0.086
Year x Cultivar		0.001	0.001	0.628	0.001	0.011

*: values within the same columns followed by different letters were significantly different ($p < 0.05$) for each parameter.

The hue^o value close to 0 indicates the color change from red to a distance from 0 indicates the change of color from yellow to green (McGuire, 1992). Dumanoglu et al. (2009) noted that the hue^o value in the fruit peel of Kalecik quince clones was 88.5-100.2 in the ecological conditions of Ankara; Gercekcioglu et al. (2014) cited that L* value varied between 69.76-81.52, a value varied -19.40 to -4.47, b value varied 54.66-63.40 of Ekmek and Limon quince grown in Tokat ecological condition; Ercisli et al. (2015) stated that the L* value varied between 79.63-81.49, a* value varied between -3.07 to -6.38, b* value varied between 56.47-65.14, chroma varied between 56.83-65.22, hue^o varied between 92.70-96.47 in quince cultivars grown in Coruh Valley. It is stated that chroma and hue^o values are the most effective parameters in defining the color characteristics, and color of the fruit skin is the most important indicator of maturity and external quality in quince (Ozcagiran et al., 2005; Ercisli et al., 2015). It can be said that the results determined in the research are compatible with previous studies carried out in similar ecology.

4. Conclusion

The quince cultivars were grafted on BA-29 quince clonal rootstock and were investigated the adaptation to the region where this research was carried out in Bafra (Samsun) ecological conditions. The highest fruit weight was obtained from 'Gördes', the highest TSS content was obtained from 'Ekmek', and the highest acidity was obtained from 'Limon' quince. The highest number of fruits per tree, yield per tree, and yield per trunk cross-section area and crown volume were obtained from the 'Limon' quince cultivar. Since the research was carried out in the 2nd and 3rd years following the sapling planting, the trees were young trees that had not yet fully yielded. In order to obtain more precise results about the performance of the cultivars, it may be appropriate to continue the trial and make a decision based on the long-term data to be obtained. As a result of the research, it can be said that the fruit yield and quality characteristics of 'Limon' quince cultivar were better than the other examined quince cultivars.

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Research Article

Some Morphological, Yield and Quality Characteristics of Cumin (*Cuminum cyminum* L.) Poulations from Different Countries

Ünal KARIK[*1]

[1]Aegean Agricultural Research Institute 35648, İzmir, Turkey

[1]https://orcid.org/ 0000-0001-6707-191X

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

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Abstract: *Cuminum cyminum* L. is one of the most widely consumed spices worldwide and in Turkey. This spice, whose cultivation sites and volume are continually changing annually, is essential, particularly in dry areas. In this research, nationwide seeds cultivated in India, Iran, Syria, Pakistan, Afghanistan, and Turkey (Denizli Province) were used. This study was carried out in the production season of 2020-2021 in Bekilli city of Denizli province as a randomized block design with three replications. In this study, plant height (cm), the number of branches per plant (number), the number of umbels per plant (number), the number of umbellates per plant (number), the number of seeds per umbellate (number), the weight of 1000 seeds (grams), the seed yield (kg/da^{-1}), fixed oil ratio (%) and fixed oil yield (%) of cumin plant were determined. The results of the two-year study have been determined as follows: plant height was 24.72 cm, the number of branches was 5.96, the number of umbels was 33.86, the number of umbellate per umbel was 3.63, the number of seeds was per umbellate 4.82, the weight of 1000 seeds was 3.4 g, seed yield was 59.88 kg/da^{-1}, fixed oil ratio was 11.28%, the fixed oil yield was 6.67 kg/da^{-1}. Türkiye (Denizli) population has reached higher values than other populations in terms of yield and quality characteristics.

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1. Introduction

Cuminum cyminum L. is the most prevalent spice worldwide after pepper (Kanani et al., 2019). This spice is among the oldest and most widely grown plants with various medicinal, nutritional, and healing properties. *Cuminum cyminum* L. is broadly used in the beverage, food, distillery, pharmaceutical, and perfumery industries (Bhatt et al., 2017). It is widely grown in dry and semi-arid areas, such as Egypt, China, Turkey, Saudi Arabia, and the Mediterranean, particularly India and Iran. India is also the largest consumer of cumin, while China is its largest exporter. *Cuminum cyminum* L. is traditionally used as an astringent, anti-flatulence, coagulant, stimulant, and effective against diarrhea, indigestion, epilepsy, toothache, pertussis, indigestion, and jaundice (Rebey et al., 2017; Bhatt et al., 2017; Thippeswamy et al., 2005; Piri et al., 2019).

Cuminum cyminum L. is approximately 30-60 cm tall, hairless, branched, and thin. It contains compound leaves and an umbel with thread-like leaflets. Each cumin branch has 3-9 umbels with 5-7 umbels composed of small white or pink hermaphrodite flowers. It consists of two mericarps and a fruit

approximately 6 mm long. The fruit is a schizocarp 1.5 mm wide with a crown-shaped calyx (Piri et al., 2019; Soltani et al., 2019).

Essential oils in cumin seeds vary between 2% to 4% depending on the area of cultivation and production materials used (Kanani et al., 2019). In studies conducted in different countries, the main component of the essential oil has been determined as *Cuminum cyminum* L. aldehyde, Y-Terpinene, 7-alp Cymene, and B-Pinene. *Cumin cyminum* L. seeds also have fixed oils. The amount of fixed oil in the seeds varies from 10% to 20%, and as the main component, it contains 60% oleic acid and 30% linoleic acid (Kanani et al., 2019; Moghaddam et al., 2015).

Cuminum cyminum L. is cultivated in late autumn and winter in temperate climates and in summer and early spring in tropical climates. About 5 to 6 months after planting, the seeds grow and bear fruit and are harvested. While in Turkey in 2019, the total cultivation area was 321 889 da and the total production was 20 245 tons, in 2020, the total cultivation area dropped to 212 132 da, and the total production decreased to 13 926 tons. The biggest issue of *Cuminum cyminum* L. production areas worldwide and in our country is root diseases and low yields. Hence, the statistics of cultivation areas and yield values show great changes every year.

This is the main reason for the decline in yield for all seeds used in areas where cultivation belongs to the local population. In this study, population seeds of important producing countries of *Cuminum cyminum* L. have been used. This study aims to determine disease-resistant populations and seed yield in cumin, prepare materials for breeding studies and determine some agronomic characteristics and quality yield in field conditions.

2. Materials and Methods

2.1. Materials

Materials studied included *Cuminum cyminum* L. seeds obtained from six different countries: India, Iran, Syria, Pakistan, Afghanistan, and Turkey (Denizli). A commercial company brings the seeds, and they all have a demographic nature.

Table 1 lists the average of many years and the amount of rainfall and temperature in 2020 and 2021 related to Bekilli city of Denizli province. According to Table 1, the region has continental climatic characteristics. The total rainfall over the years is 302 mm. Also, the total rainfall in 2020 was 286.2 mm and in 2021 was 294.5 mm. On the other hand, the average temperature was 14.1 ^{0}C for many years. Also, the average temperature in 2020 was 14.3 ^{0}C, and 14.5 ^{0}C was measured in 2021. In general, the weather data of the studied year matches the average of several years to a large extent.

Table 1. Trial years and long term mean temperature (^{o}C) and total precipitation (mm) values of Bekilli ecological conditions

Climate Factors	Years	**Months**												
		Jan.	Feb.	Mar	Apr.	May	June	July	Aug	Sep.	Oct.	Nov	Dec	Av.
Average Temperature (C^0)	2020	2.3	6.4	8.8	12.3	16.3	22.2	24.4	25.1	20.7	15.3	9.8	8.6	14.3
	2021	2.5	6.9	9.7	13.2	17.3	23.3	25.2	24.8	19.1	15.4	9.5	7.7	14.5
	Long Yeras	2.4	4.3	5.2	10.3	15.3	20.6	23.7	25.4	23.2	19.8	13.3	5.2	14.1
Total Precipitation (mm)	2020	20.2	60.3	42.4	12.8	21.5	10.7	0.0	7.2	6.3	7.6	6.4	90.8	282.6
	2021	25.2	62.4	32.8	10.8	24.6	6.2	0.0	2.3	3.7	10.5	20.7	95.3	294.5
	Long Yeras	32.4	77.6	32.5	12.3	22.7	12.6	0.7	6.9	7.2	8.8	7.5	80.8	302.0

The soil structure and content of the experimental area are given in Table 2. According to the table, the soil structure of the experimental area is loamy. There is no problem in the soil regarding the amount of salt and lime, and its pH level is moderately alkaline. At the intermediate levels in soils containing organic matter, there are no problems with phosphorus and potassium.

Table 2. Some physical and chemical properties of soil in the experimental area

Lab. No:	Depth	Saturation	EC_{25} (1:2.5)	pH (1:2.5)	Lime (%)	Alkaline matter (%)	Absorbable phosphorous	Absorbable potassium
PT-9692	0-30	42	0.19	7.39	4.4	2.83	9	448
		Loamy	Devoid of salt	Medium alkaline	Low	Medium	Medium	High

2.2. Methods

This study was performed with three replications based on the randomized blocks experimental design in the producer farm in Bekilli city of Denizli province for two years (2020 and 2021). The study was carried out in dry conditions. Seeds of demographic nature from six different countries used in this study were planted manually in six rows with a plot length of 6 m and a row spacing of 20 cm. Height, width, and total plot area were selected as 6 m, 1 m, and 6 m^2, respectively. 30 kg of 20.20.20 composed fertilizer was given per decare during seed sowing. According to the plot area, 1 kg cultivation softness was calculated and evenly distributed among the rows. Weed control was done manually, and no pesticides were used to control diseases and pests. The plants were planted in 4 rows. During the harvest, only the middle two rows were evaluated by discarding the side rows, and the harvest area was 6 m^2. In the first year, seeds were sown on February 17th and harvested on July 12th. In the second year, the seeds were sown on February 19th and harvested on July 16th. Parameters investigated in this study include plant height (cm), number of branches per plant (number), number of umbels per plant (number), number of umbellates per plant (number), number of seeds per umbellate (number), the weight of 1000 seeds (g), seed yield (kg/da^{-1}), fixed oil ratio (%) and fixed oil yield (%).

Fixed oil ratio (%)

The dried seeds were milled with a laboratory type miller (Retch GM200) then the oil of the seeds was extracted successively with petroleum ether using a soxhlet extractor (Büchi, Fat Extractor E-500) for 3 h. Oil content was calculated as % on dry matter bases.

The data obtained in the field studies as a result of the measurements and observations were subjected to variance analysis according to the randomized blocks trial design in the Costat 6.03 version package program. The LSD (5%) test was used to determine the significance level of the difference between the means.

3. Results and Discussion

The means and grouping of the obtained data are listed in Table 3. Statistical analysis showed a significant difference between populations in terms of all studied traits in both years of study Table 3.

According to Table 3, the highest heights with 27.63 cm and 27.26 cm were obtained for the first and second years of the study in Turkey (Denizli), respectively. The lowest heights of 21.26 cm and 20.73 cm were obtained for Pakistan in 2020 and 2021, respectively. A significant difference was obtained in terms of plant height between years in the study. The average plant heights in 2020 and 2021 were 25.11 cm and 24.32 cm, respectively.

According to cumin plant height in studies, Mahajan et al., (2012) in their study in India with 22 populations: from 28.21 to 33.31 cm; Bahraminejad et al., (2011) in their study in Iran with 49 different populations: from 17.24 to 35.48 cm; Mirhosseini et al., (2011), in their study in Iran with nine different populations: from 19.45 to 38.16 cm; Keskin (2015) in his study with two cultivars and one population in Isparta ecological conditions: from 23.08 to 24.42 cm. Also, according to the researchers, the height of the cumin plant varies considerably between populations. The lowest and highest plant heights obtained in this study were consistent with the results reported by the researchers.

There was a statistically significant difference between the populations used in the study regarding the number of branches per plant. The highest number of branches in the first year of the study was 6.46 in Turkey, 6.20 in Syria, and 6.16 in Iran population. In the second year, the population of Turkey with 6.36 branches was in the first group. The lowest number of branches per plant values in both experiment years were obtained from the population of Pakistan as 5.56 and 5.63 branches, respectively (Table 3). In studies conducted in previous years regarding the number of branches,

Bahraminejad et al., (2011), in a study with 49 populations in Iran, recorded the number of branches between 4.17 to 7.82. Mirhosseini et al., (2011), in their study with nine different populations in Iran, reported the number of branches between 4.82 to 27.00. Supporting the results of this study, they stated that the number of branches recorded by different researchers varied significantly depending on the cultivars and populations they used.

A significant difference was observed in the study in terms of the number of umbels per plant between populations and years (Table 3). In both years, the highest number of umbels was recorded for the population of Turkey (Denizli) 39.30 in the first year and 36.70 in the second year. The lowest number of umbels in the first and second years belonged to the population of India, 31.36 and 29.60, respectively. The average number of umbels in the first year was 34.74 and in the second year was 32.99.

In studies performed in different ecological conditions regarding the number of umbels in *Cuminum cyminum* L., Bahraminejad et al., (2011), in a study with 49 different populations of cumin in Iran, recorded the number of umbels between 19.42 and 43.74. Also, Mirhosseini et al., (2011), in a study with nine different populations in Iran, recorded the number of umbels between 22.18 and 34. Furthermore, Keskin (2015), in a study with two cultivars and one population in Isparta ecological conditions, recorded a number 11.27 to 22.73. In general, the number of umbels obtained from studies in different ecological conditions and with different populations of cumin was different from our findings.

Table 3. Average plant height, number of branches, and number of umbels in Cuminum cyminum L.

Source	Plant height (cm)			Number of branches per plant (number)			Number of umbels per plant (number)		
	2020	2021	Average	2020	2021	Average	2020	2021	Average
India	23.40c	21.76d	22.58d	5.80b	5.70c	5.75c	31.36d	29.6e	30.48e
Afghanistan	25.50b	24.40c	24.95c	5.80b	5.66c	5.73c	32.53d	31.63d	32.08d
Pakistan	21.26d	20.73d	21.00e	5.56b	5.63e	5.60c	32.10d	30.83d	30.46d
Iran	26.93ab	25.86b	26.40b	6.16a	6.13b	6.15b	35.56c	33.80c	34.68c
Syria	25.93b	25.93b	25.93b	6.20a	6.13b	6.16b	37.60b	35.40b	36.50b
Turkey	27.63a	27.26a	27.45a	6.46a	6.36a	6.41a	39.30a	36.70a	38.00a
Average	25.11A	24.32B	24.72	6.00	5.93	5.96	34.74A	32.99B	33.86
LSD (5%)	1.43	1.19	0.88	030	0.11	0.15	1.57	0.86	0.85
CV	3.21	2.76	3.00	2.88	1.12	2.19	2.55	1.46	2.10
Source	**	**		**	**		**	**	
Year	**			ns			**		
Source*Year	ns			ns			ns		

** : p<0.01; ns: non-significant

According to Table 4, in both years, a significant difference was observed between the populations regarding the number of umbellates per umbel obtained in the study. The population of Turkey (Denizli) with 3.86 and 4.03 umbellates per umbel in both years is in the first place. The lowest number of umbellates inside the umbel, with 3.36 in both years, is related to Afghanistan. Bahraminejad et al., (2011), in a study with 49 different populations in Iran, reported the number of umbellates per umbel from 2.96 to 5.13. Mirhosseini et al., (2011) in their results, show that the number of umbellates per umbel was between 3.48 and 4.96 and reported the difference between the populations in terms of the number of umbellates per umbel. Also, Mehriya et al., (2020) in their study, reported the number of umbellates inside umbels was between 4.60 and 5.07.

In the evaluations that were done in terms of the number of seeds per umbellate, a significant difference was observed between the populations in both years. The highest number of seeds (5.20 to 5.23) was recorded for the cumin population of Turkish origin in both experimental years. The number of seeds per umbellate for the population of Afghanistan with 4.53 and 4.56 seeds is the lowest in 2020 and 2021, respectively (Table 4). Mehriya et al., (2020). In their study in India, they reported the number of seeds per umbellate to be 4.70 to 5.34. Bahraminejad et al., (2011) In a study with 49 different populations in Iran, the number of seeds per umbellate was 3.18 to 6.43. Mirhosseini et al., (2011), in a

study with nine different populations in Iran, reported 4.26 to 6.12 seeds per umbellate. As a result of this study, the researchers stated that the number of seeds in the umbellate varies considerably depending on the cumin population.

There was a statistically significant difference between the populations used in the study in terms of 1000 seeds weight. In this study, the maximum weight of 1000 seeds (4.23 g-4.26 g) for both years belongs to the population of Turkey. The lowest weight of 1000 seeds in the first year belongs to Pakistan (3.73 g) and in the second year to India (3.76 g) (Table 4). In the study of 1000 seed weight, Mehriya et al., (2020) In a study in India recorded values of 3.99 to 4.67. (1999) Chaudhary, in a study in India, recorded values of 4.61 to 5.52. Azizi and Kahrizi (2008) reported 2.60 to 4.93 in their study in Iran. Mahajan et al., (2012) in their study in India, they recorded 3.68 to 4.20. Uğur (2016) recorded a value of 4.36 to 4.99 in a survey of oblique ecological conditions. Bahraminejad et al., (2011), in a study in Iran with 49 different populations, recorded values between 2.83 to 4.12. Mirhosseini et al., (2011), in a study in Iran with nine different populations, recorded the value of 3.12 to 4.08. Keskin (2015) recorded a value between 2.28 and 3.88 in a 2-cultivar, 1-population study of Isparta ecological conditions. In general, it can be seen that the values obtained in terms of the weight of 1000 seeds vary depending on the population.

Table 4. The average number of umbellates per umbel, number of seeds per umbellate, and weight of 1000 seeds

Source	Umbellates per umbel (number)			Seeds per umbellate (number)			Weight of 1000 seeds (g)		
	2020	2021	Average	2020	2021	Average	2020	2021	Average
India	3.50c	3.43c	3.46d	4.70c	4.60c	4.65c	3.76de	3.76c	3.76d
Afghanistan	3.36d	3.36c	3.36e	4.53d	4.56c	4.55d	3.83cd	3.83c	3.83cd
Pakistan	3.53c	3.43c	3.48d	4.63cd	4.60c	4.61cd	3.73e	3.86c	3.80d
Iran	3.73b	3.70b	3.71c	4.83b	4.80b	4.81b	3.90c	3.86c	3.88c
Syria	3.83ab	3.83b	3.83b	5.13a	5.13a	5.13a	4.13b	4.13b	4.13b
Turkey	3.86a	4.03a	3.95a	5.20a	5.23a	5.21a	4.23a	4.26a	4.25a
Average	3.63	3.63	3.63	4.83	4.82	4.82	3.93	3.95	3.94
LSD (5%)	0.11	0.13	0.08	0.13	0.14	0.09	0.09	0.10	0.06
CV	1.83	2.15	1.99	1.54	1.69	1.61	1.33	1.45	1.40
Source	**	**		**	**		**	**	
Year	**			ns			**		
Source*Year	ns			ns			ns		

** : p<0.01; ns: non-significant

According to Table 5, the highest seed yield was recorded in 2020 (68.30 kg/da^{-1}) and 2021 (68.73 kg/da^{-1}) related to the population of Turkey. The lowest seed yields with 53.53 kg/da^{-1} and 52.60 kg/da^{-1} for the first and second years, respectively, belong to Afghanistan.

In a study of seed yield values, Chuadhary (1999), in a study in India, recorded between 23.40 kg/da^{-1} and 33.00 kg/da^{-1}. Azizi and Kahrizi (2008), in a study in Iran, recorded the value 57.44 kg/da^{-1} to 105.00 kg/da^{-1}. Mehriya et al., (2020). In a study in India recorded values between 53.90 kg/da^{-1} and 139.50 kg/da^{-1}. Uğur (2016) reported a value between 58.50 kg/da^{-1} and 89.58 kg/da^{-1} in a survey of oblique ecological conditions. Bahraminejad et al., (2011) reported 39.50 kg/da^{-1} to 145.02 kg/da^{-1} in their study in Iran with a different population. Mirhosseini et al., (2011), in their study in Iran with a different population, recorded the value of 31.20 kg/da^{-1} to 69.74 kg/da^{-1}. Keskin (2015), in a study with a population of 1 and cultivars 2 in the ecological conditions of Isparta, reported values from 25.93 kg/da^{-1} to 97.00 kg/da^{-1}. Parashar et al., (2014) recorded a 45 kg/da^{-1} to 60 kg/da^{-1} in a 9-cultivar study in India. In studies conducted using ecological conditions and different populations with different characteristics, it is observed that cumin seed yield varies significantly depending on the population.

In terms of the amount of fixed oil obtained in the seeds of *Cuminum cyminum* L., the interaction of population, year, and year population was significant. According to the populations, it is observed that the highest percentage of fixed oil belongs to the Indian populations in 2020 (17.49%) and 2021

(18.68%). The lowest percentage of fixed oil belonged to the Iranian population (7.48% and 8.45% for the first and second years, respectively). The average population for 2020 and 2021 were 10.84% and 11.72%, respectively Table 5. In studies on the percentage of fixed oil in the seeds of *Cuminum cyminum* L., Siddarth et al., (2018) reported 17.07% in their study in India. Alfekaiki (2018) recorded 12.5 to 17.16% in their study in Iraq. Hajip et al., (2020), in a study in Morocco, recorded 16.30 to 25.70%. Singh et al., (2017), in a study in India, recorded a value of 10%. Allaq et al., (2020) recorded a value of 10% in a study in Malaysia. Al-Snafi (2016) recorded 10.00% in Iraq. Uğur (2016) recorded 4.63 to 7.41% in the study of oblique ecological conditions. Keskin (2015), in a study on the ecological conditions of Isoarta with a population of 1 and cultivars 2, recorded values of 27.07 to 29.03%. It is observed that to the materials used and the areas in which the study was performed, completely different results are obtained regarding the percentage of fixed oil in cumin.

In this study, the interaction of population, year, and year population was significant in terms of fixed oil yield in cumin. The highest fixed oil yields were recorded for the Indian population in both experimental years (9.38 and 9.88 for the first and second years, respectively). Also, the lowest fixed oil yield for the first and second years was recorded as 4.60 kg/da^{-1} and 5.23 kg/da^{-1} for the population of Iran, respectively. Looking at the years according to Table 5, we can see that the average fixed oil was 6.43 kg/da^{-1}in the first year and 6.91 kg/da^{-1}in the second year. Kesin (2015) recorded a steady-state oil yield between 7.06 kg/da^{-1}and 28.20 kg/da^{-1} in Isparta ecological conditions, and the values he obtained are higher than the values of the current study.

Table 5. Average seed yield, fixed oil percentage, and fixed oil yield for *Cuminum cyminum* L.

Source	Seed yield (kg/da^{-1})			Fixed oil percentage (%)			Fixed oil yield (kg/da^{-1})		
	2020	2021	Average	2020	2021	Average	2020	2021	Average
India	53.66e	52.90e	53.28e	17.49a	18.68a	18.08a	9.38a	9.88a	9.36a
Afghanistan	53.53e	52.60e	53.06e	9.67d	10.19d	9.93d	5.18e	5.36e	5.27e
Pakistan	57.83d	57.53d	57.68d	10.54c	11.19c	10.86c	6.09c	6.43c	6.26c
Iran	61.53c	61.90c	61.71c	7.48f	8.45f	7.96f	4.60f	5.23d	4.91f
Syria	65.70b	64.36b	65.03b	8.66e	9.65e	9.16e	5.69e	6.21c	5.95d
Turkey	68.30a	68.73a	68.51a	11.19a	12.16b	11.67b	7.64b	8.35b	8.00b
Average	60.09	59.67	59.88	10.84B	11.72A	11.28	6.43B	6.91A	6.67
LSD (5%)	1.92	1.99	1.31	0.07	0.09	0.05	0.25	0.25	0.17
CV	1.79	1.87	1.83	0.37	0.46	0.42	2.26	2.10	2.17
Source	**	**		**	**		**	**	
Year	**			ns			**		
Source*Year	ns			ns			ns		

** : p<0.01; ns: non-significant

4. Conclusion

In this research that addressed some agronomic characteristics and quality yield of *Cuminum cyminum* L. seeds cultivated in different countries, it was observed that there is a significant difference between the populations in terms of the studied parameters. As a result, the evaluation was performed according to these parameters at which the populations of Turkey ranked first: year and population, plant height (27.63 cm), number of branches (6.46), number of umbels (39.30), number of umbellates per umbellate (4.03), number of seeds per umbellate (5.23 pcs.), 1000 seed weight (3.95 g) and seed yield (68.73 kg/da^{-1}). India's population ranks first in fixed oil content (18.68%) and fixed oil yield (9.88 kg/da^{-1}). Overall, it can be seen that the agricultural value of the plant is quite low. In our country and globally, cumin is grown in rainfed areas - sometimes alternately with wheatgrass and sometimes as fallow - cumin. Since this spice is grown in areas with very low rainfall and poor soils, its yield value is also low. Yield is also very poor because it is cultivated in areas with very low rainfall and poor soils. Especially in the period between April and May, the dry season prevents the growth of cumin and causes the reproductive period to occur in a short time. As a result, performance is reduced. Another important issue in cumin cultivation is root diseases. This plant is very susceptible to root diseases. Although the

major cumin-producing countries worldwide have been working for years to develop disease-resistant cultivars, to date, no positive results have been achieved in this regard. In this study, it was found that the import was not optimal in terms of seed yield. Cumin is essential for our country, and there is a need to improve the cultivation methods in the cultivation areas and study the breeding of root disease-resistant varieties from the local population.

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Research Article

Influence of In Ovo Leptin Injection into Yolk Sac on Embryo Development, Blood Biochemical Parameters and Lipid Metabolism of Broiler Chicks during Early Post-Hatching Period

Bülent CELLAK[1], Elif BABACANOĞLU[*2]

[1,2]Van Yuzuncu Yil University, Agriculture Faculty, Animal Science Department, 65080, Van, Turkey

[1]https://orcid.org/0000-0002-0936-9141, [2]https://orcid.org/0000-0002-6329-315X

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

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Abstract: The study aimed to investigate the effect of in ovo (IO) leptin injection on yolk sac leptin concentration, embryo development, blood lipid metabolism, and biochemical parameters of broiler chicks during the early post-hatching period. Hatching eggs were weighed and placed in an incubator with 100 eggs/4 replications/group. The groups were: non-injected group (C); and 3 injected groups with 100 µl of phosphate buffer solution (PBS); 0.5 µl leptin ($L_{0.5}$), and 1 µl leptin (L_1) in 100 µl of PBS. Pure leptin hormone dissolved in PBS was injected into the yolk sac on day 7 of incubation. Yolk/yolk sac leptin concentrations at the onset of incubation, on days 7 and 15 of incubation, and at hatching were determined. Embryo/chick development was determined at all ages. Serum leptin, tri-iodothyronine (T_3), thyroxine (T_4), creatine kinase, total protein, uric acid, and lipid profiles were measured at hatching and day 7 of chick age. The leptin level of the yolk was similar to the leptin level of the yolk sac at hatching. Leptin level of a yolk sac was higher on 7th day than at other ages. IO injection* age was significant for serum leptin level, which was similar to one-day chicks in the $L_{0.5}$ at day 7 of chick age. T_3, T_4, creatine kinase, uric acid, lipid profiles, and embryo/chick development did not change with IO leptin. Serum HDL level was higher in the leptin groups than C and PBS groups at hatching. IO leptin application affects blood lipid metabolism depending on dose level for male broiler chicks at the early post-hatch period, without affecting embryo/chick development.

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1. Introduction

Leptin hormone encoded by the obesity gene (Zhang et al., 2019) is produced by fat cells and is secreted into the bloodstream. Leptin can be synthesized in the hypothalamus, pituitary gland, skeletal muscle, adipose tissue, and especially the yolk sac and liver (Ashwell et al., 1999a; Hu et al., 2008; Rao et al., 2009; Hausman et al., 2012). The first report by Ashwell et al. (1999a) concluded that the expression of leptin hormone from the egg yolk sac begins after day 3 of embryo development. Ashwell et al. (1999b) proved the presence of leptin mRNA expression in the brain, bursa, heart, muscle, and spleen of chick embryos on day 5 of embryo development. This result is confirmed by the result of

Huang et al. (2008), who indicated that leptin level is higher in the hypothalamus on days 1 and 3 of incubation compared to day 11 of incubation, and it is higher in egg yolk on days 1 and 3 of incubation compared to the beginning of incubation. Leptin hormone has a functional role in embryogenesis because leptin mRNA expression in the yolk sac of the developing broiler embryo (Ashwell et al., 1999a) activates chicken growth hormone mRNA expression and thereby enhances lipid metabolism and muscle development (Murase et al., 2016). It was reported that leptin and leptin mRNA expression in the different tissues can be affected hepatic lipid metabolism in newly hatched broiler chicks (Hu et al., 2012; Yuan et al., 2017).

In ovo (IO) leptin administration, which is an easier and faster way to understand the effects of leptin transferred from female breeder to egg yolk (Babacanoğlu and Cellak, 2019), may be a helpful application for physiological parameters and development of broiler embryos. Several studies reported that IO administration of leptin influences embryonic development (Lamoŝova et al., 2003; Su et al., 2012), skeletal muscle growth in sex-specific manner (Liu et al., 2013), the expression of growth hormone in the hypothalamus (Yuan et al., 2017), embryonic metabolism (Cellak and Babacanoğlu, 2019), hepatic leptin secretion and lipid metabolism (Hu et al., 2012). To investigate the effect of maternal leptin, IO injection into broiler breeder eggs of 0.5 µg recombinant mouse leptin dissolved in 100 µl phosphate-buffered saline at the onset of incubation changed liver leptin secretion and lipid metabolism, revealing the role of maternal leptin (Hu et al., 2012).

Leptin hormone has many physiological effects, such as the metabolism of energy, lipid, and glucose, and the development of embryos by affecting growth hormone receptors, growth factors, and protein synthesis in muscle cells (Lamoŝova et al., 2001; Lamosová et al., 2003; Hu et al., 2008; Hu et al., 2012; Liu et al., 2013; Yuan et al., 2017). However, the exact mechanism of leptin hormone level in the yolk or yolk sac on growth and development and metabolisms of lipid is not yet known for broiler embryos/chicks. Hence, this study will provide information about the activation of maternal leptin by IO leptin injection into the yolk sac in broilers. The study aimed to investigate the effect of IO leptin injection on embryo development, serum lipid metabolism, and biochemical parameters during the early post-hatch period in broilers.

2. Materials and Methods

2.1. Experimental design

The study was carried out at the Research and Application Farm of Van Yüzüncü Yıl University and approved by Van Yüzüncü Yıl University Animal Care and Use Committee (Protocol no: 2017/10).

Total 425 hatching eggs obtained from broiler breeders of Ross 308 genotype at 45 weeks- old were used as material in the experiment. At the onset of incubation, egg quality traits were measured in 25 eggs. The rest of the 400 eggs were numbered and weighed, and 4 repeats in each group (25 eggs/repeat/group) were placed in the incubator with 100 eggs/group. Prior to being placed in the incubator, mean egg weight was measured as 63.43 ± 0.39 g, and egg weights for all groups ranged from 63.00 to 63.87 g. The incubator was pre-heated 8 hours before incubation at 27 °C. The experimental design was as follows: the group without injection was control (C), and injection groups were the 100 µl phosphate buffer solution group (PBS); 0.5 µg-leptin + 100 µl PBS group ($L_{0.5}$); and 1 µg-leptin + 100 µl PBS group (L_1). One mg pure leptin hormone (recombinant rat leptin, Peprotech, CAT: 400-21, USA) was dissolved in 1 ml PBS and prepared as 0.5 µg and 1 µg-leptin solutions for 100 eggs/group.

On the 6th hour of the 7th day of incubation (at 174th hour of the incubation), in ovo injection (IO) was administered. The injection site on the eggs taken from the different repetitions in each of the three injection groups was cleaned with 70% ethanol, and the egg was pierced with a 22 G needle so that the blunt tip would slope downwards. Prepared solutions of PBS, $L_{0.5}$ + PBS, and L_1 + PBS were injected directly into the egg yolk sac using a semi-automatic injector with a 26 G needle. According to the group doses, PBS 100 µl / 100 eggs; 0.5 µg-leptin + 100 µl PBS/100 eggs; and 1µg-leptin + 100µl PBS / 100 eggs were injected into the yolk sac, respectively; After the injection, the hole opened in the egg was closed with paraffin and the injected eggs were quickly placed into the incubator. At the 178th hour of incubation, the yolk sac was separated from 8 egg samples randomly selected from each group (including the non-injection group) and stored at -20 ° C until analysis of the leptin hormone level.

On the 12th day of incubation, unfertilized eggs identified by fertility control in each group were separated. On the 18th day of incubation, the embryonated eggs were transferred to the trays, and repeats of the groups were preserved. Female and male chicks hatched between 472 and 496 hours of incubation and were left in the hatcher for drying. The dried chicks were placed in a rearing unit with 3 replicates for each group after 3 hours of their hatching time. All the traits were measured by random selection of 8 male chicks from each group to eliminate the effect of sex at both ages (at hatching and 7 days-old).

2.2. Examined characteristics

2.2.1. Egg quality traits

Egg quality traits to eliminate the effects of IO administration were measured on 25 randomly selected eggs at the onset of incubation. All quality traits (daily egg water loss, eggshell conductance, pore number and diameter, egg weight, weights of eggshell, yolk and albumen, egg shape index, length and width of egg air cell, pH and heights of yolk and albumen, eggshell surface area, eggshell thickness) were measured as described by (Peebles and McDaniel, 2004; Babacanoğlu, 2018; Babacanoğlu et al., 2018). All examined egg quality traits are presented as average values.

2.2.2. Preparation of yolk and yolk sac extracts and ELISA analysis of yolk/yolk sac and serum leptin concentrations

At the beginning of the experiment, after determining egg quality characteristics, yolk samples from 8 eggs were kept at -20 ºC until leptin analysis. After IO injection, the leptin hormone level was determined by the ELISA method in the yolk sacs obtained from embryos in each group at the 12nd hour of the 7th day of incubation (180th hour of incubation), on the 15th day of incubation, and in the residual yolk sac obtained from day-old male chicks at hatching. Yolk/yolk sac and residual yolk sac samples, randomly selected from each group (8 samples/group/day), were subjected to the extraction process before analysis. Egg yolk, yolk sac, and residual yolk sac samples were weighed to approximately 20-30 mg. Potassium chloride (KCL) solution was added as 140 mmol, which was 9 times the sample weight. After KCL was added, samples were homogenized in the homogenizer for 1 minute, and the homogenates were centrifuged at 3750 rpm for 10 minutes at 4 ºC. The obtained supernatant was transferred to eppendorf tubes as 200 µl. One ml of KCL was added to each supernatant and vortexed for 1 minute. All samples were re-centrifuged at 3750 rpm for 15 minutes at 4 °C, and after this process, 200 µl sample volume was transferred to numbered eppendorf tubes and kept at -20 ºC until analysis (Von Engelhardt and Groothuis, 2005; Babacanoğlu et al., 2013).

Two leptin hormone kits from a commercial brand (Rel Assay Diagnostics Chicken LEPTIN ELISA kit, Turkey) were used. Leptin concentration was measured by ELISA based on enzymatic immuno-sorbent assay analysis at 450 nm wavelength on a reader (Biotek ELx800 ELISA reader, USA) with units washed and studying the double antibody sandwich method. Absorbances of the standards and samples were obtained from the ELISA reader. The regression equation of the standard curve was calculated with the OD value of the graph plotted according to the standard concentrations (Hau et al., 2001; Sunwoo et al., 2011; Babacanoğlu et al., 2013). According to this calculation, when the absorbance values were placed in the formula, the yolk/yolk sac and serum leptin hormone levels were determined.

2.2.3. Embryo, yolk sac, and organ weights

On the 15th day of incubation, the shells were removed from 10 eggs randomly selected from each group, and embryo weight was measured with the embryo separated from the yolk sac and dried with a paper towel. The weights of the liver, lung, heart, brain, bursa fabricius, spleen, proventriculus + gizzard, and breast muscle dissected from the embryo were determined. At hatching and day 7 of chick age, the same measurements were repeated for 8 male chicks randomly selected from each group in order to eliminate the effect of sex on all the characteristics.

2.2.4. Serum biochemical analysis

Leptin hormone level was in blood samples taken from the vein under the left wing of 8 male chicks randomly selected from each group at hatching using the ELISA method (Hau et al., 2001; Sunwoo et al., 2011; Babacanoğlu et al., 2013). Serum from the blood samples was placed in blood

tubes and centrifuged at 3750 rpm for 10 minutes and 4 C by using a SIGMA 3 30 K brand centrifuge device. Serum-free tri-iodothyronine (T_3) and thyroxine (T_4), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) triglyceride, creatine kinase, uric acid, cholesterol, and total protein were measured by Roche CREJ2 Cobas INTEGRA 400 plus and Cobass E 411-USA autoanalyzer devices and their commercial kits (Babacanoğlu et al., 2013; Babacanoğlu, 2018). Cholesterol concentration was measured at a wavelength of 512/659 nm by the enzymatic colorimetric method using 142 µl of serum sample and cholesterol kit (CHOL2 Kit). Total protein concentration was measured at a wavelength of 512/659 nm by the colorimetric method using 152 µl of serum sample and total protein kit (TP2 Kit). HDL concentration was measured at a wavelength of 583/659 nm by a homogeneous colorimetric method using 169.5 µl of serum sample and HDL kit (HDLC4 Kit). The uric acid concentration was measured at a wavelength of 552/659 nm by the enzymatic colorimetric method using 134 µl of serum sample and uric acid kit (UA2 Kit). Triglyceride concentration was measured at a wavelength of 512/659 nm by the enzymatic colorimetric method using 150 µl of serum sample and triglyceride kit (TRIGL Kit). Creatine kinase concentration was measured at 340/552 nm wavelength by UV test method using 12.75 µl of serum sample and creatine kinase kit (CK Kit). Free T_3 concentration was measured with an autoanalyzer device using a specific anti-T_3 antibody labeled with ruthenium complex from a T_3 kit (FT_3 Kit). Free T_4 concentration was measured with an autoanalyzer device using a specific anti-T_4 antibody labeled with ruthenium complex from a T_4 kit (FT_4 II Kit). LDL and VLDL concentrations were calculated with the following equations: LDL= Cholesterol – (HDL+ Triglyceride/5) and VLDL= Triglyceride/5.

2.2.5. Performance

Chicks fed with broiler starter feed until 7 days old were raised under standard rearing conditions. The results for all the performance features were presented in Table 4.

2.3. Statistical analysis

Data were performed by using ANOVA in the SAS package program (SAS, 2009), and the main effects (group, age) and their interactions were included in the GLM procedure. Tukey's HSD test was used to compare means. The significance test was used to compare treatment groups to the control for each age on embryo and organ development. The means of serum biochemical parameters and serum lipid profiles were analyzed for treatment groups, ages, and their interaction. If group*age interaction was detected for any of these features, the mean values of this interaction were also shown on the graph.

3. Results

3.1. Egg quality traits

Eggshell conductance, pore number, pore diameter, egg weight, egg shape index, yolk weight, albumen weight, eggshell weight, width and length of the air cell, heights of albumen and yolk, pH of yolk and albumen, eggshell surface area, eggshell thickness were 8.92 mg H2O d^{-1} $Torr^{-1}$, 26.65 and 22.06 µm, 65.54 g, 80.01%, 20.17 g, 33.80 g, 6.86 g, 12.48 mm, 20.94 mm, 6.04 mm, 6.47, 9.21, 76.09 cm^2, 33.01 µm, respectively.

3.2. Yolk/yolk sac leptin concentration

At the onset of incubation, yolk leptin concentration was 658.46±35.46 ng/ml. Leptin hormone levels decreased significantly in the IO leptin injection groups compared with C at the 10^{th} hour of the seventh day of incubation (P=0.014). At hatching, injection groups were not different from C, but the yolk sac leptin level of the $L_{0.5}$ group significantly increased on the 15^{th} day of incubation. The interaction between group and age for yolk sac leptin levels was significant (P=0.025). This interaction was due to the significant decrease in the yolk sac leptin level of the L_1 group compared to the $L_{0.5}$ and PBS groups on the 15^{th} day of incubation and all groups at other embryonic ages. The effect of age was significant on yolk/yolk sac leptin concentration (P<0.001), which was the reason for the interaction of group and age (P=0.025) (Figure 1).

3.3. Embryo and organ development

Yolk sac weight of the L_1 group was significantly decreased compared to the control at hatching (P=0.012). The weights of all examined organs and embryos did not change at all ages, but the interaction between IO leptin injection and embryo/chick age was significant for liver and intestine weights. These significant interactions were due to increasing age for liver and intestine weights, but IO groups did not differ at each age (Table 1).

3.4. Blood parameters

IO injection did not affect concentrations of serum leptin, uric acid, creatine kinase, total protein, T_3, and T_4, but chick age affected significantly serum leptin, creatine kinase, total protein, T_3, and T_4 concentrations, which were higher at 7 days-old than those at hatching, except for serum T_4 concentration (Table 2). The interaction between IO injection and chick age was significant for serum leptin concentration (Figure 2). Serum leptin level of chicks in the $L_{0.5}$ injection group at one-day old was similar to serum leptin levels of all groups at 7 days of age. Therefore, this interaction was due to the increased serum leptin level of chicks in the $L_{0.5}$ injection group at hatching (Figure 2). The significant interaction between IO treatment * chick age for total protein concentration was due to age (Figure 3).

Serum triglyceride, cholesterol, HDL cholesterol, LDL cholesterol, and VLDL concentrations did not change with IO injection (Table 3). Serum triglyceride, cholesterol, LDL cholesterol, and VLDL concentrations significantly decreased with increasing chick age (Table 3). Serum HDL cholesterol concentration had significant interaction between IO treatment * chick age. Serum HDL cholesterol concentration was higher in the leptin injection groups than C and PBS at hatching and in all groups at 7-day-old chicks (Figure 4).

3.5. Performance

The results for all features representing numerical averages are presented in Table 4. Feed intake was found to be lower in IO treatment groups compared to PBS and C groups. The feed conversion ratio was found to be lower in IO leptin groups than in C and PBS groups. Sex ratio was the highest for male chicks with 63% in the $L_{0.5}$ group, and this ratio for male chicks was 55% in the PBS group and 53% in the L_1 group. The highest mortality rate was 6.45% in the PBS group, while the lowest mortality rate was 2.5% in the L_1 group.

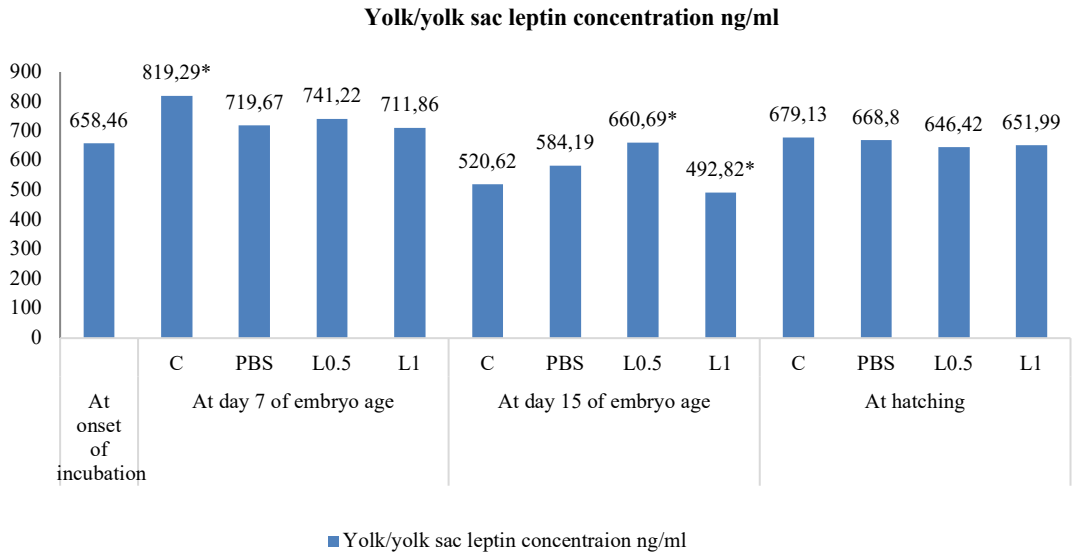

Figure 1. Interaction between IO treatment and chick age for the yolk/yolk sac leptin concentration (ng ml^{-1}).

*Means differ significantly at the same age ($P < 0.05$). C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS.

Table 1. Effects of IO leptin injection, chick age, and IO leptin injection * chick age interaction on embryo and organ development

Age (day)	IO administration[1]	Embryo weight (g)	Yolk sac weight (g)	Liver weight (%)	Lung weight (%)	Heart Weight (%)
On day 15 of incubation	**C**	14.32	15.17	1.86^{b}	0.93^{b}	0.67^{b}
	PBS	14.01	14.79	2.40^{a}	1.22^{a}	0.83^{a}
	$L_{0.5}$	13.57	16.02	2.05^{ab}	1.06^{ab}	0.73^{ab}
	L_1	14.79	13.99	1.96^{b}	1.01^{ab}	0.71^{b}
	SEM	0.53	1.14	0.10	0.07	0.03
P value		0.440	0.684	0.006	0.052	0.045
At hatching	**C**	44.79	5.73^{a}	2.43	0.90	0.62
	PBS	44.57	4.38^{b}	2.67	1.05	0.63
	$L_{0.5}$	44.73	4.78^{ab}	2.76	1.09	0.69
	L_1	44.54	4.29^{b}	2.76	1.11	0.66
	SEM	1.09	0.45	0.13	0.06	0.02
P value		0.998	0.012	0.270	0.103	0.365
At 7 days old chick age	**C**	180.55	0.28	3.70	0.92	0.65
	PBS	179.92	0.40	3.41	0.93	0.68
	$L_{0.5}$	169.04	0.25	3.65	0.83	0.65
	L_1	176.34	0.45	3.66	0.84	0.64
	SEM	5.18	0.02	0.15	0.04	0.03
P value		0.128	0.716	0.562	0.196	0.884
Group*day		0.056	0.919	0.045	0.053	0.899

Table 1. The effects of IO leptin injection, chick age and IO leptin injection * chick age interaction on embryo and organ development (continued)

Age (day)	IO administration[1]	Intestine weight (%)	Proventriculus+ gizzard weight (%)	Pancreas Weight (%)	Breast Weight (%)
On day 15 of incubation	**C**	1.18^{b}	2.79	-	-
	PBS	1.78^{a}	3.12	-	-
	$L_{0.5}$	1.35^{ab}	3.11	-	-
	L_1	1.44^{ab}	2.97	-	-
	SEM	0.15	0.16	-	-
P value		0.051	0.458	-	-
At hatching	**C**	4.61	5.71	0.15	-
	PBS	4.74	6.97	0.14	-
	$L_{0.5}$	4.03	6.08	0.15	-
	L_1	5.23	6.75	0.16	-
	SEM	0.28	0.32	0.01	-
P value		0.052	0.168	0.779	-
At 7 days old chick age	**C**	10.40	3.86	0.46	9.84
	PBS	9.44	3.84	0.39	10.89
	$L_{0.5}$	10.31	4.02	0.48	9.63
	L_1	10.64	4.14	0.48	9.98
	SEM	0.38	0.19	0.03	0.31
P value		0.192	0.715	0.219	0.199
Group*day		0.049	0.513	0.381	-

[a,b] Means within a column with a different superscript differ significantly at $P<0.05$. The significance test is the comparison of treatment groups to the control for each age.
[1]C: control (non-injection group); PBS: 100 µl phosphate buffer solution; L0.5: 0.5 µg leptin+100 µl PBS; L1: 1 µg leptin+100 µl PBS.
SEM: Standard error mean.

Table 2. Effects of IO leptin injection, chick age, and IO leptin injection * chick age interaction on concentrations of serum leptin, uric acid, creatine kinase, total protein, T_3 and T_4

	Leptin ng ml^{-1}	**Uric acid** mg dl^{-1}	**Creatine Kinase** U l^{-1}	**Total protein** mg dl^{-1}	**T_3** pg ml^{-1}	**T_4** ng ml^{-1}
IO injection(group)[1]						
C	469.31	6.62	5221.37	1.95	8.91	5.44
PBS	448.10	5.88	5132.56	1.91	11.20	6.77
$L_{0.5}$	478.37	5.13	5332.01	1.83	10.53	6.66
L_1	511.35	6.10	5309.93	1.95	11.11	7.02
SEM	31.85	0.56	533.64	0.05	0.94	0.64
Age (days)						
0	388.86^{b}	6.02	3851.37^{b}	1.51^{b}	6.79^{b}	9.76^{a}
7	564.70^{a}	5.84	6646.56^{a}	2.31^{a}	14.09^{a}	3.18^{b}
SEM	21.80	0.40	533.64	0.03	0.66	0.72
P values						
Group	0.587	0.320	0.993	0.363	0.296	0.312
Age	<0.001	0.755	<0.001	<0.001	<0.001	<0.001
Group x age	0.045	0.847	0.274	0.017	0.596	0.255

[a-d] Means within a column with a different superscript differ significantly at P<0.05. The significance test is the comparison of treatment groups to the control for each age.
[1]C: control (non-injection group); PBS: 100 µl phosphate buffer solution; L0.5: 0.5 µg leptin+100 µl PBS; L1: 1 µg leptin+100 µl PBS.
SEM: Standard error mean

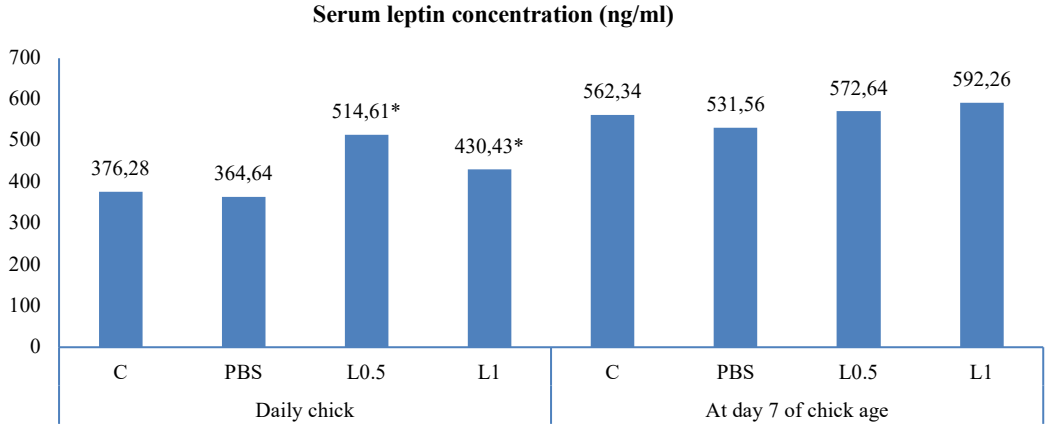

Figure 2. Interaction between IO treatment and chick age for the serum leptin concentration (ng ml^{-1}).

