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Determination of Tolerant Genotypes Against Flood Stress in Spinach

Yeşim DAL CANBAR[1], Musa SEYMEN[2], Ayşe Özgür UNCU[3], Önder TÜRKMEN[4], Banu Çiçek ARI[5]
[1]Horticulture Department of Agriculture Faculty, Selcuk University, Konya, Türkiye, [2] Horticulture Department of Agriculture Faculty, Selcuk University, Konya, Türkiye, [3]Department of Biotechnology, Necmettin Erbakan University, Konya, Türkiye, [4] Horticulture Department of Agriculture Faculty, Selcuk University, Konya, Türkiye Adresi, [5] Horticulture Department of Agriculture Faculty, Selcuk University, Konya, Türkiye
[1] https://orcid.org/ 0000-0002-3806-6465, [2] https://orcid.org/ 0000-0002-2742-137X, [3] https://orcid.org/0000-0001-6435-579X
[4] https://orcid.org/ 0000-0003-3218-6551, [5] https://orcid.org/0000-0002-1578-8561
✉: yesim.dal@selcuk.edu.tr, dalyesim@gmail.com

ABSTRACT

Abiotic stress factors generate negative effects on agricultural production daily. With the effect of global warming, the floods that have increased recently not only affected human life negatively but also caused great losses in plant development. For this reason, developing tolerant plants against flooding stress is the most critical approach reducing yield and quality losses. The present study aimed to determine the genotypes that are tolerant of flooding stress by using the agro-morphological and physiological characteristics of the commercial varieties and S5-level spinach breeding materials. In the study, 13-day flood stress was applied to 48 hybrid cultivars and 23 spinach genotypes at the S5 stage during the seedling period. As a result, in addition to the adverse effects of flood stress on plant growth, it was determined that the tolerance was different between genotypes. In the light of the results obtained, SWA0760 F1 among commercial varieties was found to be the most tolerant variety to flood stress. At the same time, genotypes 14, 9, 21, 15, 4 and 10 from breeding lines were promising genotypes that were tolerant to flooding stress. As a result, it is predicted that the inclusion of the genotypes used in the study as parents in hybrid cultivar breeding will make significant contributions to the development of tolerant cultivars against flood stress.

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ÖZET

Abiotik stres faktörleri gün geçtikçe tarımsal yetiştiricilikteki olumsuz etkilerini artırmaktadır. Küresel ısınmanında etkisi ile son zamanlarda artan sel baskınları insan hayatını olumsuz etkilediği gibi bitki gelişiminde de büyük kayıplara neden olmaktadır. Bu sebeple son zamanlarda sel baskını stresine karşı tolerant bitkilerin geliştirilmesi verim ve kalite kayıplarını azaltmada en önemli yaklaşımdır. Mevcut çalışmada bazı ticari çeşit ve gen havuzunda bulunan bazı ıspanak ıslah materyallerinin agro-morfolojik ve fizyolojik özelliklerinden sel baskını stresine tolerant genotiplerin belirlenmesi amaçlanmıştır. Çalışmada, 48 adet hibrit çeşit ve S5 kademesinde olan 23 adet ıspanak genotiplerine fide döneminde 13 günlük sel baskını stresi uygulanmıştır. Tam sulanan kontrol bitkileri ile kıyaslanan stres koşullarında bitki gelişiminin olumsuz etkilenmesinin yanı sıra ıslah hatlarında ve ticari çeşitlerin toleranslılığının farklı olduğu tespit edilmiştir. Elde edilen sonuçlar ışığında, ticari çeşitlerden SWA0760 F1 sel baskını stresine en tolerant çeşit olarak bulunurken, ıslah hatlarından 14, 9, 21, 15, 4 ve 10 numaralı genotipler sel baskını stresine tolerant ümitvar genotipler olarak bulunmuştur. Sonuç olarak, elde edilen genotiplerin hibrit çeşit ıslahında ebeveyn olarak melezleme programlarına dahil edilmesi sel baskını stresine tolerant çeşit geliştirilmesinde önemli katkılar sağlayacağı öngörülmektedir.

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INTRODUCTION

Flooding stress has become one of the important stress factors limiting productivity in agriculture day by day (Jackson & Colmer, 2005; Hirabayashi et al., 2013). In the last 50 years, flooding stress and its destructive effects have tended to increase due to global climate change (Arnell & Liv, 2001; Hirabayashi et al., 2013; Sasidharan et al., 2018). It has been reported that this situation affects plant growth negatively, as well as severely reducing yield and quality (10-40%) (Hodgson & Chan, 1982; Bange et al., 2004; Patel et al., 2014). Flooding occurs in many parts of the world due to excessive and irregular rainfall and inadequate drainage. During flooding, oxygen and/or CO_2 shortages and high levels of ethylene accumulation occur in the plant root zone (Panda et al., 2008). In the root zone, lack of oxygen causes hypoxia or anoxia around the plant tissues during flooding, causing various internal changes in plants (Ishizawa et al., 1999; Geigenberger, 2003; Bailey-Serres & Chang, 2005). Flooding can seriously impair the performance of plants due to hypoxia in the plant rhizosphere (Gibbs & Greenway, 2003; Patel et al., 2014). As a result of, metabolic activities are inhibited, and ATP production decreases (Saglio et al., 1980; Panda & Barik, 2021). Reduced ATP production restricts the energy supply for root growth; thus, plant growth slows and stops completely (Drew, 1997; Bennett, 2003). In addition, changes such as respiratory changes, leaf chlorophyll content, and photosynthetic assimilation, especially in plants whose vegetative parts are consumed, occur during the flooding stress period (Patel et al., 2014).

Spinach (*Spinacia oleracea* L.) is one of the most consumed vegetable types in winter, especially in the northern hemisphere. Spinach, which is an excellent climate vegetable, is grown in an open field in spring, winter, and autumn periods in sub-tropical regions. At the same time, it is cultivated in spring or autumn periods in areas where the continental climate is dominant. Since the frequency and amount of rainfall are high in these periods, it affects productivity negatively, especially in the bottomlands (Seymen, 2021). Plant growth stage, flood time and duration, flood water status, field characteristics, plant species, and differences among genotypes significantly affect flood tolerance (Kozlowski, 1997). Many plant species, including spinach, are affected by flash floods, and morpho-physiological changes result from stress. On the other hand, it has been stated that flood-tolerant plants have the capability of decreasing the negative impact rates of flooding with the multifaceted interactions of morphological, anatomical, and physiological adaptations (Kramer, 1951; Kozlowski & Pallardy, (1997). Investigating of flood tolerance mechanisms in plants will make essential contributions to productivity in agriculture (Xu et al., 2006; Singh et al., 2009). In this study, it was aimed to determine the tolerance rates of 71 spinach genotypes under flood stress conditions. This study will determine flood stress tolerance of spinach genotypes with a large gene pool for the first time. In addition to this, the tolerant status of commercially grown spinach varieties will be revealed. On the other hand, tolerant genotypes in the gene pool will be used as parents in cultivar breeding studies, allowing the development of tolerant cultivar candidates to flood stress.