* Means differ significantly at the same age (P < 0.05). C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS.

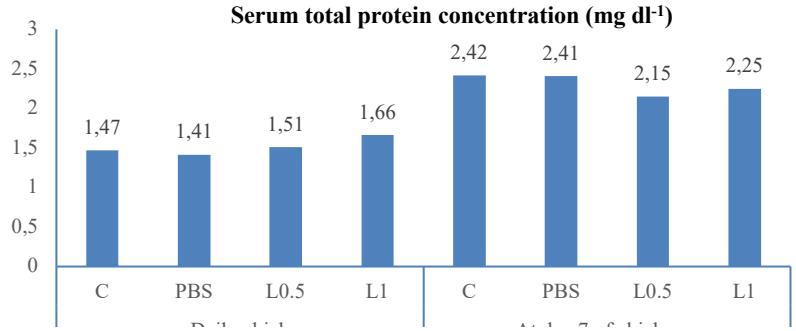

Figure 3. Interaction between IO treatment and chick age for serum total protein concentration (mg dl^{-1})

* Means differ significantly at the same age (P < 0.05). C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS.

Table 3. Effects of IO leptin injection and age on serum lipid profiles (triglyceride, cholesterol, HDL cholesterol, LDL cholesterol, and VLDL) at hatching and 7 day chick age

	Triglyceride	Cholesterol	HDL	LDL	VLDL
IO injection (group)		mg dl^{-1}			
C	74.31	256.49	120.70	120.92	14.84
PBS	60.00	255.65	123.64	121.03	11.98
L0.5	68.00	241.05	127.77	99.69	13.58
L_1	61.50	258.14	125.23	120.62	12.28
SEM	5.72	11.86	4.42	10.85	1.14
Age (day)					
0	75.03[a]	336.78[a]	128.53	120.93[a]	14.98[a]
7	56.87[b]	168.88[b]	120.14	37.37[b]	11.36[b]
SEM	4.04	8.39	3.12	7.33	0.80
P-values					
Group	0.278	0.723	0.719	0.397	0.278
Age	0.001	0.001	0.063	0.001	0.025
Group x age	0.867	0.313	0.009	0.481	0.869

[a.b.c] Means within a column with a different superscript differ significantly at P<0.05.
C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS.

Table 4. Averages for performance characteristics with IO leptin injection

	Daily feed intake	Feed conversion ratio	Sex ratio	Mortality
IO injection (group)	g	(g feed/g gain)	%	%
C	19.65	1.35	50	3.13
PBS	20.44	1.25	45	6.47
$L_{0.5}$	17.51	1.49	37	3.70
L_1	15.43	1.55	47	2.50

C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS.

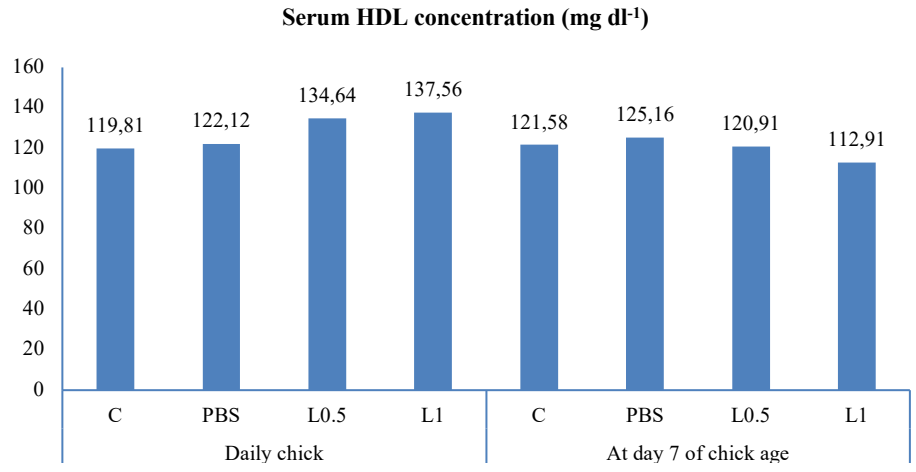

Figure 4. Interaction between IO treatment and chick age for serum HDL concentration (mg dl^{-1}).

* Means differ significantly at the same age (P < 0.05). C: control (non-injection group); PBS: 100 µl phosphate buffer solution; $L_{0.5}$: 0.5 µg leptin+100 µl PBS; L_1: 1 µg leptin+100 µl PBS

4. Discussion

This study investigated the effects of leptin hormone injected into the yolk sac of embryos on blood biochemical parameters, lipid metabolism, and development of the embryo. Leptin concentration in the yolk was determined as 658.46 ng/ml at the onset of incubation. This result indicates that leptin hormone is transferred to follicles through the plasma of the laying hen, demonstrating the maternal origin of leptin hormone in broiler breeders. Hu et al. (2012) reported that IO injection of 0.5 µg recombinant mouse leptin into broiler breeder eggs, revealing that the role of maternal leptin could change liver leptin secretion. These findings indicate the possible roles of *maternal leptin* in the *egg* on the development of an embryo. Moreover, Hu et al. (2008) determined the presence of a leptin-like immunoreactive substance in the egg yolk and albumen in broiler breeders. It was also reported that some practices related to maternal nutrition affect the storage of leptin in the egg (Hu et al., 2008; Rao et al., 2009). In this study, due to the presence of leptin hormone in egg yolk at the onset of incubation and transferral to the egg yolk from female breeders in previous studies, leptin hormone was injected into the yolk sac of the embryo by using the IO method on the 7th day of incubation. In our study, leptin hormone concentration (819.29 ng/ml) in the yolk sac of the control group on the 7th day of embryonic age revealed that leptin hormone is synthesized by chicken embryos. The presence of mRNA for leptin in the brain, bursa, heart, liver, muscle, and spleen of 5 day-old embryos (Ashwell et al., 1999b), and the presence of leptin hormone in the yolk sac of embryos in the first half of incubation indicates that this hormone is active and has a functional role during avian embryogenesis (Ashwell et al., 1999a). The result of this study indicates that egg yolk leptin concentration at onset of incubation was similar to yolk sac leptin concentration at hatching because newly hatched chick functionally utilized from leptin hormone. However, the yolk leptin level changed depending on embryonic age. Yolk sac leptin level was higher on the 7th day than on the 15th day and at hatching. Similar to the findings in our study, Huang et al. (2008) showed that egg yolk leptin level was higher on the 1st and 3rd days of incubation than on the 11th day of incubation. Huang et al. (2008) determined lower yolk sac leptin concentration at hatching and in one-day old chicks than 3 days-old chicks in two different strains of broilers. On the 15th day of embryonic age, the reduced yolk sac leptin level depends on the highest dose of leptin treatment can be a consequence of the influence of leptin gene expression at day 14 of embryonic age in this study.

Serum leptin level of 7 days-old chicks was higher than day-old chicks in the leptin injection groups, showing that serum leptin levels increased with increasing age. When the results of the interaction between age and IO application are examined, the blood serum leptin level changed and increased with age in the leptin-low dose treatment group during the early post-hatching period. Cassy et al. (2004) reported that the inhibitory effect of leptin on appetite regulation is an age dependent process in growing chicks. In the present experiment, higher serum leptin levels were determined at hatching when leptin was administered in a low dose-dependent manner than in a high dose-dependent manner in the first weeks of embryonic development. This result indicates that the leptin hormone might be changed in the early programming of appetite regulation in a dose-dependent manner because the leptin hormone may be affected by appetite regulation related to leptin dose and age of chicken (Chuang et al.,2020)

It was concluded that IO leptin injection had no effect on blood lipid profiles (serum triglyceride, cholesterol, LDL, and VLDL levels) at hatching and 7-day chick age, but the interaction of IO application and chick age on blood serum HDL level was significant. The reason for this interaction is due to the fact that there was a lower serum HDL level at day 7 of chick age than for day-old chicks after injecting 1 µg leptin. Decreased total lipid concentrations in the liver of embryos treated with leptin during incubation promote the possibility of higher lipid degradation in the first days of development in the post-hatching period (Lamosova et al., 2003). Elevated serum HDL concentration in newly-hatched chicks treated with leptin on day 7 of embryonic age may be due to affecting leptin synthesis and secretion, lipid metabolism, and mRNA expression in the liver, which is the major source of leptin in day-old broiler chicks (Hu et al. 2012).

It was reported that injection of leptin into eggs during incubation affects the levels of thyroid hormones in plasma (Mácajová et al. 2002), decreasing the T_3 level and increasing the T_4 level (Lamoŝova et al. 2003). However, IO leptin administration did not change the levels of thyroid hormones because blood lipid metabolism did not affect the liver metabolism of broiler chicks during the early

post-hatching development period in our study. In addition, leptin injection to the yolk sac of 7-day-old embryos did not affect the serum uric acid level after hatching, revealing that leptin had no effect on protein metabolism in a dose-dependent manner.

The embryo and chick weights did not change at each age examined, which may be due to the unchanged serum leptin level. ICV injection of different doses of leptin had no effect on chick weight (Mácãjová et al., 2003; Kuo et al., 2005), which is consistent with our results. While leptin application did not affect embryo and chick development, day-old chicks treated with 1 µg leptin utilized more nutrients from the yolk sac than controls. The reason for this result may also be increased digestive system efficiency due to the effect of 1 µg leptin dose in the first week after hatching because leptin plays an important role in the regulation of feed intake by inhibiting insulin secretion (Taouis et al., 2001). In this study, it was revealed that daily calculated feed intake decreased in the leptin dose groups. We can explain this result with the results of a study (Dridi et al., 2005) which reported that leptin affected the central nervous system and feed intake through selective hypothalamic neuro-peptides. Also, this result confirms that the inhibitory effect of leptin hormone on the regulation of feed intake in growing chicks is age-dependent (Cassy et al., 2004).

Conclusion

It was concluded that the leptin hormone not only originates from the maternal but it is also synthesized by the embryo. IO leptin application affects blood lipid metabolism through cholesterol metabolism of male chicks after hatching depending on leptin dose level and chick age in broilers, without affecting embryo and chick developments. These findings indicate that early programming of appetite regulation in leptin-treated embryos can alter broiler chicks in a dose-dependent manner during the early post-hatching period.

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Research Article

Commercialization of Bambara Nut Production in Nigeria

Abraham FALOLA[1], Ridwan MUKAILA*[2], Abel Ojochegbe AHMED[3]

[1,3] University of Ilorin, Faculty of Agriculture, Department of Agricultural Economics and Farm Management, 1515, Ilorin, Nigeria
[2] University of Nigeria, Faculty of Agriculture, Department of Agricultural Economics, 41001, Nsukka, Nigeria

[1]https://orcid.org/0000-0002-5265-9355, [2]https://orcid.org/0000-0001-8584-0858, [3]https://orcid.org/0000-0002-4073-9091

*Corresponding author e-mail: ridwan.mukaila@unn.edu.ng

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Household commercialization index

Abstract: The global population increases daily, which requires a considerable increase in food production. Bambara nut is an important staple food crop capable of supplying essential nutrients to the body and providing the farmers with income, yet it is underutilized. This study, therefore, assessed the commercialization of Bambara nut production in Nigeria to enhance food availability. Primary data were gathered from 240 respondents and analysed using descriptive statistics, household commercialization index (HCI), and the Tobit model. The results revealed that the mean HCI was 56%, indicating that there exists a gap of 44% for Bambara nut farmers to reach full commercialization. Access to credit, household size, age, farming experience, the quantity of fertilizer, farm output, and distance to the market were significant factors influencing the degree of Bambara nut commercialization. Inadequate access to credit/loan facilities, long-distance to market, high cost of inputs, poor road network to transport produce, and incidence of pests and diseases were the militating constraints to commercialization of Bambara nut production. This study advocates for the provision of credit and inputs, by government and financial institutions, to the farmers to improve their production and commercialization endeavours.

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1. Introduction

Owing to the ever-increasing population in Nigeria and the world in general, it becomes pertinent that food production and security should also increase progressively to meet up with the food and nutrition demands of the citizenry and as well curb the menace of malnutrition, hunger and starvation. To this end, it has become necessarily important that government, researchers and other relevant authorities seek to diversify and broaden the sources of foodstuff available to the ever-increasing population. From all indications, man is yet to exploit the full potential of nature regarding the food we eat. Food and Agriculture Organisation (2013) estimates that the major sources of human food are from less than 20 crop species. Three crops, produced at a large scale, accounted for over half of the food consumed globally (Tanzi et al., 2019). Thus, the general world food supply is very much limited to a few sources as opposed to the numerous species which are available at our disposal but yet to be recognized. With about sixty percent of the food supply coming from majorly rice, wheat, and

maize (Adzawla et al., 2016), massive underutilization exists among so many other crop species, subsequently reducing these crops to a mere subsistence level of production. A level at which production cannot cater to the needs of the teeming population and boost the nation's economy as well through production returns.

Agricultural commercialization is thus needed to boost food availability. This is achievable when all or part of the output produced by farmers is oriented toward the market, and all or part of the inputs utilized in the course of production is purchased from the agricultural markets (Agbonkpolor, 2012; Osmani and Hossain, 2015). Agricultural commercialization consequently comprises two basic sides viz output and input commercialization (Kabiti et al., 2016). It, therefore, involves a meticulous action by the farmers to make use or adopt factors of production in such a way that a larger part of the output is for sale or exchange and to also make substantial purchases from input markets to aid production (Abdullah et al., 2019; Falola et al., 2017).

Most of the foodstuffs consumed in Nigeria are limited to majorly cereals, roots, tuber crops, and a few legumes, which have, over the years, been appreciated at a price per unit of any of the food commodities. Nonetheless, there are other sources of foodstuffs that can supply nearly equal to or even more than the quality of nutrients available in the current diet, but for some reason, the potentials of some of these species are yet to be harnessed. One among such crops which have been greatly side-lined and underutilized is the Bambara nut (*Vigna subterranea*). It is a legume grain crop capable of supplying necessary and important nutrients to the population. Increasing leguminous crop production would increase protein availability which will, in turn, improve peoples' nutrition (de Jager et al., 2017). Bambara nut can be consumed by both humans and animals without any harm as long as it is well processed (DAFF, 2016). Furthermore, the Bambara nut is a complete food source due to its substantial carbohydrate (65%) and protein (about 18%) content.

Bambara nut is categorized as a crop specie whose potentials are yet to be fully exploited in terms of commercialization and utilization. The potential of this crop is still very much yet to be maximized as production at the subsistence level alone will not suffice to raise the status of the Bambara nut from underutilized to a commercially utilized specie. Commercialization of Bambara nuts on the sides of the farmers will help to improve their livelihood and welfare status as there would be significant returns through the sales of their produce. It will also bring about an improvement in food security, enhance income, and lower the rate of unemployment by creating an opportunity for massive production of the crop in contrast to the subsistence form of production that is being practiced currently.

It is the negligence and underutilization of Bambara nut, its inadequate level of commercialization, and recognition of its potential to enhance food security in Nigeria and other parts of the world that necessitated this work. Also, the available studies on the Bambara nut did not focus on its commercialisation (Adzawla et al., 2015; Ibrahim et al., 2018; Mayes et al., 2019; Oyeyinka et al., 2017). This study, therefore, aims at investigating the commercialization of the Bambara nut with the view of increasing its production. This study further examines the factors influencing Bambara nut commercialization and identifies constraints to its production. Understanding these would serve as a reference point for the policymakers to intervene in commercialising neglected and underutilised crops.

2. Material and Methods

2.1. Study area

This study was carried out in Kogi State, Nigeria. Kogi state has 21 local government areas (LGAs) and consists of three Senatorial Districts comprising of the Igala people, Okun (Yoruba), and Egbira. Other minority tribes include the Bassa-Nge, Bassa-Komo, and Idoma as well. Kogi State lies between latitude 7^0 75'N and 6^0 75'E of the equator with a total land size of 29,833km. Bambara nut is consumed majorly by the Igala people of the state; hence, cultivation is most prominent in the 9 LGAs occupied by these set of people. These are Olamaboro, Ankpa, Idah, Dekina, Ofu, Omala, Igalamela-Odolu, Ibaji, and Okehi local governments, respectively.

2.2. Sample and sampling techniques

The study population was rural Bambara nut farming households. A three-stage sampling technique was used to carry out this study. In the first stage, Ankpa, Omala, Idah, and Olamaboro LGAs

of Kogi State respectively were purposively selected. The reason is that the production of Bambara nut is highly concentrated in this region of the State. The second stage constituted a random selection of five farm villages from each of the LGAs. This was followed by the last stage using snowballing sampling technique to select Bambara nut farming households from each of the villages using the probability proportional to size technique. A total of 240 respondents were, therefore, sampled for this research.

Table 1. Sampling design

Local Governments	Villages	No of the households selected	Percentage
Ankpa	Ogodo	12	5.0
	Ikanekpo	10	4.2
	Enjema	10	4.2
	Acharane	10	4.2
	Ochi-Ofago	12	5.0
Idah	Obajadaka	10	4.2
	Idoma	10	4.2
	Etoba	12	5.0
	Okweje	10	4.2
	Alade	12	5.0
Omala	Bukami	10	4.2
	Bagana	12	5.0
	Akpacha	12	5.0
	Ajibu	20	8.3
	Ogodu	12	5.0
Olamaboro	Ofante	20	8.3
	Okpoga	12	5.0
	Emenenga	12	5.0
	Amaka	10	4.2
	Okpo	12	5.0
Total	20	240	100.0

2.3. Methods of data collection and analysis

Data were obtained from Bambara nut farmers using a structured questionnaire. The questionnaires were distributed, by the researchers and research assistants, to the selected sample of the population and supplemented with an oral interview where necessary. The data were collected between September and November 2021.

Descriptive statistics, household commercialization index (HCI), and the Tobit model were means of analysing the data. The description of the socio-economic features of the Bambara nut farmers was achieved using descriptive statistics. It was also used to examine the constraints to market-oriented Bambara nut production. The HCI was used to investigate the level of household commercialization of Bambara nut production, while the Tobit regression model was used to identify the determinants of the commercialization of Bambara nut production.

2.3.1. Household commercialization index

To assess the HCI (market sales) of Bambara nut by the farming households, the crop output market participation share (COMPS) indicator was used. It is estimated as the share of the value of crop (Bambara nut) sold and the value of total Bambara nut produced.

$$COMPSi = \frac{\sum_{k=1}^{k} \overline{P}_k \, s_{ik}}{\sum_{k=1}^{k} \overline{P}_k \, Q_{ik}} \quad (1)$$

Where S_{ik} is the amount of Bambara nut, *k*, sold by farming household *i* at the market at a mean community-level price P_k. The amount of Bambara nut sold will be equal to or less than the total amount produced. Therefore, when a farming household *i* sells all Bambara nuts produced at the market, $S_{ik} = Q_{ik}$ and $COMPS_i = 1$. If a farming household sells none of its Bambara nut produced at the market, $S_{ik} = 0$ and $COMPS_i = 0$. A value of 0 further implies a total subsistence-oriented household, while an index of 1 signifies full commercialization, and the value of the index closer to 1 means a higher degree of commercialization.

2.3.2. Tobit regression analysis

Factors influencing the level of Bambara nut household commercialization were ascertained with Tobit regression. The Tobit model is a censored model with a lower limit (0) and upper limit (1). It can perfectly predict a non-negative outcome (dependent variable) using sets of explanatory or independent variables. The model was used because the dependent variable is an index with values ranging from 0 to 1.

The model is explicitly expressed as:

$$Y = \beta_0 + \beta_1 E + \beta_2 HS + \beta_3 A + \beta_4 FE + \beta_5 FS + \beta_6 CM + \beta_7 AC + \beta_8 F + \beta_9 FO + \beta_{10} DM + \epsilon \quad (2)$$

Thus, the variables that were used in the regression analysis are measured as;
Y = Household commercialization index (0 to 1)
E = Educational level (years)
HS = Household size (Number of persons in a household)
A = Age of farmer (Years)
FE = Farming experience (years)
FS = Farm size (hectares)
CM = Cooperative membership (Yes=1 or No=0)
AC = Access to credit (₦)
F = Quantity of fertilizer (kg)
FO = Farm output (kg)
DM = Distance to market (Km)
β_0 = constant
β = estimable parameter
ϵ = Error term

2.3.3. Likert type rating scale

Likert type scale was put forward by Rensis Likert in 1932 as a technique for the measurement of attitudes (Joshi et al., 2015). A four-point Likert type scale was utilized to identify the challenges faced in Bambara nut production. The Bambara nut farmers were asked to rate their challenge on a four-point numerical rating scale. A benchmark mean of 2.50 was adopted as the decision to consider a statement as being severe or not in Bambara nut production. Any statement with a mean greater than the benchmark mean was considered a severe challenge, while those with a mean less than the decision mean were not severe, according to this study.

3. Results and Discussion

3.1. Socioeconomic and demographic profile of the farmers

Table 2 presents the socioeconomic distributions of the Bambara nut farmers. The majority (70%) of the Bambara nut farmers were males, while females also formed a reasonable proportion of the respondents. This indicated that the farming occupation was chiefly a male occupation among the respondents in the study area. Furthermore, only a few of the respondents (9.2%) were youths under 30 years of age based on the stipulation by the national youth development. The majority of the respondents (84.1%) were not older than 60 years, while only a few respondents (6.7%) were older than 60 years. Furthermore, their average age was 43 years. This indicated that Bambara nut farmers were still in their

productive and active years and therefore possessed enough physical strength to practice their farming operations. Also, the majority of the Bambara nut farmers (83.3%) were married, 12.5% of the respondents were widowed, while a few (4.2%) were single. The average household size was 11 persons. This is not far-fetched as most rural areas are extended families and employ the use of family labour to carry out farm operations; thus, they care to have many people in their household (Falola et al., 2022; Mukaila et al., 2020).

Education is an important factor in agricultural production and farmers income (Mohammed et al., 2020). About 22% of Bambara nut farmers did not have formal education, 45% had only primary education, with just a few of them (2.5%) possessing tertiary education. This shows the high level of formal illiteracy among rural farmers, which could influence their decision-making process, negatively (Mukaila et al., 2021b). Their average farming experience was 19 years suggesting a high level of knowledge and experience among them due to many years spent in farming. A few of the Bambara nut farmers were part of associations, including cooperative societies, while a majority did not participate in any form of association. There was low access to credit among the Bambara nut farmers. This implies a very low level of support rural farmers got in the areas of credit facilities. Furthermore, only a few of the respondents had contact with extension agents ranging from 2 to 3 times. The majority of the Bambara nut farmers had to travel about 8km to the market to sell their products.

Table 2. Distribution of the respondents by socioeconomic characteristics

Variables	Category	Frequency	Percentage	Mean
Gender	Male	168	70	
	Female	72	30	
Age	≤ 30	22	9.2	43
	31 – 40	80	33.3	
	41 – 50	96	40.0	
	51 – 60	26	10.8	
	> 60	16	6.7	
Marital status	Single	10	4.2	
	Married	200	83.3	
	Widowed	30	12.5	
Household size	≤ 5	48	20.0	11
	6 – 10	102	42.5	
	11 – 15	52	21.7	
	>15	38	15.8	
Education level	No formal education	52	21.7	
	Primary education	108	45.0	
	Secondary education	74	30.8	
	Tertiary education	6	2.5	
Farming experience (Years)	5 – 9	24	10.0	19
	10 – 14	50	20.8	
	15 – 19	76	31.7	
	20 – 24	18	7.5	
	≥25	72	30.0	
Membership of Association	Yes	62	25.8	
	No	178	74.2	
Access to credit facilities	Yes	16	6.7	
	No	224	93.3	
Extension contacts	Yes	76	31.7	
	No	164	68.3	
Number of contacts	≤2	70	29.2	
	>3	6	2.5	
Distance to market (km)	≤3	20	8.3	8
	4 – 8	132	55.0	
	9 – 13	66	27.5	
	>14	22	9.2	

Table 3 presents the distribution of Bambara nut farmers by their farming activities. The majority of the Bambara nut farmers (95%) acquired their land by inheritance, while a few of the respondents (5%) purchased their farmlands. The average farm size was discovered to be 2.76 hectares, with most of the farmers devoting less than or equal to one hectare to Bambara nut cultivation. This shows that Bambara nut production is on a small-scale level. Furthermore, the majority of the farmers (97.5%) still used crude implements like hoe and cutlass for farming, with just a few of the farmers (2.5%) employing the use of mechanized implements like a tractor to carry out cultivation activities. The majority of the respondents used personal funds for farming purposes. This is partly the reason for the low and subsistence level of production among the Bambara nut farmers. The commonest form of labour use among the respondents was the use of family labour. This was followed by a combination of both families and hired labour. Using motorcycles majorly as the predominant means of transportation. Transportation is; therefore, a critical issue to be considered as it affects the commercialization process of agriculture.

Table 3. Distribution of the respondents by farming activities

Variables	Category	Frequency	Percentage
Source of farmland	Inheritance	228	95.0
	Purchase	12	5.0
Size of farmland (Ha)	≤ 5.00	218	90.8
	5.01 – 10.00	20	8.3
Mean = 2.76	> 10.00	2	0.8
Means of land cultivation	Hoe and cutlass	234	97.5
	Tractor	6	2.5
Source of capital for farming	Owned funds	224	93.3
	Friends/relatives	14	5.8
	Thrift/Cooperative societies	2	0.8
Major labour use	Family	212	88.3
	Hired	6	2.5
	Family and hired	22	9.2
Means of transport to market	Motorcycle	190	79.2
	Trucks	50	20.8

3.2. Level of commercialization of Bambara nut production

Table 4 presents the summary of the Bambara nut produced, sold, or consumed by the farming households. The average quantity of Bambara nut produced by the farmers was 318.48kg, out of which 139.43kg was consumed, and 179.05kg was sold. A 100kg bag of Bambara nut which was the standard unit of measurement, was sold for ₦30 000. Thus, the values of Bambara nut produced, sold, and consumed by the households were ₦95 544, ₦53 715, and ₦41 829, respectively. About 44% of the total Bambara nut production was consumed by the farmers, and about 56% was sold in the market for public consumption.

Table 4. Breakdown of Bambara nut produced, sold, and consumed by the farmers

Variable	Mean quantity (kg)	Value (₦)
Quantity produced	318.48	95 544
Quantity consumed (as food, gifts, or stored)	139.43	41 829
Quantity sold	179.05	53 715

3.3. Distribution of the Farmers by Household Commercialization Indices

Table 5 presents the distribution of the Bambara nut farmers according to their Household commercialization indices. The household commercialization indices that range from 0 to 1 were converted to a percentage for easy understanding and interpretation. The maximum commercialization index achievable is 1 (100%) in a situation where the farmers did not consume or give Bambara nut as a gift. The household commercialization indices of the Bambara nut farmers ranged from 0 – 90.0%. The farming households whose household commercialization indices were 0 accounted for 2.5% of the

population. This implies that they grow Bambara nut mainly for household consumption (food, storage or gifts). The modal group had between 50.1 and 60% household commercialization indices. The farmers had an average household commercialization index of 56 percent. It was further revealed that 54.3 percent of the farmers had less than the average HCI, while 45.7 percent had more than or equal to the average HCI. The average HCI of 56% implies that the Bambara nut farmers still have a wide gap of 44% (100 – 56%) to attain full commercialization in the production of Bambara nut. This result implies that a little less than half (44%) of the Bambara nut produced by the farming households is consumed by the households, while the remainder (56%) is oriented towards the market for the general public through market forces.

Table 5. Distribution of the Farmers by Household Commercialization Indices

HCI	Frequency	Percentage	Minimum	Maximum
≤ 30.0	6	5.0	0	26.7
30.1 – 40.0	17	14.2	31.1	40.0
40.1 – 50.0	23	19.2	45.0	50.0
50.1 – 60.0	28	23.3	51.7	58.3
60.1 – 70.0	16	13.3	62.5	68.9
70.1 – 80.0	23	19.2	72.2	80.0
> 80	7	5.8	80.9	90.0
Sample Total:	240	100	0	90.0

3.4. Determinants of commercialization of Bambara nut production

The result of the Tobit model, determinants of commercialisation of Bambara nut production, is presented in Table 6. The model was significant at 1%, indicating its fitness. The result revealed that age, household size, access to credit, farming experience, the quantity of fertilizer used, and total farm output are significant factors that influenced the commercialization of Bambara nut production among the respondents.

The age of the Bambara nut farmers influenced the commercialisation of Bambara nut production negatively ($P<0.01$). This implies that aged farmers will have less Bambara nut output for public consumption. Thus, young, energetic, and economically active Bambara nut farmers would have more output for Bambara nut commercialization. This is because an increase in farmers' age reduces their strength and productivity (Mukaila et al., 2022).

Household size positively influenced household commercialisation of Bambara nut production ($P<0.01$). This implies that an increase in household size will result in a probability increase in output which will, in turn, lead to more Bambara nuts being sold in the market. This conforms with prior expectations. Most farming households employ the use of family labour; hence, an increase in the family size will provide manual and cheap labour to be utilized in their farming activities. This is logical as farming activities in developing countries, Nigeria inclusive, depend chiefly on physical strength (Mukaila et al. 2021a).

Farming experience also had a positive influence on household commercialization of Bambara nut production ($P<0.01$). This suggests that an increase in the number of years spent in the farming business increases the output and decision to commercialize. The experience acquired through time gives the farmers abundant knowledge on the most efficient practices that will yield the best output and as well inform their choice to become more market-oriented. Thus, well experienced Bambara nut farmers produced towards market orientation.

Access to credit was positive and significant at 10% in relation to household commercialization of Bambara nut production. This implies that the more access farmers have to credit facilities, the higher their degree of commercialization. Financial access and information also enhance investment and participation in agriculture (Achoja et al., 2020; Falola et al., 2022). Thus, access to credit paves the way for farmers to improve their production and as well channel their output to the markets for public consumption (sales). This is in line with the findings of Falola et al. (2017), who agreed that capital in the form of credit and other sources are an important stimulus and a key asset to the commercialization process of agriculture.

Furthermore, the coefficient for the quantity of fertilizer utilized in the production process was positively significant in relation to household commercialization at 5%. This implies that an increase in the quantity of fertilizer used will result in a considerable increase in the commercialization of Bambara nut production. This also conforms to *a priori* expectation as fertilizer aids the fertility of the soil on which farmers carry out their production activities; hence, an increase in the quantity utilized per production would increase their output which directly impacts the quantity of the produce channelled to the market for sales.

The result further shows that farm output was significant and positively related to household commercialization at 5%. This implies that an increase in farm output resulted in an increase in farmers' commercialization indices. This shows that an increase in total output will increase the quantity that is oriented towards the market.

The coefficient of distance to market had a negative and significant influence in relation to the commercialization of Bambara nut production at 1%. This implies that the farther the distance of the farm to the market, the lesser the farmers cultivate Bambara nut at a market-oriented level. This could be a result of high transportation costs and spoilage (post-harvest) due to a poor road network. Thus, farmers who had their farms closer to the market produced at a market-oriented level and consequently had a high level of commercialization.

Table 6. Factors affecting the level of commercialization of Bambara nut production

Variables	Coeff.	Std. error	T-value	P>t
Age	-0.0041***	0.0011	-3.86	0.000
Household size	0.0201***	0.0051	3.97	0.002
Level of education	0.0013	0.0067	0.19	0.849
Farming experience	0.0165***	0.0030	5.47	0.000
Farm size	0.0019	0.0077	0.24	0.812
Membership of Association	0.0059	0.0433	0.14	0.211
Access to credit	0.0734*	0.0402	1.82	0.071
Quantity of fertilizer	0.0009**	0.0003	2.53	0.042
Farm output	$5.92e^{-06}$**	$7.12e^{-07}$	2.14	0.034
Distance to market	-0.0004	0.0001	-3.04	0.003
Constant	0.9655***	0.1696	5.69	0.000
Pseudo R^2 = 0.7808				
Log likelihood = 21.63437				
Prob>chi2 = 0.0000 (59.83)				

Note: *** p<0.001, ** p<0.01, * p<0.05.

3.5. Constraints to commercialization of Bambara nut production

Table 7 shows the constraints to Bambara nut production as perceived by the respondents. The table shows that the respondents agreed that inadequate access to loan/credit facilities was a severe constraint they faced in their production endeavours towards commercialization. Falola et al. (2022) agreed with the importance of capital in farming when they reported that capital accumulation is an important stimulus and a key asset to investment and commercialization. Furthermore, long-distance to the market and poor road network to transport produce to the market were also severe barriers to the commercialization of Bambara nut production. Market access is crucial to the commercialization process; therefore, the long distances to the market and poor road conditions are considered external factors which impact the farmer's decision to commercialize, thereby reducing the level of production and overall market participation. Also, the high cost of inputs (labour, fertilizer, pesticides, and herbicides) was a severe constraint farmers faced in the process of commercialization of Bambara nut production. Key assets for rural households include access to physical inputs like pesticides, herbicides, and labour, amongst others. Therefore, the high prices accruing to these inputs, whereas capital used for the production process by farmers is quite meagre constitutes a great barrier to farmers' decision and ability to produce more and increase orientation towards the market. İncidence of pests and diseases was a severe constraint farmers face in the process of commercialization of Bambara nut production. This supports the reports of Ibrahim et al. (2018) that Bambara nut production was faced with the challenge of incidence of pests and disease. Mohammed (2016) reported a similar result that the severe constraints

faced in Bambara nut production were inadequate capital, high cost of input, and incidence of pests and diseases.

Table 7. Constraints to commercialization of Bambara nut production

Constraints	Very severe	Severe	Moderately severe	Not severe	Mean	Remark
Inadequate access to credit or loan facilities	64 (53.3)	56 (46.7)	0 (0)	0 (0)	3.53	Severe
Long distance to market	22 (18.3)	84 (70)	14 (11.7)	0 (0)	3.07	Severe
Inadequate access to timely market information	8 (6.7)	13 (10.8)	61 (50.8)	38(31.7)	1.93	Not severe
Poor road network to transport produce	41 (34.2)	54 (45)	24 (20)	1 (0.8)	3.13	Severe
Restriction on land usage by land tenure system	4 (3.3)	0 (0)	22 (18.3)	94 (78.3)	1.28	Not severe
High cost of inputs	44 (36.7)	74 (61.7)	2 (1.7)	0 (0)	3.35	Severe
Incidence of pest and diseases	39 (32.5)	74 (61.7)	7 (5.8)	0 (0)	3.27	Severe
On-farm or post-harvest loss	4 (3.3)	46 (38.3)	45 (37.5)	25 (20.8)	2.24	Not severe

*Figures in brackets are percentages.

Conclusion

The study concludes that the current level of Bambara nut commercialization is relatively low. About half of the Bambara nut produced by the farmers is consumed, thereby reducing the quantity that is available for sales in the market, leading to the underutilization of Bambara nut. The production of Bambara nut at a more or less subsistence level by the farmers has contributed greatly to the side-lining of the food crop and its high level of underutilization among the populace. Thus, the commercialization of Bambara nut would enhance food availability and, consequently food security as the populace would have access to cheap and nutritious food (Bambara nut). The study also showed that access to credit facilities, farming experience, household size, fertilizer usage, and farm output enhance the commercialisation of Bambara nut production, while age is an inhibiting factor to commercialisation of Bambara nut production. This study further revealed that the constraints to market-oriented Bambara nut production include inadequate access to credit facilities, long-distance to markets, high cost of inputs, poor road network, and incidence of pests and diseases.

This study advocates that the government, non-governmental organizations, and agricultural policymakers should support the farmers with inputs at a subsidized rate to curb the incidence of pests and diseases, increase their level of production, and increase and improve the use of technology. This will subsequently improve market orientation and participation. The poor state of rural roads impeded greatly the decision of farmers to become more market-oriented as this increases the time it would take to get to the market and also increases the cost of transportation. Measures that will improve infrastructure (rehabilitation and/or construction of rural roads) should, therefore, be put in place. Subsequent provision of means of efficient transportation of farm produce to both rural and urban markets is also needed. This will improve the farmer's decision to commercialize as there will be a price incentive.

There is a need for more education and enlightenment on the use of improved technology which is a major prerequisite for transiting from subsistence to a commercialized form of agriculture. It is, therefore, necessary to provide farmers with the required knowledge and technical training on mechanized and improved methods of farming. Thus, effort should be made by the government and other relevant agencies to provide extension services to the farmers to provide necessary and relevant information on new technology and its usage amongst farmers. This will greatly improve the farmer's knowledge of the need and importance of improved methods of farming and consequently commercializing their production. Inadequate access to credit was discovered to be a major constraint of Bambara nut production, while it was also discovered that credit available to the farmers would increase their production hence, the decision to commercialize. It becomes imperative that capital sources for farmers be increased to increase production. Therefore, financial institutions, as well as the

government, should provide farmers with credit facilities. This will enable the farmers to gain access to assets that will be further used to expand their production.

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Research Article

Evaluation of Quality and Some Agronomic Traits of Bread Wheat (*Triticum aestivum* L.) Lines and Cultivars under Kahramanmaraş Ecological Conditions

İlker YÜCE[1], Tuğba BAŞKONUŞ[2], Tevrican DOKUYUCU[3], Aydın AKKAYA[4] Hüseyin GÜNGÖR[5], Ziya DUMLUPINAR[*6]

[1]Sivas Science and Technology University, Agricultural Sciences and Technology Faculty, Plant Production and Technologies Department, Sivas, Turkey
[2,3]Kahramanmaraş Sutcu Imam Iniversity, Field Crops Department, Kahramanmaraş, Turkey
[4]Muş Alparslan University Applied Sciences Faculty, Plant Production and Technologies Department, Muş, Turkey
[5]Düzce University, Field Crops Department, Düzce, Turkey
[6] Kahramanmaraş Sutcu Imam Iniversity, Agricultural Biotechnology Department, Kahramanmaraş, Turkey

[1]https://orcid.org/0000-0002-9761-3561, [2]https://orcid.org/0000-0002-0744-6086, [3]https://orcid.org/0000-0002-7704-6790
[4]https://orcid.org/0000-0001-9560-1922, [5]https://orcid.org/0000-0001-6708-6337, [6]https://orcid.org/0000-0003-3119-6926

*Corresponding author e-mail: zdumlupinar@ksu.edu.tr

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Abstract: In this study, eight promising advanced bread wheat genotypes derived from breeding programs and Ceyhan-99, Sagittario, Masaccio, and Seri-82 commercial cultivars were used as materials. The experiments were carried out in 2018-2019 and 2019-2020 cropping years in a randomized complete block design with four replications. In the study, some agronomic and quality traits were investigated. Bread wheat genotypes varied significantly for all investigated traits in terms of the year, genotype, and year x genotype interactions. Based on average data, the investigated traits were determined as; plant height (PH) 90.86 cm, spike length (SL) 9.40 cm, spikelet number (SN) 18.90, grain number per spike (GNS) 56.22, grain weight per spike (GWP) 1.95 g, thousand kernel weight (TKW) 34.90 g, grain yield (GY) 654.54 kg/da, test weight (TW) 76.17 kg/hl, protein ratio (PO) 12.28%, wet gluten (WG) 34.16% and Zeleny sedimentation (ZS) 33.87 ml. According to the two-year results, the highest PH (102.8 cm) was obtained from the Ceyhan-99 cultivar, while ZDEB106 genotype had the longest spike length (9.83 cm) and the highest spikelet number (19.93). ZDEB108 genotype had the highest values in GNS (63.25), GWS (2.24 g), and test weight (77.73 kg hl^{-1}). On the other hand, ZDEB103 genotype was the highest in PO (14 %), WGR (44.28 %) and ZS (40.78 ml). Again, ZDEB101 genotype had the highest TKW (39.255 g), while the highest grain yield (754.56 kg da^{-1}) was obtained from the Massacio cultivar. On the other hand, principal component (PC1 and PC2) analysis explained 62.4 % of the total variation. Thus, a positive correlation was determined between GY with TKW, TW, and PH, while the other traits were negatively correlated with GY.

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Bazı Ekmeklik Buğday (*Triticum aestivum* L.) Çeşitleri ve İleri Hatlarının Kahramanmaraş Ekolojik Şartlarında Tarımsal Özellikler ve Kalite Bakımından Değerlendirilmesi

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Anahtar Kelimeler

Ekmeklik buğday,
Kalite,
Tane verimi,
Protein oranı,
Temel bileşenler analizi

Öz: Bu çalışmada sekiz ileri ekmeklik buğday (*Triticum aestivum* L.) hattı (ZDEB101, ZDEB102, ZDEB103, ZDEB104, ZDEB105, ZDEB106, ZDEB107 ve ZDEB108) ile dört ticari ekmeklik buğday (*Triticum aestivum* L.) çeşidi (Ceyhan-99, Sagittario, Masaccio ve Seri-82) deneme materyali olarak kullanılmıştır. Araştırma 2018-2019 ve 2019-2020 üretim sezonunda tesadüf blokları deneme desenine göre dört tekerrürlü olarak kurulmuştur. Çalışmada bazı tarımsal ve kalite özellikleri incelenmiştir. Araştırmada yıl, genotip ve yıl x genotip interaksiyonu bakımından ekmeklik buğday genotipleri arasındaki varyasyon incelenen bütün özellikler bakımından istatistiki olarak önemli bulunmuştur. İki yıllık ortalamalara göre; bitki boyu (BB) 90.86 cm, başak boyu (BU) 9.40 cm, başakçık sayısı (BS) 18.90 adet, başakta tane sayısı (BTS) 56.22 adet, başakta tane ağırlığı (BTA) 1.95 g, bin tane ağırlığı (BinTA) 34.90 g, tane verimi (TV) 656.54 kg/da, hektolitre ağırlığı (HL) 76.17 kg hl^{-1}, protein oranı (PO) % 12.28, yaş gluten (YG) % 34.16 ve Zeleny Sedimantasyon (ZS) değeri 33.87 ml olmuş; en uzun BB (102.8 cm) Ceyhan-99'dan; ZDEB106 genotipi en uzun başağa (9.83 cm) ve en çok başakçık sayısına (19.93 adet) sahip olmuş; ZDEB108 genotipi BTS (63.25 adet), BTA (2.24 g) ve HL (77.73 kg) özelliklerinde en yüksek değerlere sahip olmuştur. Bununla birlikte, ZDEB103 genotipi PO (% 14.00), YG (% 44.28) ve ZS (40.78 ml) özellikleri bakımından öne çıkmıştır. Yine ZDEB101 genotipi en yüksek BinTA (39.255 g) ağırlığına sahip olurken, en yüksek tane verimi Masaccio (754.56 kg da^{-1}) çeşidinden elde edilmiştir. Öte yandan, temel bileşenler analizi (PC1 ve PC2), toplam varyasyonun % 62.4'ünü açıklamış; buna göre, TV ile BinTA, HL ve BB özellikleri arasında pozitif bir ilişkinin olduğu, diğer özellikler ile negatif bir ilişkinin bulunduğu tespit edilmiştir.

1. Giriş

Dünyada artan nüfus oranına bağlı olarak buğday (*Triticum* spp.) tüketimi artmakta ancak üretim alanları giderek azalmaktadır (Mickelbart ve ark., 2015). Dünya nüfusunun 2050 yılında 12 milyar olacağı tahmin edilirken, artan bu nüfusa yetebilecek üretimin yapılması gerekmektedir. İstatistiklere göre, dünya buğday üretimi 1960'lı yıllarda ortalama 222 milyon ton, 2000'li yıllarda ise ortalama 586 milyon ton iken; 2020 yılında 780 milyon tona yükselmiş (FAO, 2020); 2020 yılında Türkiye'de 6.92 milyon ha'lık alanda buğday ekilerek, 20.5 milyon ton üretim yapılmış ve bunun sadece 16.5 milyon tonu ekmeklik buğdayın olmuştur (TÜİK, 2020).

İnsan ve hayvan beslenmesinde temel besin kaynağı olarak kullanılan buğdayın yüksek adaptasyon yeteneği, depolama ve taşımaya uygunluğunun yanında üretim ve işlemesinin kolaylığı gibi pek çok üstünlüğe sahip olmasından dolayı birçok ürün ve ülke için ayrı bir stratejik öneme sahiptir (Kün, 1996). Kuşkusuz, buğday üretiminin artırılabilmesi için hastalıklara dayanıklı, olumsuz çevre koşullarından daha az etkilenen, kaliteli ve yüksek verimli çeşitlerin ıslah edilerek, uygun kültürel teknikler kullanarak yetiştirilmesi gerekmektedir (Güngör ve Dumlupınar, 2019).

Günümüze kadar pek çok tarım ürününde olduğu gibi buğdayda da hem üretim hem de ıslah çalışmalarında, hedef olarak öncelikle birim alandan alınan verimi arttırmaya yönelik çalışılmalar yapılmıştır (Yağdı, 2004). Üretimi artırırken verimin yanı sıra kalitenin de artması önemli bir diğer unsurdur (Yağdı, 2004).

Hızla değişim gösteren çevre ve verim üzerinde önemli etkileri sahip canlı faktörler, üretimden tüketime kadar bütün unsurların özel isteklerinden dolayı amaca uygun buğday çeşitlerinin elde edilmesine değişen ölçü ya da ölçülerde ivme kazandırmıştır. Öyle ki, genotipik performansları ortaya çıkaran faktörlerden yağışın miktar, kalite ve vejetasyon süresine dağılımı ile minimum ve maksimum sıcaklık değerleri, toprak yapısı, verimliliği ile yetiştirme teknikleri verim ve kaliteyi etkileyen önemli faktörlerden olmakla birlikte, değişen çevreye iyi uyum sağlayan genotiplerin seçimi ekmeklik buğday ıslahının en önemli hedeflerinden birisi olarak kabul edilmektedir (Kılıç ve ark., 2016).

Ekmeklik buğdayda (*Triticum aestivum* L.) gluten ve sedimentasyon gibi kalite özellikleri, PO ile büyük ölçüde paralellik gösterir (Bonfil ve ark., 2004). Bu kalite özellikleri farklı genotip ve çevrelerde değişirken, gluten oranının önemli kalite özelliklerinden olan sedimentasyon miktarının, genotip, çevre, genotip x çevre etkileşimi, kımıl (*Aelia rostrata)* ve süne (*Eurygaster* spp.) zararı vb. bağlı olarak değişmekte (Bonfil ve ark., 2004) bundan dolayı da üretilen ve üretilecek olan buğday çeşitlerinin sadece tane verimi bakımından değil, kalite açısından da değerlendirilmesini gerektirmektedir (Bassett ve ark., 1989).

Bu çalışmada, ıslah programlarından elde edilen ileri bazı ekmeklik buğday hatlarının geniş alanlarda ekimi yapılan çeşitli ticari ekmekliklerle karşılaştırılarak kimi tarımsal ve kalite özellikleri yönünden Kahramanmaraş ekolojik koşullarında irdelenmesi amaçlanmıştır.

2. Materyal ve Yöntem

Araştırmamız, (2019-2020) ve (2020-2021) yetiştirme döneminde Kahramanmaraş'taki Doğu Akdeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsü'nün deneme tarlasında yürütülmüştür. Denemenin yürütüldüğü Kahramanmaraş 37° 35' 4.92" kuzey paralelleri ve 36° 55' 35.08" doğu meridyenleri arasında olup, denizden yüksekliği 568 m'dir. Deneme alanına ait iklim verileri Çizelge 1'de verilmiştir (Anonim, 2021).

Çizelge 1. Araştırma yıllarına ait bazı meteorolojik veriler

			Aylar									
		Yıl	**Kasım**	**Aralık**	**Ocak**	**Şubat**	**Mart**	**Nisan**	**Mayıs**	**Haziran**	**Toplam**	**Ortalama**
Yağış (mm)		2019-2020	46.4	200.2	105.8	75.2	4.6	33	23	0.3	488.2	-
		2020-2021	57.6	62.6	226.6	32.6	135.2	16.2	12	0.0	542.8	-
		Uzun Yıllar	87.5	116.6	125.4	108.3	93.4	69.8	41.2	8.4	650.8	-
Min. (°C)	**Sıcaklık**	2019-2020	5.0	3.6	-1.0	-6.7	1.0	4.3	9.8	14.6	-	3.8
		2020-2021	3.4	-0.9	-2.3	-2.3	2.3	4.6	12.4	14.5	-	4.0
Max. (°C)	**Sıcaklık**	2019-2020	25.5	17.8	14.2	18.2	23.8	27.9	38.1	37.7	-	25.4
		2020-2021	25.4	17.0	16.9	18.7	21.4	31.0	36.0	40.4	-	25.9
		Uzun Yıllar	11.5	6.8	4.9	6.4	10.6	15.5	20.3	25.3	-	12.6
Oransal Nem (%)		2019-2020	56.2	81.9	69.3	68.3	67.3	58.2	47.2	46.6	-	61.9
		2020-2021	65.9	74.4	70.2	59.8	61.2	57.5	43.3	49.0	-	60.2
		Uzun Yıllar	66.7	79.9	70.0	65.6	60.0	57.6	54.9	49.7	-	63.1

Araştırmada deneme materyali olarak, (8) ileri ekmeklik buğday hattı (ZDEB101, ZDEB102, ZDEB103, ZDEB104, ZDEB105, ZDEB106, ZDEB107 ve ZDEB108) ile (4) ticari çeşit (Ceyhan-99, Sagittario, Masaccio ve Seri-82) materyal olarak kullanılmıştır. Araştırma, tesadüf blokları deneme deseninde ve dört tekrarlamalı olarak yürütülmüştür. Ekimler birinci yıl 22.12.2019, ikinci yıl ise 14.11.2020 tarihinde; 550 m^{-2} tane bitki sıklığında ve 5 m sıra uzunluğunda, 20 cm sıra arası mesafede, 6 sıralı ve toplamda 6 m^2 parsel alanı olacak şekilde deneme mibzeri ile yapılmıştır. Denemelerde aynı gübre formları kullanılmış; ekimle birlikte (8) kg/da saf azot (N) ve (8) kg/da saf fosfor (P_2O_5) olacak şekilde taban gübresi verilmiş, kardeşlenme döneminde ayrıca (7.5 kg/da) saf azot olacak şekilde üst gübreleme yapılmış; yabancı ot savaşımı; (Mesosulfuron-methyl + Thiencarbazone-methyl + Iodosulfuron-methyl-sodium + Mefenpyr-diethyl) şeklindeki herbisit kullanımıyla gerçekleştirilmiş; hasadı ise ilk yıl 16.06.2020'de, ikinci yıl ise 13.06.2021'de ve Wintersteiger Classic marka parsel biçerdöverle yapılmıştır.