MATERIALS and METHOD

The study was carried out in the glass greenhouses of Selçuk University, Faculty of Agriculture, between September 1- December 02, 2021. In the study, 48 hybrid cultivars and 23 spinach genotypes were used in level S5 (Table 1). Seeds of these genotypes were sown in plastic pots (350 cc) filled with the peat-perlite mixture. The study was planned to have two irrigation levels, control and flood, and a single harvest time. Three seeds were planted in each pot, and the seedlings were thinned by hand so that only one plant showing homogeneous growth was left when the emergence occured. Cultural processes were applied equally to all applications throughout the experiment. Irrigation was given to all pots equally at 7-day intervals by measuring with a beaker until the flooding stress was established (18 November 2021). After this date, while the normal irrigation program was continued for control applications, in stress conditions, the drainages were closed, and flooding was created by irrigation up to the potting soil. During the 13-day flooding, the water that was lost due to the daily controls was applied to the pots again. Control and stress applications of all genotypes were harvested at once on 2 December 2021. The plant parts of the harvested plants were cut, and their roots were cleaned, and they were weighed, and the fresh plant weight (g) and root fresh weight (g) were determined. The fresh weights of the plant and root parts were dried in an oven at 72 °C. Plant dry weight (g) and root dry weight (g) were determined in the samples at a constant weight. In addition, the lengths (cm) of the root parts were determined. The fresh weight of the discs from the leaf samples was taken, and their turgor weight was determined by saturating them with water. Then, after drying the samples in an oven at 80 °C for 48 hours, their dry weights were determined, and the relative water content (%) calculations were made according to Kaya et al. (2003). Then, the discs taken from the leaves were rinsed three times with distilled water, 10 ml of water was added, and EC values were measured after shaking them in a shaker at 25 °C for 24 hours. After the same samples were kept in an autoclave at 120 °C for 20 minutes, they were cooled to 25 °C and EC measurements were made again. Then, membrane damage (%) calculations were made according to Lutts et al. (1996). In order to determine chlorophyll a, b, and carotenoids in the leaf samples, the samples were ground in 10 ml acetone and subsequently centrifuged at 15000 rpm. Then respectively readings, were made by spectrophotometric method at 663, 652, and 470 nm, chlorophyll a and b were determined according to Lichtenthaler and Buschmann (2001), and the number of carotenoids was determined according to the Jaspars formula (Witham et al., 1971).

Table 1. *The spinach genotypes and inbreeding generations in the study*
Tablo 1. Çalışmada kullanılan ıspanak genotipleri ve ıslah kademeleri

Genotype number	Genotype code	Genotype grade	Genotype number	Genotype code	Genotype grade
1	2k-13/1-1	S5	**37**	-	El Real F1
2	10b-52/3	S5	**38**	-	El Salvator F1
3	15k-1/1-2	S5	**39**	-	Yaman F1
4	19a-51/1-1	S5	**40**	-	Hudson F1
5	19b-52/1-2	S5	**41**	-	Green Gold F1
6	19c-33/1-1	S5	**42**	-	Amador F1
7	19k-42/1-2	S5	**43**	-	Şahmeran F1
8	31k-24/1-1	S5	**44**	-	Ayaz F1
9	32b-21/1-2	S5	**45**	-	Sprinter F1
10	32c-22/1-5	S5	**46**	-	Green Star F1
11	48e-12/1-2	S5	**47**	-	Reis F1
12	48k-42/1-5	S5	**48**	-	Sardes F1
13	49c-31/2-3	S5	**49**	-	Samuray F1
14	50b-51/1-1	S5	**50**	-	Apollo F1
15	51a-33/1-1	S5	**51**	-	Sayonora F1
16	53d-43/1-1	S5	**52**	-	Region F1
17	53k-12/1-3	S5	**53**	-	Revere F1
18	54k-33/2-2	S5	**54**	-	Spiros F1
19	55a-31/1-2	S5	**55**	-	Rembrant F1
20	56k-52/2-1	S5	**56**	-	Shelby F1
21	58a-2/2-1	S5	**57**	-	Ranchero F1
22	63k-21/2-2	S5	**58**	-	Matador F1
23	67c-41/1-2	S5	**59**	-	Nebraska F1
24	-	Tiger F1	**60**	-	Vena F1
25	-	Red Kitten F1	**61**	-	Catrina F1
26	-	Hynea F1	**62**	-	SV1714VC F1
27	-	Silverwhale	**63**	-	Matador
28	-	Racoon F1	**64**	-	SV1748VC F1
29	-	Parrot F1	**65**	-	S044 F1
30	-	Harrier F1	**66**	-	Midway F1
31	-	Antelope F1	**67**	-	SWA0760 F1
32	-	Pigeon F1	**68**		Java F1
33	-	Kookaburra F1	**69**		Aras F1
34	-	Manatee F1	**70**		Anemon F1
35	-	Gazelle F1	**71**		Anlani F1
36	-	El Tajin F1			

Percentage changes were determined by taking the numerical differences of agro-morphological and physiological parameters obtained from flooding stress and control applications of different spinach genotypes. On the other hand, principal component analyses were performed in the JMP-14 package program to reveal essential results in the interpretation of multiple values, and tolerant genotypes were tried to be determined as well as to determine the parameters that indicate significant changes between genotypes (Seymen, 2021).

RESULTS and DISCUSSION

The Effect of Flooding Stress on Agro-Morphological Parameters

It was observed that the flooding stress applied to the spinach genotypes in the present study had significant effects on agro-morphological and physiological parameters. When Table 1 is examined, the average plant fresh weight was found to be 2.844 g, while the applied flooding stress caused a 19% decrease in spinach cultivar and genotypes. While the genotype with the highest decrease in plant weight was obtained from the number 46 by 91%, the highest increase was obtained from the number 67 by 72%. It reduced the average subsoil fresh weight by 17% under flooding conditions in spinach. The genotype with the highest decrease was found in genotype 38 by 90%, while the highest increase was found in Revere F1 (53) hybrid variety by 86%. In addition, the effect of flooding stress did not cause a decrease in the plant and root fresh weights of genotypes 21, 62, 5 and 72. As in Table 2, the negative effect of flooding stress on subsoil dry weights was seen by a 58% decrease in genotype 8In addition, the genotype that experienced the least affected by flooding stress was hybrid number 61 with an increase of 75%. Likewise, flood stress caused a 22% decrease in average subsoil dry weight. Spinach is grown mainly for its fresh leaves, and the number of leaves per plant and leaf size; therefore, leaf weight determines the total yield. Flooding stress reveals the mechanism of hypoxia conditions and the formation of adventitious roots in the soil (Kawase, 1981). Due to this situation, the average plant root length was 13.27 cm, while the applied flooding stress caused a 36% decrease in the plant root length of spinach. While the much more decrease was observed in genotype 69 by 90%, the highest increase was obtained in genotype 11 by 33% (Table 2). Under flooding conditions, plant roots are in a state of hypoxia, their metabolic activities are inhibited, and ATP production is reduced (Saglio et al., 1980). Decreased ATP production restricts the energy supply for root growth, thereby reducing vegetative growth (Liao & Lin, 2001). This high variability for the above parameters can form the basis for the effective selection of superior genotypes. Rezvani et al. (2012), on the other hand, reported that the flooding stress had negative effects plant the fresh and dry weight of the saffron plant and the fresh and dry weight of the root. Likewise, Grichko and Glick (2001) reported that flooding stress had a negative effect on the fresh and dry weight of the plant part of tomatoes in their study on tomatoes.

The Effect of Flood Stress on Physiological Parameters

It has been determined that flooding stress significantly effects on relative water content (RWC) and membrane damage. When the impacts of flooding stress was evaluated in the control application, the amount of RWC decreased by 20% (average decrease) (Table 3). The genotype with the peak sensitivity was found to be 45 (Sprinter F1), with a decrease of 89%. While the amount of membrane damage did not change much in the control application during the harvest period, greater membrane damage was obtained in the flooding stress application (Table 3). Xu and Leskovar (2015) found that drought stress decreased the amount of RWC in their study similar to spinach. Seymen (2021) found that the RWC rate increased with flooding stress in his study on spinach. Yadav and Hemantaranjan (2017) demonstrated that flooding stress decreased membrane damage in mungbean. The obtained results are thought to be affected by the cumulative effect of plant species, soil, and environmental conditions from this discussion (Boyer et al., 2008; Parkash & Singh, 2020). It has also been reported that the effect of flooding stress varies between species (Seymen, 2021). On the other hand, physiological variables also cause significant changes in the type and duration of stress. Under flooding stress conditions, short-term stress conditions increase the RWC rate by causing less water uptake from the root, while in longer-term stress conditions, it causes a decrease in the amount of RWC due to membrane damage.

As a result of the present study, it was observed significant effect in the contents of chlorophyll a (chl a), chlorophyll b (chl b), and carotenoid physiological parameters obtained by flood stress. Compared to the control application, significant decreases were observed in the mean chl a, chl b and carotenoid contents under flood stress by 21%, 19% and 16%, respectively. Genotype 1 demonstrated the most significant difference in chl a and chl b contents, with a decrease of 75% and 74%, respectively. Studies have reported that the amount of photosynthetic pigments grouped as chlorophyll a, chlorophyll b and carotenoids in flooded plants vary depending on the species, the type and duration of stress, the stage of the plant's life cycle, the concentration of stress, and the difference in genotype (Foyer & Shigeoka, 2011; Zhigou & Derrick M, 2012; Tian et al., 2021). Therefore, they reported that chlorophyll content decreased due to flood stress, damage to the chloroplast membrane and its structure, photo-oxidation of chlorophyll, increased chlorophyllase activity, and suppression of chlorophyll biosynthesis (Kingston-Smith & Foyer, 2000; Kabiri et al., 2014). In this sense, the amount of chlorophyll decreased due to the deficiencies observed in leaf and plant development and flooding stress. Similarly, decreases in chlorophyll and carotene contents were observed under flood stress in spinach (Seymen, 2021), tomatoes (Ezin et al., 2010; Bhatt et al., 2015; Rasheed et al., 2018) and cabbage (Brazel et al., 2021). When the results obtained in this study were evaluated, it was seen that flood stress negatively affected the amount of chlorophyll.

Table 2.'%' changes obtained using agro-morphological traits among spinach genotypes under flood stress conditions
Tablo 2. Sel baskını stres koşulları altında ıspanak genotipleri arsında agro-morfolojik özellikler kullanılarak elde edilen '% 'değişimler