Çalışmada BB (cm), BU (cm), BS (adet), BTS (adet), BTA (g), BinTA (g) ve TV (kg/da) (Evlice ve ark. (2008)'e göre belirlenmiş; HL (kg/hl), PO (%), YG oranı (%) ve ZS (ml) ise Near Infrared (NIR) spektroskopi (Thermo Fisher Scientific) cihazı kullanılarak saptanmıştır; elde edilen verilere varyans analizi uygulanarak ortalama değerler Duncan testi ile karşılaştırılmıştır. Yapılan homojenite testi sonucunda yıllar arasında önemli bir farklılık olmadığı ($p>0.05$) belirlenmiş olup, bu sonuca göre varyans analizi yıllar birleştirilerek yapılmıştır (Levene, 1960). Temel bileşenler analizi ise JMP yazılımı kullanılarak yapılmıştır (JMP 15.1 SAS Institute Inc., 2020).

3. Bulgular ve Tartışma

3.1. Tarımsal Çalışmalar

Denemeye alınan genotiplerin bitki boyu, başak uzunluğu, başakçık sayısı ve başakta tane sayısı ortalama değerleri Çizelge 2'de, başakta tane ağırlığı, bin tane ağırlığı ve tane verimi ortalama değerleri Çizelge 3'de, hektolitre ağırlığı, protein oranı, yaş gluten oranı ve zeleny sedimantasyon ortalama değerleri Çizelge 4'te gösterilmiştir.

Bitki boyu bakımından yıllar, genotipler ve yıl x genotip interaksiyonu arasında önemli bir varyasyon bulunmuştur (P< 0.01). Ortalama BB değeri, çalışmanın birinci yılında 84.86 cm olurken, ikinci yılında ise 96.87 cm olarak saptanmıştır. Genotipler arasındaki BB (76.23-102.8) cm arasında değişiklik göstermiş; ZDEB108 en kısa (76.23 cm) BB'li genotip olurken, Ceyhan-99 en uzun BB (102.8 cm) çeşit olarak belirlenmiştir. Önceki çalışmalarda BB'nin temel olarak bir genotipin genetik yapısı, çevresel faktörler ve yetiştirme tekniğine göre değişiklik gösterdiği bildirilmiş olup, bildirilenler ile bulgularımız uyum içerisindedir (Aydoğan ve Soylu, 2017; Güngör ve Dumlupınar, 2019; Erdem ve ark., 2020).

Varyans analizi sonucunda başak uzunluğu bakımından yıllar, genotipler ve yıl x genotip interaksiyonu arasında önemli farklılıklar olduğu anlaşılmıştır (P<0.01). Ortalama değerler, çalışmanın birinci yılında 9.15 cm, ikinci yılında ise 9.64 cm olmuş; bu özellik genotipler arasında (8.05-9.83) cm şeklinde değişmiştir. En kısa BU, Masaccio (8.05 cm)'dan, en uzun BU ise ZDEB106 (9.83 cm)'dan elde edilmiştir. Bulgularımız; Özen ve Akman (2015), 8.3-10.4 cm, Naneli ve ark. (2015), 8-9.87 cm ve Öztürk ve Korkut (2018), 7.07-8.35 cm verileri ile benzerlik içindedir.

İstatistiksel olarak Başakçık sayısı bakımından yıllar ve genotipler P<0.01 ve yıl x genotip interaksiyonu ise P<0.05 düzeyinde önemli olarak saptanmıştır. Bu bakımdan denemenin ilk yılında 18.19 adet, ikinci yılında ise 19.61 adet olarak olan BS, genotipler arasında (16.75-19.93) adet olarak değişmiştir. BS en az Masaccio (16.75 adet)'den, en çok ise ZDEB106 (19.93 adet)'den elde edilmiştir. Bu bakımdan önceki çalışmalarda Kahrıman ve Egesel (2011), 15-20 adet, Aydoğan (2018), 17.67-25.20 adet, ve Metin (2019), 16.35-20.30 adet arasında değişen veriler elde etmişlerdir ki bu sonuçlar bulgularımızla benzerlik göstermektedir.

Başakta tane sayısı bakımdan yıllar, genotip ve yıl x genotip interaksiyonu arasında önemli bir varyasyon belirlenmiştir (P<0.01). Bu bakımdan birinci yıl 52.17 adet, ikinci yıl 60.27 adet olarak saptanmış; genotipler arasında (46.65-63.25) adet değişim olmuş; BTS en az Masaccio (46.65 adet)'den, en çok ZDEB108 (63.25 adet)'den elde edilmiştir. Başaktaki tane sayısını, Baysal (2014), 27.3 ile 44.5 adet ve Shirinzadeh ve ark. (2017), 31.34 ile 42.17 adet olarak bildirmiş olup, genel olarak değerlendirildiğinde bu karakter bakımından tarafımızca elde edilen bilgi ve bulgular genel bir benzerlik içindedir.

İstatistik analizi sonucunda başakta tane ağırlığı bakımdan yıllar (P<0.01), genotipler ve yıl x genotip interaksiyonuna (P<0.05) arasında önemli farklılıklar saptanmıştır. BTA ortalamsı birinci yıl için 1.79 g, ikinci yıl için 2.11 g olmuş; bu değer genotipler arasında (1.72-2.24) g şeklinde değişmiş; en az Masaccio (1.72 g) ile çeşidinden, en çok ZDEB108 (2.24 g) çeşidinden sağlanmıştır. Başaktaki tane ağırlığı bakımından elde etiğimiz bilgi ve bulgular; Baysal (2014), 1.05-1.75 g, Altındal ve Akgün (2018), 0.76-1.94 g ve Subaşı ve Ayrancı (2021), 0.669-1.981 g şeklinde bildirilenlerle uyum içerisindedir.

Bin tane ağırlığı bakımından, yıllara göre ortalamalar arasındaki farklar önemli bulunmazken, genotipler arasında (P< 0.01) ve yıl x genotip interaksiyonuna göre (P<0.05) önemli bir varyasyon bulunmuştur. Ortalama BinTA, ilk yıl 34.75 g, ikinci yıl 35.05 g olmuş; genotipler arasında (29.53-39.25) g şeklinde değişmiş; ZDEB103 (29.53 g) genotipi en düşük, değeri verirken, ZDEB101, (39.25 g) ile en yüksek BinTA vermiştir. Kalite ile ilgili olmasının yanı sıra, verimle de ilişkisi BinTA, genetik yapı ve çevresel faktörlerden etkilenmektedir (Mut ve ark. 2017). BinTA bakımından Kendal ve Doğan (2013), 31.0-42.4 g, Doğan ve ark. (2014), 30.9-41.6 g ve Mut ve ark. (2017), 29.2-38.4 g'nın bildirdikleriyle sonuçlarımız genelde uyum göstermektedir.

Çizelge 2. Ekmeklik buğday genotiplerinde BB, BU, BS, BTS, özelliklerine ait ortalama değerler

Yıl x Genotip	Bitki Boyu			Başak Uzunluğu			Başakçık Sayısı			Başakta Tane Sayısı		
	**			**			*			**		
	2020	2021	Ortalama	2020	2021	Ortalama	2020	2021	Ortalama	2020	2021	Ortalama
ZDEB101	83.30	92.25	87.78 ^{b}	8.77	10.35	9.56 ^{a}	18.20	20.00	19.10 ^{ab}	42.90	58.15	50.53 ^{bc}
ZDEB102	85.60	98.65	92.13 ^{b}	9.36	9.95	9.66 ^{a}	19.05	20.55	19.80 ^{ab}	56.80	64.90	60.85 ^{ab}
ZDEB103	86.10	101.05	93.58 ^{b}	9.14	9.85	9.50 ^{a}	17.80	20.10	18.95 ^{ab}	58.30	60.55	59.43 ^{ab}
ZDEB104	88.55	95.05	91.80 ^{b}	9.41	10.15	9.78 ^{a}	19.00	20.55	19.77 ^{ab}	53.05	68.05	60.55 ^{ab}
ZDEB105	88.45	98.15	93.30 ^{b}	9.52	9.85	9.69 ^{a}	18.30	20.25	19.28 ^{ab}	48.30	64.85	56.58 $^{a-c}$
ZDEB106	86.05	96.05	91.05 ^{b}	9.06	10.60	9.83 ^{a}	18.25	21.60	19.93 ^{a}	46.65	68.20	57.43 $^{a-c}$
ZDEB107	86.95	97.95	92.45 ^{b}	9.39	9.55	9.47 ^{a}	17.85	20.00	18.93 ^{ab}	53.90	62.10	58.00 $^{a-c}$
ZDEB108	63.55	88.90	76.23 ^{c}	10.24	8.80	9.52 ^{a}	19.90	18.80	19.35 ^{ab}	70.10	56.40	63.25 ^{a}
Ceyhan-99	98.85	106.65	102.8 ^{a}	9.26	10.00	9.63 ^{a}	17.70	19.10	18.40 $^{a-c}$	50.05	56.50	53.28 $^{a-c}$
Sagittario	79.15	105.85	92.50 ^{b}	8.21	9.75	8.98 ^{ab}	17.80	18.10	17.95 ^{bc}	50.95	56.50	53.73 $^{a-c}$
Masaccio	86.70	92.50	89.60 ^{b}	7.94	8.15	8.05 ^{b}	15.85	17.65	16.75 ^{c}	39.85	53.45	46.65 ^{c}
Seri-82	85.10	89.35	87.23 ^{b}	9.56	8.65	9.11 ^{ab}	18.55	18.60	18.56 $^{a-c}$	55.15	53.55	54.35 $^{a-c}$
Ortalama	**84.86^{b}**	**96.87 ^{a}**	**90,86**	**9.15^{b}**	**9.64^{a}**	**9.4**	**18.19^{b}**	**19.61^{a}**	**18.9**	**52.17^{b}**	**60.27^{a}**	**56.22**
VK (%)	**5,72**			**7.56**			**5.86**			**12.52**		

$^{a, b, c}$; Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar *:p< 0.05 ;**:p< 0.01 önemlidir.
^{öd}; Önemli değildir.
BB: Bitki boyu, BU: Başak uzunluğu, BS: Başakçık sayısı, BTS: Başakta tane sayısı, BTA: Başakta tane ağırlığı, BinTA: Bin tane ağırlığı, TV: Tane verimi.

Tane verimi bakımından yıllar arasında (P< 0.01), genotipler ve yıl x genotip interaksiyonuna göre (P<0.05) önemli farklılıklar belirlenmiş; ortalama TV birinci yıl 508.42 kg/da, ikinci yılda 804.65 kg/da olmuş; genotipler arasında ortalama TV (591.29-754.56) kg/da arasında gerçekleşmiştir. Yıllar arasındaki bu farklılığın hem toplam yağış miktarından hem de yağışların dağılımlarının farklılık göstermesinden kaynaklandığı düşünülmektedir. 2020 yılı Mart ayındaki yağışların çok az olmasının tane verimini olumsuz etkilediği düşünülmektedir (Çizelge 1). ZDEB104 genotipinde (591.29 kg/da) ile bu değer en düşük, Masaccio çeşidinde (754.56 kg/da) ile en yüksek olarak bulunmuştur. Yürüttükleri çalışmalarında TV'lerini, Kurt ve Yağdı (2013), 305.3-447.9 kg/da, Yıldırım (2019), 404.66-776.30 kg/da ve Albayrak ve ark. (2020), 341.46-511.67 kg/da olarak bildiren araştırmacıların verileriyle çalışma bulgularımız paralellik göstermektedir.

Hektolitre ağırlığı bakımından, yıllar, genotipler ve yıl x genotip etkileşimi yönünden önemli (P<0.01) varyasyonun olduğu belirlenmiştir. Ortalama HL ağırlığı ilk yıl 76.52 kg/hl, ikinci yıl 75.81 kg/hl olarak saptanmış; genotipler arasında bu değer (73.78-77.73) kg/hl arasında değişmiş; en düşük değer (73.78 kg/hl) ile ZDEB104'den, en yüksek değer (77.73 kg/hl) ile ZDEB108'den elde edilmiştir. HL ağırlığı, üretim sezonu içerisindeki yağış ve sıcaklık gibi çevre faktörlerinden etkilenirken, tane dolumu, vegetasyon süresinin uzunluğu, tane iriliği ve şekli ile karın boşluğu ve buruşukluğu gibi tanenin yapısal özelliklerinden de etkilenmektedir (Aguirre ve ark., 2002). Ortalama HL ağırlığını, Kılıç ve ark. (2016), 76.5 kg/hl, Aktaş ve ark. (2017a), 81.36 kg/hl ve Mutlu ve Taş (2020) 76.25 kg/hl olarak bildirmiş olup, çalışmadan elde ettiğimiz araştırma bulgularıyla paralellik göstermektedir.

Protein oranı bakımdan, yıllar arasındaki ortalamalar önemsiz bulunurken, genotipler ve yıl x genotip interaksiyonu arasında varyasyonların önemli olduğu (P<0.01) saptanmıştır. Ortalama PO ilk yıl % 12.27 iken ikinci yıl % 12.30 olarak elde edilmiştir. Genotipler arasındaki PO % (11.15-14.00) arasında değişmiştir. Bu karakter açısından, ZDEB107 (% 11.15) en düşük, ZDEB103 (% 14.00) ise en yüksek PO'a sahip olmuştur. Bu oran, genetik ve çevresel birçok faktöre bağlı olarak değişmekte olup, çevresel faktörlerden yağış miktar ve dağılımı, toprak yapısı, sıcaklık ve hastalıklardan oldukça etkilendiği bildirilmektedir (Cornish ve ark., 2006). Ortalama PO'nını, Mut ve ark. (2017), % 12.8 ve Karaman (2020), % 13.7 olarak bildirmiş ve bulgularımızla uyum içerisindedir.

Çizelge 3. Ekmeklik buğday genotiplerinde BTA, BinTA ve TV özelliklerine ait ortalama değerler

Yıl x Genotip	Başakta Tane Ağırlığı *			Bin Tane Ağırlığı *			Tane Verimi *		
	2020	2021	Ortalama	2020	2021	Ortalama	2020	2021	Ortalama
ZDEB101	1.83	2.09	1.96 ^{ab}	42.49	36.01	39.25 ^{a}	589.20	701.13	645.16 $^{a-c}$
ZDEB102	1.91	2.31	2.11 ^{ab}	33.85	35.66	34.75 $^{a-c}$	506.94	755.98	631.46 $^{a-c}$
ZDEB103	1.70	1.86	1.78 ^{b}	28.23	30.84	29.53 ^{c}	441.91	807.23	624.57 $^{a-c}$
ZDEB104	1.70	2.38	2.04 ^{ab}	32.07	35.08	33.58 $^{a-c}$	499.48	683.10	591.29 ^{c}
ZDEB105	1.45	2.20	1.82 ^{ab}	30.46	33.90	32.18 ^{bc}	526.60	944.52	735.56 ^{ab}
ZDEB106	1.51	2.22	1.87 ^{ab}	32.82	32.70	32.76 ^{bc}	520.87	785.54	653.21 $^{a-c}$
ZDEB107	1.81	2.18	1.99 ^{ab}	33.53	34.84	34.18 $^{a-c}$	488.40	902.42	695.41 $^{a-c}$
ZDEB108	2.30	2.18	2.24 ^{a}	33.68	38.68	36.18 ^{ab}	445.28	792.60	618.94 ^{bc}
Ceyhan-99	1.74	1.98	1.86 ^{ab}	34.77	34.99	34.88 $^{a-c}$	557.33	788.88	673.10 $^{a-c}$
Sagittario	2.06	1.96	2.01 ^{ab}	40.55	34.76	37.65 ^{ab}	458.99	792.13	625.56 $^{a-c}$
Masaccio	1.57	1.86	1.71 ^{b}	39.50	34.71	37.10 ^{ab}	615.28	893.83	754.56 ^{a}
Seri-82	1.93	2.06	1.99 ^{ab}	35.08	38.46	36.77 ^{ab}	450.80	808.40	629.60 $^{a-c}$
Ortalama	**1.79^{b}**	**2.11^{a}**	**1.95**	**34.75^{b}**	**35.05^{a}**	**34.9**	**508.42^{b}**	**804.65^{a}**	**656.54**
VK (%)		**13.59**			**10.31**			**11.84**	

$^{a, b, c}$; Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar *:p< 0.05 ;**:p< 0.01 önemlidir. ^{öd}; Önemli değildir.
BTA: Başakta tane ağırlığı, BinTA: Bin tane ağırlığı, TV: Tane verimi.

Çizelge 4. Ekmeklik buğday genotiplerinde Hektolitre ağırlığı, Protein Oranı, Yaş Glüten ve Zeleny Sedimantasyon değerleri ortalamaları

Yıl x Genotip	Hektolitre Ağırlığı **			Protein Oranı **			Yaş Gluten Oranı **			Zeleny Sedimantasyon **		
	2020	2021	Ortalama	2020	2021	Ortalama	2020	2021	Ortalama	2020	2021	Ortalama
ZDEB101	78.58	75.15	76.86 ^{b}	12.65	12.88	12.76 ^{b}	25.28	47.73	36.50 ^{d}	44.15	25.98	35.06 ^{d}
ZDEB102	75.05	74.38	74.71 ^{d}	11.03	13.08	12.05 ^{cd}	21.43	53.98	37.70 ^{c}	36.50	26.28	31.39 ^{f}
ZDEB103	79.75	74.00	76.88 ^{b}	13.43	14.58	14.00 ^{a}	26.78	61.78	44.28 ^{a}	52.30	29.25	40.78 ^{a}
ZDEB104	72.50	75.05	73.78 ^{e}	12.50	12.98	12.74 ^{b}	25.00	45.35	35.18 ^{e}	48.40	23.45	35.93 ^{c}
ZDEB105	74.58	77.25	75.91 ^{c}	12.30	11.20	11.75 ^{d}	24.48	40.98	32.73 ^{g}	47.45	22.35	34.90 ^{d}
ZDEB106	75.15	74.98	75.06 ^{d}	11.83	11.58	11.70 ^{de}	23.15	40.48	31.81 ^{h}	42.03	22.08	32.05 ^{e}
ZDEB107	76.45	77.45	76.95 ^{b}	11.33	10.98	11.15 ^{f}	22.23	36.10	29.16 ^{ı}	39.78	21.25	30.51 ^{g}
ZDEB108	76.35	79.10	77.73 ^{a}	13.23	12.25	12.74 ^{b}	26.13	38.88	32.50 ^{g}	47.10	23.55	35.33 ^{d}
Ceyhan-99	78.38	73.45	75.91 ^{c}	11.68	10.68	11.18 ^{f}	23.28	28.73	26.00 ^{k}	38.88	20.18	29.53 ^{h}
Sagittario	77.58	77.18	77.38 ^{a}	11.73	10.98	11.35 ^{ef}	23.38	31.58	27.48 ^{j}	38.55	21.03	29.79 ^{h}
Masaccio	78.33	74.05	76.19 ^{c}	12.23	12.18	12.20 ^{c}	23.90	44.18	34.04 ^{f}	40.40	24.48	32.44 ^{e}
Seri-82	75.60	77.68	76.64 ^{b}	13.35	14.25	13.80 ^{a}	26.53	58.58	42.55 ^{b}	48.45	29.00	38.73 ^{b}
Ortalama	**76.52 ^{a}**	**75.81 ^{b}**	**76.17**	**12.27 ^{b}**	**12.30 ^{a}**	**12.28**	**24.29 ^{b}**	**44.03 ^{a}**	**34.16**	**43.67 ^{a}**	**24.07 ^{b}**	**33.87**
VK (%)		**4.71**			**1.69**			**1.09**			**1.16**	

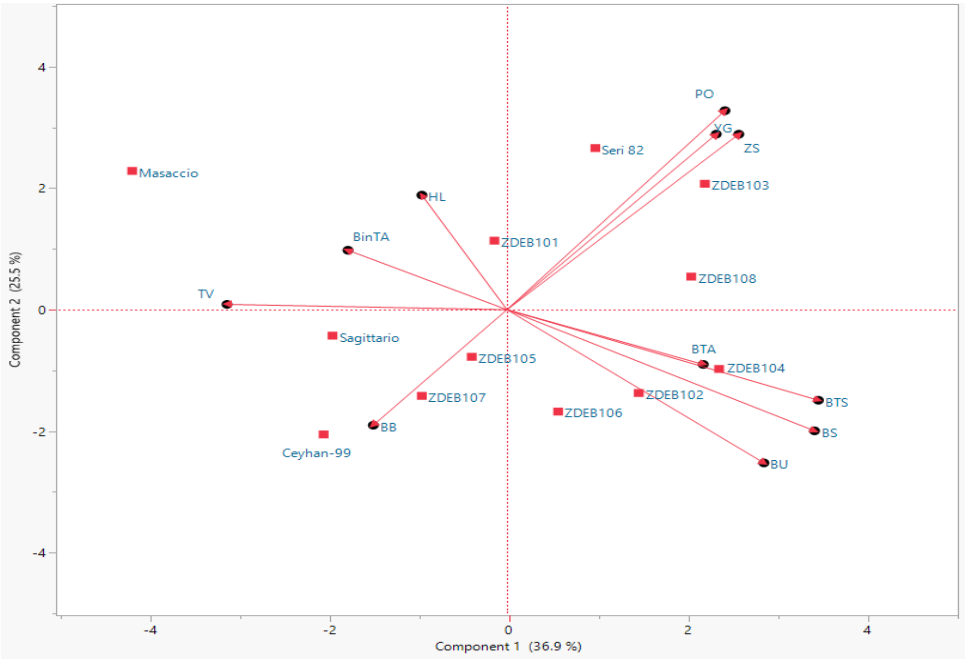
$^{a, b, c, d, f, g, h, ı, j, k}$; Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar *:p< 0.05 ;**:p< 0.01 önemlidir.
^{öd}; Önemli değildir.

Yaş gluten oranı, yıllar, genotipler ve yıl x genotip intereksiyonu arasında önemli bir varyasyon olduğu tespit edilmiştir. Ortalama YG oranı birinci yıl % 24.29, ikinci yıl ise % 44.03 olarak belirlenmiştir. Genotipler arasında YG oranı % 26.00-44.28 arasında değişiklik göstermiştir. Ceyhan-99 çeşidinde (% 26.00) en düşük olmuş; ZDEB103 genotipinde (% 44.28) en yüksek YG oranı belirlenmiştir. YG oranı yağış miktarı ve dağılımına ve gübreleme gibi faktörlerden önemli ölçüde etkilenmektedir (Şahin ve ark., 2004). Ortalama YG oranını, Egesel ve ark. (2009) % 33.8 ve Koç ve Akgün (2019), % 30.75 olarak bildirmiş ve bulgularımız ile paralellik göstermiştir.

Zeleny sedimantasyon miktarı bakımından, yıllar, genotipler ve yıl x genotip interaksiyonu arasında önemli farklılıklar tespit edilmiştir ($P<0.01$). Ortalama ZS miktarı birinci yıl 43.67 ml, ikinci yıl ise 24.07 ml olarak saptanmıştır. Genotipler arasında ZS miktarı (29.53-40.78) ml arasında değişiklik göstermiştir. Ceyhan-99 çeşidinden (29.53 ml) en düşük, ZDEB103 genotipinden (40.78 ml) en yüksek ZS miktarı elde edilmiştir. ZS miktarı gluten kalitesini gösteren, PO gibi diğer kalite özelliklerine nazaran çevresel ve yıllara ait değişikliklerden daha az etkilenen, yani kalıtım derecesi daha yüksek bir özelliktir (Şahin ve ark., 2004). ZS miktarını, Aktaş ve ark. (2017a), 41.79 ml ve Başaran ve ark. (2020), 24.5-36.0 ml olarak bildirmiştir.

3.2. Temel Bileşenler (PC) Biplot Analizi

Araştırmada incelenen bütün özelliklere ait ortalama veriler üzerinden yapılan PCA biplot analizi sonucunda, temel bileşen 1 (PC1) % 36.9, temel bileşen 2 (PC2) % 25.5, toplam olarak % 62.4 olarak saptanmıştır (Şekil 1).



Şekil 1. Denemede incelenen özellikler ile genotiplerin ilişkileri.

Analiz sonucuna göre TV ile BinTA, HL ve BB arasında pozitif bir ilişki, diğer özellikler (BU, BS, BTS, BTA, PO, YG ve ZS) ile negatif bir ilişki olduğu görülmüştür. PO ve diğer kalite özellikleri arasında pozitif bir korelasyon saptanmış; ayrıca, TV için Masaccio, PO, YG ve ZS için ZDEB103, HL için Sagittario ve BinTA için ZDEB101 öne çıkan genotipler olmuşlardır (Şekil 1). Aktaş ve ark. (2017b), yürüttükleri çalışmada tane verimi ile BinTA ve HL arasında, PO ile YG ve ZS arasında pozitif bir ilişki bildirmişlerdir.

4. Sonuç

Toplamda on iki ekmeklik buğday genotipi (ZDEB101, ZDEB102, ZDEB103, ZDEB104, ZDEB105, ZDEB106, ZDEB107, ZDEB108, Ceyhan-99, Sagittario, Masaccio ve Seri 82), Kahramanmaraş ilinin tarla koşullarında iki yıl süreyle 2020 ve 2021) incelenmiştir. Araştırmanın

sonuçlarına göre, TV yönünden Masaccio ve ZDEB105; PO bakımından ZDEB103 ve Seri 82 genotipleri öne çıkarken, yapılan temel bileşenler biplot analizinin sonucunda TV ile BinTA, Hl ve BB, PO ile HL, YG ve ZS arasında pozitif bir ilişki olduğu saptanmıştır. Buna göre, denemede kullanılan ve ümitvar oldukları önceden belirlenen sekiz adet ileri ekmeklik buğday hatlarından ıslah çalışmalarında yararlanılabileceği, ayrıca belirlenen tarımsal ve kalite özelliklerinden de yararlanılarak, daha çok sayıdaki lokasyonda ve uzun yıllar sürdürülüp, toprak analizi sonuçlarıyla desteklenmiş bilimsel çalışmaların ivedilikle yapılması gerektiği anlaşılmıştır.

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Research Article

The Effect of *Clonostachys rosea* (sch.) Schroers and Samuels Against Verticillium wilt (*Verticillium dahliae* Kleb.) and Early Blight [*Alternaria solani* (Ell. and G. Martin) Sor.] Diseases in Tomato Plants

Rojbin ÇEVİK[1], Semra DEMİR[*2], Şahimerdan TÜRKÖLMEZ[3], Gökhan BOYNO[4]

[1,2,4]Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Turkey
[3]GAP Agricultural Research Institute, Şanlıurfa, Turkey

[1]https://orcid.org/0000-0003-3064-8345, [2]https://orcid.org/0000-0002-0177-7677, [3]https://orcid.org/0000-0001-8775-5470
[4]https://orcid.org/0000-0003-3195-0749

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

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Abstract: The effectiveness of *Clonostachys rosea* against Verticillium wilt (*Verticillium dahliae*) and early blight (*Alternaria solani*) diseases, as the two most important problems in tomato cultivation with significant economic losses, was determined. It was determined that *C. rosea* was effective on *A. solani* and *V. dahliae* and suppressed mycelial growth. Also, the *C. rosea* on wheat grains inoculated to plants at 20 g, 30 g, and 40 g concentrations before and after pathogens inoculation. Then, fungal discs (2 mm in diameter) from *V. dahliae* growing colonies were inoculated on the host plant root zone. *A. solani* was also inoculated ($1x10^6$ conidia ml^{-1}) by spraying the foliar parts of the plants. Results showed that *V. dahliae* caused 76.0% disease severity in control plants, while the disease severity indices were 58.3%, 55.3%, and 25.3% at 20 g, 30 g, and 40 g *C. rosea* application, respectively. In *A. solani* x *C. rosea* treatments, the disease severities were determined as 96.6%, 63.3%, 43.6% and 46.6% in control, 20 g, 30 g, and 40 g application of *C. rosea*, respectively. The pathogen suppression rates by *C. rosea* at 30g application dose was 54.8% against *A. solani* and at 40 g application dose was 66.6% against *V. dahliae*. The effects of *C. rosea* on plant growth parameters were also determined. Results showed that *C. rosea* had a positive effect on the morphological parameters in tomato plants.

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1. Introduction

Tomato (*Solanum lycopersicum* L., Solanaceae), with a cheap and plentiful source of vitamins, is one of the most widely grown vegetables worldwide. The global tomato production is over 187 million tons (FAO, 2020). China ranked first in production (64.8 million tons), followed by India (20.6 million tons) and Turkey (13.2 million tons) (FAO, 2020). It is suffered from different plant pathogens, especially fungal ones that cause significant economic losses (Yaviç et al., 2020 Gül, 2021). Among them, *Alterneria solani* (Ellis and Martin) Sorauer causes "early blight" disease, and *Verticillium dahliae* causes Wilt" disease in tomato fields' worldwide (Jones et al., 1991; Yiğit, 1993; Shinde et al., 2018).

Alternaria solani causes early blight of tomato, which is a serious problem in warm, humid climates and semiarid locations with frequent and prolonged night dew. Early blight (EB) decreases photosynthetic area and can defoliate plants under suitable environmental conditions. It causes yield losses if no preventive measures are taken during the leaf blight stage, which is the most crucial stage in the disease's growth (Foolad et al., 2000; Koike et al., 2010). *V. dahliae*, a soil-borne fungus, is responsible for more than half of all crop losses. The disease affects the quality and quantity and can even kill the whole plant (Bletsos et al., 2003; Coşkun et al., 2021).

Regarding control strategies, chemical treatment is often suggested for *A. solani*, while for *V. dahliae*, resistant cultivars or other control measures were recommended (Demir et al., 2015; Shaban et al., 2018). The negative effects of using chemicals on different economic, social, and ecological aspects have increased using of alternative methods especially biological control techniques (Bora, 2002). Using of biological control agents on different plant diseases, including *A. solani* and *V. dahliae* has been reported in several studies (Boyno et al., 2020; Benouzza et al., 2021; Poveda and Baptista, 2021), which indicated the great importance of this technique (Naik et al., 2020; Karthika et al., 2020; Boyno et al., 2022).

Clonostachys rosea (formerly *Gliocladium roseum)*, was first described by Bainier (1907). However, Schroers et al. (1999) found that the morphology, ecology, teleomorph, and DNA sequence data of *G. roseum* were quite different from other *Gliocladium* species, so reclassified *G. roseum* as *C. rosea*. This species has been used as a biological agent against several pathogens such as *Alternaria dauci*, *A. radicina, Botrytis cinerea, B. aclada, Bipolaris sorokiniana, Drechslera teres, F. graminearum, F. verticillioides, F. croohvellense, F. culmorum, H. solani, Moniliophthora roreri, Phytophthora palmivora, Rhizoctonia solani, Rhynchosporium commune* and *S. sclerotiorum* (Krauss and Soberanis, 2001; Jensen et al., 2004; Yohalem et al., 2004; Aydın and Turhan, 2009; Kosawang et al., 2014; Schöneberg et al., 2015; Sun et al., 2015; Jensen et al. et al., 2016; Lysøe et al., 2017; Samsudin et al., 2017). The mechanisms used by *C. rosea*'s proposed to be releasing of cell wall degrading enzymes (CWDE), producing secondary metabolites, including antibiotics and toxins, as well as inducing plant resistance (Chatterton and Punja 2009; Fatema et al., 2018). The study on the effectiveness of *C. rosea* against *A. solani* and *V. dahliae* as well as its efficacy on plant growth parameters were the main objectives of this study.

2. Material and Methods

The tomato variety FDR 8516 (Seminis Tohum-Monsanto) was used in this study as the plant material. *A. solani* EAb1 (As) (Boyno, 2019) and *V. dahliae* Vd11 (Vd) (Erdoğan et al., 2014) as the pathogen isolates were provided by Mycology laboratory's culture collection, Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, and their pathogenicity had been verified by previous investigations. The biological control agent *C. rosea* MF536537 (Cr) was also obtained from the Şanlıurfa-GAP Agricultural Research Institute.

2.1. Effect of *C. rosea* against *A. solani* and *V. dahliae* at *in vitro* conditions

The inhibition rates of *C. rosea* against *A. solani* and *V. dahliae* were studied *in vitro.* The study was carried out using dual culture technique with 10 replicates. A mycelial disc (5 mm in diameter) from the margin of the one-week old *C. rosea* and pathogens (*A. solani* and *V. dahliae*) colonies were cultured at the opposite sides of the PDA plates at an equal distance (6 cm) (Figure 1). The plates with only fungal pathogen cultures were used as the controls. Then, plates were incubated at 24±2^{0}C. The fungal colonies were measured on 7th, 9th, 14th, and 21st days. The growth inhibition rate of *A. solani* and *V. dahliae* colonies were determined using the formula proposed by Royse and Ries, 1978 (Equation 1):

$$RI = [(r1 - r2) \div r1]x100 \quad (1)$$

Where RI: Inhibition Rate (%), r1: Fungal pathogen colony diamatere, r2: Fungal pathogen colony diametere in the direction of the biological control agent (Fig. 1).

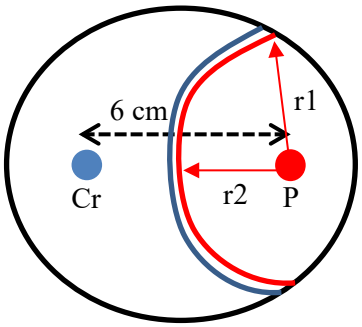

Figure 1. Schematic representation of the dual culture method. Cr: *C. rosea,* P: Pathogens (*A. solani* or *V. dahlia*), r1: Colony radius of the pathogen, r2: Growth radius of the pathogen in the direction of the biological control agent.

2.2. Preparation of *C. rosea* stock culture

C. rosea was first cultured in PDA plates Then, the sterilized wheat seeds (3 kg) were inoculated by a 5 mm diameter mycelial discs of *C. rosea* cultures. For stimulation of fungal growth, 50 g glucose was dissolved in 300 ml of sterile water and poured into the storage containers, and kept at 25 °C until the fungus covered the wheat seeds. The *C. rosea* fungus was then inoculated in plants before and after the pathogens inoculation with different doses (20, 30 and 40 g).

2.3. Effects of *C. rosea* against *A. solani* and *V. dahliae* at *in vivo* conditions

The study was carried out in a growth chamber (for weeks at 24±2 ^{0}C, 60-70% RH, 16 hours light, and 8 hours dark). The mixture of Peat: perlite (1:1) and vermiculite was used as the seedling growing medium. The growing seedlings were transplanted after 2 weeks (observing the emergence of the first leaves) into pots containing 3 kg of sterilized soils and different concentrations of *C. rosea* Then, 5 days later, Pathogens inoculation was carried out. For this purpose, a 2 mm disc of *V. dahliae* colony was inoculated in the root zone of each plant. The *A. solani* was also inoculated to the foliar parts of the plants (50 ml spore suspension containing $1x10^{6}$ conidia ml^{-1}) by hand sprayer. Ten days after the pathogens' inoculation, again, the *C. rosea* was applied, similar to the first inoculation. The study was carried out in a completely randomized design (CRD) experiment with 10 replicates for 9-weeks up to host plants fully developed.

2.3.1. Disease severity index assessment

Disease symptoms were examined 3th, 4th, 5th and 6th weeks after both pathogens' inoculation. For measuring the disease severity index in *V. dahliae*, a 0-4 scale was used for wilting symptoms in leaves (Zeise and Tiedemann, 2002) as well as a 0-3 scale was used for symptoms in stem (Erwin et al., 1976). For *A. solani* disease severity index, a 0-4 scale was used (Devanathan and Ramanujam, 1995). The measured scale values were then converted to disease severity (DS) by using the Townsend Heuberger formula (Townsend, 1943) as follows:

$$DS\ (\%) = [\sum(S \times L) \div (M \times Smax)] \times 100 \qquad (2)$$

Where: S = scale value, L = the number of plants evaluated in the scale (number of leaves for *A. solani*), M = the total number of the plant (number of total leaves for *A. solani*), and Smax = the highest scale value.

2.3.2. Plant growth parameters Assessment

The experiment was finished after nine weeks, and some growth parameters of the plants, including shoot dry and fresh weight (g), root dry and fresh weight (g), total plant length (cm), and stem diameter (mm), were also measured. The seedlings were cut from the root collar for the shoot and fresh root weights, and the upper parts were weighed directly after washing with tap water. Then were dried

at 70°C for 48 hours for measuring dry weights. The stem diameter was also determined with a digital calliper (Insize-1112-150, Germany). The total plant height was recorded by measuring with a ruler.

2.4. Statistic analyses

The recorded data were statistically analyzed using IBM SPSS v21 (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) program (SPSS, 2012). The mean values were also compared using the DUNCAN multiple range test at 5%. The data values measured based on studied times were also converted into graphs using the Microsoft Excel program, and the means were calculated.

3. Results and Discussion

3.1. The Effect of *C. rosea* against *A. solani* and *V. dahliae in vitro* conditions

The *C. rosea* inhibited the growth of *A. solani* and *V. dahliae* fungal pathogens on 7th, 9th, 14th, and 21st days compared to controls (Cr, As, and Vd). Also, in Vd+Cr treatment, the inhibition rates were 34.05% and 25.48% on the 9th and 14th days of postinoculation, respectively (Figure 2).

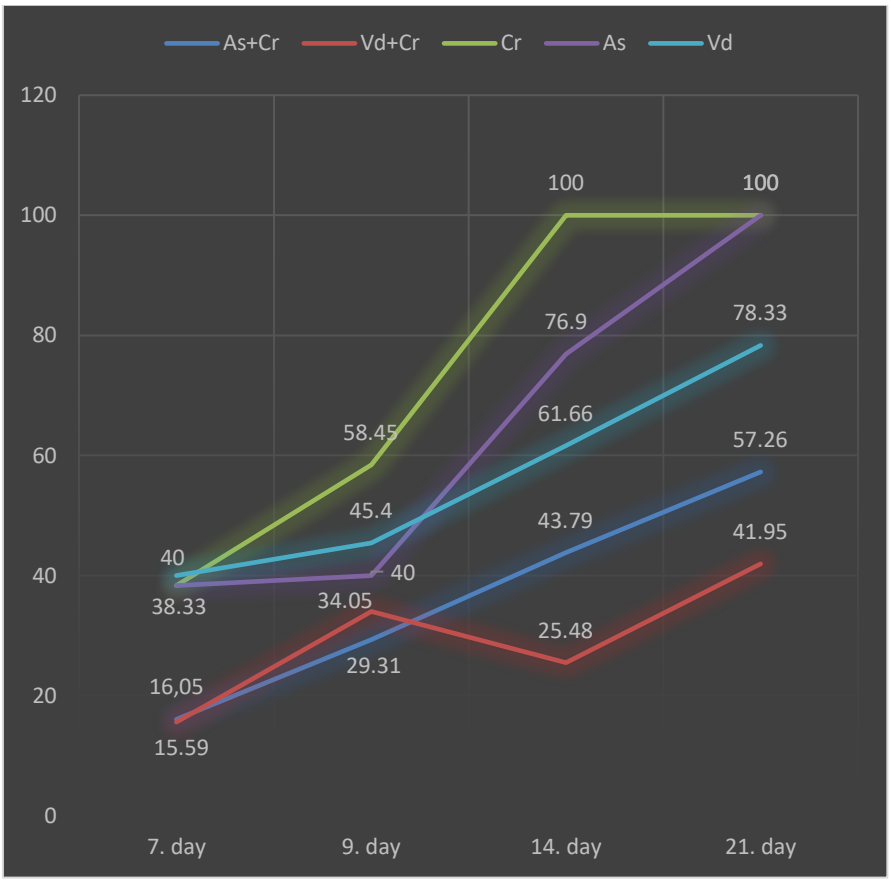

Figure 2. The growth inhibition of *A. solani* (As) and *V. dahliae* (Vd) fungal pathogens by *C. rosea* (Cr).

Generally, Cr decreased fungal pathogen growth (As+Cr and Vd+Cr) compared to control treatments (Cr, As, Vd). *C. rosea* is a highly potential mycoparasitic fungus with biological control ability against several plant pathogenic fungi (Roberti et al., 2008; Nygren et al., 2018; Sun et al., 2020). However, there are no reports on the effect of *C. rosea* against the *V. dahliae in vitro.* In a study conducted by Flores et al. (2015) under *in vitro* conditions, it was reported that *C. rosea* had a significant antagonistic effect on *Fusarium oxysporum*, *A. solani,* and *Botrytis cinerea*.

3.2. The Effect of C. rosea on the A. solani-infected plants growth parameters

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the growth parameters of *A. solani*-infected plants were statistically significant ($p<0.05$). The As treatment decreased significantly the total plant height (12.34 cm) compared to the NK treatment (26 cm). The other plant growth parameters decreased compared to control treatments but not significantly (Table 1). *A. solani is* reported to cause damages on plants in all growth stages (Faheed et al., 2005). Furthermore, it has been found that this fungus affects plant growth as well as host plant yield by decreasing photosynthesis and pigment contents(Agamy et al., 2013). Results showed that different doses of Cr, especially Cr_{40} had a significant increase on all plant's growth parameters in both NK and As treatments. Also, Cr_{40}, Cr_{40}+As, and Cr_{30} treatments significantly improved the stem diameters (1.23 cm, 1.01 cm, 1.00 cm, respectively). All doses of *C. rosea* (20 g, 30 g, and 40 g) were also promoted plant growth in As-infected plants. The highest amounts of total plant height (68.42 cm), shoot dry weights (48.53 g), and root dry weights (4.94 g) could be observed in Cr_{40}+ AS treatment (Table 1).

Using of *C. rosea* increased the growth parameters of both infected and uninfected plants with *A. solani* (Table 1). Generally, it is reported that biological control agents promote plant growth parameters (Murphy et al., 2003; Harman, 2006; Woo et al., 2006). Goh et al. (2020) reported that the leaf area, stem diameter, and plant height were significantly increased 5 months after inoculation by *C. rosea* compared to the controls. It has also been reported that some biological control agents increase the growth parameters of plants infected with *A. solani* (Fritz et al., 2006; Chowdappa et al., 2013; Boyno et al., 2020; 2022). Lahlali and Peng (2014) was also determined that *C. rosea* stimulates the growth of pathogen-infected plants and induced plant resistance

Table 1. The effects of *C. rosea* on the morphological growth parameters of the plants infected with *A. solani*

Treatments	Plant total length (cm)	Stem diameter (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
NC*	26.00±3.46^{d}**	0.46±0.05^{de}	6.07±1.99^{e}	2.59±0.52^{e}	1.12±0.11^{f}	0.13±0.06^{d}
As	12.34±1.52^{e}	0.27±0.01^{e}	2.88±0.13^{e}	0.15±0.03^{e}	0.93±0.25^{f}	0.06±0.04^{d}
Cr_{20}	66.33±3.21^{b}	0.86±0.05^{bc}	186.41±6.70^{b}	47.12±3.98^{b}	42.35±2.00^{b}	5.47±0.48^{ab}
Cr_{30}	65.01±5.00^{b}	1.00±0.30^{ab}	191.39±14.50^{b}	41.29±2.72^{c}	40.46±3.28^{bc}	3.19±0.27^{c}
Cr_{40}	80.66±9.01^{a}	1.23±0.20^{a}	212.49±7.73^{a}	58.77±3.83^{a}	48.83±3.66^{a}	5.78±0.33^{a}
Cr_{20}+As	48.66±3.51^{c}	0.60±0.17^{cd}	145.41±12.86^{d}	34.01±4.01^{d}	32.27±2.13^{e}	3.32±0.60^{c}
Cr_{30}+As	54.33±4.04^{c}	0.73±0.05^{bcd}	161.16±7.19^{c}	36.99±2.95^{cd}	35.20±1.36^{de}	3.72±0.26^{c}
Cr_{40}+As	68.42±3.64^{b}	1.01±0.11^{ab}	164.30±6.12^{c}	48.53±3.44^{b}	37.51±2.29^{cd}	4.94±0.47^{b}

*NC: Negative Control, As: *Alternaria solani*, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

3.3. The effect of *C. rosea* on the *V. dahliae*-infected plants growth parameters

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the growth parameters of the plants infected with *V. dahliae* were found to be statistically significant ($p<0.05$). Results showed that Vd treatment reduces (not significantly) all growth parameters compared to NC treatment except for root fresh (not significantly) and dry weight (significantly), which increased (Table 2). It is found that the hyphae of *V. dahliae* colonize the internal tissues and spread systemically in the plant (Robb, 2007). It has also been demonstrated that vascular wilt is generally observed in plants susceptible to the disease (Veronese et al., 2003), which decreases plant height and fresh/dry weight values (Veronese et al., 2003; Robb, 2007; Demir et al., 2015).

Results showed that all Cr treatments significantly increased all the plant growth parameters except of stem diameter and root dry weight (Table 2). There are significant increases in stem diameter parameters among the Cr_{20} (0.86 cm), Cr_{30} (1.00 cm), Cr_{40} (1.23 cm), and Cr_{40}+Vd (0.73 cm) treatments with NC and Vd treatments. While the Cr_{20} (5.47 g), Cr_{40} (5.78 g), and Cr_{40}+Vd (4.09) treatments increased root dry weight significantly more than Vd (2.60 g), all other treatments increased this parameter significantly compared to NC. Also, it was found that Cr_{40} treatment had the highest values of all growth parameters. All doses of *C. rosea* (20 g, 30 g, and 40 g) enhanced total plant height, shoot

fresh/dry weight, and root fresh weight in Vd-infected plants; however, only Cr_{40}+Vd treatment significantly increased stem diameter and root dry weight parameters (Table 2).

C. rosea was observed to promote the growth parameters of plants infected or uninfected with *V. dahliae* (Table 2). The previous studies showed that *C. rosea* promoted plant growth parameters infected by different pathogens (Lahlali and Peng, 2014; Goh et al., 2020). Although there is no information regarding the effect of *C. rosea* on plants infected with *V. Dahliae,* it has been reported that different biological control agents may increase plant growth parameters infected by *V. dahliae* (Demir et al., 2015; Gómez-Lama Cabanás et al., 2018).

Table 2. The effects of *C. rosea* on the morphological growth parameters of the plant infected with *V. dahliae*

Treatments	Plant total length (cm)	Stem diameter (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
NC*	26.00±3.46^{e}**	0.46±0.05^{e}	30.07±1.99^{e}	12.59±0.52^{f}	1.12±0.11^{e}	0.13±0.06^{e}
Vd	20.34±1.02^{e}	0.40±0.10^{e}	28.12±3.36^{e}	10.27±2.37^{f}	3.69±0.51^{e}	2.60±0.52^{cd}
Cr_{20}	66.33±3.21^{b}	0.86±0.05^{bc}	186.41±6.70^{b}	47.12±3.98^{b}	42.35±2.00^{b}	5.47±0.48^{a}
Cr_{30}	65.01±5.00^{b}	1.00±0.30^{ab}	191.39±14.50^{b}	41.29±2.72^{c}	40.46±3.28^{b}	3.19±0.27^{c}
Cr_{40}	80.66±9.01^{a}	1.23±0.20^{a}	212.49±7.73^{a}	58.77±3.83^{a}	48.83±3.66^{a}	5.78±0.33^{a}
Cr_{20}+Vd	34.33±3.52^{d}	0.47±0.05^{e}	102.30±11.47^{d}	24.36±2.94^{e}	22.73±2.81^{d}	2.34±0.48^{d}
Cr_{30}+Vd	45.45±4.32^{c}	0.56±0.05^{de}	123.03±9.09^{c}	30.82±2.42^{d}	26.57±2.04^{cd}	2.80±0.26^{cd}
Cr_{40}+Vd	50.57±1.49^{c}	0.73±0.06^{cd}	112.42±9.23^{cd}	39.49±0.65^{c}	29.85±0.51^{c}	4.09±0.16^{b}

*NC: Negative Control, Vd: *Verticillium dahliae*, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

3.4. Effect of *C. rosea* on disease severity index

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the disease severity index were significant ($p<0.05$). The positive control treatments (PC) in both fungal pathogens were found to have the maximum disease severity rates (Table 3).

A. solani causes serious diseases in tomatoes if no control measure is made (Grigolli et al., 2011; Gannibal et al., 2014; Shinde et al., 2018). The Cr_{30} and Cr_{40} treatments decreased significantly the disease severity index (43.66% and 46.67%, respectively) rather than *A. solani* positive control. Also, the Cr_{20} treatment was effective against the disease compared to the control treatment, with a disease severity rate of 63.33% and a suppression rate of 34.72% rather than *A. solani* positive control treatment. All doses of *C. rosea* (20 g, 30 g, and 40 g) were significantly effective on *A. solani* (Table 3).

Table 3. The effect of *C. rosea* on disease severity and suppression rates of *A. solani*

Treatments	*A. solani*		*V. dahliae*	
	Disease severity (%)	Suppression rates (%)	Disease severity (%).	Suppression rates (%)
PC*	96.67±3.05^{c}**	-	76.00±5.29^{c}	-
Cr_{20}	63.33±4.63^{b}	34.72	58.33±10.4^{0b}	23.25
Cr_{30}	43.66±11.15^{a}	54.83	55.34±5.50^{b}	27.18
Cr_{40}	46.67±15.27^{ab}	51.72	25.33±6.11^{a}	66.67

*PC: Positive Control, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

It was reported that the *V. dahliae* infected tomato plants had a 91.25% disease severity index after 12 weeks (Ait-Rahou et al., 2020). This vascular pathogen with a wide host range was distributed worldwide and could be able to survive in the soil for several years (Acharya et al., 2020). In this study, the most effective treatment against *V. dahliae* was Cr_{40}, with 25.33% disease severity index and 66.67% suppression rate. However, the Cr_{20} and Cr_{30} treatments were also effective, with 58.33% and 55.34% disease severity indices and 23.25% and 27.18% suppression rates, respectively. On the other hand, it

was found that all doses of *C. rosea* (20 g, 30 g, and 40 g) were significantly effective on *V. dahliae* (Table 3).

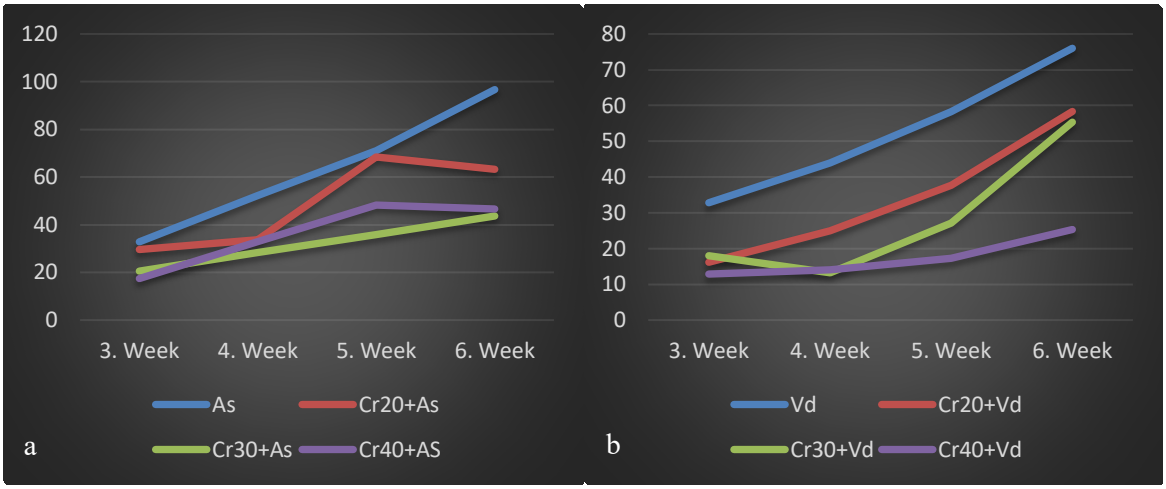

Figure 3. The effect of *C. rosea* (Cr) on disease severity indices of the plants infected with (a) *A. solani* (As) and (b) *V. dahliae* (Vd) during the times.

The disease severity indices were at high rates in As inoculated plants at different times (3rd, 4th, 5th, and 6th) (Figure 3a). Boyno et al. (2022) showed that the disease severity index exceeded 50% and reached the highest level five weeks after inoculation by *A. solani.* All doses of *C. rosea* (20 g, 30 g, and 40 g) were observed to reduce disease severity indices compared to As treatment. The Cr_{40}+As treatment was the most effective treatment against the disease at all times (Figure 3a). It has been observed that *C. rosea* has an endophytic nature in plant tissues, colonizes plants quickly, and works against a variety of fungal plant pathogens (Jensen et al., 2004; Sun et al., 2020; Silva et al., 2021). During the recognition of the biocontrol agent by the host plant, several defense mechanisms such as different hormone and enzyme activities were activated and reached the maximum level during this period (Azcón-Aguilar and Barea, 1996; Morandi, 1996). Although the mechanisms and their activation are unknown, it is proposed that lignification, formation of hydroxyproline-rich cell walls, hypersensitive reactions, antifungal enzyme production, and activated physical barriers, which occur as a result of localized rapid biochemical defense mechanisms, are effective (Demir, 2005). Silva et al. (2021) reported that *Clonostachys* species significantly reduced the disease severity index of *A. solani* in potatoes. Also, it was reported that *C. rosea* suppresses disease by activatingthe defense mechanisms of plants against many fungal plant pathogens such as *A. dauci, A. radicina,* and *Botrytis cinerea*, as well as *A. solani* (Jensen et al., 2004; Sun et al., 2020).

Vd inoculated plants had the highest levels of a disease severity index in all studied times (3rd, 4th, 5th, and 6th weeks). All doses of *C. rosea* (20 g, 30 g, and 40 g) reduced disease severity rates compared to Vd positive control treatment. Also, Cr_{40}+Vd was the most effective treatment against the disease in different studied times (Figure 3b). The biological control agents are quite effective against pathogens (Amin et al., 2010; Tapwal et al., 2011; Sun et al., 2018; Boyno et al., 2020). The *C. rosea* has been reported to be effective against several soil-borne pathogens, especially *Fusarium graminearum, F. verticillioides, F. crookwellense, F. culmorum, Phytophthora palmivora, Rhizoctonia solani,* and *Sclerotinia sclerotiorum* (Krauss and Soberanis, 2001; Yohalem et al., 2004; Kosawang et al., 2014; Schoneberg et al., 2015; Lysøe et al., 2017; Samsudin et al., 2017). Although there is no study on the effect of *C. rosea* on *V. dahliae*, it was found that it inhibited the germination of pathogen microsclerotia in the soil (Keinath et al., 1991; Varo et al., 2016). In addition, it is supposed that *C. rosea* inhibits mycelial growth and microsclerotia formation with its non-volatile secondary metabolites (Rodriguez et al., 2011).

4. Conclusion

In conclusion, it was observed that *C. rosea* could be an effective biological control agent due to its positive effects on both the disease severity index as well as promoting plant growth. Therefore, this biological control agent could be used as an alternative Plant Growth Regulator. However, it is supposed that further studies are needed to fully elucidate its mechanisms.

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Research Article

Assessment of Some Selected Cultivars of Almond on GF677 Rootstock in Drought Stress Conditions

Saeed PIRI[*1], Shahram SEDAGHATHOOR[2]

[1,] Agriculture Faculty, Department of Horticulture, Abhar Branch, Islamic Azad University, Abhar, Iran
[2] Agriculture Faculty, Department of Horticulture, Rasht Branch, Islamic Azad University, Rasht, Iran

[1]https://orcid.org/ 0000-0002-9314-39022, [2]https://orcid.org/ 0000-0002-2438-2299

*Corresponding author e-mail: sedaghathoor@yahoo.com

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Water stress

Abstract: Drought stress is the most important factor limiting the successful production of agricultural crops worldwide. The response of some almond cultivars grafted on GF677 rootstock was studied to drought stress at a research greenhouse in the Horticultural Science Research Institute of Karaj, Iran, in the 2016-2017 growing season. The plant materials included 12 almond (*Prunus dulcis* Mill.) genotypes/cultivars, including 'Sh10', 'Saba', 'A1-16', 'Shokofeh', 'Kh1', 'A230', 'Mamaie', 'A13-40', 'A9-7', 'A8-24', 'Fragiolu', and 'Sh17', which were grafted onto Gf677 rootstock. The results showed that the treated almonds differed significantly. When all studied traits are considered, it can be concluded that genotypes 'Kh1' and 'A13-40' outperformed all other genotypes and cultivars in terms of the studied morphological and physiological traits and exhibited far more tolerance to drought stress. In normal conditions, cultivars Kh1 and Mamaei had maximum proline (2.35 µmole/g), but A8-24 still showed the lowest content of proline (1.20 µmole/g). The highest K content under drought stress was obtained in A1-16 (2.8 %) and Mamaei (2.71 %) cultivars.

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1. Introduction

Almond (*Prunus dulcis* Mill.) is one of the oldest nuts with a high production rate in the world. The crop plays a significant role in the agricultural economy of the arid and semi-arid regions (Rouhi et al., 2007; Egea et al., 2009). On the other hand, drought is the most limiting factor of viable crop production in the world. It happens when a mix of physical and environmental factors creates stress inside plants and impairs their production. This impairment results from delayed or no plant establishment, debilitation or killing of the established plants, plant exposure to diseases and pests, and physiological and biochemical changes in plant metabolism (Scholz et al., 2008).

Although almond is a drought-resistant crop, it requires irrigation over the growing season to guarantee their economic crop production (Romero et al., 2004). The loss of water potential of almonds induced by water stress entails the reduction of tree growth, high leaf shedding, lower kernel weight, and the change in pericarp color. In addition, stomatal conductance and carbon metabolism are reduced (Isaakidis et al., 2004). In a study, rootstocks were exposed to daily irrigation treatments up to field capacity and drought stress for five days in July and August. The results showed that the GF677

rootstock was the most adapted to different soil moistures. This rootstock could maintain water availability for photosynthesis even in the extended droughts by gradual stomatal closure (Yadollahi et al., 2011).

A study of the response of some *Prunus* species to drought stress indicated that specific leaf area could be a morphological indicator for the assessment of drought resistance of the species. Also, the ratio of root dry weight to leaf area and the ratio of root length to leaf area exhibited high correlations with drought resistance (Rieger et al., 2003). Genotypes vary in their drought resistance. It has been documented that Masbovera is more appropriate for rain-fed farming (De Herralde et al., 2001). De Herralde et al. (2003) reported that the cultivars 'Nonpareil', 'Marcona', and 'Giarrigues' had a strong drought resistance trait.

Genotypes and ecotypes of almonds that show more root development are more tolerant of water stresses. The roots of *Amygdalus scoparia* ecotypes are not influenced by water stress extensively when compared to cultivated almonds, but when the seedlings of *Prunus dulcis* genotypes are irrigated adequately, their roots show more extension than the seedlings of *A. scoparia* (Sardabi et al., 2003). According to Camposeo et al. (2011), environmental conditions, especially temperature, and seasonal variations, can change leaf development and stomatal characteristics. They focused on the effect of water reduction on stomatal and leaf parameters in cultivars of almond and wild almonds (*Amygdalus webbii*) in field conditions. The results revealed broad differences in leaf area between the wild and cultivated almonds. The wild species lost its leaf area to a greater extent compared to cultivated species (% 31 vs. % 14). Almond cultivars have shown diverse responses in their leaf anatomic and physiological traits to water stresses. Some cultivars like 'Masbovera' have highly sensitive stomatal aperture whilst some varieties like 'Guara' produce thick cuticles. Accordingly, the leaf area is a genetic trait that can tolerate slight changes in environmental conditions (Gispert et al., 2011).

Zamani et al. (2002) studied the response of some almond seedlings selected from Iranian almond populations to drought stress. The results showed that the leaf area, stem length, root dry weight, and leaf water potential were lost with an increase in the irrigation interval, whereas proline content and stomatal resistance were increased. At lower levels of drought stress, drought adaptation was observed with a decrease in stomatal resistance after an initial increase. Leaf area was also decreased under the drought stress, and this reduced net photosynthesis. Likewise, the drought stress adversely influenced mesophyll conductance which is a non-stomatal factor underpinning the photosynthesis rate. Furthermore, chlorophyll a and b contents exhibited a decline in the stressful plants. However, these changes were a function of the cultivar (Javadi et al., 2006). The present study aimed to explore the tolerance of different cultivars of almonds to drought stress using physiological and morphological indicators in order to estimate the drought tolerance of resistant cultivars.

2. Material and Methods

2.1. Experimental site and plant material

The study was carried out in the Horticulture Research Station of Karaj, Iran, in 2016 on 12 almond cultivars and genotypes based on their main morphological and physiological traits related to drought resistance. The almond cultivars were obtained at the experimental orchard of the Plant and Seed Research Organization (PSRO) in Kamalabad of Karaj (Iran). The trees were assessed in drought stress and normal conditions. The cultivars were subject to the measurement of all vegetative traits, including growth and physiological parameters, so that data analysis could reveal the best combination of scion- rootstock and tolerant cultivar. After the planting of the trees in pots and their exposure to drought stress, the following morphological and physiological traits that had been shown to have close relationships with drought resistance/tolerance were examined.

2.2. Growth parameters

Growth was measured with a caliper. Also, the relative water content (RWC) of the leaves was assessed. To measure leaf RWC, four full leaves were detached from the upper part of the branch and four from the lower part. Then, after their fresh weight (FW) was recorded, they were placed in distilled water in the darkness at 4°C for 24 hours to swell. Then, they were taken out of the distilled water and were drained, and their turgidity weight (TW) was recorded. Then, the samples were oven-dried at

105°C for 24 hours to find out their dry weight (DW). Leaf RWC was calculated by the following equation:

RWC (%) = (FW- DW/ TW- DW) ×100

2.3. Proline measurement

First, 0.5 g of fresh plant material was crushed in a mortar, poured into 15-mL tubes, added with 10 mL of sulfosalicylic acid 3%, and placed in an ice-water solution for 10 minutes. Then, the tubes were centrifuged at 15000 rpm at 4°C for 10 minutes. After that, 2 mL of the supernatant was poured into 15-mL tubes and was mixed with 2 mL of ninhydrin acid and 2 mL of pure acetic acid. At the same time, 2 mL of standard 0, 4, 8, 12, 16, and 20 mg L^{-1} proline was added to new tubes and was well mixed with 2 mL of ninhydrin acid and 2 mL of glacier acetic acid. The main and standard samples were first placed in a hot water bath at 100°C for 1 hour and then in an iced water bath for 10 minutes to cool down and have all the reactions stop. Next, 4 mL of toluene was added to the solution and was mixed with a vortex for 20 seconds. The absorption of the samples was read at 528 nm with a spectrophotometer (BT600 Plus, Canada). Finally, proline content (in µmol g^{-1} FW) was found by the absorption rate of the samples and its comparison with a standard curve according to the following equation ((Bates et al., 1973):

µmole proline/g FW = (µg proline/ml × ml toluene) /[115.5µg/µmole) / (g sample/5])

2.4. K^+ measurement

To determine leaf K content, a sample of 15-20 leaves was taken from the branches grown from the middle part of the stems for leaf analysis in the plants exposed to drought. After they were washed and dried, they were placed at 70°C for 48 hours. Then, they were prepared for digestion by fresh oxidation method using sulfuric acid 96%, salicylic acid, hydrogen peroxide, and selenium (Jaiswal, 2014). An amount of 0.3 g of the plant sample was poured into a digestion tube and was mixed and shaken with 2.5 mL of the acid mixture so that all particles were soaked. After two hours, the digestion tubes were placed on a heater at 100°C for 2 hours. Then, they were cooled down and were added with 1 mL of hydrogen peroxide three times. Each time, the tube was shaken thoroughly for the reaction with hydrogen peroxide to complete. Then, they were placed on the heater again, but this time at 330°C. The digestion was conceived to be complete when the extract was bleached or turned into light yellow (2 h). The tubes were then cooled down, were added with 48.3 mL of distilled water, and were infiltrated after shaking. The K content was measured by flame photometry.

2.5. Statistical analysis

The experiment was analyzed as a randomized complete block design with three replications, each replication with two trees. 10-12 years old trees were evaluated under two conditions drought and normal stress (control). The cultivars and genotypes included A8-24, Sh17, Kh1, Shokufeh, Saba, A1-16, Sh10, A230, Fragiolu, Mamaei, A9-7, and A13-40 on a GF677 (*Prunus amygdalus* × *Prunus persica*) rootstock. Finally, the collected data were statistically analyzed by the SAS-9.1 software package. Means comparison was performed for the effect of treatments by Duncan's Multiple Range Test to select the best tolerant cultivar.

3. Results

3.1. Growth parameters

The results of the analysis of variance are presented in Table 1. Accordingly, almond cultivars exposed to drought stress and control plants (normal condition or without stress) differed significantly ($P < 0.01$ and $P < 0.05$). Means comparison for branch growth between the drought-exposed plants showed that water shortage in arid regions is a major factor limiting the growth of the trees considerably. This response was examined by measuring branch growth. All studied genotypes and cultivars exhibited significant differences between normal irrigation and drought stress conditions. The trees exposed to drought stress had lower average growth than those grown under normal irrigation conditions. This response may arise from the high demand for transpiration and/or the shortage of water that is required

to build the compounds for growth. Although water deficiency during the drought stress period affected the growth of the branches, some cultivars and genotypes were influenced by this stress to a lesser extent.

The means comparison revealed significant differences in growth. The highest growth was observed in 'Kh1' and the lowest in 'K7-9'. The results showed that in similar drought stress conditions, some genotypes of almonds outperformed others and showed better growth responses whilst other genotypes were severely influenced by the drought stress so that their growth was slowed down or even stopped (Fig 1 and 2). According to the comparison of the means for leaf yellowing in different irrigation treatments (Fig 3), this response was observed in all cultivars and genotypes exposed to severe drought stress, but it was stronger in some cultivars. As can be seen, 'A9-7' and 'A13-40' exhibited the highest and lowest leaf shedding, respectively. It is likely to reflect the capability of these cultivars in keeping their leaves, which is crucially important for their vital activities such as assimilation and the supply of nutrients for different parts of the plant, including fruits.

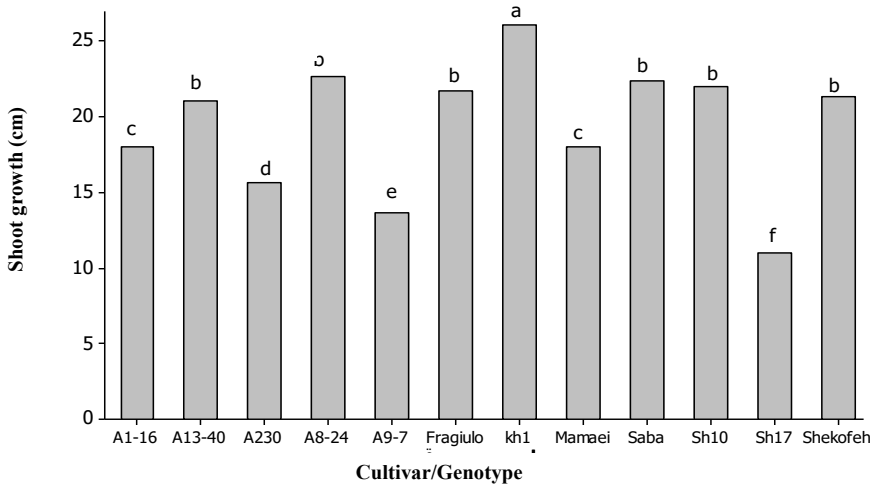

Figure 1. Means comparison for branch growth under drought stress.
Means followed by the similar letter(s) are not significantly different by Duncan test ($P < 0.01$).

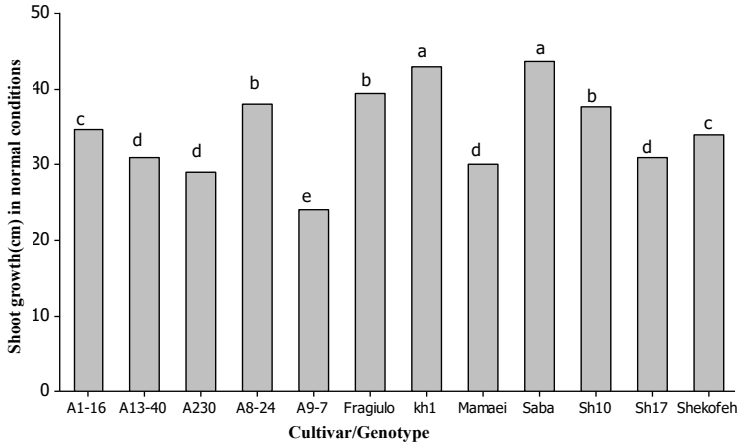

Figure 2. Means comparison for branch growth under normal conditions.
Similar letter on the bars show insignificant differences at the $P < 0.01$.

Table 1. The analysis of variance of morphological and physiological traits of almond cultivars in drought stress and normal conditions

Sources of variations	df	Mean of squares								
		Leaf yellowing	Growth under stress	Growth under normal conditions	RWC under stress	RWC under normal conditions	Proline under stress	Proline under normal conditions	Potassium under stress	Potassium under normal conditions
Cultivars	11	787.54**	55.17**	105.62**	38.87*	43.75*	0.33**	2.04**	1.11**	0.138**
Replication	2	3.69ns	0.69ns	6.36ns	4.11ns	3.17ns	0.007ns	0.003ns	0.004ns	0.0001ns
Error	2	5.02	2.30	4.81	3.50	2.86	0.01	0.02	0.003	0.02
C.V. (%)	4	5.97	7.80	6.34	2.49	3.30	5.45	3.52	2.23	1.67

*: Significance at the P < 0.05; **: Significance at the P < 0.01; ns: non-significance.

3.2. Physiological traits

The results of the comparison of the means revealed significant differences in the leaf relative water content (RWC) between the plants exposed to drought stress and those grown in normal conditions (Fig 4 and 5). Leaf RWC was one of the most important parameters that varied among the cultivars under similar drought stress conditions. This parameter represents the variations in the water content of leaf cells under stressful conditions. The plants exposed to the drought stress had lower leaf RWC, but the extent to which the leaf RWC was lower was different among different cultivars. In addition, the difference in the leaf RWC between normal irrigation and water deficit conditions was slighter in some genotypes, whereas it was much greater and even statistically significant in other genotypes. Similarly, some cultivars or genotypes had higher leaf RWC in their cells, and others had lower. The variation trend of leaf water showed that the leaf water content was different between the stressful conditions and the normal conditions, so it was much lower in the stressful conditions.

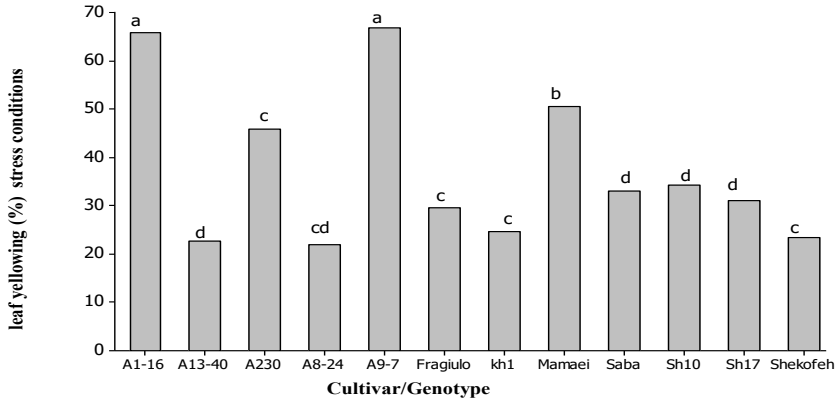

Figure 3. Leaf yellowing of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

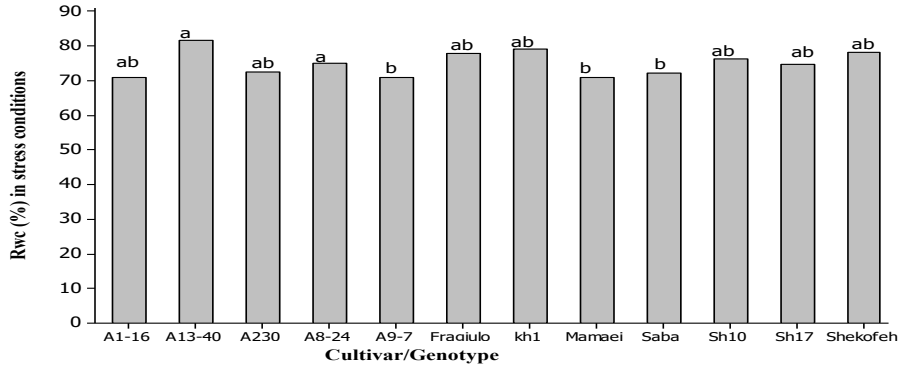

Figure 4. Leaf relative water content of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

3.3. Leaf proline

Table 1 revealed that the proline content of experimental cultivars differs in both stress and normal conditions significantly. The results of the means comparison for proline content (µmol g^{-1} FW) of different almond cultivars exposed to drought stress or normal conditions are depicted in Figures 6 and 7. The cultivars that were exposed to the drought stress differed significantly in this trait. In stress conditions, the highest amount of proline was obtained in cultivars A1-16 and then Mamaei. While cultivar A8-24 showed the minimum proline content among all cultivars (Figure 6). In normal conditions, cultivars Kh1 and Mamaei had maximum proline, but A8-24 still showed the lowest content of proline (Figure 7).

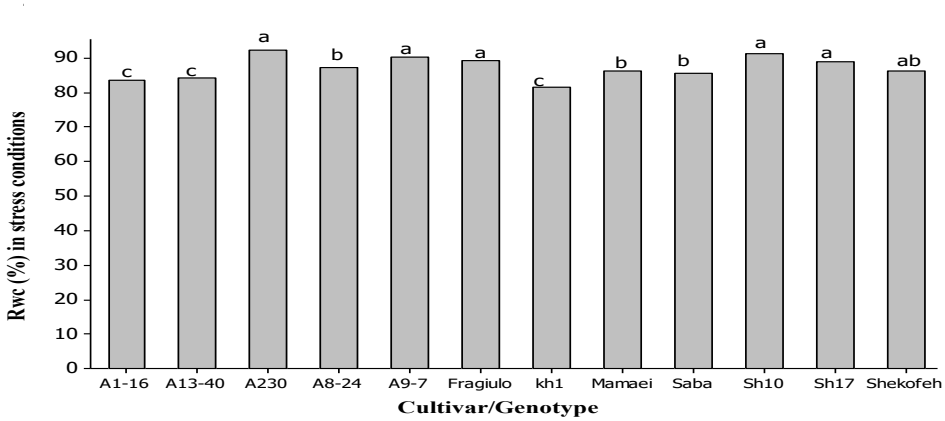

Figure 5. Leaf relative water content of almond cultivars under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

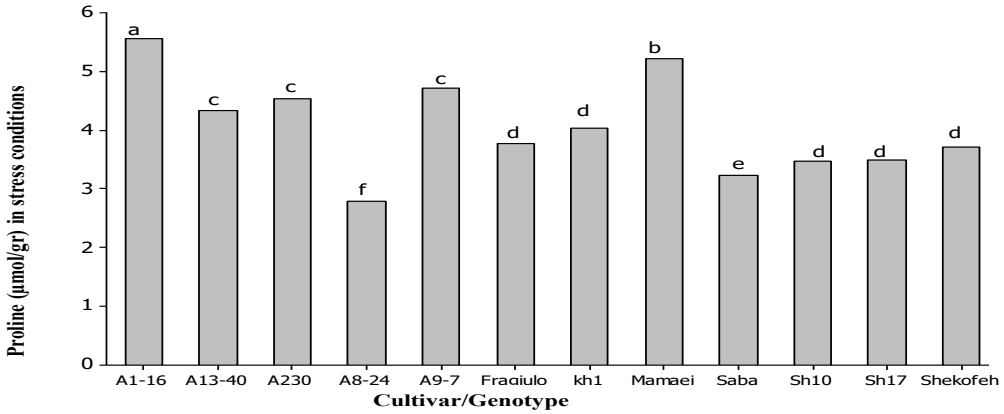

Figure 6. Proline content of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

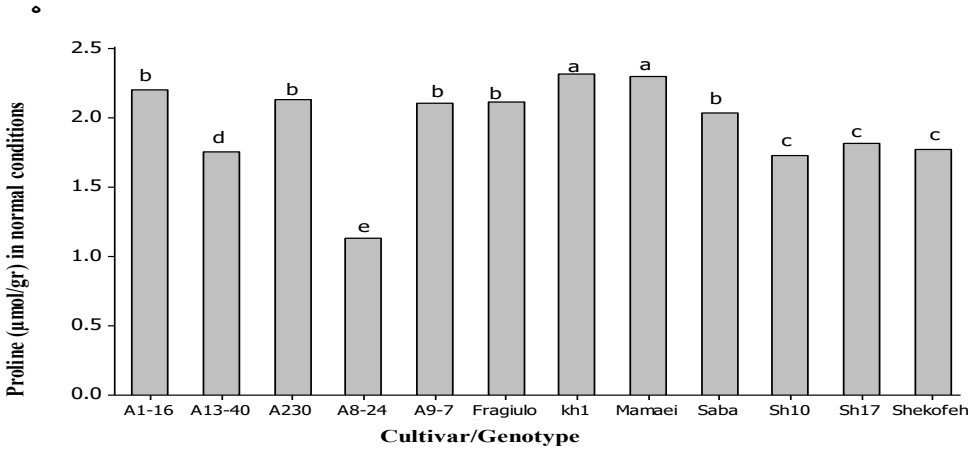

Figure 7. Proline content of almond cultivars under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

3.4. K content

Based on the analysis of variance (Table 1), the potassium content of leaves was a significant difference under stress and normal condition. The highest K content under drought stress was obtained in A1-16 and Mamaei cultivars, while the least K content was related to Saba.

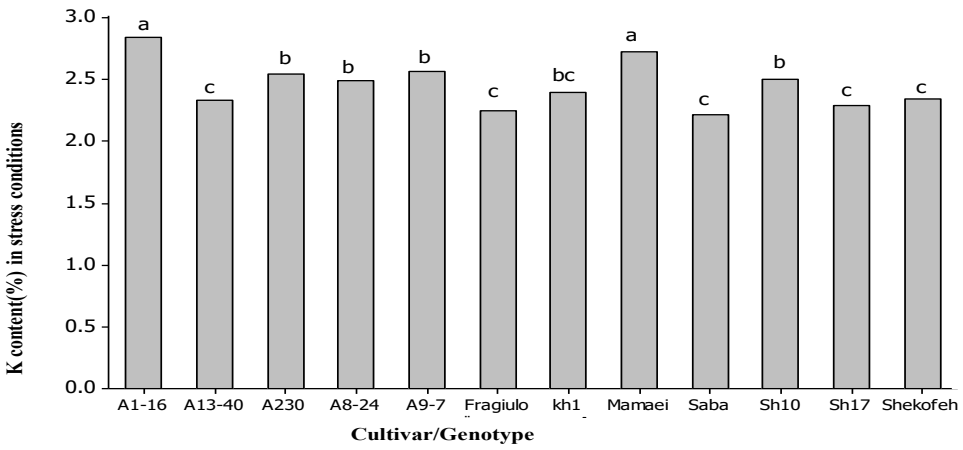

Figure 8. Leaf potassium content in studied genotypes under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

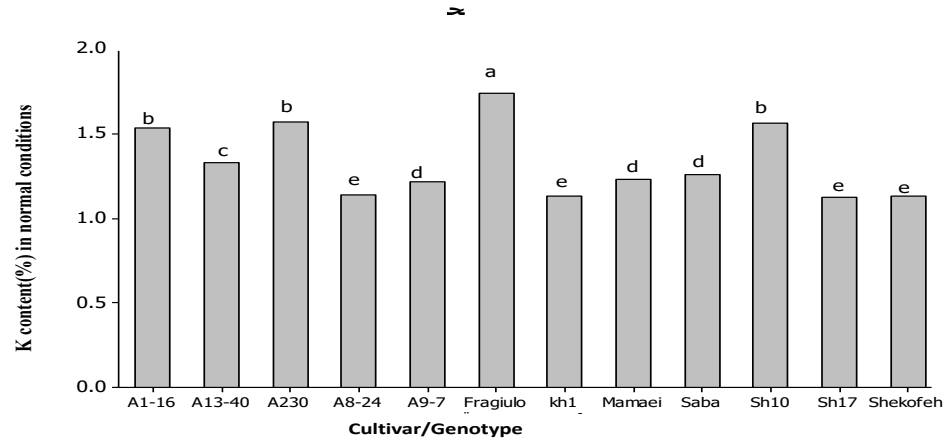

Figure 9. Leaf potassium content in studied genotypes under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

4. Discussion and Conclusion

Plant growth under environmental stresses can be important for breeding programs of drought-tolerant cultivars. It should be noted that these findings are consistent with De Herralde et al. (2001) and Nortes et al. (2005) reported significantly different yields of almond cultivars and genotypes exposed to drought stress. Daudet et al. (2005) found that the variations in tree growth were related to the variations in water uptake and thermal expansion (Fig 1 and 2). Zokaee-Khosroshahi et al. (2014) reported that *Prunus eburnea* had the highest relative water content under drought stress compared to the other species, the least decline in the weight of roots, stems, and the whole plant, and quick abscission of leaves under stress. Based on their results, it can be concluded that *P. eburnea* has a higher level of resistance to drought stress. Akbarpour et al. (2017) studied almond cultivars under in vitro drought stress and found that drought stress caused an increase in electrolyte leakage and proline content while it reduced the RWC in almond.

The accumulation of the amino acid proline in plant tissues in response to various abiotic stresses plays a remarkable role in protecting the plants against oxidative damage of reactive oxygen

species (ROS). Proline has several functions in stress adaptation, the most important ones being the osmotic adjustment and the storage of carbon, nitrogen, and energy (Liang et al., 2013). When exposed to drought stress, some cultivars synthesize a high amount of proline, and some synthesize a low amount. It has been documented that drought stress induces proline accumulation and synthesis and hinders the binding of proline to proteins and their decomposition (Pagter et al., 2005; Türkan et al., 2005). Osmotic adjustment compounds are known to include many micro-molecules, e.g. potassium, dissolved sugars, proline, and betaine. These molecules are major physiological markers for the ability of osmotic adjustment and drought resistance in plants exposed to drought stress (Wang et al., 2009). Liang et al. (2013) reported that when drought stress was applied to the roots, Abscisic acid (ABA) acted as a signal to induce proline synthesis to help the plants adapt to the environmental variations.

Studies have displayed that the amino acid proline increases in most almond genotypes or cultivars that are exposed to drought stress (Karimi et al., 2012). This is supported by our results, too. However, the extent of proline increase varies among cultivars and genotypes. Overall, as stress is intensified, more proline is synthesized. Although the cultivars in which more proline is synthesized exhibit moderate to high resistance to drought stress, the amount of proline in drought-tolerant cultivars is not necessarily more than that in other cultivars under similar stress conditions. Therefore, it seems that cultivar A8-24 has a lower proline content than other cultivars under any circumstances (drought or normal conditions). According to Barzegar et al. (2012), in almond, accumulation of proline in response to drought stress is a common trait and cannot be used as an indicator for introducing the tolerant cultivars.

Figures 8 and 9 depict that the total K content of the leaves was higher in most cultivars exposed to drought stress. This shows that potassium-containing compounds are accumulated in leaves under stressful conditions to contribute to the osmotic adjustment of the leaves. The results indicated that Saba and Fragiolu had the lowest potassium content among all the studied cultivars. Based on our results, trial almond cultivars had K content of about 1.1 and 1.8%, while the content of potassium under drought stress reached about 2,2-2.8%. According to Wang et al. (2013), plants under drought stress have a greater internal need for potassium. The supply of K can overcome the limiting effect of water stress in conditions of drought stress (Damon and Rengel, 2007; Bahrami-Rad and Hajiboland, 2017). Under water deficit stress, more potassium is required to maintain CO2 fixation of photosynthesis and protection of chloroplasts from oxidative damage (Cakmak, 2005; Bahrami-Rad and Hajiboland, 2017). Sufficient amounts of potassium can improve the biomass accumulation of plants under drought stress in comparison to minor K concentrations (Egilla et al., 2001; Wang et al., 2013). Additionally, adequate K prompts solute accumulation, thus lowering osmotic potential and assisting in maintaining plant cell turgor under osmotic stress. An adequate K status may help osmotic adjustment, which maintains higher turgor pressure, relative water content, and lower osmotic potential, thus improving the capacity of plants to tolerate drought stress (Kant and Kafkafi, 2002; Egilla et al., 2005; Wang et al., 2013)

Cultivars' genotypes differ in strategies they adopt against environmental stresses. These strategies can vary in effectiveness in different conditions and stresses depending on the conditions. The more the stress avoidance and/or confrontation methods are in a genotype, the more viable it is in adverse environments. The results revealed that no single genotype had all optimum responses to drought stress; rather, some optimal traits were observed in some of them. When all traits are considered together, it is concluded that the genotypes 'Kh1' and 'A13-40' were more drought tolerant than other genotypes.

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Research Article

Determination of Soil Moisture and Temperature Regimes with the Newhall Simulation Model: Example of Van Province

Siyami KARACA[*1], Bulut SARĞIN[2]

[1,2]Van Yuzuncu Yıl University, Agriculture Faculty, Soil Science and Plant Nutrition Department, Van, Turkey

[1]https://orcid.org//0000-0002-2434-1171, [2]https://orcid.org//0000-0002-4752-4333

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

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Abstract: The aim of this study was to determine Van province and districts soil, temperature, and humidity regimes, which contain different geographical and climatic characteristics, with the Newhall Simulation Model. In the study, long-term average precipitation and temperature data obtained from Van center,Bahçesaray, Başkale, Çaldıran, Çatak, Erciş, Gürpınar, Gevaş, Muradiye, Özalp and Saray meteorological stations were used. In addition, Thorntwhaite and Erinç climate classifications were used to find the climate classification of the province and districts. Looking at the Thornthwaite climate classification, Bahçesaray and Çatak districts are in the region's southwest and in the semi-humid climate class. In contrast, the other locations show semi-arid climate characteristics. According to the Erinç climate classification, Bahçesaray, Çatak, and Muradiye are classified as semi-humid, Gürpınar in the arid climate class, and the districts located in the northern and eastern parts of the province are categorized as semi-arid. With the Newhall Simulation model, the soil temperature regime of the province and all districts was determined as "Mesic." The moisture regime of the soil of Bahçesaray, Çatak, Gevaş, and Muradiye districts and the Van region was seen as Dry Xeric. The soil moisture regime of Başkale, Çaldıran, Erciş, Gürpınar, Özalp and Saray districts was found to be "Typic Aridic". Calculation of the soil water budget, determination of water deficiency, and preparation of drought action planning will be beneficial in the effectiveness of all necessary physical, chemical, and biological activities of the soil and in determining the groups in soil classification.

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Newhall Simülasyon Modeli ile Toprak Nem ve Sıcaklık Rejimlerinin Belirlenmesi: Van İli Örneği

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Öz: Bu çalışmanın amacı, farklı coğrafi ve iklim özelliklerini içinde barındıran Van il ve ilçe topraklarının Newhall Simülasyon Modeli ile toprak sıcaklık ve nem rejimlerinin belirlenmesidir. Çalışmada, Bahçesaray, Başkale, Van Bölge, Çaldıran, Çatak, Erciş, Gürpınar, Gevaş, Muradiye, Özalp ve Saray meteorolojik istasyonlarından temin edilen uzun yıllar ortalama yağış ve sıcaklık ölçümlerinden yararlanılmıştır. Ayrıca, il ve ilçelerin iklim sınıflamasını bulmak için

Anahtar Kelimeler

Newhall Simülasyon Modeli, Toprak nem ve sıcaklık rejimi, Van

Thorntwhaite ve Erinç iklim sınıflaması kullanılmıştır. Thornthwaite iklim sınıflamasına bakıldığında ilin güneybatısındaki Bahçesaray ve Çatak ilçeleri yarı nemli iklim sınıfında yer alırken, diğer ilçeler ise yarı kurak iklim özelliği göstermektedir. Erinç iklim sınıflamasına göre, Bahçesaray, Çatak ve Muradiye yarı nemli, Gürpınar kurak iklim sınıfında ve ilin kuzey ve doğu kısımlarında yer alan ilçeler ise yarı kurak olarak kategorize edilmiştir. Newhall Simülasyon modeli ile il ve ilçelerin tamamının toprak sıcaklık rejimi "Mesic" olarak belirlenmiştir. Bahçesaray, Çatak, Gevaş ve Muradiye ilçeleri ile Van bölge topraklarının nem rejimi Dry Xeric olarak sınıflandırılmıştır. Başkale, Çaldıran, Erciş, Gürpınar, Özalp ve Saray ilçelerinin ise toprak nem rejimi "Typic Aridic" olarak bulunmuştur. Nem ve sıcaklık rejiminin belirlenmesi; su bütçesinin hesaplanması, su noksanlığının tespiti, kuraklık eylem planlamasının hazırlanması, toprakta meydana gelen fiziksel, kimyasal ve biyolojik olan tüm önemli faaliyetlerin etkinliğinde ve toprak sınıflandırmasındaki grupların belirlenmesinde fayda sağlayacaktır.

1. Giriş

Yaşamın temel ögelerinden biri olan ve yer yüzündeki doğal kaynak olarak kabul gören toprak, bilim insanlarının araştırma konusu olmuş ve pek çok bilim disiplini tarafından detaylı bir şekilde ele alınıp incelenmiştir (Alaboz ve ark., 2021, Demir ve Başayiğit 2021., Kızılkaya ve ark., 2019). Toprak, çeşitli kayaçların fiziksel parçalanması ve organik maddenin kimyasal ve biyolojik ayrışma olayları sonucu oluşan, sürekli değişkenlik gösteren, yer kabuğunun en üst tabakasını oluşturan içinde çeşitli flora ve fauna barındıran, dinamik bir denge yapısına sahip, üç fazlı ve boyutlu canlı ana materyale olarak tanımlanabilir. Toprağın her 1 cm'nin meydana gelebilmesi için, uzun yıllar içerisinde ana materyalin parçalanabilmesi, bunun yerine çeşitli canlıların topluluğunun nüfuz etmesi, yıkanma ve birikme olaylarının cereyan etmesi gereklidir. Farklı coğrafik koşullar altında ve çeşitli iklim ve vejetasyon tesiri altında farklı topraklar meydana gelmektedir (Atalay, 2012., Tolunay, 2017). Ana materyalin ayrışmasından başlayarak, toprağın olgun bir yapıya ulaşmasına kadar süregelen toprak oluşum evresinde çeşitli faktörler etkilidir. Bu faktörler aktif ve pasif olmak üzere ikiye ayrılır. Aktif olan faktörleri iklim ve mikro organizmalar oluştururken, pasif olanları ise topografya, ana materyal ve zaman faktörleri oluşturmaktadır. İklim, yer kabuğunun oluşmasında etkili olan ayrışma, taşınma, birikme ve yeryüzünün şekil alması olaylarında son derece önem arz etmektedir. Kayaların ayrışmasına doğrudan etki eden iklim, bitki örtüsünü de dolaylı yollardan etkilemektedir. İklimin yeryüzüne ve toprak oluşumuna olan büyük etkisi diğer bütün faktörlerin etkisini yok edecek kadar bir güce sahip olabilir. Toprak oluşumunda iklim önemli bir etken olarak ele alındığında, bu oluşuma etki eden iki maddenin göz önünde bulundurulması gerekebilir. Bunlar, iklimin doğrudan doğruya etkisi ve dolaylı etkisidir. Toprağın meydana gelmesinde iklim doğrudan etki etmektedir. Bu durum, topraktaki yağış ve sıcaklık rejimlerinin farklı niteliklerdeki tesiriyle bariz bir şekilde ortaya çıkmaktadır. Toprağın periyodik olarak ısınması, soğuması, ıslak ve kuru oluşu fiziksel, kimyasal ve biyolojik olaylara önemli ölçüde etki etmesinin yanında, evaporasyon değerinin de artış veya azalışına etki eder. Sıcaklık ve nem değişimleri, aynı zamanda toprak içerisinde oldukça faal olan toprak kolloidlerinin belirlenmesini, kendine özgü nitelik kazanmasında, gelişim göstermesinde oldukça etki göstermektedir. Ayrıca, topraktaki mikroorganizmalarının yaşamsal faaliyetleri ve makro floranın da büyümesine etki etmektedir. Bundan dolayı, nem ve sıcaklık rejimleri toprakta oluşan tüm aktif etkenleri belirler ve bu sebeple toprağın meydana gelmesinde temel kavramlar içerisinde önemi büyüktür (Mater, 1986). Sıcaklık ve toprak nemi birbiriyle yakın bir ilişki içerisindedir. Toprak nemi duyarlı ve gizli bir şekilde ısı akışını ve toprak yüzeyine yakın olan hava olaylarını kontrol etmektedir (Huang ve ark., 2016). Toprak yüzeyindeki ve aşağı derinliklerdeki katmanların (örneğin 20 cm, 50cm ve 100 cm vb) sıcaklığı günlük, aylık, yıllık ve mevsimlik olarak önemli değişimler göstermektedir (Mater, 1986, Ekberli ve ark 2005, Ekberli ve Sarılar, 2015, Dengiz ve Ekberli, 2017., Turan ve ark, 2018). Toprağın niteliksel ve niceliksel özelliklerini temel alan Toprak Taksonomisi, toprağın oluşum yansıması olarak morfolojiyi göstermektedir (Başayiğit ve Dinç, 2005). Toprakların ordo, alt ordo, büyük grup ve familya düzeyleri belirlenirken nem ve sıcaklık rejimleri ile toprağın oluşumu arasında bağlantı kurulabilir (Almaraz ve Eswaran, 1997, Soil Survey Staff, 1999). Toprak taksonomisi yapılırken, Aridisol bir toprağın kategorize edilmesinde Aridic toprağın nem rejimi ordo düzeyinde sınıflandırmaya etki eden bir etmen

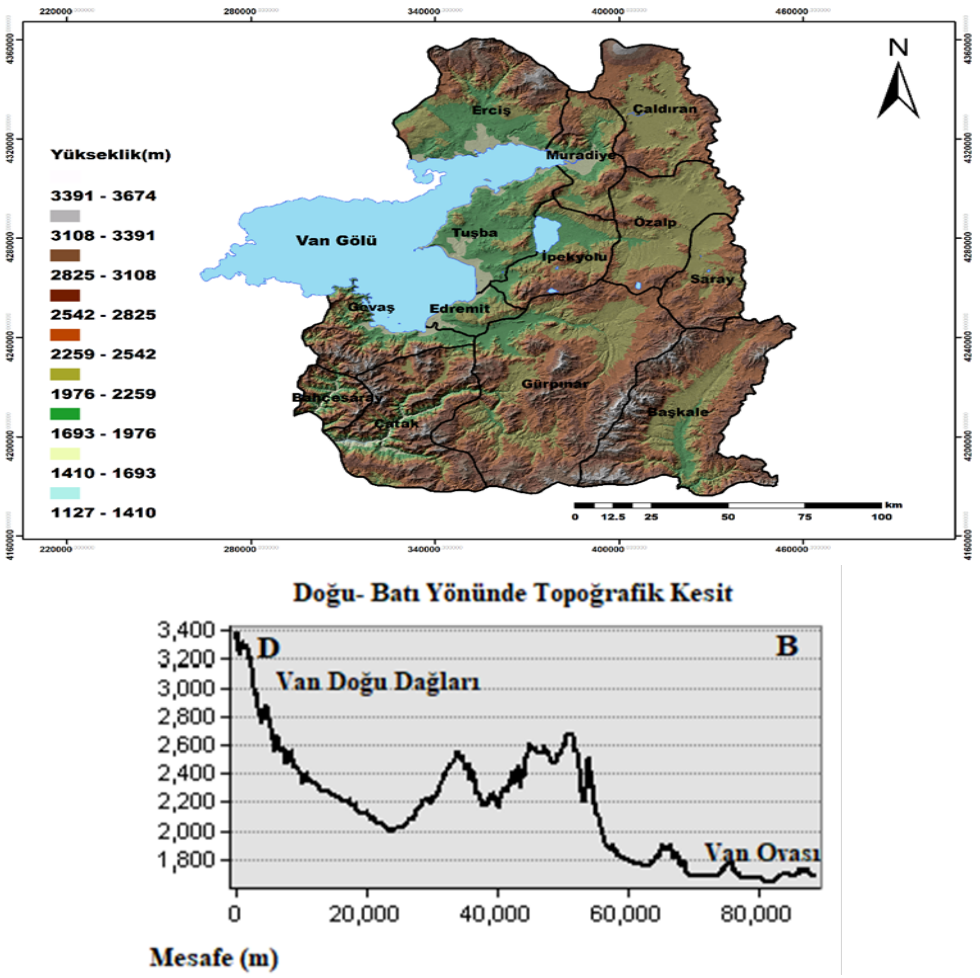
olarak değerlendirilirken, Gelisol bir toprağın sınıflandırılmasında ise hem nem hem de sıcaklık rejimleri ayrı bir kategori olarak kullanılmıştır (Başayiğit ve Dinç, 2005). Günümüzde, küresel ısınmadan dolayı kullanılabilir su potansiyelindeki düşüş ve giderek artmakta olan kontrolsüz su kullanımı da eklenince bilhassa kurak ve yarı kurak yerlerde su gereksiniminin sağlanmasında problemlere neden olmaktadır. İklim değişiklikleri neticesinde meydana gelmesi muhtemel olan sıcaklık artışı ve yağışın yetersizliği toprakların nem rejimini doğrudan etkileyen parametreler olarak bilinmektedir. İklim tiplerinin sınıflandırılması için yapılan çalışmalarda farklı yöntemler kullanılmaktadır. Bunun nedeni, sınıflandırma kurallarının ya da hareket noktalarının farklı olmasından kaynaklanır. İklim sınıflandırmalarında kullanılan yöntemlerin arasında Erinç , Thornthwaite, De Martonne , Aydeniz ve Köppen-Geiger gibi yöntemler bulunmaktadır. Yapılan çalışmada yaygın olarak kullanılan iki yöntem, Erinç (1965) ve Thornthwaite (1948) yöntemi kullanılmıştır. Bu çalışmada, farklı coğrafi ve iklim özelliklerini içinde barındıran Van il ve ilçe topraklarının Newhall Simülasyon Modeli ile toprak sıcaklık ve nem rejimlerinin tespit edilmesi amaçlanmıştır.