Genotype no	Genotype code	Plant fresh weight(g)			Root fresh weight (g)			Plant dry weights (g)			Root dry weights (g)			Root lengths (cm)		
		Control	Flooding	Difference(%)	Control	Flooding	%	Control	Flooding	%	Control	Flooding	%	Control	Flooding	%
1	2k-13/1-1	2.90	1.88	-0.35	0.36	0.31	-0.16	0.49	0.38	-0.22	0.06	0.05	-0.05	6.33	5.83	-0.08
2	10b-52/3	2.84	2.46	-0.14	0.38	0.25	-0.33	0.42	0.36	-0.15	0.05	0.04	-0.09	7.17	5.67	-0.21
3	15k-1/1-2	3.67	2.92	-0.20	0.37	0.33	-0.10	0.58	0.47	-0.19	0.06	0.05	-0.20	7.83	5.00	-0.36
4	19a-51/1-1	3.26	3.48	0.07	0.33	0.29	-0.11	0.49	0.54	0.08	0.05	0.05	-0.06	5.58	7.25	0.30
5	19b-52/1-2	3.26	1.92	-0.41	0.24	0.24	0.00	0.49	0.32	-0.35	0.05	0.03	-0.33	6.42	4.50	-0.30
6	19c-33/1-1	2.72	1.75	-0.36	0.41	0.28	-0.31	0.46	0.35	-0.24	0.07	0.03	-0.54	7.83	3.25	-0.59
7	19k-42/1-2	2.56	1.51	-0.41	0.37	0.25	-0.33	0.28	0.28	0.00	0.05	0.03	-0.46	7.83	3.50	-0.55
8	31k-24/1-1	3.92	1.35	-0.66	0.29	0.15	-0.48	0.59	0.25	-0.58	0.06	0.03	-0.51	10.50	5.08	-0.52
9	32b-21/1-2	2.66	3.14	0.18	0.24	0.38	0.63	0.37	0.43	0.16	0.04	0.04	0.09	6.65	3.65	-0.45
10	32c-22/1-5	2.45	2.00	-0.18	0.25	0.25	0.01	0.42	0.44	0.05	0.04	0.05	0.17	8.17	5.25	-0.36
11	48e-12/1-2	3.08	2.11	-0.32	0.24	0.30	0.23	0.44	0.46	0.03	0.04	0.05	0.25	5.00	6.67	0.33
12	48k-42/1-5	2.59	2.18	-0.16	0.19	0.19	-0.05	0.38	0.45	0.17	0.03	0.03	0.01	6.88	5.75	-0.16
13	49c-31/2-3	2.45	2.96	0.21	0.18	0.31	0.77	0.37	0.46	0.25	0.03	0.06	0.82	7.25	5.00	-0.31
14	50b-51/1-1	1.60	1.57	-0.02	0.14	0.25	0.82	0.21	0.27	0.27	0.02	0.03	0.40	7.50	9.50	0.27
15	51a-33/1-1	2.92	3.23	0.11	0.39	0.44	0.12	0.47	0.57	0.21	0.06	0.08	0.37	16.00	13.08	-0.18
16	53d-43/1-1	3.20	2.66	-0.17	0.28	0.37	0.34	0.65	0.49	-0.25	0.06	0.07	0.18	5.75	7.20	0.25
17	53k-12/1-3	3.83	4.77	0.24	0.29	0.51	0.73	0.63	0.75	0.19	0.05	0.08	0.61	9.25	10.10	0.09
18	54k-33/2-2	3.84	2.73	-0.29	0.26	0.32	0.23	0.66	0.55	-0.17	0.05	0.02	-0.49	6.58	4.92	-0.25
19	55a-31/1-2	2.80	2.23	-0.20	0.26	0.23	-0.13	0.38	0.48	0.27	0.05	0.01	-0.80	6.25	3.33	-0.47
20	56k-52/2-1	2.11	1.68	-0.20	0.12	0.10	-0.17	0.32	0.28	-0.15	0.03	0.02	-0.29	5.33	4.00	-0.25
21	58a-2/2-1	2.35	2.35	0.00	0.51	0.55	0.09	0.39	0.58	0.46	0.07	0.08	0.09	19.50	7.17	-0.63
22	63k-21/2-2	3.55	2.00	-0.44	0.29	0.20	-0.31	0.53	0.52	-0.03	0.06	0.05	-0.12	9.42	5.33	-0.43
23	67c-41/1-2	4.47	2.62	-0.41	1.29	0.29	-0.77	0.65	0.54	-0.17	0.16	0.05	-0.70	25.33	5.25	-0.79
24	Tiger F1	3.81	1.18	-0.69	0.91	0.11	-0.88	0.54	0.26	-0.53	0.09	0.02	-0.82	19.80	2.83	-0.86
25	Red Kitten F1	3.40	2.36	-0.31	1.13	0.36	-0.68	0.60	0.38	-0.37	0.14	0.06	-0.57	18.20	5.58	-0.69
26	Hynea F1	2.05	1.00	-0.51	0.34	0.19	-0.43	0.36	0.23	-0.36	0.04	0.02	-0.50	17.00	21.80	0.28
27	Silverwhale	2.80	1.98	-0.29	0.86	0.35	-0.59	0.32	0.24	-0.24	0.10	0.04	-0.56	25.00	9.08	-0.64
28	Racoon F1	3.37	1.92	-0.43	1.15	0.27	-0.77	0.53	0.33	-0.38	0.12	0.04	-0.69	18.42	4.25	-0.77
29	Parrot F1	2.26	2.37	0.05	0.17	0.21	0.21	0.36	0.43	0.18	0.03	0.03	0.23	8.92	6.33	-0.29
30	Harrier F1	2.59	2.15	-0.17	0.18	0.20	0.06	0.45	0.45	0.01	0.04	0.02	-0.53	7.67	4.92	-0.36
31	Antelope F1	2.66	2.41	-0.10	0.46	0.37	-0.18	0.45	0.45	-0.01	0.09	0.06	-0.36	21.10	6.50	-0.69
32	Pigeon F1	2.32	1.99	-0.14	0.56	0.23	-0.59	0.38	0.36	-0.06	0.08	0.04	-0.50	17.92	4.67	-0.74
33	Kookaburra F1	1.81	2.33	0.29	0.41	0.18	-0.56	0.33	0.49	0.51	0.06	0.04	-0.24	15.33	4.25	-0.72
34	Manatee F1	2.63	2.16	-0.18	0.62	0.24	-0.61	0.50	0.39	-0.22	0.09	0.04	-0.56	18.92	5.75	-0.70
35	Gazelle F1	2.08	2.14	0.03	0.45	0.46	0.02	0.38	0.40	0.06	0.07	0.05	-0.18	18.42	6.92	-0.62