2. Materyal ve Yöntem

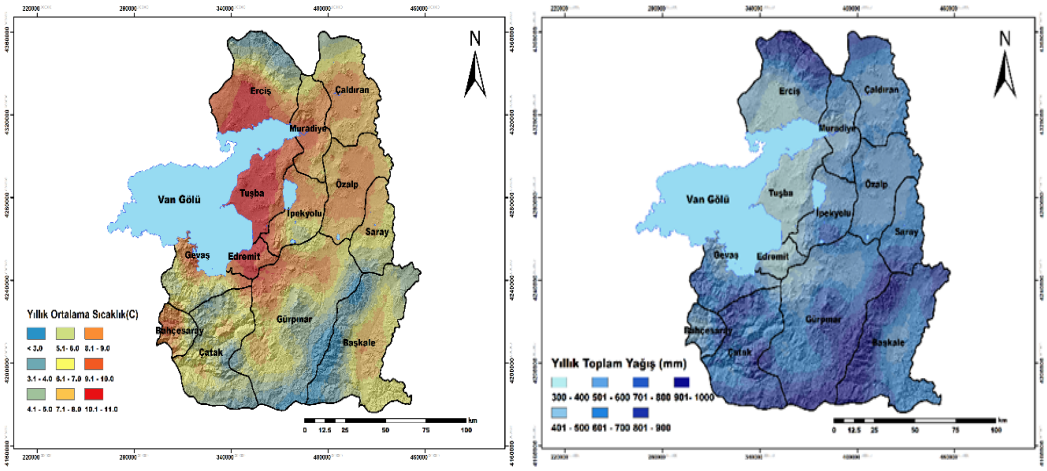
2.1. Materyal

Çalışma, Van il sınırları içinde yer, alan hem çeşitli fizyografik niteliklere hem de coğrafi bir öneme sahip olan 10 ilçe (Bahçesaray, Başkale, Çatak, Çaldıran, Erciş, Gevaş, Gürpınar, Muradiye Özalp ve Saray) ve il merkezinin bulunduğu alanları kapsamaktadır. Doğu Anadolu bölgesinde yer alan Van ili, 42° 40' ve 44° 30' doğu boylamları ile 37° 43' ve 39° 26' kuzey enlemleri arasındadır. İlin doğusunda İran Devlet sınırı, kuzey kesiminde Ağrı ilinin Doğubayazıt, Diyadin ve Hamur ilçeleri, batısında Ağrı ilinin Patnos ilçesi, Bitlis ilin Tatvan, Adilcevaz ve Hizan ilçeleri, güneyinde ise Siirt ilin Pervari ilçesi, Hakkâri ilinin Beytüşşebap ve Yüksekova ilçeleri yer almaktadır. 19.069 km^2 olan yüz ölçümüyle, Van ili Türkiye'nin yüz ölçümü bakımından 6. büyük ilidir. Van ili deniz seviyesinden 1725 m yükseklikte olup, il genelinde karasal iklim hüküm sürmektedir (Karaca ve ark 2019). En düşük yıllık ortalama sıcaklık 5.9 ℃ ile Çaldıran ilçesinde gözlemlenmiştir. Van ilinin doğusunda ve kuzeyinde yer alan Erciş, Muradiye, Çaldıran, Özalp ve Saray ilçeleri 300-450 mm arasında yağış alırken, ilin güneyinde yer alan Başkale, Gevaş, Gürpınar, Çatak ve Bahçesaray ilerinde ise 275 -750 mm arasında yağış gerçekleşmektedir. En düşük toplam yağış 275.9 mm ile Gürpınar ilçesinde görülmüştür. (Tablo 1.)

Şekil 1. Çalışma alanı yer buldur haritası.



Şekil 2. Van il ve ilçelerinin yükseklik dağılımı haritası ve topoğrafik kesiti.



Şekil 3. Van il ve ilçelerine ait sıcaklık ve yağış dağılım haritaları.

Çalışmada, Van 14. Meteoroloji Bölge Müdürlüğünden Bahçesaray (2013-2020), Başkale (1980-2020), Van Bölge (1980-2020), Çaldıran (2013-2020), Çatak (2015- 2020), Erciş (2009-2020), Gürpınar (2013- 2020), Gevaş (2009- 2020), Muradiye (2009- 2020), Özalp (2009- 2020) ve Saray (2015- 2020) ilçelerine ait meteoroloji gözlem istasyonlarından temin edilen uzun yıllara ait ortalama yağış ve sıcaklık datalarından yararlanılmıştır (Tablo 1.).

Tablo 1. Van il ve ilçe sınırları içerisinde yer alan meteoroloji istasyonlarından alınan aylık toplam yağış ve sıcaklık verileri

	Yıllar	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran	Temmuz	Ağustos	Eylül	Ekim	Kasım	Aralık	Yıllık
Bahçesaray sıcaklık	2013-2020	-1.5	0.4	4.4	8.8	13.7	19.1	23.7	23.5	19.1	12.4	5.7	0.8	10.8
Bahçesaray yağış	2013-2020	86.4	54.6	131.2	113.6	88.6	19.0	4.7	9.5	5.6	63.7	73.7	89.6	740.4
Başkale sıcaklık	1980-2020	-6.5	-5.7	-1.3	4.8	9.9	15.3	19.6	19.5	15.4	8.5	1.4	-4.0	6.4
Başkale yağış	1980-2020	28.3	22.0	50.9	60.9	53.2	24.7	14.6	11.8	5.5	27.2	28.6	26.8	354.3
Bölge sıcaklık	1980-2020	-2.7	-1.9	2.4	8.2	13.2	18.5	22.6	22.4	18.1	11.7	5.0	0.0	9.8
Bölge yağış	1980-2020	34.4	34.3	49.2	54.5	48.7	17.2	6.8	7.2	16.1	46.4	50.0	42.0	407.0
Çaldıran sıcaklık	2013-2020	-10.9	-8.7	-0.9	5.5	10.9	16.0	20.3	20.2	15.6	8.4	1.5	-7.3	5.9
Çaldıran yağış	2013-2020	29.5	15.3	37.1	34.8	56.7	30.6	11.5	12.8	15.1	43.2	29.4	35.5	351.5
Çatak sıcaklık	2015-2020	-1.7	0.8	5.6	9.8	14.3	19.1	23.0	22.6	18.7	12.7	6.2	1.0	11.0
Çatak yağış	2015-2020	82.4	77.0	130.3	115.9	72.5	14.1	4.1	8.6	1.8	63.9	60.9	109.0	740.4
Erciş sıcaklık	2009-2020	-5.5	-4.6	0.7	7.1	12.3	17.7	21.8	21.2	16.2	9.7	3.1	-2.4	8.1
Erciş yağış	2009-2020	25.5	22.0	54.9	65.7	57.4	15.5	2.5	2.4	7.1	31.1	21.5	30.1	314.9
Gevaş sıcaklık	2009-2020	-3.1	-2.4	2.0	8.1	12.8	17.7	21.6	21.1	16.9	10.6	4.2	-0.6	9.1
Gevaş yağış	2009-2020	49.6	36.7	54.3	60.6	71.4	20.2	8.2	6.6	13.0	43.5	36.8	47.4	422.5
Gürpınar sıcaklık	2013-2020	-3.9	-2.5	3.7	8.5	13.6	18.7	22.7	22.3	17.9	10.9	4.3	-1.7	9.5
Gürpınar yağış	2013-2020	29.6	18.2	29.3	39.7	49.8	15.8	4.8	6.8	3.9	28.2	20.5	29.3	275.9
Muradiye sıcaklık	2009-2020	-4.8	-3.9	1.1	7.7	13.0	18.5	23.0	22.6	17.5	10.8	3.8	-1.7	9.0
Muradiye yağış	2009-2020	41.7	38.2	58.9	63.9	61.2	15.9	12.2	9.0	22.9	33.7	36.5	50.7	444.2
Özalp sıcaklık	2009-2020	-9.5	-7.9	-1.3	5.8	11.0	16.2	20.7	20.4	15.3	8.5	1.1	-5.7	6.2
Özalp yağış	2009-2020	21.8	17.7	29.7	48.9	59.0	28.2	10.1	9.2	3.7	28.7	26.6	23.1	306.6
Saray sıcaklık	2015-2020	-7.8	-5.6	0.7	5.7	11.2	16.7	21.0	21.2	16.8	9.7	2.2	-4.2	7.3
Saray yağış	2015-2020	25.2	24.5	37.6	43.8	50.8	31.1	24.2	11.5	9.3	42.6	22.9	30.7	354.0

2.2. Yöntem

Van il ve ilçelerinin uzun yıllara ait aylık ortalama sıcaklık, aylık ortalama yağış, enlem-boylam bilgileri ve rakım verileri Meteoroloji 14. Van Bölge Müdürlüğü'nden temin edilmiştir. Van bölge ve Başkale istasyonları dışında diğer istasyonlar sonraki dönemlerde kurulduğu için kullanılanan dataların tarih aralıkları değişkenlik göstermiştir. Temin edilen bu datalar kullanılarak Thorntwhaite (1948) ve Erinç (1965)'e göre evapotranspirasyon hesaplanmıştır. Erinç (1965) metodu yağış etkinliği indis değerini (Im) belirlemede, yağışa (P) ve buharlaşmayla su kaybına yol açan esas etmen olan maksimum sıcaklığa (To) dayanmakta ve Im= P/To formülü ile ifade edilmektedir.

Van il ve ilçe topraklarının nem ve sıcaklık rejimlerinin elde edilmesi için Java Newhall Simulasyon Modeli 1.6.0 (JNSM) yazılımdan yararlanılmıştır (Newhall ve Berdanier, 1996; Van Wambeke vd. 1986 ve 1992; Van Wambeke, 2000). Toprağın sıcaklık ve nem rejimleri sınıflarının tespitinde ise toprak taksonomisi (Soil Survey Staff, 1999) kullanılmıştır.

3. Sonuçlar ve Tartışma

3.1. Van il ve ilçelerin Erinç (1965)'e ve Thornthwaite (1948)'e göre iklim sınıfları

İklim, uzun bir süreç içerisinde meydana gelen gözlemler sonucunda bir yerin genel durumu hakkında incelemelerle elde edilen sonuçlardır. Bir başka tanımla, bir bölgeye ait meteorolojik olayları genel özelliklerini ve bitki örtüsünü de tayin eden en önemli veri iklimdir. Toprak oluşumun da iklim faktörünün en önemli iki unsuru yağış ve sıcaklıktır. Toprak oluşumuna etki eden iklim faktörünün diğer temel değişkenleri de rüzgâr ve nemdir. Toprakta periyodik olarak meydana gelen ısınma, nemlilik, kuraklık ve soğuma vb. değişimler toprak yapısında fiziksel parçalanma, kimyasal ve biyolojik olayların artmasında ve azalmasında etkin rol oynamaktadır (Brady ve Weil, 2000). Kayaların parçalanması, ayrışması ve bir toprak profili niteliğinin oluşabilmesi için sıcaklık ve yağış büyük bir öneme sahiptir (Dinç ve ark., 1987). Bunun yanı sıra bitki örtüsünün ve mikro organizmaların yaşam faaliyetleri de sıcaklık ve yağışa bağlıdır. Van ili çeşitli coğrafi ve iklim özelliklerine sahip olduğu için farklı iklim sınıfları içerisinde yer almaktadır. Erinç iklim sınıflandırması (1965)' göre, Van bölge ve ilçeleri (doğu, güney ve kuzey kesim içerisinde yer alan kısımlar) yarı nemli iklim sınıflandırmasına girmektedir. Thornthwaite İklim Sınıflandırması (1948)'e göre ise Tablo 2'de gösterilmiştir.

Tablo 2. Van il ve ilçelerinin Thornthwaite (1948)'e göre iklim sınıfları

İstasyonlar	İklim İndeksleri	Thornthwaite İklim Özellikler
Bahçesaray	C2, B'2, s2, b'2	Yarı nemli 2. derece mezotermal, su noksanı yaz mevsiminde ve çok kuvvetli olan tali iklim
Başkale	D, B'1 d, b3	Yarı kurak, 1. derece mezotermal, su fazlası olmayan veya pek az olan tali iklimi
Bölge	D, B'2, s, b'3	Yarı kurak, 2. derece mezotermal, su fazlası kış mevsiminde ve orta derecede olan
Çaldıran	D B'1 d b'2	Yarı kurak, 1. derece mezotermal, su fazlası olmayan ve pek az olan
Çatak	C2, B2, s2, b'3	Yarı nemli, 2. derece mezotermal, su noksanı yaz mevsiminde ve çok kuvvetli olan
Erciş	D, B'1, d, b'3	Yarı kurak, 1. derece mezotermal, su fazlası olmayan ve pek az olan
Gevaş	C1, B'2, s, b'2	Yarı kurak -az nemli, 2. derece mezotermal, su fazlası kış mevsiminde ve orta derece olan
Gürpınar	D, B'1, d, b'3	Yarı kurak,1. derece mezotermal, su fazlası olmayan veya pek az olan
Muradiye	C1, B'2, s, b'3	Yarı kurak -az nemli, 2. derece mezotermal su fazlası kış mevsiminde ve orta derece olan
Özalp	D, B'1, d, b'3	Yarı kurak, 1. derece mezotermal, su fazlası olmayan ve pek az olan tali iklim
Saray	D, B'1, d, b'3	Yarı kurak, 1. derece mezotermal, su fazlası olmayan ve pek az olan

Thornthwaite İklim Sınıflandırması (1948)'e göre ise, Bahçesaray ve Gevaş iklimi C2, B'2, s2, b'2 ile belirtilen; yarı nemli 2. derece mezotermal, su noksanı yaz mevsiminde ve çok kuvvetli olan tali iklime sahiptir. Başkale, Erciş, Gürpınar, Özalp ve Saray ise D, B'1 d, b3 ile belirtilen; yarı kurak, 1. derece mezotermal, su fazlası olmayan veya pek az olan tali iklime sahiptir. Van bölgesinin iklimi D, B'2, s, b'3 ile belirtilen; yarı kurak, 2. derece mezotermal, su fazlası kış mevsiminde ve orta derecede olan iklime sahiptir. Çaldıran ilçesinin iklimi D B'1 d b'2 ile belirtilen; yarı kurak, 1. derece mezotermal, su fazlası olmayan ve pek az olan iklime sahiptir. Çatak ilçesinin iklimi C2, B2, s2, b'3 ile belirtilen; yarı nemli, 2. derece mezotermal, su noksanı yaz mevsiminde ve çok kuvvetli olan tali iklime sahiptir. Muradiye ilçesinin iklimi C1, B'2, s, b'3 ile belirtilen; yarı kurak -az nemli, 2. derece mezotermal su fazlası kış mevsiminde ve orta derece olan iklime sahiptir.

Erinç (1965) iklim sınıflamasına göre, Van il ve ilçelerinde 3 farklı iklim özelliği görülmüştür. Bunlar; kurak, 'yarı kurak' ve "yarı nemli iklim sınıflarıdır (Tablo 3).

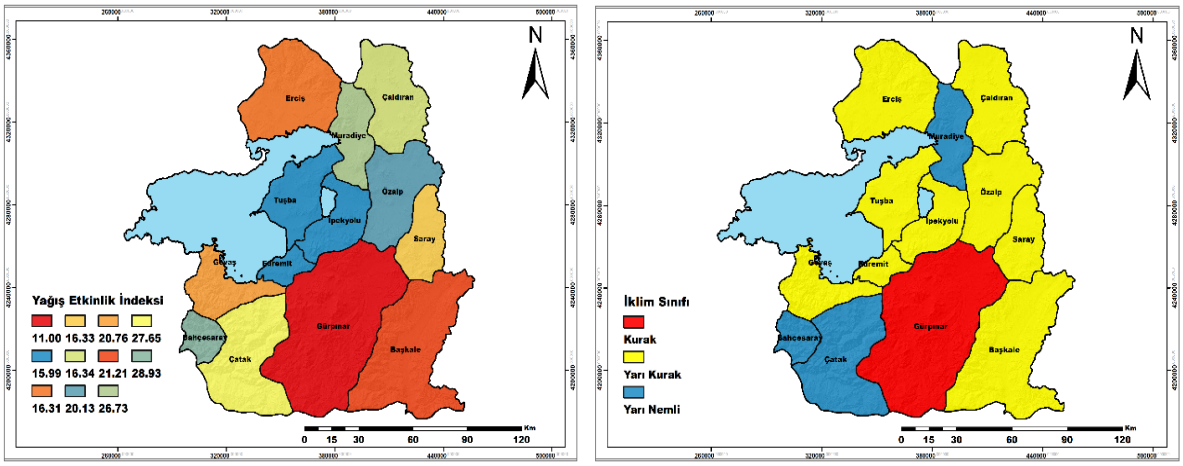
Tablo 3. Erinç (1965)'e göre yağış etkinlik indeksi değerleri, iklim niteliği ve bitki örtüsü

Yağış etkinlik indeksi	İklim Özelliği	Bitki Örtüsü
<8	Tam Kurak	Çöl
8-15	Kurak	Çöl-Step
15-23	Yarı Kurak	Step
23-40	Yarı Nemli	Park görünümlü kuru orman
40-55	Nemli	Nemli orman
55>	Çok Nemli	Çok nemli orman

Van il ve ilçelerinin yağış etkinlik indeksleri incelendiğinde en düşük değerli istasyon; Gürpınar (11.00) görülürken, en yüksek değerli istasyon ise Bahçesaray (28.93) olarak bulunmuştur (Tablo 4).

Tablo 4. Van il ve ilçelerinin Erinç (1965)'e göre İklim Tipleri

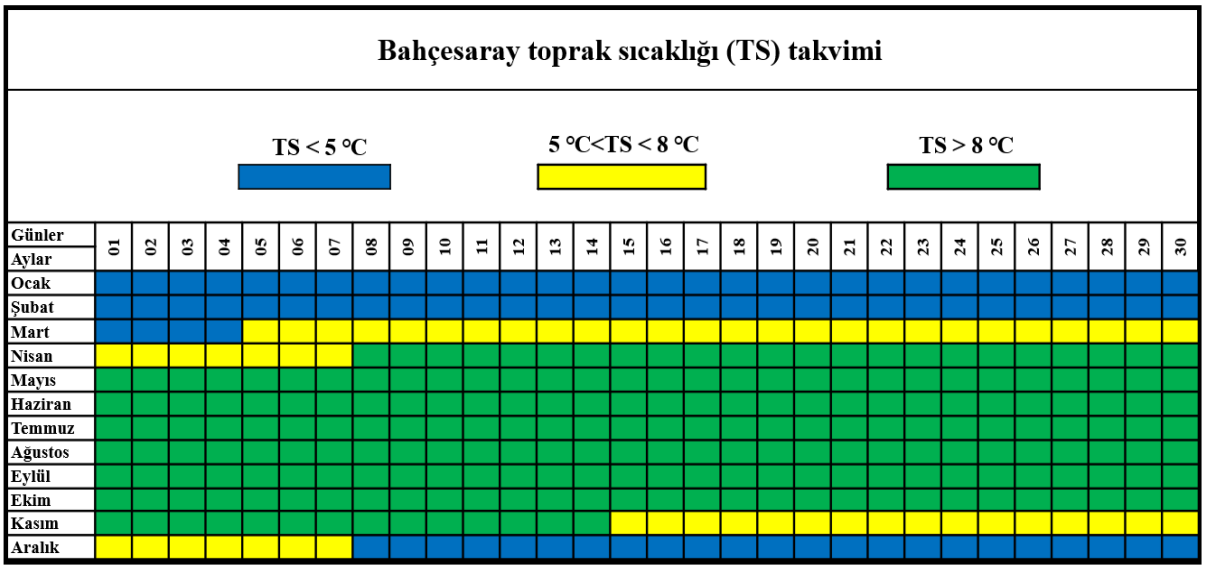
İstasyonlar	Yağış Etkinlik İndeksi	İklim Sınıfı
Bahçesaray	28.93	Yarı Nemli
Başkale	21.21	Yarı Kurak
Van Bölge	15.99	Yarı Kurak
Çaldıran	16.34	Yarı Kurak
Çatak	27.65	Yarı Nemli
Erciş	16.31	Yarı Kurak
Gevaş	20.76	Yarı Kurak
Gürpınar	11.00	Kurak
Muradiye	26.73	Yarı Nemli
Özalp	20.13	Yarı Kurak
Saray	16.33	Yarı Kurak



Şekil 4. Van il ve ilçelerinin Erinç (1965)'e göre yağış etkinlik indeksi ve iklim sınıfları.

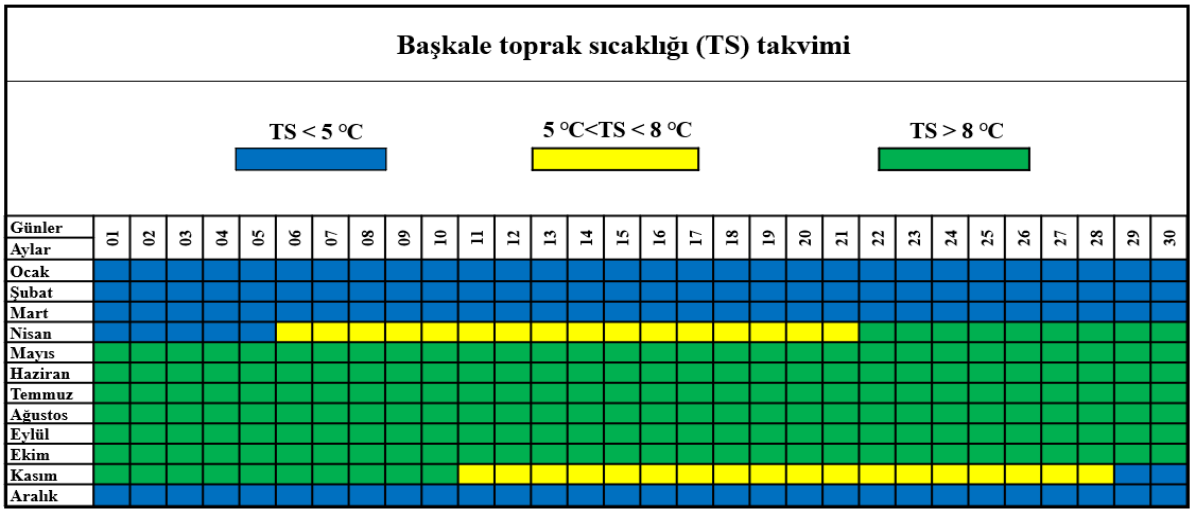
3.2. Van ve ilçelerinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık dağılımı

Van il ve ilçe sınırları içeresinde mevcut bulunan 11 Meteoroloji İstasyon 'undan elde edilen uzun yıllara ait sıcaklık parametrelerinin Java Newhall simülasyon modeli (JSNM) kullanılarak toprak sıcaklık rejimleri bulunmuştur. Kullanılan simülasyon modeliyle, Bahçesaray Meteoroloji İstasyonu'ndan sağlanan uzun yıllar (2013-2020 yıllarına ait) ölçümleri dikkate alındığında, yıllık ortalama sıcaklık 10.8°C olarak gözlemlenmiştir. Bahçesaray ilçe toprağının sıcaklık değerleri 8 Aralık ile 4 Mart tarihleri arasında 5 °C altına inmektedir. 5°C ile 8°C arasında değişen ilçe toprağının sıcaklığı, 5 Mart ile 7 Nisan ve 15 Kasım ile 7 Aralık tarihlerinde iken, ilçenin 8°C üzerinde olan toprak sıcaklığı ise 8 Nisan ile 14 Kasım arasında olduğu belirlenmiştir (Şekil 5). Kullanılan modele göre, Bahçesaray ilçesi toprak sıcaklık rejiminin "Mesic" olduğu belirlenmiştir.



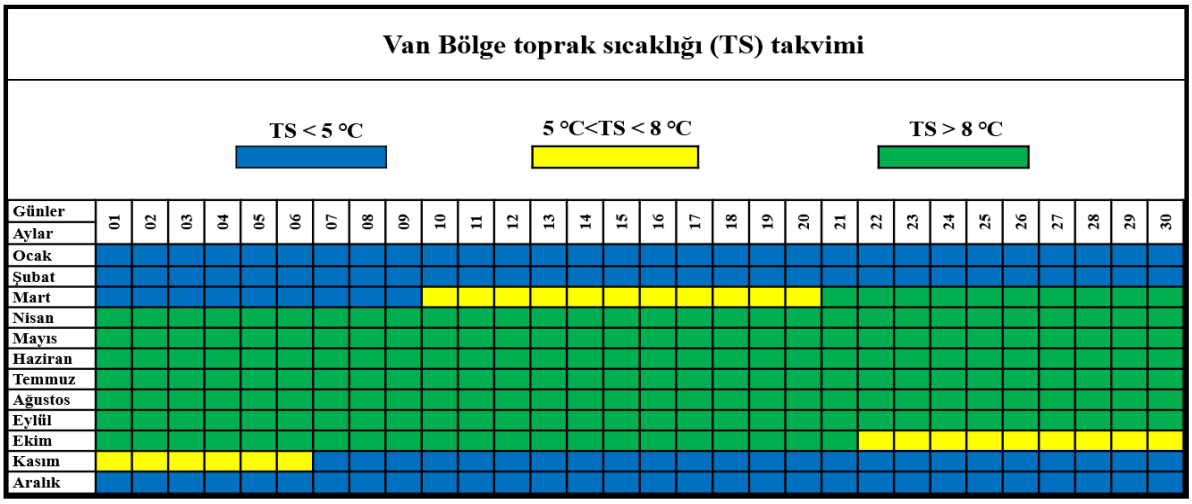
Şekil 5. Bahçesaray ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Başkale Meteoroloji İstasyonu'ndan sağlanan uzun yıllar ortalama (1980-2020 yıllarına ait) değerlerine göre yıllık ortalama sıcaklık 6.4°C olarak gözlemlenmiştir. 29 Kasım – 5 Nisan tarihleri arasında Başkale ilçesinin ortalama toprak sıcaklığı 5°C altındadır. İlçenin 5°C ile 8°C arasındaki ortalama toprak sıcaklığı 6 Nisan ile 21 Nisan ve 11 Kasım ile 28 Kasım tarihlerinde iken, uzun yılların ortalamasının 8°C'in üstündeki günler ise Nisan ayının 22'si ile Kasım ayının 10 olarak gözlemlenmiştir (Şekil 6). Modele göre, Başkale ilçesinin toprak sıcaklık rejiminin "Mesic" olduğu belirlenmiştir.



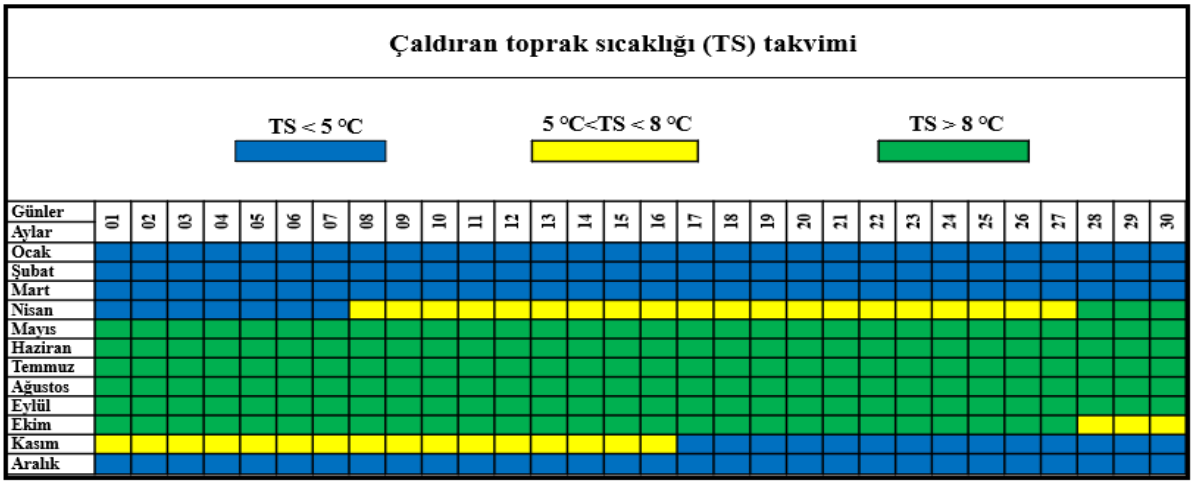
Şekil 6. Başkale ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Van Bölge Meteoroloji İstasyonu'ndan temin edilen uzun yıllar (1980-2020) iklim parametrelerine göre, yıllık ortalama sıcaklık 9.8°C olarak tespit edilmiştir. Thornthwaite iklim modeline göre, Van ilinin potansiyel Evapotranspirasyon 756.31 mm olarak hesaplanmıştır. Yağış miktarının fazla olduğu Şubat, Mart ve Nisan aylarında fazla su (90.2 mm) yüzey akışı ile uzaklaşırken, diğer aylarda ise su noksanlığının (439.37 mm) olduğu görülmektedir (Soil Taxonomy 1999). Toprak sıcaklığının 7 Kasım ile 9 Mart tarihleri arasında 5°C'nin altında olduğu tespit edilmiştir. 5°C- 8°C arasındaki toprak sıcaklığının ise model yardımıyla 10 Mart -20 Mart tarihleri ve 22 Ekim ile 6 Kasım tarihleri arasında olduğu belirlenmiştir. 8°C'nin üzerinde olan toprak sıcaklığı günleri 21Mart ile 21 Ekim tarihleridir (Şekil 7). Van il toprak sıcaklık rejiminin model sonucuna göre "Mesic" olduğu bulunmuştur.



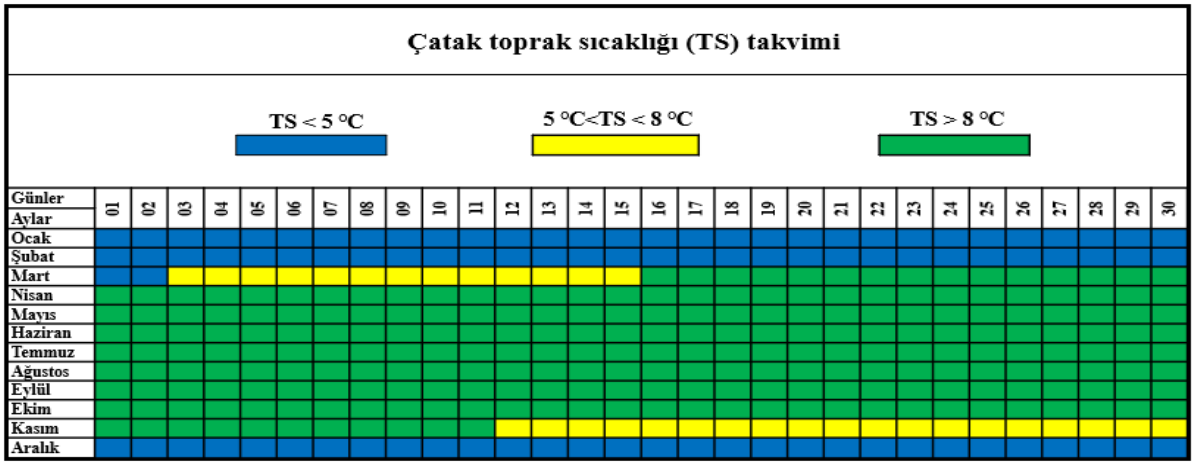
Şekil 7. Van Bölge Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Çaldıran Meteoroloji İstasyonundan temin edilen uzun yıllar (2013-2020) meteorolojik verilere göre, yıllık ortama sıcaklığı diğer tüm ilçelere göre en düşük değere sahip olup 5.9°C olarak tespit edilmiştir. İlçenin Thornthwaite iklim modeline göre bulunan yıllık potansiyel Evapotranspirasyon miktarı 622.39 mm'dir. 17 Kasım ile 7 Nisan tarihlerinde Çaldıran ilçesinin toprak sıcaklığı 5°C'nin altına düşmektedir. 8 Nisan ile 27 Nisan tarihleri arasında ve 28 Ekim ile 16 Kasım tarihleri arasında toprak sıcaklığı 5°C ila 8°C arasındayken, 28 Nisan ile 27 Ekim tarihlerinde ise sıcaklığın 8°C üstüne çıkmıştır (Şekil 8). Modelden elde edilen sonuca göre, Çaldıran ilçesinin toprak sıcaklık rejiminin "Mesic" olduğu belirlenmiştir.



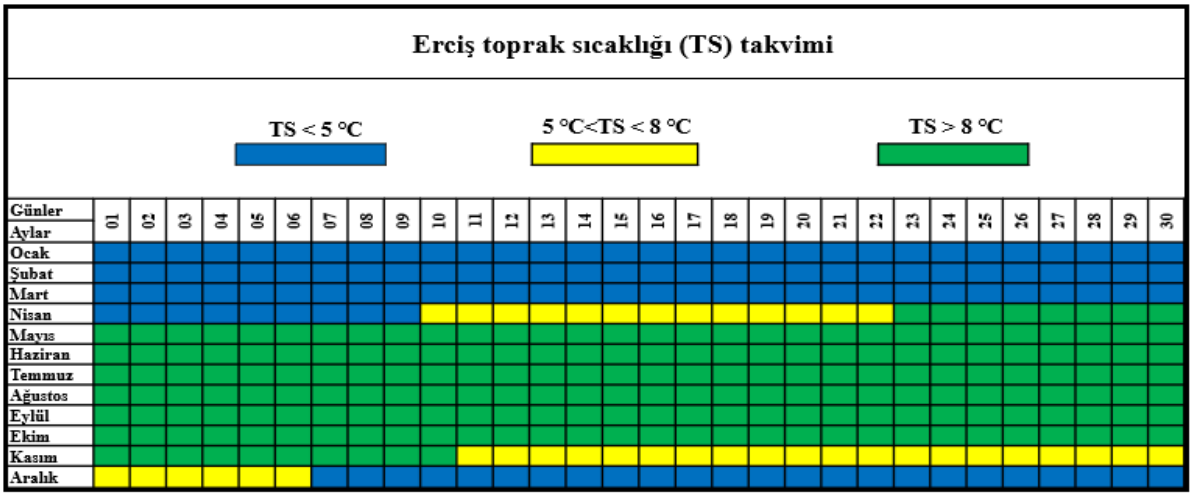
Şekil 8. Çaldıran ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Çatak Meteoroloji İstasyonu'ndan temin edilen uzun yıllara ait (2015-2020) meteorolojik veriler göz önüne alındığında, ilçenin yıllık ortalama toplam sıcaklığı diğer tüm ilçelere göre en yüksek değere sahip olup, 11.0°C olarak gözlemlenmiştir. İlçenin meteorolojik verileri Thornthwaite iklim modeline göre hesaplandığında, yıllık potansiyel evapotranspirasyon miktarı 755.53 mm olarak görülmüştür. İlçenin toprak sıcaklığına bakıldığında, 1 Aralık ile 2 Mart tarihleri arasında 5°C'nin altına inmiştir. 5°C ila 8°C arasındaki toprak sıcaklığı 3Mart ile 15 Mart ve 12 Kasım ile 30 Kasım tarihleri arasında gözlemlenirken, 8°C'nin üstündeki toprak sıcaklığına ise 16 Mart ile 11 Kasım tarihleri arasında çıkmıştır (Şekil 9). Çatak ilçesinin toprak sıcaklık rejimi, model sonucuna göre "Mesic" olarak bulunmuştur.



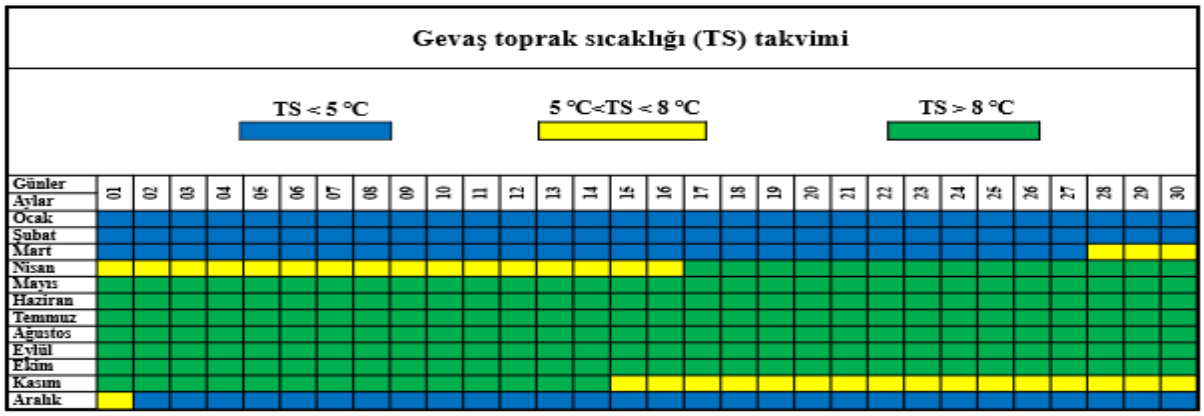
Şekil 9. Çatak ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Erciş Meteoroloji İstasyonu'ndan temin edilen uzun yıllar ortalamasına ait (2009-2020) meteorolojik ölçümler incelendiğinde, ilçenin yıllık ortalama sıcaklığı 8.1°C olarak tespit edilmiştir. Erciş ilçesi ortalama meteorolojik verileri Thornthwaite iklim modeline göre hesaplandığında, ilçenin toplam 402.07mm'lik su noksanlığı olduğu görülmektedir. Ortalama toprak sıcaklığına bakıldığında, 7 Aralık ile 9 Nisan tarihleri arasında 5°C'nin altında görülmüştür. 5°C ile 8°C arasındaki toprak sıcaklığı 10 Nisan ile 22 Nisan ve 11 Kasım ile 6 Aralık tarihleri arasında gözlemlenmiştir. 23 Nisan ile 10 Kasım tarihleri arasında ise toprak sıcaklığının 8°C'nin üstüne görülmüştür (Şekil 10). Erciş ilçesinin toprak sıcaklık rejimi, model sonucuna göre "Mesic" olarak bulunmuştur.



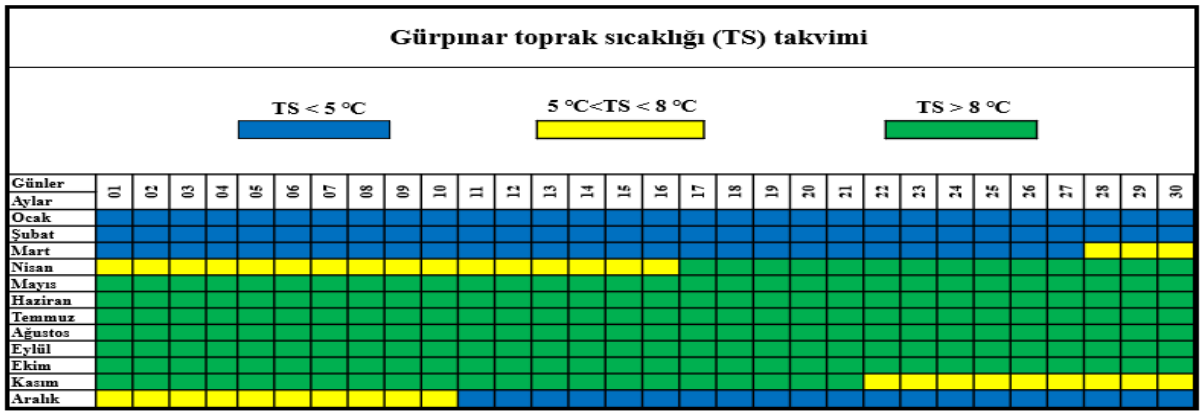
Şekil 10. Erciş ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Gevaş Meteoroloji İstasyonu'ndan alınan uzun yıllara ait (2009-2020) meteorolojik ölçümlere bakıldığında, ilçesinin yıllık ortalama sıcaklığı 9.1°C olarak tespit edilmiştir. Thornthwaite iklim modeline göre, Gevaş ilçesinde potansiyel evapotranspirasyon 739.19 mm ve yağışın fazla olduğu Ocak, Şubat, Mart, Nisan ve Mayıs aylarındaki su fazlalığı ise 124.66 mm olarak görülmüştür. 5°C'nin altındaki uzun yıllar ortalama toprak sıcaklığı, 2 Aralık ile 27 Mart tarihleri arasında bulunmuştur. İlçenin 5°C ile 8°C arasındaki uzun yıllar ortalama toprak sıcaklığı 28 Mart ile 16 Nisan ve 15 Kasım ile 1 Aralık tarihleri arasında görülürken. 28 Mart ile 14 Kasım tarihleri arasında ise toprak sıcaklığı 8°C'nin üstüne çıkmıştır (Şekil 11). Model sonucuna bakıldığında, Gevaş ilçesinin toprak sıcaklık rejiminin "Mesic" olduğu belirlemiştir.



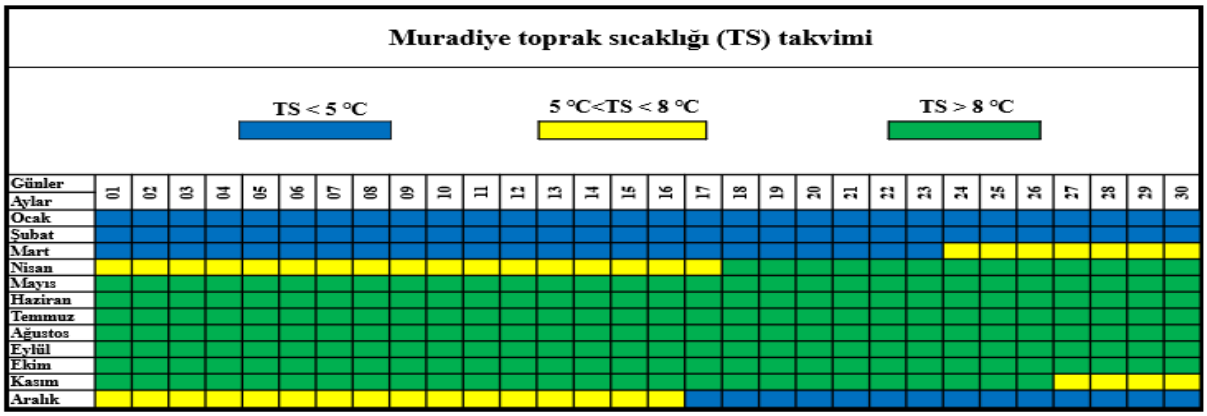
Şekil 11. Gevaş ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Gürpınar Meteoroloji İstasyonu'ndan alınan uzun yıllara ait ortalama (2013-2020) meteorolojik ölçüm parametreleri incelendiğinde, ilçenin yıllık ortalama sıcaklığı 9.5°C olduğu belirlenmiştir. Thornthwaite iklim modeline göre, Gürpınar ilçesinin ortalama potansiyel Evapotranspirasyon 705.17 mm, gerçek Evapotranspirasyon 429.26 mm ve su fazlalığı olmadığı görülmüştür. Uzun yıllar ortalama 5°C'nin altındaki toprak sıcaklığı, 11 Aralık ile 27 Mart tarihleri arasında bulunmuştur. Uzun yıllar ortalamasına göre 5°C ile 8°C arasındaki toprak sıcaklığı 28 Mart ile 16 Nisan ve 22 Kasım ile 10 Aralık tarihleri arasında görülürken, 17 Nisan ile 21 Kasım tarihleri arasında ise toprak sıcaklığının 8°C'nin üstüne çıktığı görülmüştür (Şekil 12). Model sonucuna bakıldığında, Gürpınar ilçesinin toprak sıcaklık rejiminin "Mesic" olduğu belirlemiştir.



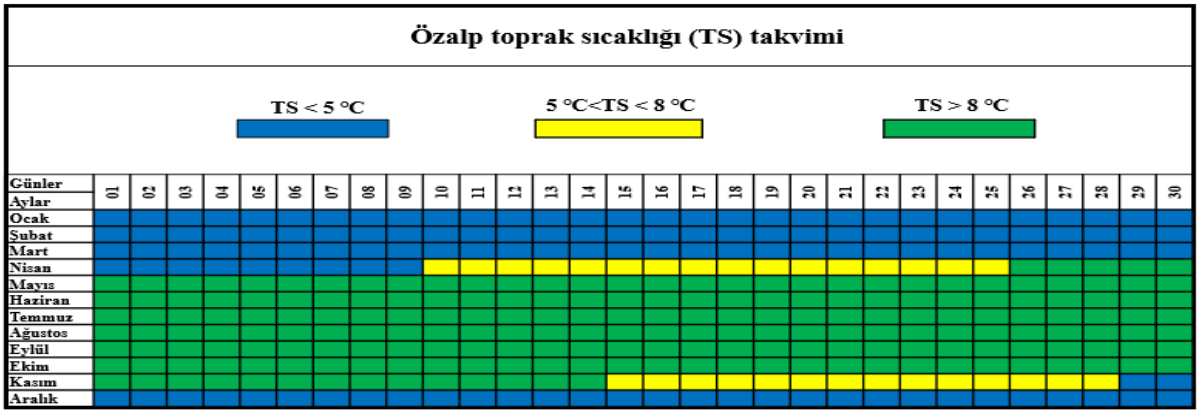
Şekil 12. Gürpınar ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Muradiye Meteoroloji İstasyonu'ndan temin edilen uzun yıllar ortalamasına ait (2009-2020) meteorolojik ölçümler incelendiğinde, ilçenin yıllık ortalama sıcaklığı 9.0°C olarak tespit edilmiştir. Muradiye ilçesinin meteorolojik verileri Thornthwaite iklim modeline göre değerlendirildiğinde, ilçenin potansiyel Evapotranspirasyon 729.83 mm olmasına rağmen, gerçek Evapotranspirasyon değerinin 285.61 mm olarak hesaplanmıştır. Yağışın fazla olduğu Şubat, Mart ve Nisan aylarında yüzey akışı görülürken, Temmuz, Ağustos, Eylül ve Ekim aylarında ise su noksanlığı olduğu görülmektedir. Uzun yıllar ortalama toprak sıcaklığına bakıldığında, 17 Aralık ile 23 Mart tarihleri arasında 5°C'nin altında görülmüştür. 5°C ile 8°C arasındaki toprak sıcaklığı 24 Mart ile 17 Nisan tarihleri arasında ve 27 Kasım ile 17 Aralık tarihleri arasında gözlemlenmiştir. 18 Nisan ile 26 Kasım tarihleri arasında ise uzun yıllar ortalama toprak sıcaklığı 8°C'nin üstüne çıktığı görülmüştür (Şekil 13). Muradiye ilçesinin toprak sıcaklık rejimi, model sonucuna göre "Mesic" olarak bulunmuştur.



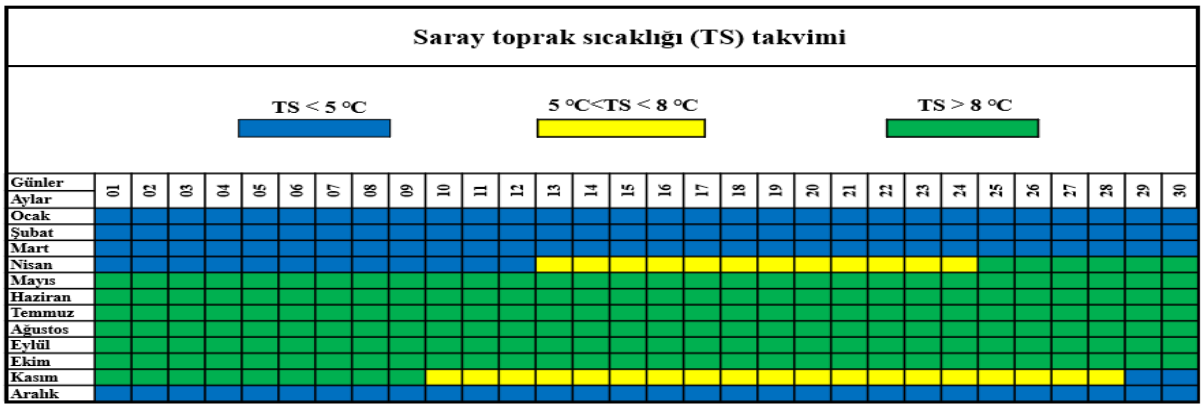
Şekil 13. Muradiye ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Özalp Meteoroloji İstasyonu'ndan temin edilen uzun yıllara ait (2009-2020) meteorolojik ölçümlere incelendiğinde, ilçenin yıllık ortalama sıcaklığı 6.2°C olarak tespit edilmiştir. Özalp ilçesinin meteorolojik verileri Thornthwaite iklim modeline göre uzun yıllar ortalama iklim verileri değerlendirildiğinde, ilçenin potansiyel evapotranspirasyon 679.14 mm olduğu görülürken, Temmuz, Ağustos, Eylül ve Ekim aylarındaki toplam su noksanlığı 381.74 mm olarak tespit edilmiştir. 28 Kasım ile 9 Nisan tarihleri arasında uzun yıllar ortalama toprak sıcaklığı, 5°C'nin altına görülmüştür. 10 Nisan ile 25 Nisan tarihleri ve 15 Kasım ile 28 Kasım tarihleri arasında uzun yıllar ortalama toprak sıcaklığı 5°C ila 8°C arasında gözlemlenirken, toprak sıcaklığı 8°C'nin üstüne ise 26 Nisan ile 14 Kasım tarihleri arasında çıkmaktadır (Şekil 14). Özalp ilçesinin toprak sıcaklık rejimi, model sonucuna göre "Mesic" olarak bulunmuştur.



Şekil 14. Özalp ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

Saray Meteoroloji İstasyonu'ndan temin edilen uzun yıllara ait ortalama (2015-2020) meteorolojik ölçümlere incelendiğinde, ilçenin yıllık ortalama sıcaklığı 7.3°C ve yıllık toplam yağış ise 354 mm olarak tespit edilmiştir. Saray ilçesinin meteorolojik verileri Thornthwaite iklim modeline göre incelendiğinde, ilçenin potansiyel evapotranspirasyon 673.62 mm olduğu görülmüştür. 29 Kasım ile 12 Nisan tarihleri arasında Saray ilçesinin uzun yıllar ortalama toprak sıcaklığı 5°C'nin altına düşmüştür. 5°C ila 8°C arasındaki toprak sıcaklığı 13 Nisan ile 24 Nisan tarihleri ve 10 Kasım ile 28 Kasım tarihleri arasında görülürken, 8°C'nin üstündeki uzun yıllar ortalama toprak sıcaklığı ise 25 Nisan ile 9 Kasım tarihleri arasında olduğu belirlenmiştir (Şekil 15). Saray ilçesinin de toprak sıcaklık rejimi, model sonucuna göre "Mesic" olarak bulunmuştur.



Şekil 15. Saray ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak sıcaklık takvimi.

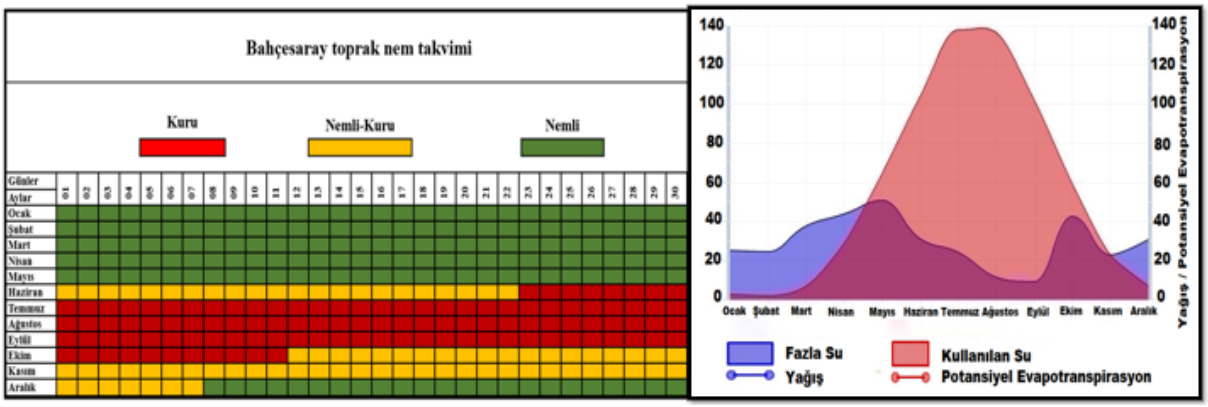
3.3. Van ve ilçelerinin Newhall Simülasyon Modeline (NSM) göre toprak nem dağılımı

Bahçesaray Meteoroloji İstasyonundan temin edilen uzun yıllara ait iklim parametrelerine göre, ilçenin yıllık toplam yağışı 740.35 mm olup, Thornthwaite modeline göre potansiyel evapotranspirasyon miktarı ise 705.17 mm'dir. İlçenin toprak nem takvimine bakıldığında; son bahar ve kış aylarında yağışların başlaması ile toprakta 8 Kasım'da nemli durum başlamış ve 30 Mayıs'a kadar da devam etmiştir. 1 Haziran- 7Aralık tarihleri arasında ise toprak "nemli-kuru" ve "kuru" dönem olarak görülmüştür. Bahçesaray'da toprak 109 gün "kuru", 78 gün "nemli-kuru" ve 173 gün ise "nemli" kalmıştır. Topraktaki su akışı Aralık ayı ortalarında başlayıp ve Mayıs ayının ilk haftasına kadar gerçekleşmiştir. Su akışının sona erdiği günden itibaren Evapotranspirasyon ile kullanılan su, düşük miktardaki yağış ile karşılanamamıştır. Bundan dolayı, Temmuz ayında 151.93 mm'lik su noksanlığı görülmüştür (Şekil 16). Yıllık toplam 400.46 mm su yetersizliği belirlenmiştir. Bu istasyon için "Dry Xeric" toprak nem rejimi belirlenmiştir.

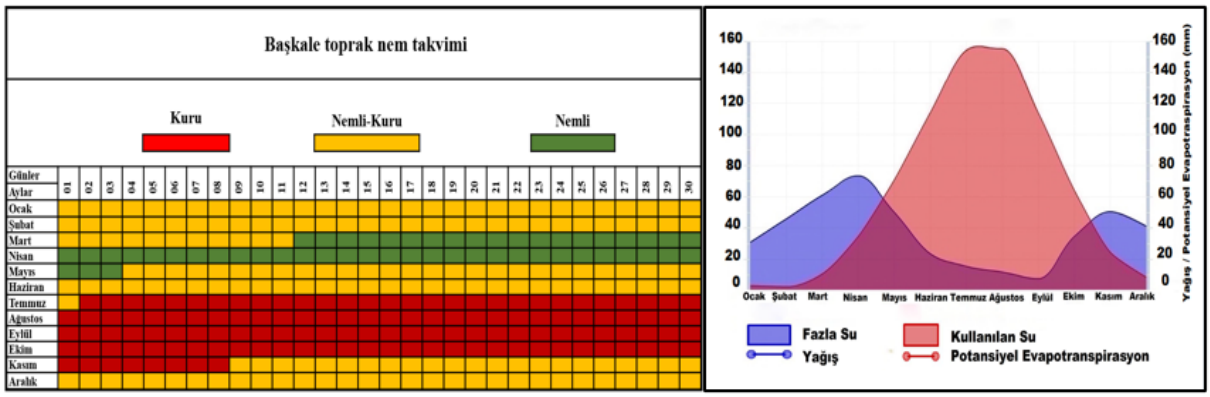
Başkale ilçesinin 1980 ile 2020 yılları arasındaki iklim verileri incelendiğinde, ilçenin yıllık toplam yağışı 354.31mm'dir. Başkale ilçesinin toprak nem takvimine bakıldığında; nemli durum 12 Mart ile 3 Mayıs tarihleri arasındadır. Nemli-kuru dönem ve kuru dönem ise 4 Mayıs ile 11 Mart tarihleri arasında gözlemlenmiştir. Topraktaki yüzey akışı, Mart ayının ortasından başlayıp Nisan ayının sonuna kadarki dönemde 59.76 mm olarak gerçekleşmiştir. Yıllık toplam 367.13 mm su açığı belirlenmiştir. En fazla su yetersizliği ise Ağustos ayında 122. 79 mm olarak görülmüştür. Başkale ilçesinin toprak nem rejimi "Typik Aridic" olarak bulunmuş olup, nem rejiminde toprak 52 gün nemli, 181 gün nemli-kuru ve 127 gün kuru dönem kalmıştır (Şekil 17).

Bölge Meteoroloji İstasyonundan sağlanan 1980-2020 yılları arasındaki ölçüm değerlerine bakıldığında yıllık toplam yağış 407 mm'dir. Toprak nem takvimi incelendiğinde Şubat, Mart ve Nisan aylarında toplam 90.02 mm yüzey akışı gözlemlenirken, Haziran, Temmuz, Ağustos, Eylül ve Ekim aylarında ise toplam 439.37 mm su yetersizliği görülmüştür. 14 Kasım-30 Ocak tarihleri ve1 Mayıs-21 Haziran tarihleri arasında toprak "nemli-kuru" dönemde iken, 22 Haziran ile 13 Kasım tarihleri arasında ise toprak "kuru" döneme rastlamıştır Topraktaki "nemli" durum 1 Şubat'tan başlayıp 30 Nisan kadar devam etmiştir. Bölge istasyonu için ortaya çıkan "Dry Xeric" nem rejiminde toprak 142 günü kuru, 128 günü nemli-kuru ve geriye kalan 90 günde nemlidir (Şekil 18).

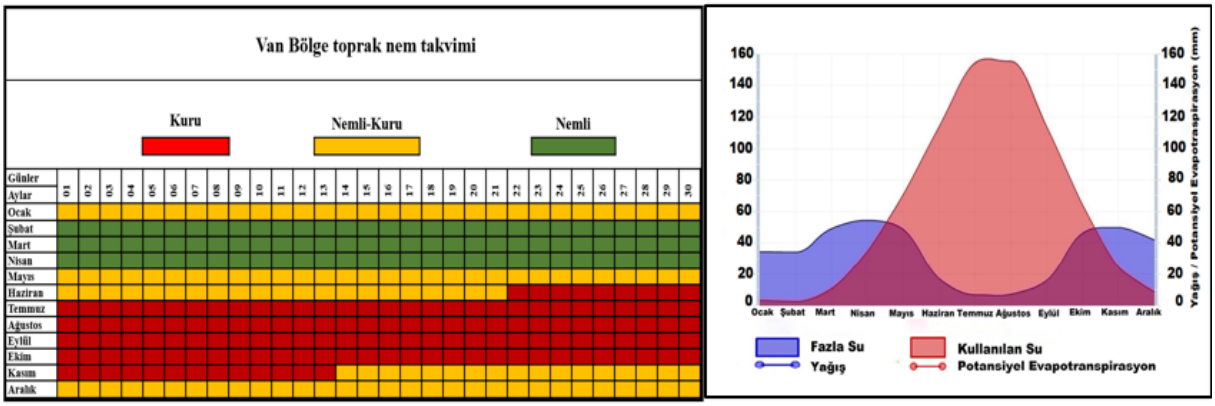
Çaldıran istasyonundan alınan 2013 -2020 yılı ölçüm değerlerine göre, toplam yıllık yağışın 351.53 mm olduğu görülmüştür. İlçenin toprak nem takvimine bakıldığında, Mart ve Nisan aylarında 31. 72 mm yüzey akışı görülürken, Temmuz, ila Ekim aylarında ise 302.56 mm su noksanlığı görülmüştür. Topraktaki "nemli" dönem 2 Mart ile 1Mayıs tarihleri arasında görülmüştür. 2 Mayıs ile 1 Mart tarihleri arasında "nemli-kuru" ve "kuru" dönem olduğu gözlemlenmiştir. İlçe toprağı 60 gün "nemli", 118 gün "kuru" ve 182 günde "nemli-kuru" kalmıştır (Şekil 19). Toprak nem rejimi ise "Typic Aridic" olarak bulunmuştur.



Şekil 16. Bahçesaray ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



Şekil 17. Başkale ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



Şekil 18. Van Bölge Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.

Çatak istasyonundan elde edilen 2015-2020 yıllarına ait iklim parametrelerine incelendiğinde, ilçenin yıllık toplam yağışı 740.40 mm olup, en yüksek yağışa sahip olan ilçedir. İlçenin toprak nem takvimi hesaplandığında; toprakta "nemli" durum 12 Aralık'tan başlayarak ve 3 Mayıs'a kadar devam etmiştir. 4 Mayıs-22 Haziran tarihleri ve 3 Ekim-11 Aralık tarihleri arasında toprak "nemli-kuru" dönem görülürken, 23 Haziran ile 2 Ekim tarihleri arasında "kuru" dönem olarak görülmüştür. Çatak ilçesinde toprak 100 gün "kuru", 118 gün "nemli-kuru" ve 142 gün ise "nemli" kalmıştır. Topraktaki su akışı Aralık ayı ilk günleri meydana gelmiş ve Nisan ayının son haftasına kadar görülmüştür. Topraktaki su fazlalığının sona erdiği günden itibaren Evapotranspirasyon ile kullanılan su, düşük miktardaki yağış ile karşılanamamıştır. Bundan dolayı, 146.36 mm'lik su yetersizliği temmuz ayında saptanmıştır. Yaz

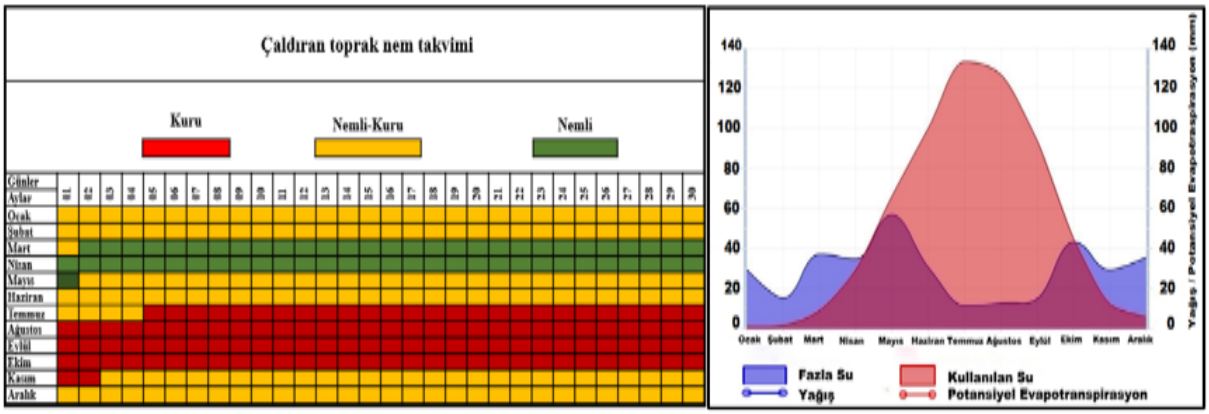
ayları boyunca toplam 403.46 mm su yetersizliği belirlenmiştir (Şekil 20). Bu istasyon için "Dry Xeric" toprak nem rejimi belirlenmiştir.

Erciş ilçesinin 2009-2020 yıllarına ait meteorolojik verilerine bakıldığında, ilçenin yıllık toplam yağış miktarı 314.9 mm'dir. Erciş ilçesinin toprak nem takvimine bakıldığında, 4 Aralık-16 Mart tarihleri arasında toprak "nemli-kuru" dönem görülmesine rağmen, kış aylarında yağışların başlaması ile toprak 17 Mart'ta "Nemli" döneme girmiş ve bu durum 6 Mayıs'a kadar devam etmiştir. 7 Mayıs-23 Haziran tarihleri arasında toprak tekrar "nemli-kuru" dönem geçiş yapmıştır. 24 Haziran ile 3 Aralık tarihleri arasında toprakta "kuru" dönem gözlemlenmiştir. Toprağın yüzey akış miktarı 55.92 mm iken, su noksanlık miktarı ise 402.07 mm olarak hesaplanmıştır (Şekil 21). Toprak nem rejimi "Typic Aridic" olarak bulunan ilçenin toprakları 160 gün kuru, 150 gün nemli- kuru ve 50 günü de nemli geçirmiştir.

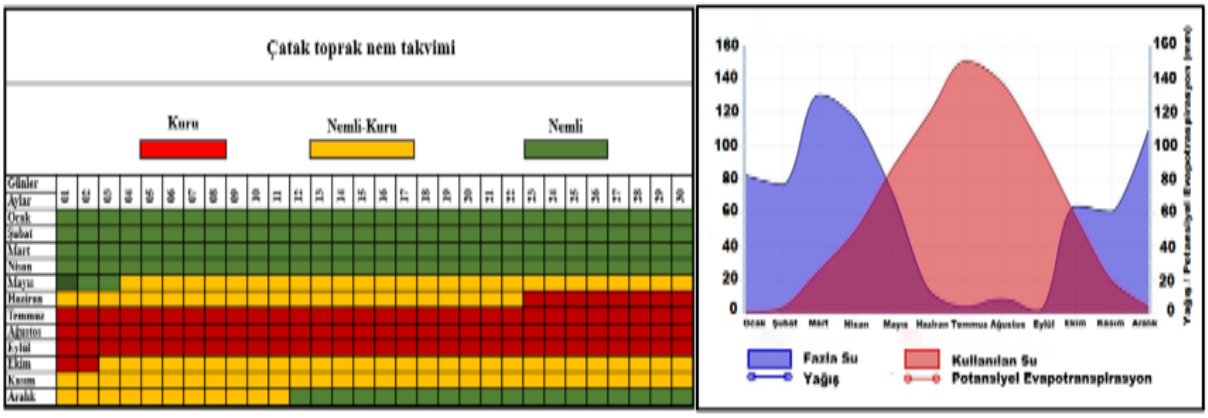
Gevaş ilçesi meteorolojik istasyonundan alınan 2009-2020 yıllarına ait ölçüm değerleri incelendiğinde, ilçenin yıllık toplam yağış miktarı 422.47 mm'dir. Gevaş ilçesinin toprak nem takvimi değerlendirildiğinde, 15 Kasım-11 Ocak tarihleri arasında toprak "nemli-kuru" dönem görülmesine rağmen, sonbahar ve kış aylarında yağışların başlaması ile toprak 12 Ocak'ta "nemli" dönem görülmeye başlamış ve 2 Haziran'a kadar bu durum devam etmiştir. Hava sıcaklığının 8°C üstünde seyrettiği 3 Haziran ila 27 Haziran tarihleri arasında "nemli-kuru" dönem ve 28 Haziran ile 14 Kasım tarihleri arasında ise toprakta "kuru" döneme görülmüştür. Ocak ile Mayıs ayları arasında toprakta yüzey akışı gerçekleşirken, en yüksek akış miktarı ise 46.49 mm ile Mart ayında olmuştur. Su noksanlığı miktarı ise 413.77 mm olarak bulunmuştur (Şekil 21). İlçe toprakları sırasıyla 137 gün "kuru", 83 gün "nemli- kuru" ve 140 günü de "nemli" dönem geçirmiştir (Şekil 22). Gevaş ilçe toprakları Newhall modeline göre, "Dry Xeric" toprak nem sınıfı olarak belirlenmiştir.

Gürpınar ilçesinin 2013 ile 2020 yılları arasındaki iklim verilerine bakıldığında, ilçenin yıllık toplam yağışı 275.90 mm ile en düşük yağış miktarına sahip istasyonudur. Gürpınar ilçesinin toprak nem takvimine bakıldığında; "nemli-kuru" dönem 15 Aralık-26 Nisan tarihleri ve 6 Mayıs-23 Haziran tarihleri arasında görülürken, "kuru" dönem ise 24 Haziran- 14 Aralık tarihleri arasında bulunmuştur. Nemli dönem ise 27 Nisan-5 Mayıs tarihleri arasında gözlemlenmiştir. Evapotranspirasyon ile kullanılan su yağış ile karşılanamadığı için toprak yüzey akışı görülmemiştir. Bundan dolayı, Haziran ile Kasım ayları arasında toprakta 429.26 mm'lik su noksanlığı belirlenmiştir. Gürpınar ilçesinin toprak nem rejimi "Typic Aridic" olarak bulunmuş olup, nem rejiminde toprak 9 gün "nemli", 180 gün "nemli-kuru" ve 179 gün "kuru" durum bulunmaktadır (Şekil 23).

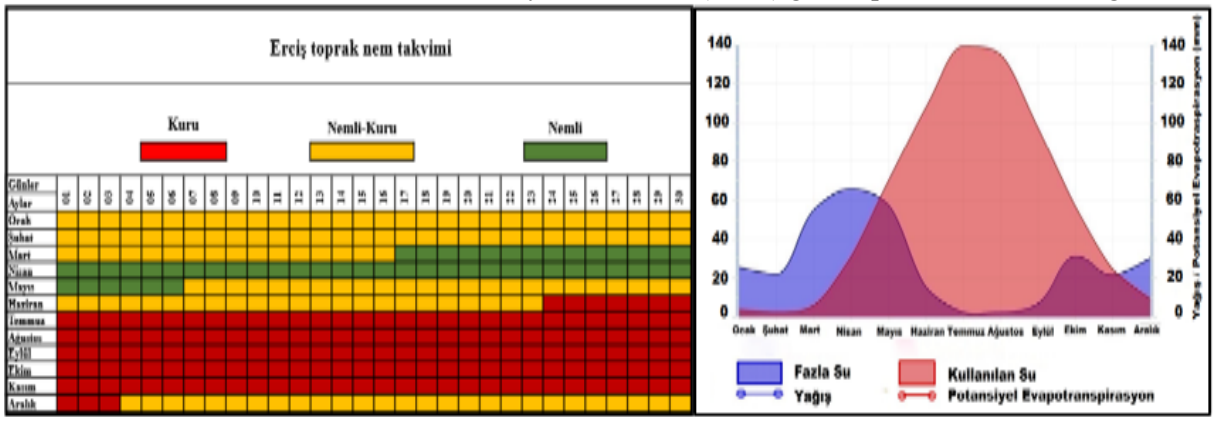
Muradiye meteoroloji istasyonundan elde edilen 2009-2020 yıllarına ait ölçüm değerleri hesaplandığında, ilçenin yıllık toplam yağışı 444.22 mm'dir. İlçenin toprak nem takvimi incelendiğinde; ilkbahar aylarındaki yağışlarının artması ile "nemli" dönem 7 Şubat'ta başlayıp 23 Mayıs tarihine kadar devam etmiştir. 24 Mayıs'tan başlayıp 6 Şubat' kadar toprakta "nemli-kuru ve "kuru" dönemler görülmüştür. Muradiye ilçesinde toprak 135 gün "kuru", 118 gün "nemli-kuru" ve 107 gün ise "nemli" kalmıştır. Topraktaki su akışı Şubat ayının ikinci haftası başlayıp, Nisan ayının son haftasına kadar görülmüştür. Topraktaki evapotranspirasyon ile kullanılan su, düşük miktardaki yağış ile karşılanamamıştır. Bundan dolayı, Temmuz ile Ekim ayları arasında toprakta toplam 393.40 mm'lik su eksikliği yaşanmıştır (Şekil 24). Muradiye istasyon için "Dry Xeric" toprak nem rejimi belirlenmiştir.



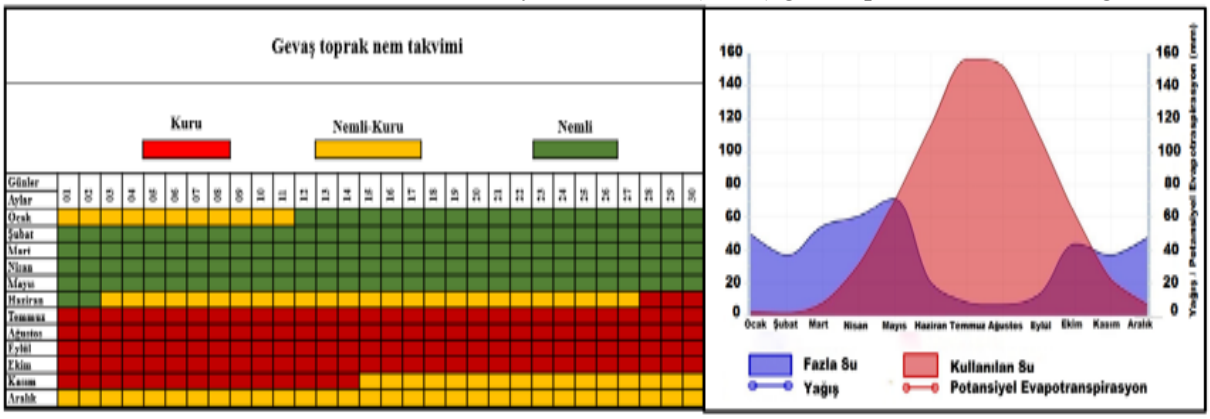
Şekil 19. Çaldıran ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



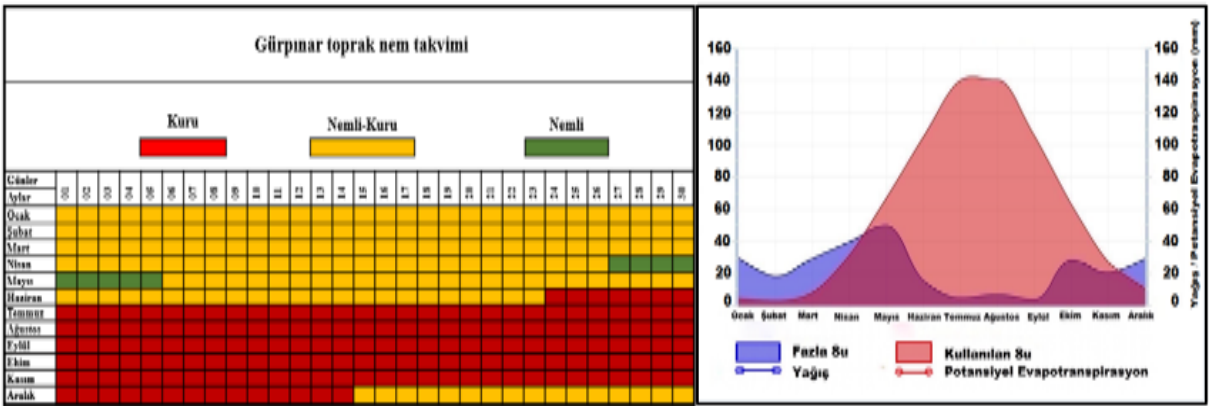
Şekil 20. Çatak ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



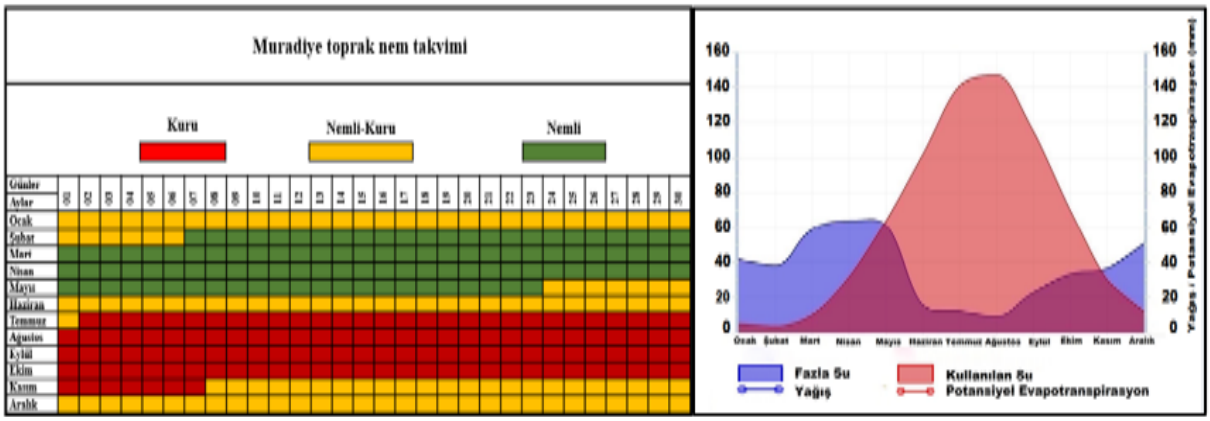
Şekil 21. Erciş ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



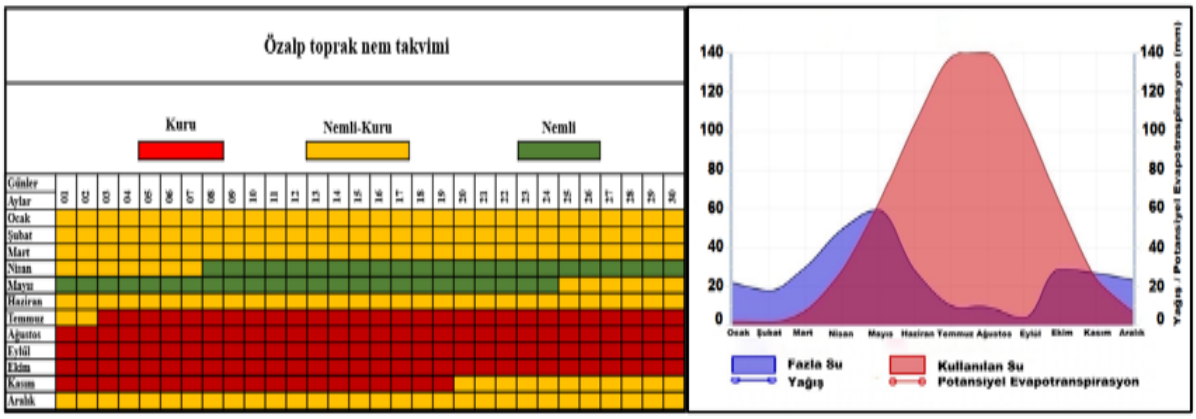
Şekil 22. Gevaş ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



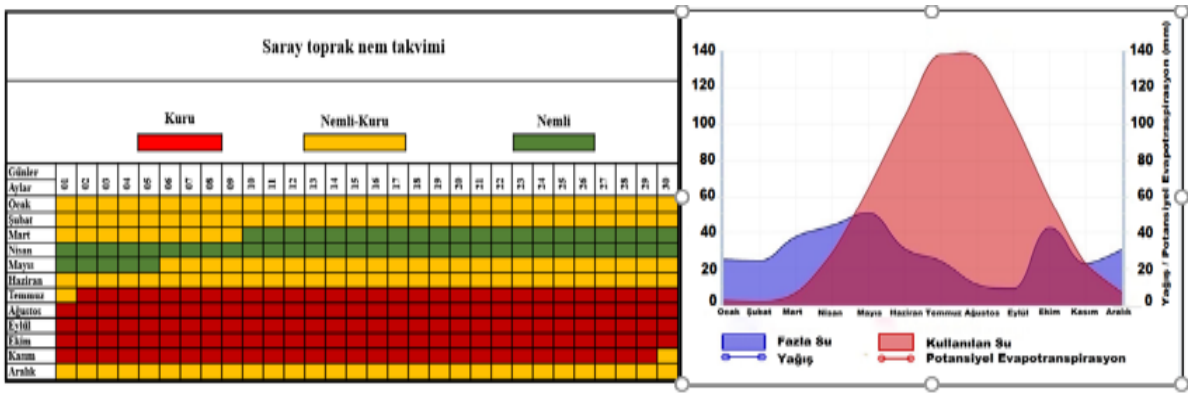
Şekil 23. Gürpınar ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



Şekil 24. Muradiye ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



Şekil 25. Özalp ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.



Şekil 26. Saray ilçesinin Newhall Simülasyon Modeline (NSM) göre toprak nem bütçesi dağılımı.

Özalp meteoroloji istasyonundan alınan 2009 -2020 yılı ölçüm parametrelerine değerlendirildiğinde, yıllık toplam yağışı 306.60 mm'dir. İlçenin toprak nem takvimine bakıldığında, Nisan ayında 9.33 mm yüzey akışı görülürken, Temmuz ila Ekim aylarında ise toprakta 381.87 mm su noksanlığı görülmüştür. Topraktaki "nemli" dönem 8 Nisan'da başlamış 24 Mayıs'a kadar devam etmiştir. 25 Mayıs ile 7 Nisan tarihleri arasında "nemli-kuru" ve "kuru" dönem olduğu görülmüştür. İlçe toprağı 47 gün "nemli", 137 gün "kuru" ve 176 günde "nemli-kuru" kalmıştır (Şekil 25). Toprak nem rejimi ise "Typic Aridic" olarak bulunmuştur.

Saray ilçesi Meteoroloji İstasyonundan temin edilen 2015-2020 yıllarına ait iklim verilerine göre, ilçenin yıllık toplam yağışı 353.98 mm olup, en yüksek yağış miktarı ise 50.77 mm ile Mayıs ayıdır. İlçenin toprak nem takvimine bakıldığında; 10 Mart'ta nemli durum başlamış ve 5 Mayıs'a kadar da devam etmiştir. 6 Mayıs-1 Temmuz tarihleri ve 30 Kasım-9 Mart tarihleri arasında ilçe toprağı "nemli-kuru" görülürken, 2 Temmuz ila 29 Kasım tarihleri arasında ise toprak "kuru" dönem olarak

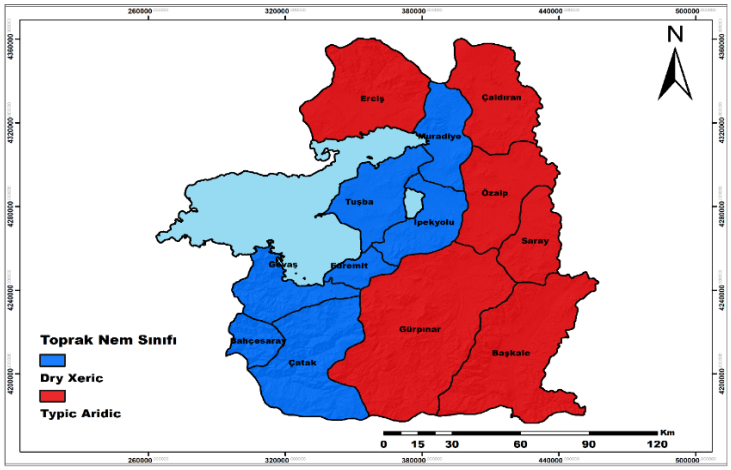
bulunmuştur. Saray ilçesinin toprağın da su akışı Mart ve Nisan aylarında toplam 24.37 mm olarak gerçekleşmiştir. Su akışının sona erdiği günden itibaren ise Evapotranspirasyon ile kullanılan su, düşük miktarlardaki yağış ile geri dönüşümü sağlanamamıştır. Bundan dolayı, ilçe toprağında Temmuz-Ekim ayları arasında su noksanlığı görülmüştür. En yüksek su eksikliğinin ise 124.75 mm olarak Ağustos ayında olduğu hesaplanmıştır Yıllık su noksanlığı ise toplamda 344 mm olarak belirlenmiştir. Saray ilçesinde toprak 148 gün "kuru", 156 gün "nemli-kuru" ve 56 gün ise "nemli" kalmıştır (Şekil 26). Bu istasyon için "Dry Xeric" toprak nem rejimi belirlenmiştir.

Toprak sınıflandırılmasında, iklim rejimlerinin belirlenmesi ve sınıflandırma sistemi içerisinde kullanımının önemli bir yeri bulunmaktadır. Toprak sıcaklık ve nem rejimleri farklı sınıflandırma kategorilerinde bir faktör olarak kullanılmakta ve iklim öğelerinin her biriyle doğrudan ilişkili olduğu için değişkenlik göstermektedir (Başayiğit ve Dinç, 2005). Toprak taksonomisine uygun olarak nem ve sıcaklık rejimlerini model kullanılarak belirlemek, iklim verileri sınıflandırmasında karışıklığa neden olmadan toprak sınıflandırmasında kolaylık sağlayacaktır. Toprak sınıflandırılmasında ve toprak etütlerinde kullanımı kabul gören Newhall modeliyle yapılan çalışmalarda, benzer şekilde toprakların sıcaklık ve nem rejimleri belirlenerek gerekliliği ve önemi vurgulanmıştır (Angel ve ark. 2014, Turan ve ark, 2018).

Küresel ısınma nedeni ile iklim değişikliğinin etkilerinin hissedileceği tarımsal alanlarda, toprak nem rejimi üzerine yapılan modelleme çalışmaları bulunmaktadır. Bu çalışmalarda benzer şekilde iklim rejimleri belirlenerek, ileri tarihli senaryo analizlerinde kullanılmıştır. Bu çalışmaların sonuçlarına göre, sıcaklık artışı ile birlikte evapotranspirasyonun potansiyel olarak önemli oranda artacağı vurgulanmıştır (Kayam ve Aydın 2017; Deveci ve ark., 2019). Elde edilen bulguların, günümüzde önemi gittikçe artan küresel ısınma ve iklim değişikliğinin tarımsal açıdan etkilerini belirlemek amacıyla yapılacak çalışmalar için faydalı olacağı görülmektedir.

Sonuç

Van il ve ilçe sınırları içerisinde bulunan 11 meteorolojik veri ölçüm istasyonundan temin edilen iklim parametreleri (uzun yıllara ait aylık ortalama sıcaklık ve aylık ortalama yağış), Newhall Simülasyon modeli kullanılarak il ve ilçe topraklarının sıcaklık ve nem rejimleri bulunmuştur. Elde edilen sonuçlara bakıldığında, il ve ilçelerin tamamının toprak sıcaklık rejimi "Mesic" olarak belirlenmişken, toprakların nem rejimleri ise" Dry Xeric" ve "Typic Aridic" sınıf içerisinde yer almıştır. Bahçesaray, Çatak, Gevaş ve Muradiye ilçeleri ile Van bölge topraklarının nem rejimi Dry Xeric olarak görülmüştür. Başkale, Çaldıran, Erciş, Gürpınar, Özalp ve Saray ilçelerinin ise toprak nem rejimi "Typic Aridic" olarak bulunmuş ve sonuç haritası olarak Şekil 27'de verilmiştir.



Şekil 27. Van il ve ilçe topraklarının Newhall Simülasyon Modeline göre nem sınıfları.

Çalışmada Newhall modeliyle nem ve sıcaklık rejimlerinin elde edilmesi; Özellikle küresel ısınmanın etkin bir şekilde görüldüğü son yıllarda yaşanan kuraklıktan dolayı bölgedeki su bütçesinin doğru hesaplanmasında, toprakta su noksanlığının etkin olduğu zamanların tespitinde ve kuraklık eylem

planlamalarının hazırlanmasında, toprak mikroorganizmalarının yaşamsal faaliyetlerinde etkili olan makro floranın incelenmesinde, toprakta cereyan eden fiziksel, kimyasal ve biyolojik olan tüm önemli faaliyetlerin etkinliğinde ve toprak sınıflandırmasındaki grupların belirlenmesinde fayda sağlayacaktır.

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Research Article

Preparation and Physicochemical Properties of Cucurbit Protein Isolate from *Lagenaria siceraria* Defatted Seed Flour

Abdalbasit A. MARIOD[*1], Makarim M. MUSTAFA[2], Abdelazim A. M. NOUR[3] Mahmood A. ABDALLAH[4]

[1]Ghibaish College of Science and Technology, Indigenous Knowledge Center, Ghibaish, Sudan
[1]University of Jeddah, College of Sciences and Arts, Alkamil, Saudi Arabia
[2]Department of Food Science, University of Bahri, Khartoum North, Sudan
[3]Department of Food Science & Technology, Faculty of Agriculture, University of Khartoum, Sudan
[4]Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia

[1]https://orcid.org/ 0000-0002-9593-7941, [2]https://orcid.org/ 0000-0003-3237-7948, [4]https://orcid.org/0000-0003-0372-4541
Third author was passed away

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

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Isolate,
Lagenaria siceraria,
Functional properties

Abstract: Cucurbit seeds including *Lagenaria siceraria* seeds have been used from early times as a source of food. The protein constitutes about thirty-five percent of the weight of the decorticated seeds. Isolates are defined as the kind of protein that forms with a high concentration of protein that contains no dietary fiber. This study aims to investigate the preparation of protein isolated from *Lagenaria siceraria* defatted seed flour and to study their physicochemical properties. Protein was isolated from seed flours by using distilled water, salt solution, an alkaline solution, then precipitated with 0.1 N hydrochloric acid followed by centrifugation and freeze-drying. The findings reflected a high protein content (ranged from 80.67-91.04 g $100g^{-1}$) with the absence of fats. The functional properties of the protein isolate are said to be acceptable exhibiting a considerable range for all examined properties, water holding capacity 2.4-3.9 mL g^{-1} protein, fat binding ability 2.7-3.7 mL g^{-1} protein, emulsion capacity 51.0-57.8 mL of oil per g of protein, and bulk density 0.39-0.56 g mL^{-1}, for all samples. The results of foaming stability indicated that the seeds were possibly utilized in food applications such as beverages and cakes processing. The maximum protein solubility (95%) occurred at the alkaline range pH 10 and11. The SDS page test detected that the three major polypeptides are those with molecular weights 10, 23, and 38 KDa. This study is the first to take place on the protein isolation from eight Lagenaria siceraria defatted seeds grown in Sudan, opening the way for further studies on these seeds

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1. Introduction

Bottle gourds are available in two varieties, one is bitter bottle gourds and another is a sweet variety, a useful bottle gourd used as a vegetable or as a medicinal plant. The bitter variety is available as a wild plant and used for pharmacological applications (Shah et al., 2010). The bitterness in such bitter varieties is due to the presence of complex tetracyclic triterpenoid compounds known as cucurbitacin (Chen, et al. 2005). All the parts of *Lagenaria siceraria* involved fruits, leaves, stems, flowers, roots, and seeds have been traditionally used in the ointment for the ailment of various diseases throughout the world (Kumar, et al., 2012). The protein amount of *Lagenaria siceraria* seeds is like that of soybeans and pumpkin seeds and much more than groundnut (Fokou and Tchounguep, 2004). Due to the considerable space between the human populace and protein flexibly, it's necessary to look for and examine unconventional oilseeds as a future protein source (Fagbemi, 2007). Ogunbusola, et al. (2010) studied the composition of protein fractions and amino acids of *Lagenaria siceraria* defatted seed flours and found that the seed flours contained enough essential amino acids for young and adults, so, they suggested that the seeds have the importance as protein added value product in cereal diets or to be a protein source in animal proteins in well-known foods. Protein isolates were produced early in America in the late fifties (Jay and Michael, 2004). It is a high percentage of protein with the benefit of natural and useful qualities making it an ideal crude component to be utilized in refreshments, baby diets and kid's milk food, finished protein items, and specific kinds of functional foods (Olaofe, 1998). Protein isolates of the seeds of *Lagenaria siceraria* and other melons with regards to their functional characteristics have been investigated by many scientists (Ogundele, et al., 2015; Oyeleke, 2012). DeMan, (1999) defined the useful characteristics of proteins that influence the protein found in food frameworks during handling, storage, preparation, and utilization. That can refer to the texture, solubility, viscosity, water holding capacity, and fat binding ability, among different properties. According to their role in food systems, functional characteristics are classified into three groups including properties because of hydration, for example, dissolvability and wettability, properties identified with protein structure, for example, viscosity, and properties identified with protein surface, for example, emulsifying and foaming (Siong, et al., 2011). Ogunbusola, et al. (2013) determined the in-vitro protein digestibility of protein isolate prepared from white melon seed flours they reported the highest digestibility which is due to the removal of protease inhibitors during extraction. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) can determine the protein quantity laying on their molecule weights from 1 to 1,000 kDa. The proteins are typically dissolved in either a non-reducing or reducing buffer (Mohamed et al., 2009).

2. Material and Methods

2.1. Materials

2.1.1. Plant materials

Lagenaria siceraria dry fruits, (8 varieties) four of them are sweet gourds including African Kettle (AK), African Zulu (AZ), Snake Gourd (SG), Water Jug (WJ), and the other four are bitter gourds including Basket Ball (BB), Long Handle Dipper (LHD), Calabash Hybrid (CH) and Curved Dipper (CD) were collected from Omdurman market, Sudan.

2.1.2. Chemicals

All the chemicals utilized in this research are of a high analytical grade and quality. They were purchased either from (Sigma-Aldrich, UK) or (Thermo Fisher Scientific, USA) by the University of Malaya.

2.2. Seed preparation

The seeds were separated from the eight different types of *Lagenaria siceraria* dry fruits using a 4.8 mm knife, then, the obtained seeds were cleaned and washed off any adhering residue and sun-dried for 24 h. The dried seeds were then grounded to flour using a home blender. The grounded seeds were packed in plastic containers and kept in a deep freezer for further investigations.

2.2.1. Preparation of the seed extract

100 grams of the crushed materials of each seed flour were extracted sequentially into 500 ml of n-hexane, and the resulting extracts were evaporated and concentrated under reduced pressure using a Rotary evaporator at 50°C. The extracts were then kept in dark glass bottles for further use (Nyakudya et al., 2013).

2.2.2. Preparation of protein isolate

The protein isolates of eight *Lagenaria siceraria* varieties of defatted seed flour was processed following Adebowale et al. (2009) method. 500 mL of distilled water were added to 50 g of defatted seed flour, and the slurry 1:10 one part of the sample to 10 part of distilled water was stirred continuously with a magnetic stirrer for 2 hours at room temperature. The pH of the blend was controlled to pH 10 by the addition of 1% NaOH to make protein solubility to a maximum at pH 10. The slurry was centrifuged at 1800 rpm for half an hour.

The supernatant was collected into a plastic flask, and the protein in the residue was additionally extracted two times using distilled water. The supernatants were collected, and the pH changed with 1 M HCl to already determine the isoelectric points of the seeds.

The extract was additionally centrifuged at 6 000 rpm in a cold centrifuge at 4°C for a quarter an hour. The supernatant was decanted, and the protein isolate was dialyzed against distilled water for one day and afterward freeze-dried. The protein isolates were kept in airtight polythene bags.

2.2.3. Proximate analysis

Moisture, crude protein, fat, and ash contents were determined according to AOAC (2000). Total carbohydrate was determined by the difference for the total as follows:
total carbohydrate [g $100g^{-1}$] = 100 [g $100g^{-1}$] - moisture [g $100g^{-1}$] - protein [g $100g^{-1}$] - lipids [g $100g^{-1}$] - ash [g $100g^{-1}$]

2.3. Functional properties of protein isolates

2.3.1. Determination of water holding capacity

This was calculated utilizing the method reported by Rodriguez et al. (2005); 100 mg of each sample was blended with 1.0 mL of distilled water utilizing a magnetic stirrer. The protein suspension was then centrifuged at 1 800 rpm for 20 min at room temperature. The supernatant was collected, and the tube was drained at 45 angles for 10 min. Water holding capacity was determined by classifying the volume of water absorbed by the weight of the protein sample.

2.3.2. Fat binding ability (FBA)

Kaushik et al. (2016) method was followed to investigate FBA where a hundred milligrams of protein isolate were vortex-mixed with 1 mL of sunflower oil for 30 seconds. The emulsion was incubated at 25ºC for half an hour and afterward centrifuged at 13 600 rpm for 10 min. The supernatant was decanted and drained at a 45 angle for 20 min. The volume of oil absorbed was separated with the weight of the protein sample, to obtain the fat or oil binding ability of the sample.

2.3.3. Determination of foaming properties

The method of Vani and Zayas, (1995) was used to calculate Foaming Capacity (FC), where two grams of protein isolate were mixed with 50.00 cm^3 clean water in a mixer. The mixture was directly put into a 100.00 cm^3 measuring cylinder. The foam volume was measured directly after mixing. The Foaming Stabilities (FS) of the samples were calculated as a function of time for 0-2 hrs.

2.3.4. Determination of emulsifying properties

The method of Pedrocheet al. (2004) was used to measure the Emulsifying capacity (EC), where one gram of each sample was homogenized at a speed of 160 rpm for 1.0 min at room temperature in 25 cm^3 distilled water. The protein solution was mixed with 25 cm^3 of edible oil followed by homogenization at 160 rpm for 1.0 min and finally, the emulsion was centrifuged for 5 min. Emulsifying capacity was determined by measuring the height of the layer separated in the centrifuge tube, expressed as a percent of the total height of the liquid.

2.3.5. Protein solubility

The method of Klompong et al. (2007) was used, whereby 200 mg of the sample was dispersed in 20 mL of deionized water, and the pH was controlled to 2, 4, 6, 8, 10, and 12 with 1.0 or 0.1 N HCl and 1.0 or 0.1 N NaOH. The mixture was stirred at 20 °C for half an hour and then centrifuged at 7 500 g for 15 min. The protein percentage in the supernatant was calculated using the Bradford kit method (Bradford, 1976). Soluble protein was calculated as g $100g^{-1}$ DW sample.

2.3.6. Determination of in-vitro protein digestibility (IVPD)

The method of Hsu et al. (1977) was followed to determine the IVPD of the studied samples where Multienzyme solution was used. The IVPD was calculated using Hsu et al. (1977): IVPD = 65.66 + 18.1ΔpH 10min.

2.3.7. SDS–PAGE analysis SDS–polyacrylamide gel electrophoresis

Laemli (1970) method was followed to report SDS–PAGE by using a 4% stacking gel and a 15% separating gel. 60 µg of protein concentrate were added to the gel. Proteins were stained with 0.125% Coomassie brilliant blue R-250 and detained with 25% ethanol and 10% acetic acid. Electrophoresis was reported on plates of 100 mm 82 mm 1 mm at 25º C, for one hour.

2.4. Statistical analysis

All tests were run out in triplicate unless otherwise stated; results were reported as means ± SD. Statistical analysis was calculated utilizing a one-way ANOVA with a significance level of $P \leq 0.05$. SPSS for Windows statistical package (v.10.0.6; SPSS, Chicago, IL, USA) was used for analysis.

3. Results and Discussion

3.1. Proximate composition of gourd seed protein isolates

The proximate analysis of protein isolates prepared from eight Sudanese varieties of *Longenara siceraria* seeds is shown in (Table 1).

Table 1. Proximate composition (g $100g^{-1}$) of protein isolate of eight Sudanese *Lagenaria siceraria* gourd seeds (mean ±SD)

Sample	Moisture content	Protein content	Oil content	Ash content	Total CHO
AK	4.10 ± 0.13^{b}	90.32 ± 0.29^{a}	0.57 ± 0.28	2.88 ± 0.27^{c}	2.13 ± 0.08^{d}
AZ	4.96 ± 0.06^{b}	88.22 ± 0.16^{b}	1.41 ± 0.13^{a}	3.79 ± 0.02^{b}	1.62 ± 0.31^{e}
SG	4.00 ± 0.08^{b}	91.04 ± 0.07^{a}	0.68 ± 0.22^{b}	3.09 ± 0.18^{b}	1.19 ± 1.0^{e}
WJ	5.75 ± 0.11^{a}	85.93 ± 0.18^{c}	1.2 ± 0.16^{a}	4.33 ± 0.11^{a}	2.79 ± 0.47^{d}
BB	5.98 ± 0.16^{a}	79.50 ± 0.09^{e}	1.71 ± 0.14^{a}	3.21 ± 0.12^{b}	9.60 ± 0.39^{a}
LHD	5.03 ± 0.12^{a}	83.24 ± 0.19^{d}	1.14 ± 0.17^{a}	4.00 ± 0.17^{a}	6.59 ± 0.25^{b}
CH	5.27 ± 0.09^{a}	86.39 ± 0.20^{c}	1.66 ± 0.14^{a}	2.93 ± 0.15^{c}	4.29 ± 0.33^{c}
CD	5.44 ± 0.23^{a}	80.67 ± 0.15^{e}	0.86 ± 0.20^{b}	3.38 ± 0.17^{b}	9.65 ± 0.43^{a}

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a column indicate significant differences at $p \leq (0.05)$. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

The moisture content of the samples ranged between 4.10 g $100g^{-1}$ and 5.98 g $100g^{-1}$. The moisture content of the seed protein isolate depends on the drying conditions after protein isolation. The protein contents of the eight isolates (Table 1) were significantly different ($P \leq 0.05$) from each other, ranging from 80.67 for sample CD to 91.04 g $100g^{-1}$ for sample SG. This variation may be due to the variation in the protein contents of the starting raw gourd seeds (Mariod et al., 2015). On the other hand, the protein of the eight isolates (Table 1) was the predominant constituent, and in the range of protein contents of seed, protein isolates were reported in other works (Ahmed 1998; Wani et al., 2015). The low oil content of the eight protein isolates (0.57-1.66 g $100g^{-1}$) may be due to the defatting process of

gourd seeds prior to the preparation of the protein isolates. The ash content of the gourd seed protein isolates (Table 1) ranged from 2.88 to 4.33 g $100g^{-1}$, the ash content of the isolates reflects the presence of minerals in these products. The carbohydrate content of the eight isolates (Table 1) was significantly variable among the samples and may be inversely related to the protein content of these isolates as found by Ahmed (1998) for *Hibiscus sabdariffa* seed products.