36	**El Tajin F1**	2.18	2.03	-0.07	0.75	0.37	-0.50	0.44	0.41	-0.08	0.10	0.06	-0.40	21.90	8.83	-0.60
37	**El Real F1**	1.79	1.31	-0.27	0.66	0.14	-0.79	0.30	0.26	-0.13	0.08	0.02	-0.72	14.08	7.08	-0.50
38	**El Salvator F1**	1.92	0.90	-0.53	1.41	0.14	-0.90	0.32	0.16	-0.49	0.16	0.01	-0.91	17.25	5.50	-0.68
39	**Yaman F1**	3.93	2.27	-0.42	0.48	0.36	-0.25	0.63	0.49	-0.23	0.09	0.06	-0.29	16.00	5.75	-0.64
40	**Hudson F1**	4.08	4.18	0.02	0.55	0.80	0.44	0.69	0.79	0.14	0.09	0.10	0.09	16.08	17.17	0.07
41	**Green Gold F1**	3.49	2.11	-0.40	0.64	0.27	-0.57	0.54	0.36	-0.33	0.08	0.04	-0.49	16.83	11.58	-0.31
42	**Amador F1**	2.94	2.62	-0.11	0.65	0.39	-0.40	0.50	0.49	-0.01	0.09	0.07	-0.26	18.83	8.33	-0.56
43	**Şahmeran F1**	3.57	0.32	-0.91	0.57	0.35	-0.39	0.67	0.56	-0.17	0.10	0.07	-0.36	12.08	10.08	-0.17
44	**Ayaz F1**	4.45	3.41	-0.23	0.56	0.41	-0.26	0.78	0.59	-0.24	0.10	0.09	-0.13	16.42	13.30	-0.19
45	**Sprinter F1**	2.64	3.10	0.17	0.30	0.51	0.69	0.48	0.51	0.06	0.06	0.06	0.03	10.92	13.25	0.21
46	**Green Star F1**	3.48	2.69	-0.23	0.20	0.37	0.85	0.54	0.51	-0.07	0.10	0.06	-0.40	11.08	6.58	-0.41
47	**Reis F1**	3.39	2.94	-0.13	0.46	0.34	-0.25	0.54	0.60	0.10	0.06	0.06	-0.08	10.30	5.60	-0.46
48	**Sardes F1**	1.86	1.39	-0.25	0.41	0.17	-0.58	0.32	0.25	-0.20	0.05	0.03	-0.46	14.33	4.70	-0.67
49	**Samuray F1**	2.31	1.22	-0.47	0.76	0.18	-0.76	0.42	0.22	-0.49	0.09	0.03	-0.68	18.20	5.58	-0.69
50	**Apollo F1**	2.44	2.61	0.07	0.66	0.32	-0.51	0.39	0.44	0.12	0.08	0.05	-0.43	19.10	7.00	-0.63
51	**Sayonora F1**	3.51	1.79	-0.49	0.58	0.21	-0.63	0.56	0.42	-0.24	0.08	0.04	-0.50	16.75	3.83	-0.77
52	**Region F1**	2.84	1.78	-0.37	0.53	0.31	-0.41	0.47	0.36	-0.23	0.08	0.05	-0.37	13.20	6.58	-0.50
53	**Revere F1**	2.86	2.71	-0.05	0.22	0.41	0.86	0.45	0.50	0.11	0.04	0.06	0.54	9.70	9.17	-0.05
54	**Spiros F1**	2.75	1.88	-0.32	0.19	0.27	0.39	0.43	0.40	-0.08	0.04	0.05	0.40	5.97	5.67	-0.05
55	**Rembrant F1**	2.76	1.98	-0.28	0.25	0.26	0.04	0.48	0.44	-0.10	0.05	0.05	-0.01	7.25	6.00	-0.17
56	**Shelby F1**	3.35	2.95	-0.12	0.67	0.42	-0.37	0.53	0.44	-0.18	0.08	0.06	-0.20	13.33	8.58	-0.36
57	**Ranchero F1**	3.25	2.89	-0.11	0.37	0.35	-0.05	0.54	0.47	-0.13	0.04	0.06	0.24	10.08	7.58	-0.25
58	**Matador F1**	2.73	1.89	-0.31	0.33	0.25	-0.26	0.48	0.39	-0.20	0.06	0.04	-0.30	13.80	8.17	-0.41
59	**Nebraska F1**	2.17	2.18	0.00	0.38	0.29	-0.23	0.39	0.40	0.05	0.06	0.05	-0.25	18.12	8.40	-0.54
60	**Vena F1**	2.45	3.04	0.24	0.46	0.42	-0.07	0.46	0.44	-0.05	0.06	0.08	0.32	16.42	12.83	-0.22
61	**Catrina F1**	1.95	3.35	0.72	0.46	0.48	0.05	0.31	0.54	0.75	0.06	0.07	0.24	12.25	15.92	0.30
62	**SV1714VC F1**	2.78	1.48	-0.47	0.93	0.40	-0.57	0.50	0.32	-0.36	0.11	0.06	-0.44	24.42	5.20	-0.79
63	**Matador**	2.17	1.46	-0.33	0.89	0.11	-0.87	0.35	0.22	-0.37	0.10	0.02	-0.81	21.75	2.25	-0.90
64	**SV1748VC F1**	2.68	1.86	-0.30	1.06	0.17	-0.84	0.41	0.31	-0.24	0.11	0.03	-0.75	20.25	3.92	-0.81
65	**S044 F1**	2.97	2.12	-0.28	1.06	0.90	-0.15	0.46	0.31	-0.33	0.13	0.09	-0.35	20.50	12.83	-0.37
66	**Midway F1**	3.29	2.84	-0.14	0.81	0.81	0.00	0.58	0.49	-0.15	0.11	0.10	-0.11	12.25	13.50	0.10
67	**SWA0760 F1**	2.78	2.80	0.01	0.56	0.65	0.16	0.46	0.41	-0.12	0.06	0.05	-0.20	12.75	9.25	-0.27
68	**Java F1**	2.78	2.07	-0.26	0.53	0.61	0.14	0.42	0.33	-0.21	0.07	0.08	0.10	14.25	13.00	-0.09
69	**Aras F1**	2.36	2.04	-0.14	0.40	0.37	-0.09	0.37	0.33	-0.10	0.07	0.05	-0.22	8.00	8.50	0.06
70	**Anemon F1**	2.34	1.84	-0.21	0.36	0.24	-0.33	0.37	0.31	-0.17	0.05	0.04	-0.34	15.67	7.67	-0.51
71	**Anlani F1**	1.86	2.26	0.21	0.26	0.25	-0.04	0.78	0.37	-0.52	0.04	0.03	-0.19	15.08	8.92	-0.41
	mean±SE	**2.84±0.08**	**2.25±0.09**	**-19±3%**	**0.50±0.03**	**0.33±0.02**	**17±5%**	**0.47±0.01**	**0.41±0**	**-9±3%**	**0.07± 0**	**0.05±0**	**22±4%**	**13.33±0.66**	**7.43±0.43**	**36±4%**
	SD	**0.66**	**0.74**	**26%**	**0.29**	**0.16**	**45%**	**0.12**	**0.12**	**25%**	**0.03**	**0.02**	**37%**	**5.54**	**3.65**	**32%**
	min.	**1.60**	**0.32**	**-91%**	**0.12**	**0.10**	**-90%**	**0.21**	**0.16**	**-58%**	**0.02**	**0.01**	**-91%**	**5.00**	**2.25**	**-90%**
	max.	**4.47**	**4.77**	**72%**	**1.41**	**0.90**	**86%**	**0.78**	**0.79**	**75%**	**0.16**	**0.10**	**82%**	**25.33**	**21.80**	**33%**

Table 3: '%' changes obtained using physiological characteristics between spinach genotypes under flood stress conditions
Tablo 3: Sel baskını stres koşulları altında ıspanak genotipleri arsında fizyolojik özellikler kullanılarak elde edilen '%' değişimler