3.2. Functional properties of protein isolate

3.2.1. Water holding capacity

The water holding capacity (WHC) of foods can be defined as the ability to hold its own and added water during the application of forces, pressing centrifugation, or heating (Zayas 1997). Table 2 shows the water holding capacities of eight protein isolates prepared from Sudanese gourd seeds, they ranged from 2.4 to 3.9 mL g^{-1} protein. The lower water holding capacity can be due to less availability of polar amino acids. The quinoa protein showed an absorption capacity of 3.94 mL g^{-1} protein (Elsohaimy et al., 2015), they commented that the good ability of quinoa protein isolates to absorb water encourages its use in bakery products to enhance their functional properties. Referring to the results in Table 2, the water absorption capacity of the isolates may be affected by conformation and environmental factors. Kinsella and Melachouris (1976) related that conformational changes in the protein molecules may expose previously enclosed amino acid side chains, thereby making them available to interact with water. The Sudanese gourd seeds protein isolates in this study are good at absorbing water, they can be used in bakery products to improve their functions.

3.2.2. Fat binding ability

Fat binding ability (FBA) is a very important functional characteristic that contributes to improving the mouth feel while preserving the flavor of the food (Iwe et al., 2016). The fat binding ability of protein isolates of gourd seeds of eight Sudanese varieties are shown in Table 2, they range from 2.7 to 3.7 mL oil g^{-1} protein. These results are higher than that reported by Ashraf et al. (2012) for the fat binding ability of quinoa protein, wheat protein, and soy protein which were 1.88, 1.58, and 2.1 mL g^{-1}, respectively. The FBA is always related to the presence of hydrophilic and polar amino acids on the surface of the protein molecules (Garba, & Kaur, 2014). The variation in FBA of seed proteins because of protein percentage, surface area, hydrophobicity, the solubility of the oil, and the method of analysis utilized, as far as to lay on protein capacity to capture the oil (Wani et al., 2015).

3.2.3. Emulsion capacity

Emulsion capacity (EC) of foods is related to the quantity of oil, non-polar amino acid residues on the surface of the protein, water, and other food ingredients (Godswill, et al. 2019). Emulsion capacity is a crucial factor in deciding which protein to utilize in industrial food processing (Elsohaimy, et al. 2015). The emulsion capacity of the protein isolates in this study (Table 2) ranged from 51.0 for sample CD to 59.0% for sample SG. These results are comparable to those of other works (Tang, 2007; Yust, et al., 2010; Malomo and Aluko, 2015).

3.2.4. Bulk density

Bulk densities of seed protein isolates of eight Sudanese gourd varieties are reported in Table 2. They ranged from 0.39 to 0.56 g mL^{-1}. Freeze dried lentil protein isolate has a bulk density of 0.28 g mL^{-1} (Joshi et al., 2011). A high bulk density value of 0.94 g mL^{-1} was reported by Rajesh and Prakash (2008) for the albumin fraction of lentil. Bulk density depends on particle size. It is also depending on the method of measurement, and surface properties (Iwe et al., 2016).

Table 2. Functional properties of seeds protein isolates of eight Sudanese *Lagenaria siceraria* gourds

Sample	Water holding capacity (g $100g^{-1}$)	Fat binding ability (g $100g^{-1}$)	Emulsion Capacity (mL of oil per g of protein)	Bulk Density (g mL^{-1})
AK	3.7±0.43[a]	3.5±0.72[a]	57.8±0.74[b]	0.51±0.26[b]
AZ	3.5±0.87[a]	3.5±0.61[a]	55.3±0.36[c]	0.47±0.88[c]
SG	3.9±0.90[a]	3.7±0.52[a]	59.0±0.62[a]	0.56±.0.94[a]
WJ	3.2±0.46[b]	3.0±0.66[b]	55.0±0.17[c]	0.45±.92[d]
BB	2.4±0.81[c]	2.8±0.36[b]	51.5±0.82[e]	0.40±0.16[e]
LHD	2.8±0.74[b]	3.0±0.78[b]	51.7±1.0[e]	0.43±0.28[e]
CH	3.0±0.43[b]	3.2±0.33[b]	53.6±076[d]	0.49±0.76[d]
CD	2.4±0.29[c]	2.7±0.66[c]	51.0±0.33[e]	0.39±0.75[e]

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a column indicate significant differences at $p \leq (0.05)$. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid and CD=Curved Dipper.

3.2.5. Foaming stability

Table 3 shows values of foaming stability as a function of time for protein isolates provided from gourd seeds. Foam stability results are expressed in terms of foam height measured in milliliter (mL), for every sample foam stability corresponding to a time interval (from 0.00 to 120.00 minutes) was calculated. Foaming stability for the AK sample was ranged from 67.0±0.74 in zero time to 58.5±0.28, 57.5±0.33, 56.0±0.80, and 54.5±0.26 after 30, 60, 90, 120 min, respectively (Table 3). The difference between 0.00 to 120.00 min with respect to foam height (mL) for AK, AZ, SG, WJ, BB, LHD, CH, and CD samples was as follows: 12.5, 12.0, 12.0, 9.0, 6.0, 7.5, 10.5 and 6.0 mL, respectively, these results may indicate good foam stability for the samples. Foaming stability was ranged from more than 65% at zero time to more than 55% after 60 min for AK, AZ, and SG. These results were found less than foaming stability for quinoa protein that was ranged from 83.55 as zero time to 54.54% after 60 min. The result of the high ability of quinoa protein to form foam with high stability qualifies it for use in food processing (Elsohaimy et al., 2015). Further, Wani et al. (2015) commented that the formation of foam is much needed in food processing techniques such as syrups, beverages, cakes, and whipped toppings.

Egg albumin is an excellent foaming agent which is considered the standard reference, its foaming capacity, and foaming stability were reported in the literature (Lomakina and Mikova, 2006). So, we can say that the protein isolates from gourd seeds had the ability to make foam less than egg albumin but showed foam stability like it.

3.2.6. Protein solubility

Good protein solubility is important to use a product in manufacturing foods. The improvement of other functional properties is essential, underpinning the appropriate initial solubility of proteins (Yust et al., 2010). Figure 1. shows the protein solubility profiles of eight protein isolates prepared from gourd seeds. SG, AK, AZ, and WJ samples have very low solubility between pH 4 and 5 (the approximate isoelectric point for the proteins). Samples CD and CH have 25-30% solubility at pH 4 and 5. An increase in protein solubility is remarkable at pH 2 (Acidic range) for all the samples, but the maximum solubility (≈95%) occurred at pH 10 and 11 (alkaline range). The same solubility examples were shown in other seed protein isolates (Tang, 2007; Yust et al., 2010; Malomo and Aluko, 2015; and Wani et al., 2015). Protein solubility at different pH values shows the importance of the protein isolates to be applied to different food systems.

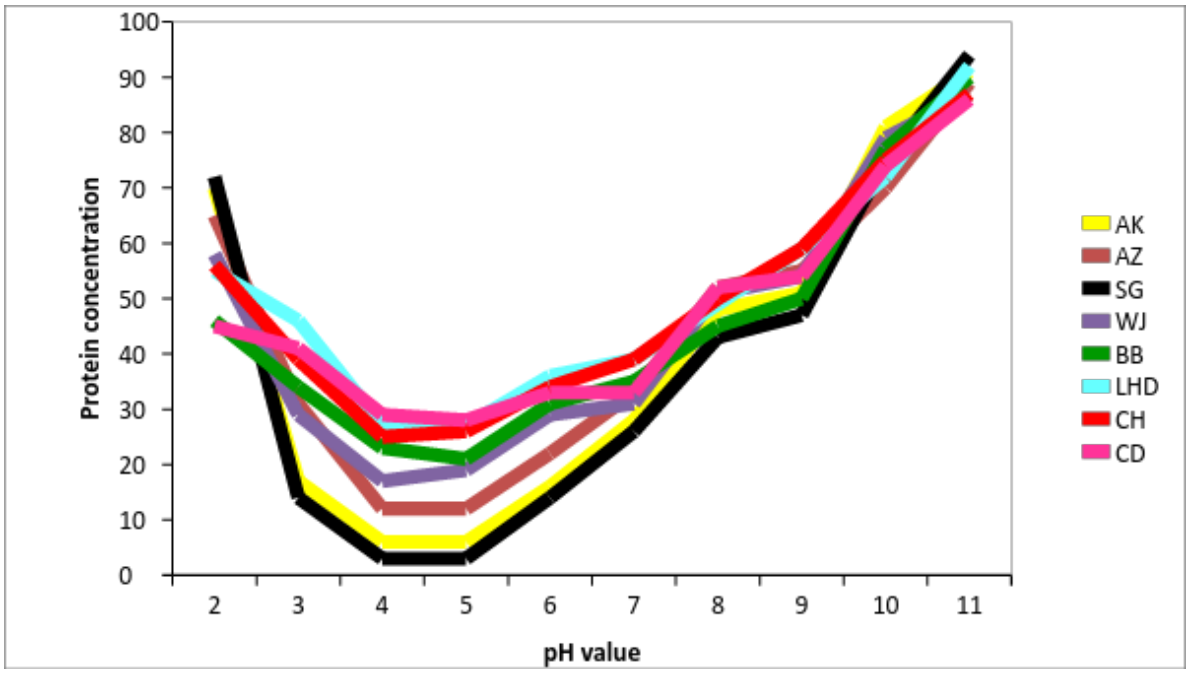

Fig. 1. Protein solubility of eight Lagenaria siceraria seeds flour protein isolate. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basket Ball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

Table 3. Foaming stability as a function of time for *Lagenaria siceraria* seeds flour protein isolate

Time (min)	Foam height (mL) of the seed flours Protein isolates							
	AK	AZ	SG	WJ	BB	LHD	CH	CD
0.00	67.0±0.74^{b}	65.0±0.36^{a}	68.0±0.62^{a}	60.5±0.17^{a}	56.5±0.82^{a}	59.0±1.0^{a}	62.5±076^{a}	57±0.33^{a}
0.25	65.0±0.46^{a}	63.0±0.66^{b}	66.5±0.74^{b}	58.0±0.90^{b}	53.5±0.59^{b}	57.0±0.8^{b}	60.0±0.6^{b}	54.5±0.1^{b}
0.50	63.5±0.58^{c}	62.0±0.59^{c}	64.5±0.33^{c}	55.5±0.44^{c}	52.5±0.62^{c}	56.0±0.5^{c}	58.5±0.1^{c}	53.5±0.6^{c}
1.00	62.5±0.36^{c}	61.0±1.50^{d}	63.5±0.49^{d}	55.0±0.66^{c}	51.5±083^{d}	55.5±0.6^{d}	57.5±0.7^{d}	52.5±0.4^{d}
1.50	62.5±0.77^{c}	61.0±0.55^{d}	63.5±0.71^{d}	55.0±1.10^{c}	51.5±0.47^{d}	55.5±0.9^{d}	57.5±0.2^{d}	52.5±0.4^{d}
2.00	62.5±0.66^{c}	61.0±0.66^{d}	63.5±0.47^{d}	55.0±0.69^{c}	51.5±0.4^{d}	55.5±0.1^{d}	57.5±0.6^{d}	52.5±0.6^{d}
15.00	60.0±0.64^{d}	59.5±0.15^{d}	60.5±0.76^{e}	53.0±0.85^{d}	51.0±0.66^{d}	53.0±0.6^{e}	55.0±0.7^{e}	52.5±0.9^{d}
30.00	58.5±0.28^{e}	59.5±2.00^{d}	59.0±0.29^{e}	52.0±0.77^{d}	50.5±0.57^{e}	52.0±0.4^{f}	54.5±0.6^{f}	52.0±0.4^{d}
60.00	57.5±0.33^{e}	56.5±0.95^{e}	58.5±0.18^{e}	51.5±0.33^{e}	50.5±0.8^{e}	51.0±0.3^{g}	53.0±0.5^{f}	52.0±0.8^{d}
90.00	56.0±0.80^{e}	54.5±0.37^{f}	57.0±1.30^{f}	51.5±0.63^{e}	50.5±0.77^{e}	51.5±0.7^{g}	52.0±03^{g}	51.0±0.6^{e}
120.00	54.5±0.26	53.0±0.88	56±.0.94	51.5±.92^{e}	50.5±0.16^{e}	51.5±0.2^{g}	52.0±0.7^{g}	51.0±0.7^{e}

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a row indicate significant differences at p ≤ (0.05). AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid and CD=Curved Dipper.

3.2.7. In vitro protein digestibility

In vitro protein digestibility is rapid and inexpensive compared to animal assays, in vitro assays are suitable for routine monitoring of the nutritional quality of protein foods (Ahmed, 1998). The in vitro protein digestibility of eight gourd seed protein isolates is AK 94.78, AZ 93.66, SG 95.35, WJ 92.96, BB 92.03, LHD 92.24, CH 93.11, CD 91.86, these values ranged from 91.86 for sample CD to 95.35% for the sample SG. The in vitro protein digestibility of the eight samples is high compared to quinoa seed protein isolate which was 78.37% (Elshohaimy et al., 2015), and compared to in vitro protein digestibility of *Hibiscus sabdariffa* seed protein isolate (87.09%) as reported by Ahmed (1998). High in vitro digestibility indicates high susceptibility of the protein to the proteolytic enzymes used and a high percentage of readily digested protein. The presence of trypsin and chymotrypsin amino acids, which act as inhibitors, in addition to the nature of the structure of *Lagenaria siceraria* seed proteins, undoubtedly, all of them limit the work of digestive enzymes, which affects protein digestion (Clemente, et al. 2019).

3.2.8. SDS-PAGE profiles

The SDS-PAGE profiles of protein constituents of the eight protein isolates are shown in Fig 3. The electrophoretic pattern of the eight samples was almost identical, with some minor variation among the samples. Four major polypeptides with molecular weights 10, 23, 38, and 47 KDa were identified in samples AK, AZ, WJ, LHD, and CD. On the other hand, three major polypeptides with molecular weights of 10, 23, and 38 KDa were identified in samples SG, BB, and CH. The protein band with MW 23 KDa in samples AZ and WJ has higher intensity, indicating the high concentration of the polypeptide in the mentioned samples. The disappearance of protein components of high MW (>70 KDa) was observed in the eight samples (Fig. 2). Chango et al. (1995) stated that the increase in heating temperature, used for coagulation of soluble lupin proteins, resulted in the removal of high molecular weight proteins.

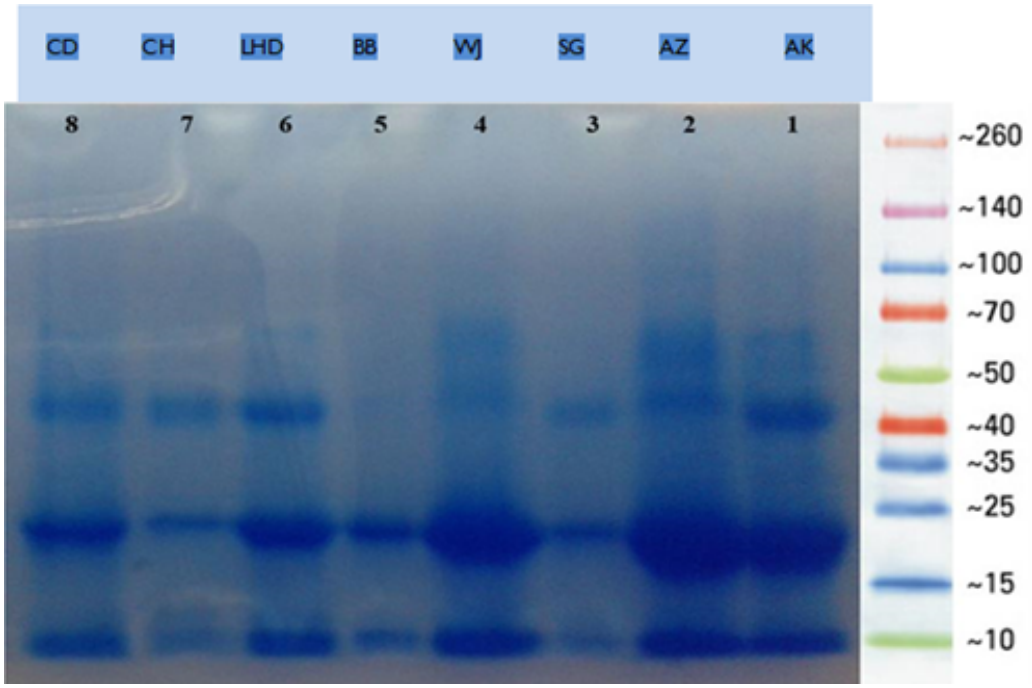

Figure 2. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of eight *Lagenaria siceraria* Seeds flour protein isolate. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basket Ball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

4. Conclusion

The protein isolates prepared from bottle gourd seeds are of good functional properties, such as water holding capacity, fat binding ability, foaming stability, protein solubility, and emulsion capacity. These findings gave basic information on the nutritional and potentiality of these isolates and achieving the hypothesis of the study and ensuring that they can be utilized as supplemented materials in food products to enhance their nutritional values especially for improving protein content and quality.

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Research Article

Validity Determination of Some Molecular Markers Used in Melon Breeding

Mürşide HATİPOĞLU*[1], Suat ŞENSOY[2]

[1]Van Yüzüncü Yıl University, Institute of Natural and Applied Sciences, Horticultural Sciences, Van Türkiye
[2]Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Horticulture, Van Türkiye

[1]https://orcid.org/0000-0002-1514-8951, [2]https://orcid.org/0000-0001-7129-6185

*Corresponding author e-mail: mursidehatipoglu2017@gmail.com

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Abstract: The present work aimed to validate some molecular markers (AM, FM, Fom2-R408, Fom2-S342, SCAPB051046, SCOPE14541, T1, T1ex, M3A and M3a SCAR markers and Fom1R-Fom1S, CAPS-Dde I, CAPS2, CAPS3 and EX1-C170T CAPS markers) developed for melon breeding in the literature on some melon cultivars and genotypes in Turkey with the aid of marker-assisted selection. For this purpose, these molecular markers developed for resistance to Fusarium oxysporum f. sp. melonis (FOM), Cucumber mosaic virus (CMV) and powdery mildew or sex determination have been tested. AM, FM, Fom2-R408 and Fom2-S342, (SCAR) and Fom1R-Fom1S, CAPS2, and CAPS3 (CAPS) markers for FOM; SCAPB051046 and SCOPE14541, (SCAR) for CMV; CAPS-Dde I for powdery mildew; T1, T1ex, CAPS EX1-C170T, M3a and M3A markers for sex determination were employed. These selected markers were examined in 24 melon genotypes, 11 of which were commercial F1 cultivars. The results were obtained from FM, Fom2-R408 and Fom2-S342 SCAR markers and Fom1R-Fom1S CAPS marker for Fusarium wilt disease and SCAR SCOPE14541 for CMV. In this context, it is seen that the resultant SCAR and CAPS markers could be used effectively in MAS studies.

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Kavun,
MAS,

Öz: Bu çalışmada, literatürde kavunda geliştirilen AM, FM, Fom2-$R_{408,}$ Fom2-$S_{342,}$ SCAPB$05_{1046,}$ SCOPE$14_{541,}$ T1, T1ex, M3A, M3a, SCAR belirteçleriyle; Fom1R-Fom1S, CAPS-Dde I, CAPS2, CAPS3, EX1-C170T CAPS belirteçlerinin, markör destekli seleksiyon yardımıyla ülkemizdeki bazı kavun çeşitleri ve genotipleri üzerinde geçerliliğini tespit etmek amaçlanmıştır. Bu amaçla *Fusarium oxysporum* f. sp. *melonis* (FOM), hıyar mozaik virüsü (CMV), külleme hastalığı ve cinsiyet tanımlanması için geliştirilmiş olan bu moleküler belirteçler test edilmiştir. FOM için; AM, FM, Fom2-$R_{408,}$ Fom2-$S_{342,}$ (SCAR) ile Fom1R-Fom1S, CAPS2, CAPS3, (CAPS) belirteçleriyle testleme yapılmıştır. CMV için; SCAPB$05_{1046,}$ SCOPE14_{541}, (SCAR), külleme için, CAPS-Dde I, cinsiyet belirleme için T1, T1ex, CAPS EX1-C170T, M3a, M3A markörleri

SCAR

kullanılmıştır. Bu seçilen belirteçlerin, 11'i F1 çeşit olmak üzere ve bazı egzotik tipleri de içeren toplam 24 kavun genotipinde etkinliği incelenmiştir. Çalışılan belirteçlerden Fusarium solgunluğu için, SCAR; FM, Fom2-$R_{408,}$ Fom2-S_{342} ve CAPS Fom1R-Fom1S, CMV için SCAR SCOPE14$_{541}$ markörlerinden sonuç alınabilmiştir. Bu bağlamda sonuç alınan SCAR ve CAPS belirteçlerinin kavunda MAS çalışmalarında etkin bir şekilde kullanılabileceği öngörülmüştür.

1. Giriş

Cucurbitaceae familyasının Cucumis cinsine giren *Cucumis melo* L. türü olarak sınıflandırılan kavun, dünya çapında ticari değeri nedeniyle tarımsal açıdan en önemli ekonomik Cucurbitaceae türlerinden biridir (McCreight ve ark., 1993; Şensoy, 2005; Cakmakci et al., 2017). Kavun genotipleri arasında büyük bir çeşitlilik olduğu tespit edilmiştir (Pitrat ve ark., 2000; Sensoy ve ark., 2007a). Türkiye yerel kavun genotipleri ile Türkiye'de farklı kurumlarda toplanma ve karakterizasyon çalışmaları yürütülmektedir (Sensoy ve ark., 2007a). Dünya kavun üretiminde ise Çin 17.082.608 milyon ton üretimiyle ilk sırada yer alırken, Çin'i sırasıyla Türkiye 1.813.422 milyon ton ile ve İran 1.591.414 milyon ton ile takip etmektedir (Faostat, 2019).

Bitkiler yaşadıkları ortamda çeşitli hastalık ve zararlı etmenler (virüs, bakteri, fungus, nematod vb.) tarafından saldırıya uğramaktadırlar (Baker ve ark., 1997). Kavun hastalıkları arasında toprak kökenli patojenler ve bunlardan biri olan *Fusarium oxysporum* f. sp. *melonis* (FOM)'in neden olduğu kök çürüklüğü ve solgunluk hastalığı ilk sırada yer almaktadır. Hastalığın 0, 1, 2 ve 1-2 olmak üzere farklı 4 ırkı olup, ülkemizde yaygın olarak görülmektedir. Hastalığın kalıtımında Fom-1 ve Fom-3 genleri 0 ve 2 ırklarına karşı Fom-2 geni ise 0 ve 1 ırklarına karşı dayanıklılık sağlamaktadır. Fusarium % 100 kayıplara neden olan epidemiler oluşturabilmektedir (Zitter, 1999). Hastalıkla mücadelede en pratik ve etkin metot dayanıklı çeşitlerin üretimde kullanılmasıdır (Martyn ve Gordon, 1997). Biyotik etmenler içerisinde boyutu, kimyasal ve fiziksel yapısı, enfeksiyon şekli, bitki içerisindeki çoğalması, taşınması, simptom oluşturması ve etkin bir mücadelesinin olmayışı gibi yönleri ile virüs hastalıklarının özel bir önemi vardır (Agrios, 1997). CMV (Hıyar mozaik virüsü), tüm dünyada yetiştirilen ürünlerde hastalık gelişimine ve zararlara neden olmaktadır (Zitter ve Murphy, 2009). Virüs bitkilerin yapraklarında hafiften şiddetliye doğru değişen mozaik, yaprak deformasyonları ile ya da sadece bodurlaşma, yapraklarda eğrelti otu şeklinde yaprak deformasyonlarına yol açmaktadır (Kearney ve ark., 1990). Öncelikle yaprağın üst yüzeyinde parça parça, nispeten yuvarlak lekeler belirir, sonradan bu lekeler birleşerek yaprağın her iki yüzeyini, yaprak sapını ve gövdeyi kaplar. Yapraklar kuruyup dökülür ve bitkide gelişme durur. Bunun sonucunda ürün kaybı meydana gelir (Anonim, 2022). Bu hastalık etmenlerinden dolayı ticari firmalar bu hastalıklara dayanıklı genleri MAS yardımıyla kültür çeşitlerine aktararak bu verim kaybını önlemeyi amaçlamışlardır. Ayrıca, kavunda cinsiyet belirlemede monoik ve andromonoik bitkilerin erken aşamada belirlenmesi, ıslah programlarının etkin bir şekilde yürütülmesini sağlamaktadır.

Geliştirilen markörler ile ıslah programlarında dayanıklılık özelliğini taşıyan bireyler belirlenebilmektedir. Islah çalışmalarında, hastalığa dayanımı sağlayan gene yakın moleküler markörlerin kullanımı, daha kısa sürede ve daha güvenilir sonuçlar elde etmemizi sağlamaktadır (Oumouloud ve ark., 2008). Moleküler belirteçler, son yıllarda kavun çeşitliliği araştırmalarında giderek daha fazla kullanılmaktadır (Sensoy ve ark., 2007a,b; Erdinc ve ark., 2013; Ibrahim ve Erdinç, 2020). Bu amaçla, ülkemizde de sebze ıslahında birçok ıslahçı markör yardımlı seleksiyon (MAS) çalışmalarıyla moleküler markörlerden yararlanmaktadır (Pınar ve ark., 2013). RAPD belirteçleri, kavun genotipleri arasındaki genetik benzerliğin çözümlenmesinde başarılı olmuş ve diğer moleküler DNA markörleri ile uyumlu olduğu anlaşılmıştır (Sensoy ve ark., 2007a; Yıldız ve ark., 2011). Bir bitkiyi veya bitki grubunu morfolojik anlamda diğerlerinden ayıran özellikler morfolojik belirteç (marker, markır, markör, vb.) olarak değerlendirilir. Meyve kabuğu, çiçeğin şekli, yaprağın şekli, bitki, meyve ve tohum gibi özellikler bu grup markörleri meydana getirir (Gülşen ve Mutlu, 2005). Morfolojik tanının bazı durumlarda etmenlerin belirlenmesinde tek başına yeterli olmaması, araştırıcıları moleküler düzeyde tanıya sevk etmiştir (Hosseinalizadeh ve ark., 2021). Morfolojik markörler yerine moleküler markörlerin kullanılması bitki ıslahçılarına daha yüksek etkinlikle çalışma imkanı vermektedir (Üstün ve ark., 1996). DNA temelli markör teknolojisiyle birlikte ıslahçılar Mendelci genetik yaklaşımı ıslah programının tamamlayıcısı olarak kullanabilmekte ve bu sayede kısa sürede yeni bir çeşit

geliştirebilmektedir. DNA belirteçleri, bitkilerin genetik çeşitliliğin araştırılmasında, yakın türlerin, çeşitlerin belirlenmesi ve tanımlanmasında araştırmacılara yoğun çalışma imkanı veren güçlü bir metottur (Akakaçar, 2001). Islah programlarında istenilen genle bağlantılı markörlerin kullanılmasının potansiyel faydaları yön değiştirmiş ve araştırmacılar 1970'lerin sonuna doğru DNA markörlerinin geliştirilmesiyle yeni bir çalışma alanı ortaya çıkmıştır (Grube ve ark., 2000).

Belirtilen hastalıklara karşı ürün kaybını önlemek için yapılan kimyasal ve kültürel önlemler yetersiz kaldığı ve dezavantajlarının görüldüğü noktada dayanıklı çeşit geliştirme metotları ön plana çıkmıştır. Dayanıklı çeşit geliştirmede kullanılan yöntemlerden olan klasik ıslah yöntemlerine göre daha kısa zamanda, etkili güvenilir ve maliyetinin düşük olmasıyla MAS yöntemi klasik ıslah yöntemine destek olmaktadır. Bu çalışmada Türkiye'deki kavun çeşitlerinde dayanıklı çeşit geliştirmede kullanılan belirteçlerin (markörlerin, işaretleyicilerin) çalışıp, çalışmadığı ve ıslah çalışmalarına kolaylık sağlayıp, sağlayamayacağı etkin bir şekilde kontrol edilmeye çalışılmıştır.

2. Materyal ve Yöntem

Çalışmada, 11 F_1 hibrit kavun (Şıra F_1, Serin F_1, Balözü F_1 , Napolyon F_1, Digital F_1, VCR-601 F_1, Polidor F_1, Dragon F_1, Favori F_1, Medetli F_1, Galina F_1 ile 13 standart ve egzotik kavun genotipiyle (CU-129, CU-305, Y15 Isabelle, Y63, CU-269, T4 Hasanbey, Y9, Topatan Kavunu, Düvlek Muz Kavunu, Musakka Kavunu, Çilli Kavun, Beyaz Gönen Kavunu ve T6 Acur) toplamda 24 genotip kullanılmıştır. Çalışmada Fusarium solgunluğu, cinsiyet, CMV ve külleme için 10 SCAR markörü ve 5 CAPS markörü olmak üzere toplam 15 markör çifti (AM, FM, Fom2-R_{408}, Fom2-S_{342}, SCAPB05$_{1046}$, SCOPE14$_{541}$, T1, T1ex, M3A, M3a SCAR Fom1R-Fom1S, CAPS-Dde I, CAPS2, CAPS3 ve EX1-C170T CAPS) kullanılmıştır. Çeşitli bölgelerden temin edilen yerli ve hibrit çeşitlere ait tohumlar iklim odasında gruplandırılarak saksılara ekilmiştir. Yaklaşık 15-20 gün sonra bitkiler 2-3 gerçek yaprak dönemindeyken genç yapraklardan örnek alınmıştır. DNA izolasyonu için yaprak örnekleri alınarak liyoflizatöre konulmuştur. Düşük basınçta liyoflize edilen yapraklar öğütülerek toz haline getirilip, izolasyon aşamasına geçilmiştir. Kavun yapraklarından DNA izolasyonu Doyle ve Doyle (1990)'a göre modifiye edilmiş olan CTAB metoduna göre yapılmıştır. Reaksiyon ortamı için gereken kalıp DNA, DNA'ya yapışacak sentetik markörler, DNA'nın çoğalması için gerekli enzim olan Taq polimeraz, Taq polimerazın kofaktörü olan $MgCl_2$, ddH_2O eklenmesiyle PCR reaksiyonu hazırlanmıştır (Çizelge 1). PCR ürünlerinin görüntülenmesi için agaroz jel eletroforezi ve kapiller elektroforez olmak üzere iki farklı sistemden faydalanılmıştır. PCR ürünleri % 2.5'lik agaroz jelde 120 V 3.5 saat süreyle agaroz jel elektroforezinde yürütülmüştür. Çalışma kapsamında fusarium solgunluğu, hıyar mozaik virüsü, külleme hastalığı ve cinsiyet belirlemede kullanılan bazı SCAR ve CAPS markörlerinin 11'i F1 çeşit olmak üzere ve bazı egzotik tipleri de içeren toplam 24 kavun genotipinde etkinliği araştırılmıştır. SCAR ve CAPS markör yöntemi çalışmamızda belirlenen hastalıklara ve cinsiyet belirlemelerine göre sekansa özgü markörler PCR ortamında çoğaltılmasıyla gerçekleşmiştir. CAPS markör tekniğinde gen spesifik markör çiftleriyle PCR ortamında çoğaltılan amplifikasyon ürünleri kesme enzimleri ile kesme sağlanmıştır (Jiang ve ark., 1997).

Çizelge 1. PCR reaksiyonu

Kullanılan Kimyasallar	Her örnek için Kullanılan Miktar (µl)
ddH_2O	6.3
TBE Buffer	2
$MgCl_2$	2.5
dNTP	2
Primer (forward ve reverse)	5+5
Taq	0.2
DNA	2
Toplam Hacim	25

3. Bulgular ve Tartışma

Fungal patojenin ırk 0 ve 1'e direnç içeren Fom-2 genleriyle bağlantılı markörlerini tanımlamak için geliştirilen ko-dominant SCAR FM markörü 24 kavun genotipi üzerinde analiz edilmiştir. Bu ko-dominant markörün dayanıklılık bantı 564 bç'de görüntü vermiştir (Şekil 1). Onbir F_1 ticari kavun çeşidinde 1 adet duyarlı, 4 adet dayanıklı, 4 adet heterozigot bant elde edilirken, 2 adet F_1 çeşidinde bant elde edilmemiştir. Yerli ve egzotik kavun genotiplerinde 5 adet duyarlı, 4 adet dayanıklı 2 adet ise heterozigot olarak toplam 11 F_1 çeşitten bant elde edilirken, 2 yerli çeşitten bant elde edilmemiştir (Çizelge 2). Aynı zamanda 24 kavun genotipinin hastalıklara dayanımlarıyla elde edilen sonuçlar birbiriyle karşılaştırılmıştır. Wang ve ark. (2000), yaptıkları çalışmada direnç genine bağlantılı moleküler markörleri tanımlayarak direnç genotiplerinin seçimi için önemli bir araç olabildiğini ortaya koymuşlardır. Fom-2 genleriyle bağlantılı markörleri tanımlamak için AFLP'den bu iki dominant (FM, AM) markör, ebeveynler arasında polimorfizm uzunluğu üreterek spesifik PCR primerlerinin dizayn edilmesiyle SCAR ko-dominant markörlere dönüştürmüşlerdir. Fom-2 ile bağlantılı olan SCAR markörleri tanımlamak için 200 ECORI/MseI ve 240 PstI/MseI primer kombinasyonlarını kullanarak 564 bç'de dayanıklı genotipler görüntülenmiştir. Kırkbeş genotipin direnç fenotipleri ve parça boyutları arasında yüksek bir korelasyon göstermiştir. Ayrıca birkaç kavun genotipi (Kırkağaç 637 ve Galia) Fom-1 ırkına kadar direnç gösterirken, Güney Anadolu genotipleri ve Yuva çeşidinin direncinin olmadığı belirlenmiştir (Demir ve ark., 2006). Bu markörlerin MAS' ta kullanımının faydalı olabildiği ortaya konulmuştur.

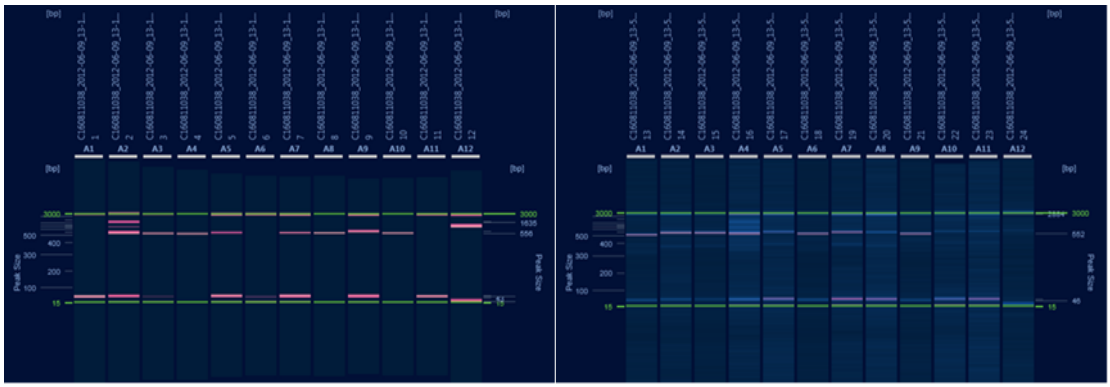
Türkiye'de kabakgil yetiştirme alanlarında bulunan en önemli virüslerden birinin hıyar mozaik virüsü (cucumber mosaic virus=CMV), olduğu araştırıcılar tarafından bildirilmiştir (Nogay, 1983) CMV, 80'den fazla yaprak biti türü ile non-persistent olarak taşınır (Palukaitis, 1992). En yaygın vektörleri *Myzus persicae* ve *Aphis gossypii'dir*. CMV-B2 direnç genini tespit etmek için dizayn edilen (Staub ve ark.,1996), dominant SCAR markörlerinin 24 kavun genotipinde etkinliği araştırılmıştır. APB-05'ten türetilen $SCAPB05_{1046}$ marköründen bant elde edilmezken, OP-14'ten türetilen $SCOPE14_{541}$'te 11 F_1 çeşitten 7 adet, 13 yerel ve egzotik çeşitten de 8 adet bant görüntüsü elde edilmiştir (Şekil 2, Çizelge 3).

Kavunda CMV-B2 direnç geni ile bağlantılı iki RAPD marköründen, kavunda CMV-B2 direnç genini tespit etmek için SCAR markörleri dizayn edilmiştir ve Yamatouri çeşidinden klonlanmıştır. APB-05'ten türetilen SCAPB05 marköründen bir DNA bantı elde edilirken, OP-14'ten türetilen SCOPE14'te 16 bant vermiştir. Sonuç olarak SCAR analizlerinin sadece kavunlardaki etkinliği değil aynı zamanda RAPD markörleri tarafından gözlemlenen belirsiz direnç sonuçlarına da açıklık getirmiştir (Daryono ve ark., 2009). Fom2 R_{408} ve Fom2 S_{342} SCAR markörleri, Fom-2 genine karşı dayanıklılık ve duyarlılık allelleri için geliştirilmiştir (Oumouloud ve ark., 2012). Geliştirilen bu markörler 24 kavun genotipi üzerinde analiz edilmiştir (Şekil 3-4). Bu markörlerin dayanıklılık bantı 408 bç, duyarlılık bantı ise 342 bç'dir. Dayanıklılık geni ile ilişkili olan bant, 11 F_1 ticari çeşitten herhangi birinde elde edilmezken, 13 yerel ve egzotik genotipten 2 adet dayanıklılık bantı elde edilmiştir. Duyarlılık geni ile ilişkili olan bant, 11 F_1 ticari çeşitten 5 adet duyarlılık bantı elde edilirken, 13 yerel ve egzotik çeşitten 4 adet duyarlılık bantı elde edilmiştir (Çizelge 4-5). Yerel ve egzotik çeşitlerden Musakka kavununda hem duyarlılık bantı hem de dayanıklılık bantı görüntüsü elde edilmiştir. Bundan dolayı ko-dominant özellik taşımış olup, heterozigot bir genotip olduğu görülmüştür. Genotip özellikleri ile uyuşumlu olmayan durumlarda, markör ile dayanıklı gen arasında ilişkinin kesilmiş olabileceği varsayılmaktadır.

Oumouloud ve ark. (2012), yaptığı çalışmada dayanıklı ve duyarlı alleller olmak üzere iki SCAR (Fom2-R_{408} ve Fom2-S_{342} Fom2) markörü, geliştirilmiştir. Bu alleller çoklu PCR için kombine edildiğinde ko-dominant olarak kullanılabilmiştir. Fom2-R408 markörü 27 kavun genotipinde içerisinde 13 dayanıklı bant elde edilirken, Fom2-S342 Fom2 marköründe ise 17 duyarlı bant görüntüsü elde edilmiştir. Kavunda Fom-2 geni için bu iki markörün maliyet etkinliği ve güvenilirliği açısından geliştirmesini ifade etmişlerdir.

Fom-1 dayanıklılık genine bağlı olarak geliştirilmiş olan Fom1-R / Fom1-S markörünün (Oumouloud ve ark., 2015), 24 kavun genotipi üzerinde taraması yapılmıştır. CAPS, Fom1-R / Fom-1-S marköründe kesme enzim olarak dayanıklılık bantı eldesi için *Bsp*CNI ve duyarlılık bantı eldesi için *Bsp*HI enzimi kullanılmıştır. Dirençli *Bsp*CNI enziminde kesme görülmeyip, sadece bant aralığı 568 bç'de 5 tane F_1 çeşitten ve 5 tane de yerel ve egzotik çeşitten sonuç alınırken, *Bsp*HI kesme enziminde

bant aralıkları ise 262+306 bç ve 568 bç bant görüntüsü elde edilmiştir (Şekil 5). *Bsp*HI kesme enzimiyle sadece CU 305 (*Cucumis melo* subp. *agrestis*) genotipinde her üç bantı da verdiği belirlenmiştir (Şekil 6). F_1 çeşitlerden, 9 adet 568 bç'de görüntü elde edilirken, yerel ve egzotik çeşitlerden 7 adet, görüntü elde edilmiştir (Çizelge 6). Oumouloud ve ark. (2015), yaptıkları çalışmada kavunun Fom ırk 2'ye olan direncinin moleküler ıslah için, markörlerin gelişimi ve Fom-1 geninin moleküler karakterizasyonu çalışılmıştır. Bu çalışmada, araştırmacılar bu gen içinde dizayn edilen primerlerinin üçünü kullanarak birçok duyarlı ve dayanıklı kavun genotiplerinden Fom-1'in tam genomik sekansını üretmişler ve çoğaltmışlardır. Fom-1 lokusunun kod bölgesi içerisinde tek nükleotidli polimorfizme dayalı olarak 2 CAPS markörü üretilmiştir. Fom-1R 182+386 bç ve 568 bç de bant aralığı vermiş ve Fom-1S CAPS markörü için 262+306 bç ve 568 bç bant aralığı vermiştir. Kavun ıslah programları için moleküler yardımlı seleksiyonda her iki CAPS markörünün açık bir şekilde kullanışlı olduğunu kanıtlanmıştır (Oumouloud ve ark., 2015).

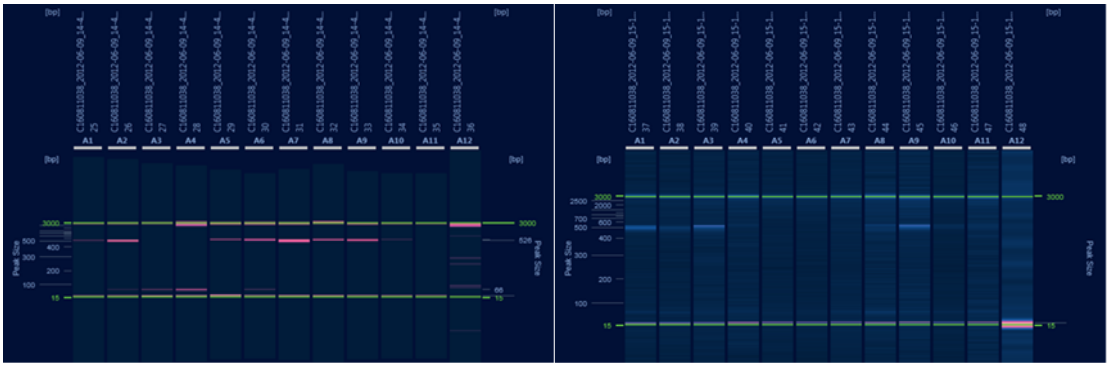


Şekil 1. FM ko-dominant markörü ile elde edilen dayanıklı 564 bç'deki kapiller elektroforez bant görüntüsü.

Çizelge 2. FM belirtecine ait sonuçlar

GENOTİP#	FOM DAYANIKLILIK (FOM-2 GENİ) MARKÖR MEVCUDİYETİ (564bç)
1. CU 129	?
2. CU 305 (*Cucumis melo* subp. *agrestis*)	S
3. Y15 Isabelle	R
4. Y63 (*C. melo* subsp. melo *var. conomon*)	R
5. CU 269 (*C. melo* subsp. melo *var. dudaim*)	S
6. T4 Hasanbey	?
7. Y9 (*C. melo* subsp. melo *var. conomon*)	R
8. Şıra F_1	R
9. Serin F_1	S
10. Balözü F_1	R
11. Napolyon F_1	?
12. Digital F_1	?
13. Vcr-601 F_1	R
14. Polidor F_1	H
15. Dragon F_1	H
16. Favori F_1	H
17. Medetli F_1	H
18. Galina F_1	R
19. Topatan	H
20. Düvlek Kavunu	H
21. Musakka	R
22. Çilli	S
23. Beyaz Gönen	S
24. T6 Acur (*C. melo* subsp. melo *var. flexuosus*)	S

*: H: heterozigot dayanıklı; R: homozigot dayanıklı; S: homozigot duyarlı ?: Bant elde edilmemiştir.
#Çizelgede alt tür ve çeşit grupları belirtilen egzotik kavun genotipleri dışındaki tüm genotipler C. melo subsp. melo var. cantalupensis veya C. melo subsp. melo var. inodorus çeşit grupları içinde yer almaktadır.

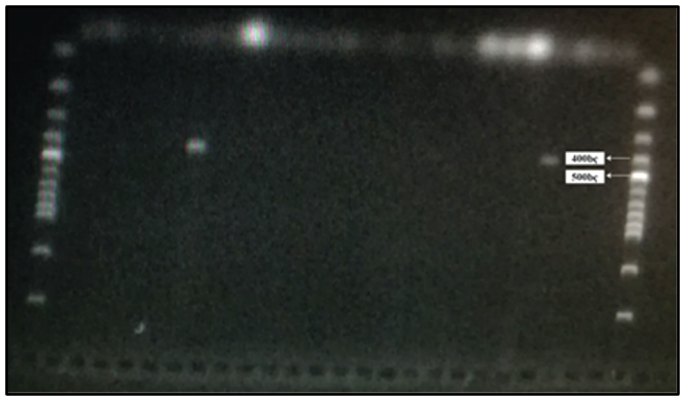


Şekil 2. SCOPE14$_{541}$ markörü ile elde edilen 541 bç'deki kapiller elektroforez bant görüntüsü.

Çizelge 3. SCOPE14541 belirtecine ait sonuçlar

GENOTİP	CMV DAYANIKLILIK (CMV-B2 GENİ) MARKÖRÜ MEVCUDİYETİ
1. CU 129	+
2. CU 305	+
3. Y15 Isabelle	-
4. Y63	-
5. CU 269	+
6. T4 Hasanbey	+
7. Y9	+
8. Şıra F$_1$	+
9. Serin F$_1$	+
10. Balözü F$_1$	+
11. Napolyon F$_1$	-
12. Digital F$_1$	+
13. VCR-601 F$_1$	+
14. Polidor F$_1$	+
15. Dragon F$_1$	+
16. Favori F$_1$	-
17. Medetli F$_1$	-
18. Galina F$_1$	-
19. Topatan	-
20. Düvlek Kavunu	+
21. Musakka	+
22. Çilli	+
23. Beyaz Gönen	-
24. T6 Acur	-

*: (+): bant mevcut * :(-): bant mevcut değil.

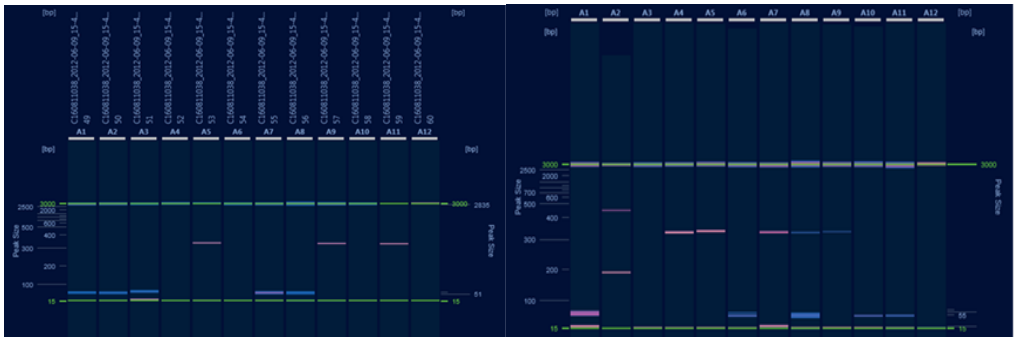


Şekil 3. Fom2 R408 dayanıklı markör ile elde edilen 408 bç'de agaroz jelde bant görüntüsü.

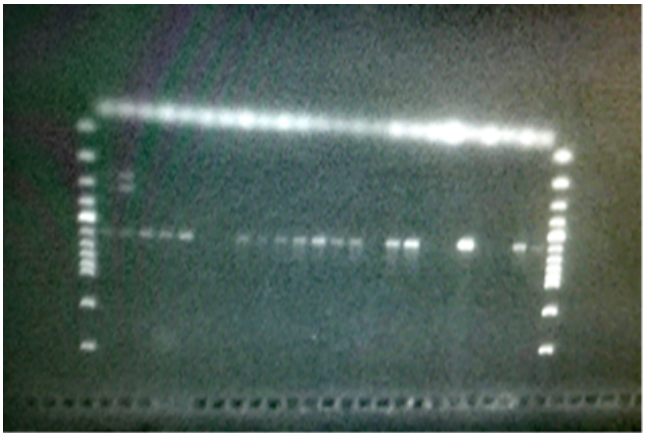
Çizelge 4. Fom2 R_{408} belirtecine ait sonuçlar

GENOTİP	FOM DAYANIKLILIK (FOM-2 GENİ) MARKÖRÜ (408bç)
1. CU 129	-
2. CU 305	-
3. Y15 İsabelle	-
4. Y63	-
5. CU 269	-
6. T4 Hasanbey	R
7. Y9	-
8. Şıra F_1	-
9. Serin F_1	-
10. Balözü F_1	-
11. Napolyon F_1	-
12. Dıgıtal F_1	-
13. VCR-601 F_1	-
14. Polidor F_1	-
15. Dragon F_1	-
16. Favori F_1	-
17. Medetli F_1	-
18. Galina F_1	-
19. Topatan	-
20. Düvlek Kavunu	-
21. Musakka	R
22. Çilli	-
23. Beyaz Gönen	-
24. T6 Acur	-

*R: homozigot dayanıklı



Şekil 4. Fom2 S342 duyarlı markör ile elde edilen 342 bç'de kapiller elektroforez bant görüntüsü.