Genotype number	Genotype code	Membran damage			RWC			chl a			chl b			carotenoid		
		Control	Flooding	%	Control	Flooding	%	Control	Flooding	%	Control	Flooding	%	Control	Flooding	%
1	2k-13/1-1	17.49	17.94	3%	104.42	43.58	-58%	22.32	5.54	-75%	14.74	3.83	-74%	3.41	1.17	-66%
2	10b-52/3	13.62	13.77	1%	82.01	47.14	-43%	21.77	21.28	-2%	14.01	14.09	1%	3.14	3.23	3%
3	15k-1/1-2	12.42	11.85	-5%	75.01	27.32	-64%	20.13	9.38	-53%	13.20	6.63	-50%	2.61	1.78	-32%
4	19a-51/1-1	16.40	10.50	-36%	90.78	23.12	-75%	12.96	9.22	-29%	9.11	6.18	-32%	1.78	1.92	8%
5	19b-52/1-2	11.24	10.09	-10%	85.76	23.18	-73%	17.31	16.42	-5%	11.45	10.96	-4%	2.24	2.73	22%
6	19c-33/1-1	14.33	18.41	28%	88.57	66.42	-25%	26.34	8.54	-68%	18.80	5.86	-69%	5.51	1.40	-75%
7	19k-42/1-2	11.23	17.75	58%	179.09	124.11	-31%	12.78	6.65	-48%	8.09	4.52	-44%	1.93	1.47	-24%
8	31k-24/1-1	13.54	8.43	-38%	132.96	63.59	-52%	13.90	11.57	-17%	9.87	8.01	-19%	2.21	2.64	20%
9	32b-21/1-2	10.99	14.58	33%	136.64	100.89	-26%	11.02	8.68	-21%	6.97	5.40	-22%	1.77	1.63	-8%
10	32c-22/1-5	13.32	26.51	99%	133.88	63.56	-53%	8.93	9.54	7%	6.27	6.16	-2%	1.40	1.90	36%
11	48e-12/1-2	12.19	15.71	29%	128.20	116.78	-9%	19.93	6.49	-67%	13.88	4.14	-70%	3.03	1.59	-48%
12	48k-42/1-5	11.57	12.19	5%	117.14	152.26	30%	22.26	16.97	-24%	28.50	22.71	-20%	-0.81	-0.34	-59%
13	49c-31/2-3	13.40	11.07	-17%	73.67	106.14	44%	23.19	18.07	-22%	31.45	22.78	-28%	-5.39	-0.33	-94%
14	50b-51/1-1	12.48	12.99	4%	137.37	112.44	-18%	15.05	9.40	-38%	9.35	6.19	-34%	2.38	1.58	-33%
15	51a-33/1-1	11.26	10.99	-2%	82.85	115.39	39%	15.24	16.31	7%	9.28	15.12	63%	2.20	1.24	-44%
16	53d-43/1-1	12.17	14.89	22%	110.23	101.13	-8%	20.96	13.03	-38%	13.24	8.00	-40%	3.30	2.44	-26%
17	53k-12/1-3	11.52	10.58	-8%	110.79	110.11	-1%	22.11	9.70	-56%	17.88	6.55	-63%	2.48	1.80	-27%
18	54k-33/2-2	10.96	20.77	90%	124.03	85.29	-31%	19.17	16.70	-13%	12.84	10.61	-17%	2.87	3.11	8%
19	55a-31/1-2	10.48	8.77	-16%	123.31	79.51	-36%	17.90	14.38	-20%	11.36	9.38	-17%	2.71	3.10	15%
20	56k-52/2-1	10.73	11.03	3%	163.60	118.55	-28%	24.28	11.97	-51%	15.79	6.38	-60%	3.57	2.25	-37%
21	58a-2/2-1	17.05	13.55	-20%	161.69	98.49	-39%	26.44	19.80	-25%	19.56	13.79	-30%	3.35	3.77	12%
22	63k-21/2-2	15.92	15.91	0%	84.51	95.91	13%	25.20	21.06	-16%	18.64	13.90	-25%	3.13	3.22	3%
23	67c-41/1-2	13.42	11.56	-14%	145.56	109.15	-25%	25.20	25.08	0%	17.96	19.22	7%	3.47	4.34	25%
24	Tiger F1	11.47	11.76	3%	135.86	101.63	-25%	32.36	18.92	-42%	30.99	14.45	-53%	3.64	2.35	-35%
25	Red Kitten F1	11.65	17.73	52%	129.15	103.00	-20%	22.50	11.56	-49%	15.23	9.26	-39%	3.29	1.88	-43%
26	Hynea F1	10.26	12.64	23%	139.16	99.81	-28%	24.64	16.97	-31%	17.45	11.71	-33%	3.35	2.73	-18%
27	Silverwhale	10.82	17.82	65%	132.61	90.29	-32%	26.69	15.11	-43%	20.61	12.77	-38%	3.29	2.28	-31%
28	Racoon F1	16.08	27.75	73%	124.89	107.23	-14%	29.41	16.83	-43%	31.73	12.12	-62%	2.77	1.24	-55%
29	Parrot F1	10.90	21.36	96%	138.96	92.65	-33%	20.69	7.57	-63%	13.90	5.30	-62%	3.25	1.49	-54%
30	Harrier F1	10.60	20.99	98%	143.96	98.68	-31%	30.97	12.48	-60%	24.48	8.90	-64%	4.30	1.58	-63%
31	Antelope F1	13.05	13.46	3%	129.07	122.10	-5%	12.50	10.63	-15%	8.45	7.45	-12%	2.10	1.94	-8%
32	Pigeon F1	11.42	21.79	91%	122.58	71.01	-42%	19.89	11.49	-42%	14.18	8.03	-43%	2.91	2.52	-13%
33	Kookaburra F1	12.60	23.82	89%	119.40	66.06	-45%	13.88	10.68	-23%	9.26	7.48	-19%	2.26	2.25	-1%
34	Manatee F1	11.82	16.37	39%	142.24	104.25	-27%	25.45	15.06	-41%	18.21	9.94	-45%	4.06	2.85	-30%
35	Gazelle F1	15.37	17.22	12%	120.51	81.21	-33%	21.65	11.08	-49%	14.05	6.54	-53%	3.82	2.97	-22%

36	**El Tajin F1**	12.31	12.08	-2%	135.35	122.41	-10%	22.80	18.68	-18%	14.59	11.21	-23%	3.79	3.28	-14%
37	**El Real F1**	18.94	10.73	-43%	109.14	133.91	23%	11.30	15.71	39%	5.63	8.23	46%	2.98	3.13	5%
38	**El Salvator F1**	11.14	11.48	3%	96.31	112.24	17%	19.76	19.23	-3%	10.35	10.81	4%	3.97	3.65	-8%
39	**Yaman F1**	10.82	15.31	41%	77.31	82.81	7%	17.11	17.65	3%	9.14	9.56	5%	3.55	4.10	16%
40	**Hudson F1**	11.28	13.45	19%	78.13	75.03	-4%	24.21	17.81	-26%	13.05	8.55	-35%	4.45	3.72	-16%
41	**Green Gold F1**	12.01	12.14	1%	71.50	42.83	-40%	13.48	17.42	29%	6.05	8.92	47%	3.10	3.81	23%
42	**Amador F1**	13.68	23.57	72%	75.04	66.08	-12%	24.55	19.50	-21%	14.26	10.79	-24%	5.02	3.67	-27%
43	**Şahmeran F1**	15.52	14.59	-6%	77.42	61.86	-20%	32.43	27.73	-14%	32.67	19.66	-40%	4.25	4.19	-1%
44	**Ayaz F1**	15.68	15.44	-2%	77.01	64.59	-16%	24.30	12.46	-49%	16.10	8.38	-48%	3.49	1.98	-43%
45	**Sprinter F1**	11.24	17.03	51%	497.38	55.82	-89%	20.28	13.29	-34%	13.24	9.12	-31%	2.97	2.45	-18%
46	**Green Star F1**	16.98	12.27	-28%	72.40	72.22	0%	16.38	17.39	6%	10.86	11.40	5%	2.71	2.81	4%
47	**Reis F1**	15.42	17.05	11%	78.12	52.23	-33%	27.36	15.03	-45%	19.93	10.03	-50%	3.98	2.75	-31%
48	**Sardes F1**	14.42	26.85	86%	73.34	61.27	-16%	25.04	11.20	-55%	16.92	7.80	-54%	3.53	2.26	-36%
49	**Samuray F1**	13.92	23.98	72%	71.74	61.23	-15%	15.88	10.01	-37%	10.66	7.22	-32%	2.58	2.28	-12%
50	**Apollo F1**	15.71	13.75	-12%	82.24	70.32	-14%	10.83	12.18	12%	7.59	8.41	11%	1.73	1.90	10%
51	**Sayonora F1**	14.76	28.04	90%	80.52	56.58	-30%	23.17	13.52	-42%	15.53	9.46	-39%	3.32	2.38	-28%
52	**Region F1**	10.87	14.60	34%	81.77	58.24	-29%	12.18	15.64	28%	7.94	13.81	74%	1.99	1.61	-19%
53	**Revere F1**	13.88	10.27	-26%	76.78	69.38	-10%	19.69	7.74	-61%	12.08	5.09	-58%	2.98	1.24	-58%
54	**Spiros F1**	10.92	12.46	14%	80.80	67.48	-16%	12.29	16.66	36%	8.36	11.20	34%	1.90	2.71	43%
55	**Rembrant F1**	11.45	12.31	7%	80.55	65.40	-19%	11.45	10.15	-11%	7.42	6.68	-10%	1.76	1.65	-6%
56	**Shelby F1**	11.62	15.22	31%	76.94	77.42	1%	21.97	22.45	2%	14.50	18.49	28%	3.58	3.22	-10%
57	**Ranchero F1**	10.14	14.27	41%	77.44	72.00	-7%	15.17	13.78	-9%	10.40	9.51	-9%	2.59	2.53	-2%
58	**Matador F1**	19.91	9.63	-52%	80.23	58.06	-28%	14.96	18.52	24%	9.47	13.97	48%	2.36	2.42	2%
59	**Nebraska F1**	14.73	10.39	-29%	75.57	68.77	-9%	21.65	14.78	-32%	14.67	9.96	-32%	3.50	2.37	-32%
60	**Vena F1**	12.64	10.89	-14%	82.25	76.77	-7%	22.90	13.72	-40%	14.71	9.30	-37%	3.64	2.07	-43%
61	**Catrina F1**	10.70	11.80	10%	90.46	73.65	-19%	17.80	20.65	16%	12.53	12.92	3%	2.69	3.18	18%
62	**SV1714VC F1**	11.62	12.47	7%	83.77	68.50	-18%	16.17	11.39	-30%	10.43	7.70	-26%	2.49	2.00	-20%
63	**Matador**	11.97	17.05	42%	80.15	55.70	-31%	12.71	12.48	-2%	8.71	7.92	-9%	2.93	2.22	-24%
64	**SV1748VC F1**	19.44	12.40	-36%	84.04	59.57	-29%	15.33	16.74	9%	10.10	11.46	13%	2.45	2.97	21%
65	**S044 F1**	10.82	12.05	11%	80.83	72.99	-10%	12.05	14.67	22%	7.15	9.07	27%	2.08	2.50	20%
66	**Midway F1**	12.01	12.69	6%	81.66	80.99	-1%	18.64	21.06	13%	11.37	19.53	72%	3.13	2.64	-16%
67	**SWA0760 F1**	14.14	11.89	-16%	67.68	73.60	9%	16.20	12.45	-23%	9.35	6.95	-26%	3.13	2.47	-21%
68	**Java F1**	11.09	12.17	10%	78.98	70.79	-10%	22.96	10.13	-56%	16.10	5.16	-68%	3.28	2.19	-33%
69	**Aras F1**	11.19	21.63	93%	75.22	56.75	-25%	12.80	19.78	55%	6.65	10.93	64%	2.68	3.95	47%
70	**Anemon F1**	10.78	11.08	3%	85.17	67.49	-21%	12.98	16.19	25%	6.10	8.10	33%	2.86	3.23	13%
71	**Anlani F1**	13.23	14.48	9%	82.96	71.58	-14%	25.08	12.80	-49%	13.99	6.08	-57%	4.57	2.82	-38%
	mean±SE	***13±0,28***	***15.07±0.56***	***19±5%***	***107±6.5***	***80±3.12***	***-20±3%***	***19.6±0.67***	***14.43±0.54***	***21±3%***	***13.8±0.74***	***10±0.5***	***-19±4%***	***2.9±0.16***	***2.42±0.11***	***-16±3%***
	SD	***2,34***	***4.76***	***39%***	***54.74***	***26.31***	***24%***	***5.67***	***4.56***	***29%***	***6.26***	***4.19***	***37%***	***1.36***	***0.91***	***29%***
	min.	***10,14***	***8.43***	***-52%***	***67.68***	***23.12***	***-89%***	***8.93***	***5.54***	***-75%***	***5.63***	***3.83***	***-74%***	***-5.39***	***-0.34***	***-94%***
	max.	***19,91***	***28.04***	***99%***	***497.38***	***152.26***	***44%***	***32.43***	***27.73***	***55%***	***32.67***	***22.78***	***74%***	***5.51***	***4.34***	***47%***

PcA Analysis

PCA has been reported to be a very practical statistical analysis as it simultaneously analyzes quantitative and qualitative features and is used by many researchers (Dehghani et al., 2008; Eftekhari et al., 2010; Everitt & Dunn, 2010; Sabaghnia et al., 2011). In the current study, when we examined the genotypes one by one in terms of agro-morphological and physiological parameters, the study was explained in two components. According to the evaluated criteria, PC produces the critical value of each factor called "Eigenvalue", "Eigenvalue" values greater than one were taken into (Brejda et al., 2000) account and helped to reduce data complexity in two factors. In this study, the variations among spinach genotypes are explained in two components, with PC1= %34.31 and PC2= %25.59, and the principal component (PC) representing approximately 59.90% of the total variability. In order to use PC analysis in a study, it has been reported that more than 25% of the variance of the first two components should be enough to explain variations among factors (Mohammadi & Prasanna, 2003; Mozafari et al., 2019; Seymen et al., 2019). Generally, it has been stated that the first component contributes to the maximum variance, while the rest of the factors justify the remaining amount of variance (Fereidoonfar et al., 2018). Therefore, the PC analysis was found to strongly explain the study.

Using the PC1 and PC2 components, a Biplot plot was created to examine the relationship between the morphological and physiological observations-based parameters of the genotypes affected by flood stress. The degree of association between the two traits is based on the multiple traits being compared between genotypes and the identification of genotypes that can be used as parents in breeding programs because they are particularly good in certain respects (Figure 1). According to this biplot, the cosine of the angles formed by the vectors connected to each variable and represents the degree of relationship among variable (Yan & Rajcan, 2002; Dehghani et al., 2008). In this context, if the angle between the vectors in the figure is <90°, there is a positive relationship, and if it is >90 °, there is a negative relationship, if the angle between the vectors is 180 °, it has been reported that there is no significant relationship (Yan & Kang, 2002). Angles formed by biplot analysis under flooding stress conditions showed a high correlation between UDW, UFW, AFW, ADW and RL. Therefore, the higher AFW, the higher ADW was found. A strong correlation was found between plant dry weight after flood stress and plant survival under critical flooding conditions. Flooding inhibits new leaf formation, reducing the total leaf area and promoting leaf senescence (Kato et al., 2014). In the light of this information, genotypes 67 and 14 were found to be highly tolerant in terms of growth parameters under flooding stress conditions. Singh et al. (2014) observed that the dry weight of leaves decreased sharply by 70% in varieties sensitive to flood stress, while it decreased by only 30-40% in tolerant varieties under 17-day flood stress. A similar study was conducted by Arif et al. (2013) and Sabaghnia et al. (2015) and show the relationship between different morphological features. Moreover, it was determined that the higher the chl a and chl b values, the higher the carotene content. Therefore, a highly positive relationship was found between chl a, chl b, and carotene. Singh et al. (2014) also stated that maintaining integrity of chlorophyll content during and after flooding is essential for plant survival as it aids photosynthesis continuity under flooding and aids faster recovery in case of flooding. Accordingly, it can be stated that genotypes 2, 53, 59, and 75 are more tolerant to flooding stress in terms of chlorophyll content (Figure 1). These results support studies evaluating the correlation between agricultural variables in similar studies (Sarkar et al., 2006; Seymen, 2021). A significant negative correlation was found between RWC and membrane damage among spinach genotypes. Membrane damage content is significantly higher in susceptible variety during flooding and after exposure to air (Kawano et al., 2002; Damanik et al., 2012; Panda & Sarkar, 2013). For this reason, the genotypes that revealed the most membrane damage are less productive. In this context, genotypes 58 and 60 were determined to be highly sensitive to flooding stress.