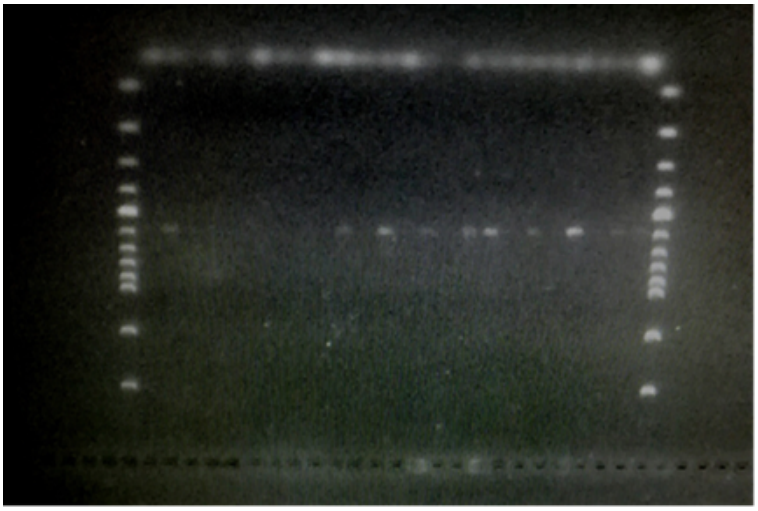


Şekil 5. CAPS Fom1-R / Fom1-S (BspHI kesme enzimi) markörü ile elde edilen 262+306 bç ve 568 bç'nin agaroz jeldeki bant görüntüsü.

Çizelge 5. Fom2 S342 belirteçlerine ait sonuçlar

GENOTİP	FOM DUYARLILIK (FOM-2 GENİ) MARKÖRÜ (342bç)
1. CU 129	-
2. CU 305	-
3. Y15 İsabelle	-
4. Y63	-
5. CU 269	S
6. T4 Hasanbey	-
7. Y9	-
8. Şıra F_1	-
9. Serin F_1	S
10. Balözü F_1	-
11. Napolyon F_1	S
12. Dıgıtal F_1	-
13. VCR-601 F_1	-
14. Polidor F_1	S
15. Dragon F_1	-
16. Favori F_1	S
17. Medetli F_1	S
18. Galina F_1	-
19. Topatan	S
20. Düvlek Kavunu	S
21. Musakka	S
22. Çilli	-
23. Beyaz Gönen	-
24. T6 Acur	-

*S: homozigot duyarlı.



Şekil 6. CAPS Fom1-R / Fom1-S (*Bsp*CNI kesme enzimi) markörü ile elde edilen 568 bç'nin agaroz jeldeki bant görüntüsü.

Dünyada, kavunda tek ve birden çok özelliği barındıran genler veya QTL'lerle ilgili moleküler belirteçler (markörler) geliştirilmeye devam edilmektedir. Moleküler belirteçler kavunda tarımsal açıdan birçok önemli özellik için genlerin ve QTL'lerin belirlenmesinde veya haritalanmasında moleküler yardımlı seleksiyon yöntemiyle kullanışlı ve faydalı olabilmektedir. Morfolojik ve biyokimyasal markörler yapılarından kaynaklanan dezavantajlarından dolayı günümüzde yerini ya moleküler markörlere bırakmaktadır ya da moleküler markörler ile birlikte karşılaştırılmalı çalışmalarda kullanılmaktadır. DNA esas alınarak yapılan teşhislerde gözlemlenen polimorfizm morfolojik ve biyokimyasal markör tekniklerinde gözlemlenenlere oranla daha yüksektir. Ayrıca moleküler yöntemlerde küçük bir miktar DNA yöntemin uygulanabilmesi için yeterli olmaktadır. Bununla birlikte,

literatürde belirtilen tüm markörler kavun ıslah programlarında uygulanamamaktadır. Markörleri yeniden tanımlayabilmek, belirli ıslah hatlarında faydalılık, çoğaltılabilirlik, yeni polimorfik markörleri tanımlamak ve geliştirmek için genellikle ek çabalar gereklidir. Bu çalışmada, fusarium solgunluğu, hıyar mozaik virüsü, külleme ve cinsiyet belirleme için literatürlerde belirtilen geliştirilmiş olan markörler içinden 15 tanesi seçilmiştir. Seçilen CAPS ve SCAR markörleri 11'i F1 çeşit olmak üzere ve bazı egzotik tipleri de içeren toplam 24 kavun genotipi üzerinde test edilmiştir.

Çizelge 6. CAPS Fom1-R / Fom1-S markörüne ait duyarlı ve dayanıklı kesme enzimiyle elde edilen sonuçlar

GENOTİP	**FOM DUYARLILIK (FOM-1 GENİ) VE DUYARLILIK (*BspCNI* KESME ENZİMİ) MARKÖRÜ MEVCUDİYETİ (568 bç)**	**FOM DAYANIKLILIK (FOM-1 GENİ) VE DUYARLILIK (*BSP*HI KESME ENZİMİ) MARKÖRÜ MEVCUDİYETİ (262+306 ve 568 bç)**
1. CU 129	-	+ (568 bç)
2. CU 305	+ (568bç)	H (262+306+568 bç)
3. Y15 İsabella	-	+ (568 bç)
4. Y63	-	+ (568 bç)
5. CU 269	-	+ (568 bç)
6. T4 Hasanbey	-	-
7. Y9	-	-
8. Şıra F_1	-	+ (568 bç)
9. Serin F_1	-	+ (568 bç)
10. Balözü F_1	+ (568 bç)	+ (568 bç)
11. Napolyon F_1	-	+ (568 bç)
12. Dıgıtal F_1	+ (568 bç)	+ (568 bç)
13. VCR-601 F_1	-	+ (568 bç)
14. Polidor F_1	+ (568 bç)	+ (568 bç)
15. Dragon F_1	-	+ (568 bç)
16. Favori F_1	+ (568 bç)	-
17. Medetli F_1	+ (568 bç)	+ (568 bç)
18. Galina F_1	-	+ (568 bç)
19. Topatan	+ (568 bç)	-
20. Düvlek Kavunu	-	+ (568 bç)
21. Musakka	+ (568 bç)	-
22. Çilli	-	-
23. Beyaz Gönen	+ (568 bç)	+ (568 bç)
24. T6 Acur	+ ((568 bç)	+ (568 bç)

*: H: heterozigot dayanıklı *: (+): bant mevcut (568 bç) * :(-): bant mevcut değil.

Fusarium solgunluğu için, FM, Fom2 R408, Fom2 S342, Fom-1R / Fom-1S markörleri 24 kavun genotipi üzerinde test edilerek sonuç alınmıştır. Bu sonuçlar Oumouloud ve ark. (2013; 2015)'nın çalışmaları ile Wang ve ark. (2000)'nın yaptığı çalışmalar sonucunda paralellik gösterdiği tespit edilmiştir. Bu markörlerin kavun ıslahında etkin bir şekilde kullanılabileceği düşünülmektedir. Ayrıca RAPD belirteçleriyle (E07- G17) 8 yerli genotipinde yapay inokulasyonla fusariuma duyarlılık düzeyleri tespit edilmiştir (Şensoy, 2005). Bu çalışmada ortak olarak kullanılan genotiplerde benzer sonuçlar elde edilmiştir.

Fom2 R408, Fom2 S342 SCAR belirteçlerinde elde edilen sonuçlar 408 bç dayanıklılık bantı ve 342 bç duyarlı bant elde edilmiştir. Fom2 R408, belirtecinde 2 yerli genotipte bant görüntüsü elde edilmiştir. Çalışmada yer alan hibrit, yerel veya egzotik 24 kavun genotipinin dokuzunda duyarlılık ile ilişkili bant elde edilmiştir. E07 RAPD belirteciyle egzotik kavun genotiplerinde CU-269'un fusariuma duyarlı olduğu tespit edilmiştir. Bu genotipin Fom2-S342 SCAR markörüne göre de duyarlı olduğu tespit edilmiş olup, E07 RAPD belirtecine göre eşdeğerdedir. T4 Hasanbey genotipi fusariuma duyarlı iken, Fom2-R408 SCAR markörüne göre dayanıklı olduğu görülmüştür. E07 RAPD belirtecine göre T6 Acur, Gönen Beyazı ve CU 269 yerli genotiplerde fusariuma duyarlı bant elde edilmiştir. SCAR Fom2-S342 duyarlı marköründe T6 Acur, Gönen Beyazı'ndan bant elde edilmezken, CU 269 genotipinde duyarlı bant elde edilmiştir (Şensoy, 2005).

Hıyar mozaik virüsü için test edilen SCAR $SCOPE14_{541}$ ve $SCAPB05_{1046}$ markörü 24 kavun genotipi üzerinde tespit edilmiş ve $SCOPE14_{541}$ için sonuç alınmıştır. Bu sonuç, Daryono ve ark. (2009), yaptığı çalışmayla paralellik göstermiştir ve klasik testlemeye alternatif olabileceği düşünülmektedir.

Fom-1 genine bağlı olarak geliştirilmiş olan Fom1-R / Fom1-S markörü ise 24 kavun genotipi üzerinde taraması yapılmıştır (Oumouloud ve ark., 2015). CAPS, Fom1-R / Fom-1-S marköründe kesme enzim olarak dayanıklılık bantı eldesi için, *Bsp*CNI ve duyarlılık bantı eldesi için, BspHI enzimi kullanılmıştır. Kesilme sadece duyarlılık bandında olmuştur. Duyarlı çeşit olan bant duyarlı olan kesme enzimiyle uyuşmakta olduğu görülmüştür. Duyarlı BspHI kesme enziminin güvenle kullanılabileceğini göstermiştir. *Bsp*HI enzim kesiminde bant aralıkları ise 262+306 bç ve 568 bç bant görüntüsü elde edilmiştir. Sadece bir duyarlı çeşitte kesme enzimi ile kesim sağlanmıştır.

Cinsiyet belirlemeyi sağlayan andromonoik ve monoik T1, T1ex, M3A, M3a SCAR markörleriyle EX1_C170T CAPS marköründen olumlu bir sonuç alınamamıştır. Belirteçler ile ilgili bantlar görülmemiş ve genotipler hakkında herhangi bir görüşe varılmamıştır. Külleme için; CAPS Pm-2F genini tanıyan CAPS marköründe de güvenilir sonuç alınamamıştır. Markörün bant aralığı görülmüş olup, kesme enzimiyle kesme işlemi gerçekleşmemiştir. Fom-2 genini tanıyan DNA belirteci olan CAPS2 ve CAPS3, CAPS markörlerinden de sonuç alınamamıştır.

4. Sonuç ve Öneriler

Elde edilen sonuçlar gen kaynaklarının korunmasında kolaylık sağlayacağı ve klasik ıslah yöntemlerine kaynak oluşturacağı düşünülmektedir. Türkiye'nin sahip olduğu zengin kavun populasyonlarının günümüz biyoteknoloji imkanlarının modern kavun ıslahına katkı sağlaması ve kavunda ileride değişik hastalık ve zararlılara karşı reaksiyonların da önceden bilinmesi kavun ıslahına önemli katkılar sağlayacağı da aşikardır.

Dayanıklılık ıslah programında en büyük zorluk ebeveynlerin (duyarlı ve dayanıklı bireyler) melezlenmeleri sonucu elde edilen dayanıklı bireylerin belirlenmesidir. Başta birden fazla gen olmak üzere, birkaç gen ve tek gen tarafından idare edilen dayanıklılık özelliklerini (karakterleri) normal şartlarda geleneksel ıslah yöntemleri ile belirlemek zaman almakta, fazla iş gücü gerektirmekte ve çok güç olmaktadır. Bununla birlikte bir bitkiyi aynı anda birden fazla hastalık veya zararlı etmenle testlemek geleneksel yöntemlere göre hemen hemen imkansızdır. Bütün bu zorluklar moleküler markörlerin devreye girmesiyle üstesinden gelinebilmektedir (Lu ve ark., 1999).

Bu çalışmada literatürde kavun ıslah programlarında moleküler belirteç yardımlı seleksiyon amaçlı geliştirilmiş markörlerden sadece üçte birinde (5/15) etkin bir sonuç alınmıştır. Bu markörler Çizelge 7'de gösterilmiştir.

Çizelge 7. Bu çalışmada sonuç veren moleküler markörlere ait primer dizilimleri

Gen	Markör	Markör tipi	Markör ID	Markör dizilimi
Fom-2	SCAR	Ko-dominant	FM	F=GAAGATGCAAAGAAAAAGAGAAGG R=TCAATTAAACATTCTGATGCC
CMV-B2	SCAR	Dominant	SCOPE14 541	F= TGCGGCTGAGGACGGTTGGAGGTC R= TGCGGCTGAGCATTCTCGAGCAG
Fom-2	SCAR	Dominant	Fom2-R408	F=GAGAAATTTGCAATGGGTGG R=TTACACTATTATTGCTCAACTTGC
Fom-2	SCAR	Dominant	Fom2-S342	F=ATGAAAAGAAAAGATAACGACGA R=ATTGCTCTAAGTTGATCATATTCTG
Fom-1	CAPS	Ko-dominant	Fom1-R Fom1-S	F=ATGAGTTTTGATAGTTTCATAAG R=GAACACTCCCTTAGATACTT

Çalışmada ayrıca kapiller elektroforezin agaroz jel elektroforezden daha etkin olduğu görülmektedir. Markörlerin seleksiyonda etkili olabileceği fakat markörlerin gene uzaklık durumunun, dikkate alınması; klasik hastalık testleme çalışmaları ile de testleme yapılmasının faydalı olacağı düşünülmektedir. Kapiller yöntemin hem zamandan tasarruf hem de 1 µl örnekte bile güvenilir sonuç alınabilmesinin yanında yüzlerce örneği kısa bir zaman diliminde test edebilmesinden dolayı avantajları oldukça yüksektir.

Bu yüzden literatürde farklı populasyonlar kullanılarak elde edilmiş belirteçlerin ülkemiz populasyonlarında kullanılmasında sıkıntılar olabilecektir. Klasik hastalık bulaştırma yöntemlerinden yararlanılarak, kavun genotiplerinin dayanıklılıkları yapay inokulasyonla da hastalık belirlenmesi ve genotiplerin dayanıklılıkları tespit edilmesi önem arz edecektir. Elde edilecek MAS sonuçların klasik hastalık bulaştırma yöntemleri ile desteklenmesi faydalı olacaktır.

Entansif tarım uygulamaları yüzünden hastalık ve zararlı etkenleri sürekli kendilerini değiştirme kapasitesine sahiptirler. Bu yüzden hastalık ve zararlılara dayanıklılık sağlayan yeni gen kaynaklarının bulunması ve bunlarla ilişkili güvenilir ve etkin belirteçlerin ıslah programlarına gelecekte yoğun bir şekilde kazandırılması önem arz etmektedir.

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Research Article

Use of Brown Seaweed Extracts as Bio-fertilizers and their Effects on the Ice-ice Disease Occurrence, Carrageenan Yield, and Growth Rate of the Red Seaweed *Kappaphycus striatus*

Albaris B. TAHILUDDIN[*1], Sitti Sheha H. IRIN[2], Katrina S. JUMADIL[3]
Radzwina S. MUDDIHIL[4], Ertugrul TERZI[5]

[1,2,3,4]Mindanao State University-Tawi-Tawi College of Technology and Oceanography, College of Fisheries, Sanga-Sanga, Bongao, 7500, Tawi-Tawi, Philippines
[5]Kastamonu University, Faculty of Fisheries, 37200, Kastamonu, Turkey

[1]https://orcid.org/0000-0002-3237-3552, [2]https://orcid.org/0000-0002-6409-1007, [3]https://orcid.org/0000-0002-0832-6019
[4]https://orcid.org/0000-0003-3061-7426, [5]https://orcid.org/0000-0003-2811-6497

*Corresponding author e-mail: albarist20@gmail.com

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Seaweed liquid extract,
Turbinaria

Abstract: *Kappaphycus striatus* is one of the most important eucheumatoid species that is widely farmed worldwide. In the southern Philippines, where the initial farm was established, sluggish growth of farmed *Kappaphycus* species brought about by the poor quality of planting materials and extensive farming resulting in unproductive farms and frequent ice-ice outbreaks have been a hindrance in increasing the seaweed production. As a result, farmers have led to the application of inorganic fertilizers as nutrient enrichment for *Kappaphycus*. However, inorganic or chemical fertilizers always pose negative impacts on the environment. Hence, in this study, a preliminary investigation on the potential use of extracts of brown seaweeds *Sargassum cristaefolium* and *Turbinaria conoides* as bio-fertilizers was tested on *K. striatus* for their growth rate, carrageenan yield, and ice-ice disease occurrence. Seaweed liquid extracts (SLE): *S. cristaefolium* (SC), *T. conoides* (TC), combination of SC and TC (MX), and control (C) were utilized as bio-fertilizers for *K. striatus*. SLE-enriched *K. striatus* seedlings were cultivated in a seaweed farm using the fixed-off bottom method for 45 days. Results revealed that the specific growth rates of all SLE treatments were significantly higher than no SLE treatment at day 45. The percentage of ice-ice disease and the yield of carrageenan did not differ among treatments. Enrichment of *K. striatus* using SLE of two selected brown seaweeds before out-planting could improve growth rates while not affecting the ice-ice disease occurrence and carrageenan yield. Hence, formulated SLE from brown seaweeds *S. cristaefolium* and *T. conoides* can be used as potential bio-fertilizers for *Kappaphycus* cultivation.

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1. Introduction

The genus of *Kappaphycus* is an economically important red seaweed cultured in both tropical and subtropical waters. This red seaweed is highly marketable worldwide owing to its carrageenan content, which is extensively utilized in food and non-food products as a binder, emulsifier, gelling, and

thickening agents (Hurtado et al., 2000; Hurtado et al., 2015; Tahiluddin and Terzi, 2021a). The Philippines is a major seaweed producer, ranking fourth globally (FAO, 2020). However, one significant hurdle in increasing the seaweed production in the Philippines is the unproductive seaweed farms which now yield only minimal production compared to before. Reasons could be attributed to lowering seedling stock quality or seaweed overstocking, leading to nutrient depletion and slow seaweed growth. Hence, water fertility is tremendously crucial in regulating the yield and sustainability of seaweed production (Luhan et al., 2015).

The cultivation of *Kappaphycus* species depends primarily on the sea's natural fertility (Hurtado et al., 2001; Muñoz et al., 2004; Hayashi et al., 2007; Luhan et al., 2015). One way of improving seaweed production to meet the phycocolloids demand and improve seaweed farmers' earnings is through the nutrient enrichment of seaweeds (Luhan et al., 2015). Previous studies that utilized inorganic fertilizers and organic biostimulants as nutrient enrichment showed promising results in increasing the growth and ameliorating the health and condition of seaweeds (Neish et al., 1977; Rui et al., 1990; Lavilla-Pitogo, 1992; Dawes et al., 1994; Menéndez et al., 2002; Nagler et al., 2003; Loureiro et al., 2010; Martins et al., 2011; Borlongan et al., 2011; Loureiro et al., 2012; Luhan et al., 2015; Umanzor, 2019; Ali et al., 2020).

Seaweed liquid extracts (SLE) are formulated as a source of bio-fertilizer with huge nutrient contents that affect the diverse physiological processes like seed germination, vegetative growth, and productivity, resistance against pathogens of various crops (Sathya et al., 2010). As originally initiated by Milton in 1952, SLE is now well utilized to enrich crops in agriculture and horticulture (Hurtado et al., 2009). *Sargassum* and *Turbinaria* species have been utilized as SLE for crops, which according to studies, showed remarkable growth, yield, quality, and other properties of crops (Zodape et al., 2008; Erulan et al., 2009; Kumari et al., 2011; Kavipriya et al., 2011; Vijayanand et al., 2014; Selvam and Sivakumar, 2014; Nabti et al., 2017; Layek et al., 2018; Manea and Abbas, 2018; Silva et al., 2019; Sunarpi et al., 2020; Karthik et al., 2020; Ali et al., 2021).

Another problem that hinders eucheumatoid production is the occurrence of ice-ice disease (Ward et al., 2022), which is typically characterized by the appearance of soft and white portions on the infected branches (Tahiluddin and Terzi, 2021a; Tahiluddin et al., 2021b). This disease is brought about by fluctuations of environmental parameters like light intensity, salinity, and temperature resulting in stress on the seaweeds, thus weakening its immune defense system against harmful bacteria *Cytophaga-Flavobacterium* and *Vibrio-Aeromonas* (Largo et al. 1995a and 1995b; Tahiluddin and Terzi, 2021a). In the Philippines, the occurrence of the ice-ice disease is widespread and caused a severe decline in the production of *Kappaphycus* species (Mendoza et al., 2002; Faisan et al., 2021); thus, it is considered a significant threat to the seaweed industry (Tahiluddin et al., 2021c). Nutrient deficiency has been suspected as another factor that may trigger ice-ice disease in the farm (Maryunus, 2018). Moreover, Luhan et al. (2015) reported that nutrient-enriched *K. alvarezii* had significantly lower ice-ice disease occurrence than untreated seaweed.

In Tawi-Tawi, southern Philippines, the ice-ice disease occurrence and slow growth have led to the rampant use of inorganic fertilizers as nutrient enrichment for *Kappaphycus* (Tahiluddin, 2018; Tahiluddin et al., 2021a and 2021b). The diversity of seaweed in Tawi-Tawi is high, with a reported 79 species, including cultured *Kappaphycus* species and wild brown seaweeds (Puig-Shariff, 2015; Yangson et al., 2022). *S. cristaefolium* and *T. conoides* are among the most abundant brown seaweed species. Therefore, exploring the potential use of these seaweeds as alternative environmental-friendly fertilizers is worth investigating. This is also to diminish the use of inorganic fertilizers, which may not only harm the marine environment but also pose negative health risks to consumers since cultivated *Kappaphycus* species are also consumed as salads by local communities. Besides, no studies have been conducted on the potential use of extracts of these brown seaweeds as bio-fertilizer for *K. striatus*. Hence, this study investigated the use of brown seaweed extracts (*S. cristaefolium* and *T. conoides*) as potential bio-fertilizers by evaluating their effects on the ice-ice disease occurrence, growth rate, and carrageenan yield of *K. striatus*.

2. Material and Methods

2.1. Study area

This study was carried out at the seaweed farm of Panglima Sugala municipality, province of Tawi-Tawi, southern Philippines (Fig 1), for 45 days from December 30, 2018, to February 13, 2019.

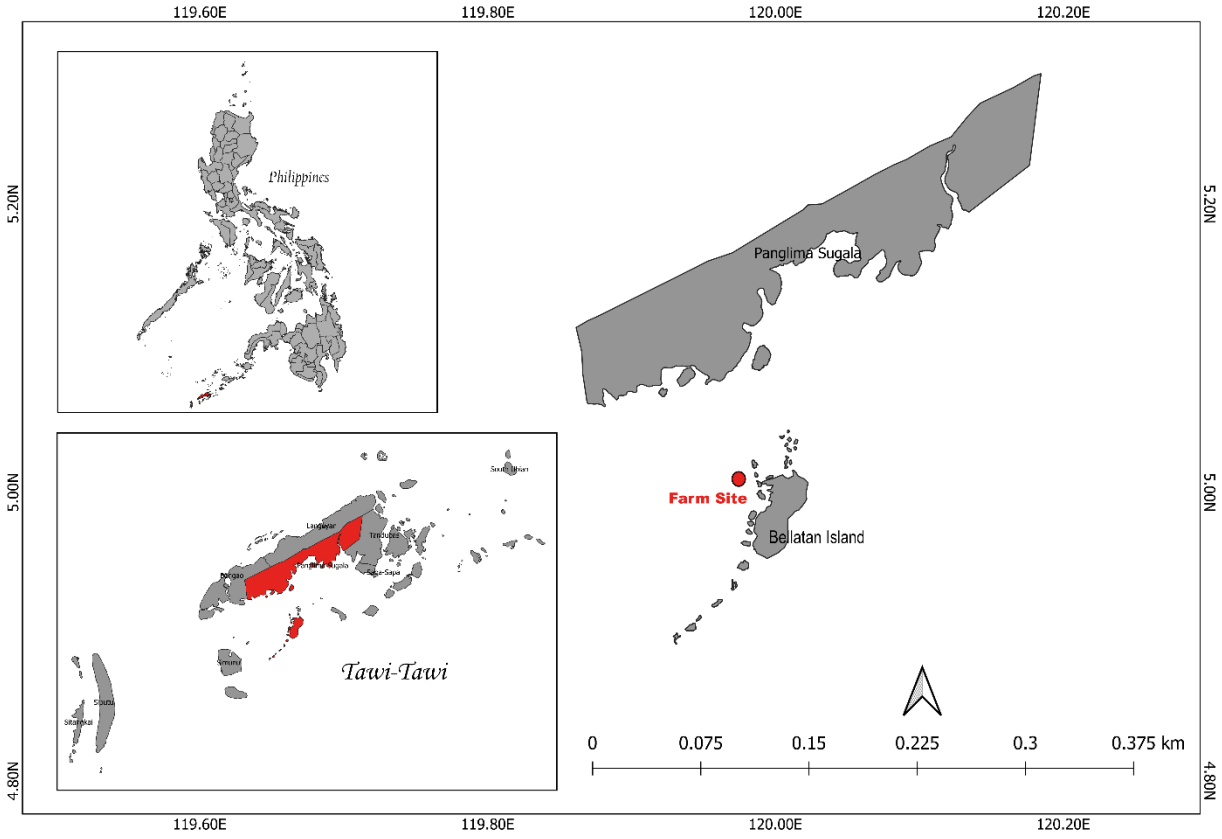

Figure 1. Study area.

2.2. Source and preparation of seedlings

The seedlings of *K. striatu*s were bought from the farmer at the study site. Healthy, disease-free, and untreated seedlings were used in this study. The seedlings were prepared by cutting into 50 g each. These were tied into a rope line measuring 5 m with an interval of 25 cm. There were 20 points per line. A total of 12 lines were prepared to represent the 3 SLE treatments and control, with 3 replicates each.

2.3. Formulation of seaweed liquid extract fertilizers

Formulation of SLE as bio-fertilizers followed the method of Vijayanand et al. (2014). The brown seaweeds *S. cristaefolium* and *T. conoides* were gathered from the rocky area of Sunkist, Pahut, Bongao, Tawi-Tawi, and southern Philippines. The collected seaweeds were cleaned by washing with fresh water 3 times in order to remove any foreign materials like sand, epiphytes, and other associated flora and fauna. SLE was formulated as follows: SC = *S. cristaefolium*, TC = *T. conoides*, MX = combination of SC and TC at a 50:50 ratio. The brown seaweeds were chopped into small pieces. Approximately a kg of each brown alga was sun-dried for 5 min to remove excess moisture. The weight of the chopped seaweeds was determined. Three liters of distilled water were added to chopped seaweeds and boiled for approximately 2 hr. The crude aqueous extracts were cooled and filtered with a muslin cloth to get the SLE. SLE fertilizers were placed in clean bottles and stored in a cool and dry place.

2.4. Immersion of seedlings to SLE solution and planting

The immersion method of seedlings to SLE solution was done in the afternoon from 4-6 pm following Tahiluddin (2018) as practiced by the seaweed farmers in the province. Three bio-fertilizer solutions using SLE (SC = *S. cristaefolium*, TC = *T. conoides*, MX= combination of SC and TC) were prepared at 8.82 mL L^{-1} concentration [average concentration practiced by the farmers (Tahiluddin, 2018)] for each treatment, while only seawater was used for C as control. Three (3) cultivation lines (serving as replicates) were simultaneously dipped into each fertilizer solution for 30 s, then covered with canvas, and left overnight. Before out-planting, the seedlings were immersed in seawater for 10 min to avoid stress, and then these were transported via a small boat to the farming site. Cultivation lines were randomly set up by the fixed-off bottom method, 30 cm above the seabed.

2.5. Monitoring of ice-ice disease occurrence, growth rate, and environmental parameters

Ice-ice disease occurrence and growth rate were determined on days 14, 30, and 45. Growth rate sampling was done by removing tagged branches, patting with a clean cloth, and weighing. Sampled seaweeds were re-tied to their original positions. Ice-ice disease occurrence was monitored through visual inspection. The whitening and softening of the seaweed branches were considered ice-ice disease symptoms (Luhan et al., 2015; Tahiluddin et al., 2021b). The specific growth rate (SGR) was calculated using the formula adopted by Luhan et al. (2015), and the specific growth rate (SGR) was calculated. Ice-ice disease occurrence (%) was calculated by dividing the total number of infected branches by the total of branches per line and multiplying by 100 (Largo et al., 1995).

Seawater conditions in the farm site like salinity, temperature, and pH were monitored using a refractometer, thermometer, and pH meter, respectively, every 7 days. The water current was determined using an improvised drogue, and depth was measured using a calibrated rope every 7 days. In addition, the cultivation setup was maintained every 7 days by removing silt, debris, and predator attached to the farmed seaweeds.

2.6. Carrageenan yield determination

Determination of carrageenan quality was done after 45 days of culture. First, samples were dried under the sun for 3 days. Next, dried seaweeds were cleaned by washing with water to remove silts and other debris. Fifteen (15) g of dried seaweeds were boiled in approximately 250 mL of purified water until all the seaweeds had been dissolved. The dissolved seaweeds were immediately filtered while the slurry was still hot. The filtrate was allowed to cool and freeze overnight. The frozen filtrates of seaweeds were thawed, then dried at 60 ºC in the oven. The resulting product was the native carrageenan. Carrageenan yield was calculated using the formula (Luhan et al., 2015).

$$CY = \frac{Wc}{Wds} x100 \quad (1)$$

Where: CY = Carrageenan yield
Wc = Weight of carrageenan
Wds = Weight of dried seaweeds

2.7. Data analysis

Using IBM SPSS software version 20, data on ice-ice disease, growth rate, and carrageenan yield were subjected to One-way Analysis of Variance (ANOVA). Post hoc (Duncan) was used if significant differences exist among treatments. The data were given as mean ± SE. The statistical significance level was set to 0.05.

3. Results

The specific growth rates (SGRs) of *K. striatus* enriched with different bio-fertilizers are shown in Figure 2. On day 14, the SGRs of SC, TC, MX, and C were 5.1±0.27, 5.23±0.16, 5.01±0.31, and 3.65±0.55% day^{-1}, respectively. On day 30, the SGRs of SC, TC, MX, and C were 4.33±0.14, 4.14±0.17, 4.09±0.3, and 2.87±0.38% day^{-1}, respectively. On day 45, the SGRs of SC, TC, MX, and C were

4.33±0.14, 4.14±0.17, 4.09±0.3, and 2.87±0.38% day^{-1}, respectively. Analysis revealed that the SGRs of *K. striatus* enriched with different bio-fertilizers (SC, TC, and MX) were significantly higher than the control (C) on days 14, 30, and 45 (p<0.05). The mean weights (MWs) of cultured SLE-enriched *K. striatus* are shown in Figure 3. On day 14, MWs of SC, TC, MX, and C were 103.13±4.04, 104.33±2.24, 101.87±4.42, and 86.67±6.04 g, respectively. One-way ANOVA revealed that all SLE-treated *K. striatus* was significantly higher (p<0.05) than control. Higher MWs were also observed on day 30, where SC (185.4±7.49 g), TC (176.2±8.61 g), and MX (180.27±15.13 g) were significantly higher (p<0.05) than C (125.2±15.17 g). On day 45, MWs of SC (238.67±13.12 g), TC (192.07±26.85 g), and MX (180.27±27.47 g) were significantly higher (p<0.05) than C (116.67±22.06 g).

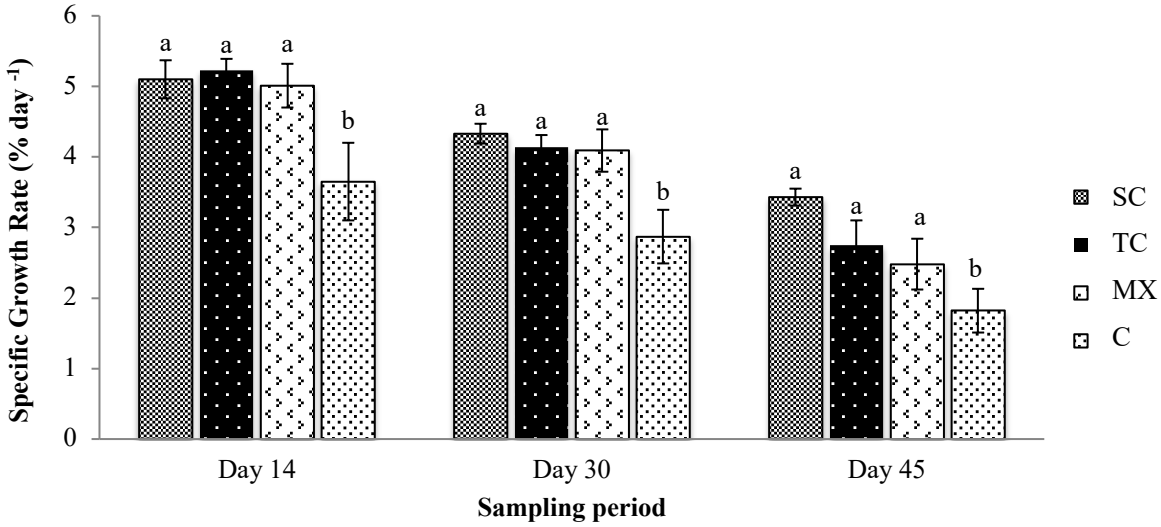

Figure 2. SGR (% day^{-1}) of *K. striatus* in every sampling period. SC= *S. cristaefolium*, TC = *T. conoides*, MX = combination of SC and TC, and C = control. Bars with different letters are significantly different (p<0.05). Error Bars in SEM (standard error of the mean), n=13-15.

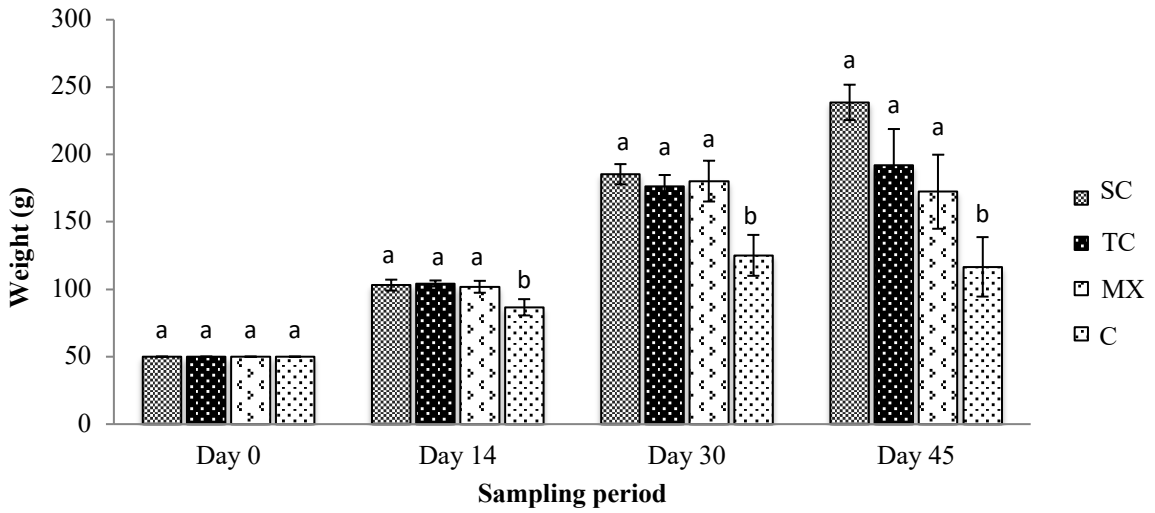

Figure 3. Mean weight of *K. striatus* in every sampling period. SC= SLE (*S. cristaefolium*), TC= SLE (*T. conoides*), MX= SLE (combination of SC and TC), and T_4=Control. Bars with different letters are significantly different (p<0.05). Errors bars in SEM (standard error of the mean), n=13-15.

Ice-ice disease occurrence of all SLE-enriched *K. striatus* treatments did not differ from the control throughout the sampling period, which ranged from about 5-12%, 65-62%, and 8-25% on days 14, 30, and 45, respectively (Fig 4). The percentage occurrence of SC (6.67±6.41%), TC (5±7.38%),

MX (10±2.85%), and C (11.67±1.45%) showed no significant difference (p>0.05) on day 14. On day 30, ice-ice disease occurrences in SC, TC, MX, and C were 45.92±9.96%, 48.09±16.56%, 60.94±10.79%, and 61.81±5.93%, respectively. On day 45, ice-ice disease occurrences in SC, TC, MX, and C were 7.62±6.36%, 20.24±6.07%, 12.31±4.69%, and T4 27.31±3.46%, respectively. No significant differences (p>0.05) in ice-ice disease occurrence were observed on days 30 and 45 among treatments. Change in the occurrence of ice-ice disease of cultured SLE-enriched *K. striatus* is shown in Figure 5. From day 0 to 30, the ice-ice disease occurrence for all treatments significantly increased (p<0.05) but significantly dropped (p<0.05) after 45 days of culture (Fig 5).

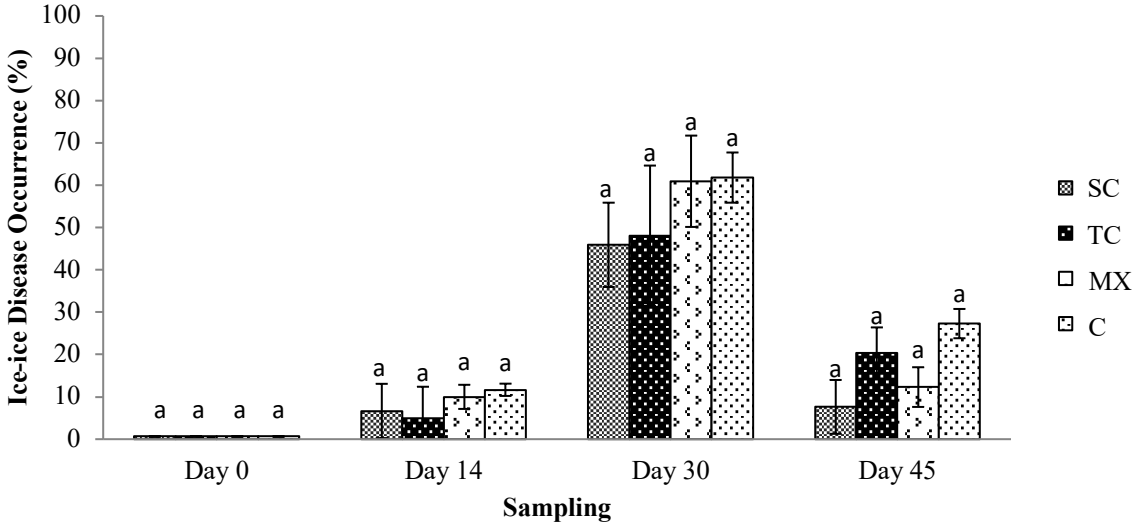

Figure 4. Occurrence of ice-ice disease of *K. striatus* in every sampling period. SC = *S. cristaefolium*, TC = *T. conoides*, C = combination of SC and TC, and C = control. Bars with the same letters are not significantly different (p>0.05). Errors bars in SEM (standard error of the mean), n=14-20.

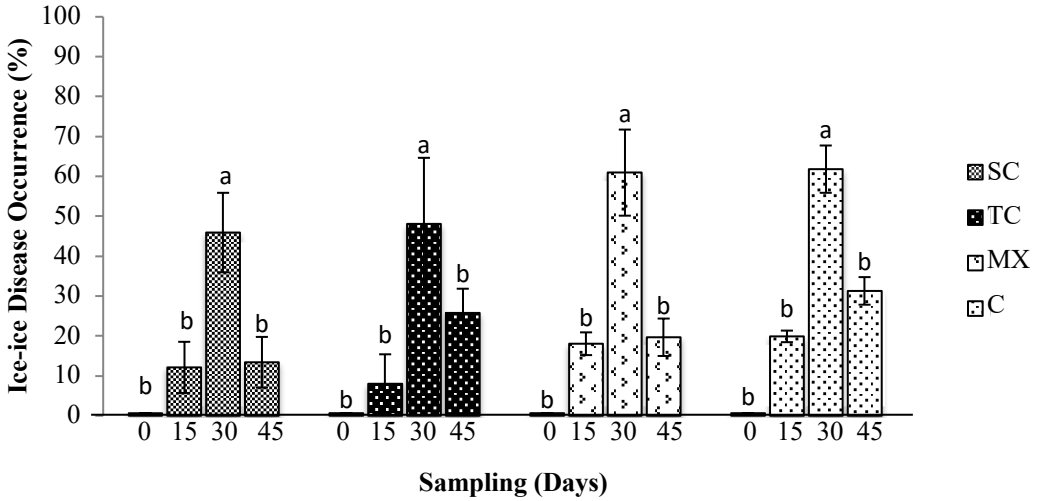

Figure 5. Change in ice-ice disease occurrence of *K. striatus.* SC= SLE (*S. cristaefolium*), TC= SLE (*T. conoides*), MX= SLE (combination of SC and TC), and C=Control. Bars with different letters are significantly different (p<0.05). Errors bars in SEM (standard error of the mean), n=14-20.

Figure 6 shows the carrageenan yield of SLE-enriched *K. striatus* after 45 days (SC, TC, MX, and C), which were 35.07±1.58, 33.44±5.52, 32.98±0.79, and 32.24±1.36 %, respectively, indicating no significant difference (p>0.05) among treatments.

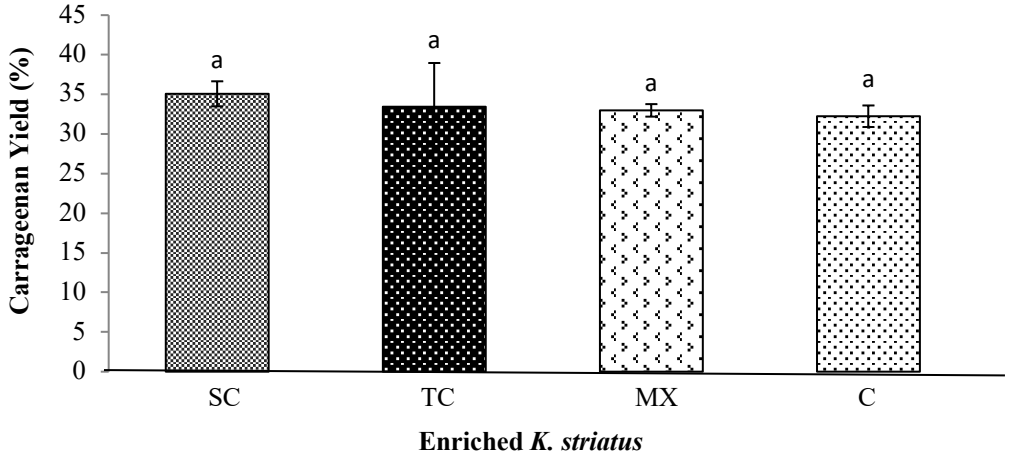

Figure 6. Carrageenan yield of 45-day old *K. striatus*. SC = *S. cristaefolium*, TC = *T. conoides,* MX = combination of SC and TC, and C = control. Bars with the same letters are not significant different (p>0.05). Errors bars in SEM (standard error of the mean), n=3.

The temperature of the farm ranged from 27.23±0.03 to 32.87±0.18 °C, salinity was from 33.33±0.33 to 35±1.0 ‰, pH was from 7.02±0.04 to 8.69±0.01, water current velocity was from 0.08±0.03 to 0.2±0.08 m s^{-1}, and water depth was from 30.67±0.33 to 188.12±0.96 cm throughout the culture period.

4. Discussion

The potential use of brown seaweeds *S. cristaefolium* and *T. conoides* or its combination as liquid extract biostimulants enriched in *K. striatus* before out-planting has demonstrated promising results. Typically, the availability of nutrients in the seawater in the environment allows the seaweed's cell walls to absorb and assimilate additional nutrients (nitrogen) needed to obtain a better growth rate farmed in nutrient-deficient conditions (Luhan et al., 2015). Although we did not determine the nutrient contents of these SLE, studies have found that brown seaweeds such as *T. conoides* and *Sargassum* spp. contain a vast array of nutrients and minerals such as phosphorus, nitrogen, potassium, zinc, magnesium, manganese, iron, calcium, and copper (Santoso et al., 2006; Sutharsan et al., 2017). SLE used in this study may have utilized the nutrients from these extracts or contain biostimulatory properties favoring higher growth than untreated. Besides, the methods and concentration used in this study have proven efficient in providing additional nutrients to *K. striatus*, thereby increasing its growth (Tahiluddin, 2018; Tahiluddin et al., 2021a; Sarri et al., 2022). Ali et al. (2021) reported that seaweed-based bioproducts have phytostimulatory properties that result in improved plant yield and growth parameters in various crops. For instance, SLE of *S. wightii* enriched in cluster bean plant at low concentration (1.5%) promoted growth (Vijayanand et al., 2014). The extract of *S. johnstonii* applied to *Lycopersicon esculentum* showed a positive influence on its yield, growth, and quality (Kumari et al., 2011). The growth and biochemical constituent of pigeon pea *Cajanus cajan* exhibited better results using extract of *S. polycystum* (Erulan et al., 2009). Also, extracts from *S. muticum* and *Ascophyllum nodosum* at 25% concentration showed positive effects in lettuce and rice plants (Silva et al., 2019). On the other hand, a combination of *T. ornata* and *Ulva reticulata* as seaweed liquid fertilizer showed better plant growth and seed germination of *Phaseolus vulgaris* (Green Pea), *Raphanus sativus* (Radish), and *Vigna radiata* (Mung) (Karthik et al., 2020). Similarly, the liquid extract of *T. murayana* has been shown as an effective bio-fertilizer on tomato plants (*Lycopersicum esculentum*) by significantly improving the fruit and flower numbers (Sunarpi et al., 2020).

The application of brown seaweed *Sargassum* and *Turbinaria* as nutrient enrichment for *Kappaphycus* farming has not been explored. Previous investigations focused on using Acadian Marine Plant Extract Powder (AMPEP) to improve the growth of *Kappaphycus*. AMPEP is extracted from

brown alga *A. nodosum*. For instance, *K. alvarezii* enriched with AMPEP obtained an SGR of 1.3 – 4.1 % day^{-1} (Borlongan et al., 2011), lower than the SGR obtained in this study. Loureiro et al. (2010; 2012) reported that brown seaweed *A. nodosum* (liquid form) as enrichment increased the daily growth rate (5.5 – 5.6%) of cultured *K. alvarezii,* which is similar to our result with SGR of 5.23% in enriched *K. striatus*. Also, AMPEP significantly increased the growth rate (7.3%) in *K. alvarezii* (Loureiro et al., 2014), relatively higher than our study.

The ice-ice disease of *Kappaphycus* is usually associated with changes in environmental factors like light intensity, salinity, and temperature manifested with extensive whitening of the branches, which are further degraded by the presence of opportunistic bacteria (Largo et al., 1995a and 1995b; Tahiluddin and Terzi, 2021a and 2021b; Tahiluddin et al., 2021c). Likewise, marine-derived fungi were reported to be a potential causative agent of this disease (Solis et al., 2010). Another factor that seemed to trigger the occurrence of the ice-ice disease is the lack of nutrients in the nutrient-deficient environment, as Luhan et al. (2015) demonstrated. The authors planted the nutrient-enriched *K. alvarezii* in a bamboo raft net cage without maintenance for 45 days and revealed that enriched seaweed had significantly lower ice-ice disease occurrence (8.75%) than control (97%). However, in this study, since enriched *K. striatus* seaweeds and control were cleaned regularly, the effect of used bio-fertilizers in ice-ice disease occurrence was not detected, although a high incidence of ice-ice disease occurred on day 30 but eventually declined on day 45. On the other hand, AMPEP lessened ice-ice disease development in *Kappaphycus* farming (Hurtado and Critchley, 2013). Besides, AMPEP-enriched *K. striatus* with a concentration of 0.01 g L^{-1} and 8.82 g L^{-1} using the same method used in this study significantly decreased ice-ice disease occurrence (Illud, 2020). Moreover, the utilization of AMPEP in *K. alvarezii* as mitigation against epiphytes has been successfully tested (Ali et al., 2020; Borlongan et al., 2011; Hurtado and Critchley, 2013; Loureiro et al., 2017).

The reported carrageenan yield of *Kappaphycus* in the Philippines ranged from 15 to 64% (Hurtado-Ponce, 1995; Mendoza et al., 2002; Luhan et al., 2015; Robles, 2020; Sarri et al., 2022). In this study, the carrageenan yield of nutrient-enriched *K. striatus* (33-35%) did not differ from the untreated seaweed (32%). These findings are parallel to the studies of Loureiro et al. (2012 and 2014), in enriched *K. alvarezii* with AMPEP was about 33 and 38%, respectively. Conversely, in sodium nitrate-enriched *K. alvarezii*, the carrageenan yield was 42% (Luhan et al., 2015), relatively higher than our study. The utilization of SLE of brown seaweeds in *K. striatus* did not influence the carrageenan yield suggesting that these seaweeds can be used as potential bio-fertilizers for *Kappaphycus* farming.

Conclusion

In conclusion, this study revealed that the experimental liquid extracts from the brown seaweeds *S. cristaefolium* and *T. conoides* could be utilized as potential bio-fertilizers that may work as biostimulants since the growth of *K. striatus* improved while not affecting the ice-ice disease and carrageenan yield. Although this is a preliminary study, this practical approach may benefit seaweed farmers who attempt to increase production for better profit and livelihood. However, application refinement, like using different concentrations, mode of application, and soaking period may be explored for enrichment efficiency. In addition, the nutrient contents of these SLE may also be further investigated based on their seasonal availability.

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