The biplot plot can provide knowledge to the plant breeder with flexibility in finding the number of plants to be evaluated (Yan & Rajcan, 2002). The plant breeder can use multivariate methods by first identifying the combination of traits that make up an ideal genotype. In this context, it can be reported that genotype 67 (SWA0760 F1) is the most ideal line to be used in spinach breeding studies. Besides, genotypes 14, 9, 21, 15, 4, and 10 belong to the S5 level and were found to be flooding stress tolerant. In the study, it was determined that the genotypes 24, 25, 26, 27, 28, 52, 54 and 69 were the most sensitive to flooding stress (Figure 1). It can be stated that the determined genotypes can be ignored in future studies. This research was conducted for only one growing season and thus genotype-environment interactions may cause some fluctuates, but the results showed that multidimensional methods can be sufficiently informative in the selection of breeding directions.

CONCLUSION

It was determined that the tolerance levels of 23 breeding materials and 48 commercial spinach varieties under different flood stress conditions. In addition to the negative effects of the 13-day flood stress applied on the spinach seedling period, it was observed from the agro-morphological and physiological parameters that the tolerance of the breeding lines and commercial varieties were different. As a result of PCA, commercial cultivar SWA0760 F1 (genotype 67) was found to be the most tolerant line against flooding stress. Other cultivars were found to be sensitive or moderately tolerant. In this context, the development of tolerant varieties against flood stress in spinach is among the important issues. The spinach genotypes 14, 9, 21, 15, 4 and 10 in the S5 level were determined to be tolerant lines against flood stress. It is thought that these genotypes will be used as parents in hybrid breeding and included in hybridization programs and will give important results in the development of flood stress-tolerant variety. The obtained variety and variety candidates will contribute to the reduction of yield and quality losses in the agricultural lands where spinach is grown, due to the flooding stress, which increases its negative effects day by day.

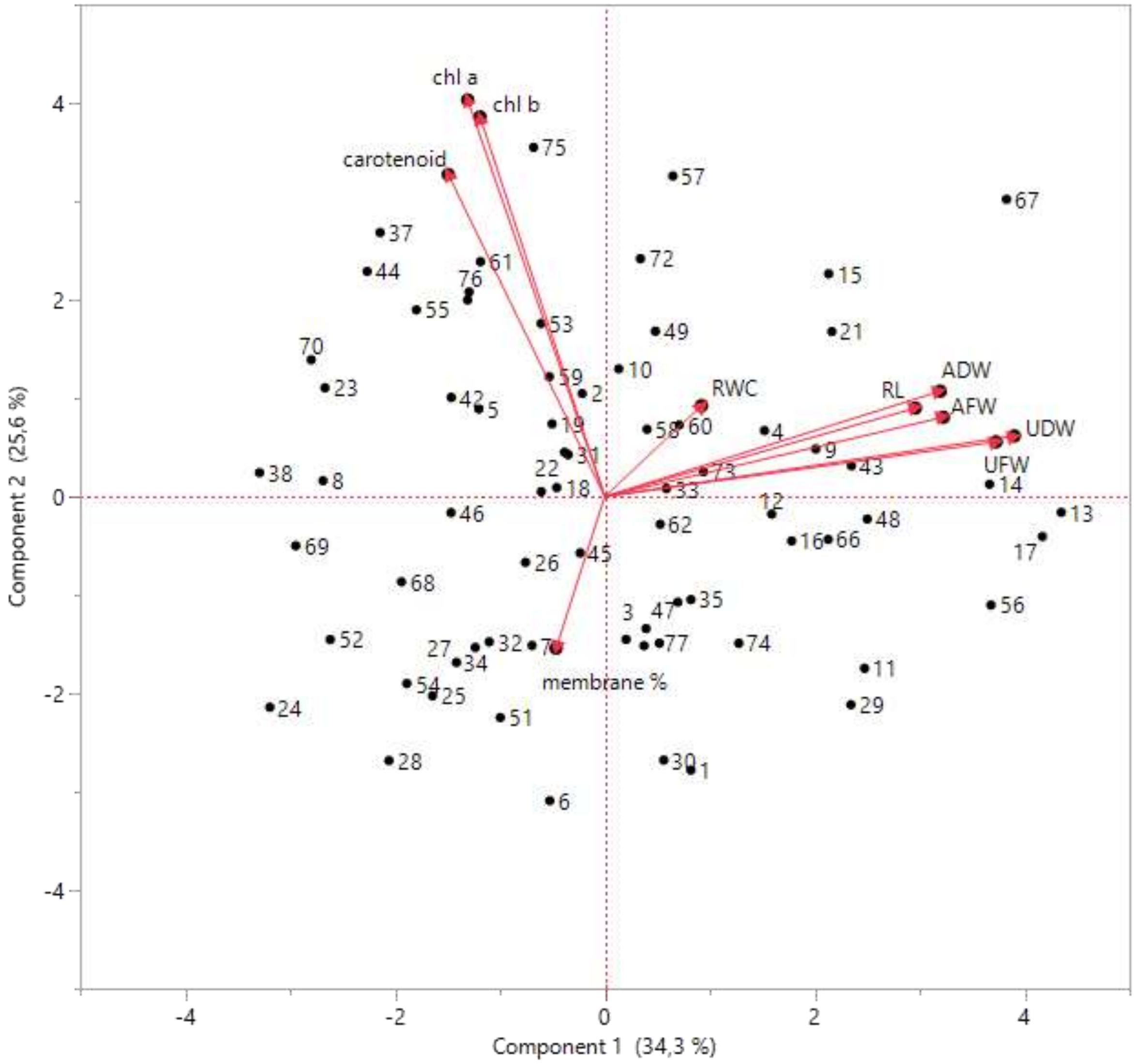

Figure 1. Biplot plot based on PC 1 and 2 obtained from PCA using agro-morphological traits among spinach genotypes under flood stress conditions

Şekil 1. Sel baskını stres koşulları altında ıspanak genotipleri arsında agro-morfolojik özellikler kullanılarak PCA'dan elde edilen PC 1 ve 2'ye dayalı biplot grafiği

Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The article authors declare that they do not have any conflict of interest.

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