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ORIGINAL PAPER

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IDENTIFICATION OF WATER STRESS-TOLERANT EDIBLE PUMPKIN SEED (*Cucurbita pepo*) GENOTYPES USING SEED YIELD-BASED TOLERANCE INDICES

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ABSTRACT

Pumpkin is usually cultivated in arid and semiarid regions, and the lack of water stress-tolerant cultivars is a major limiting factor. Therefore, this study was carried out to identify superior water stress-tolerant genotypes. For this purpose, 44 inbred lines with superior agronomic traits were selected from the gene pool. In addition, two hybrids (G1-Mert Bey F1 and G2-Sena Hanim F1) and two landraces (G3-Hatun Tırnağı and G4-Cercevelik) with high commercial value were used as commercial cultivars. The water stress indices were calculated from seed yields from the pumpkin genotypes grown in irrigated and water stress conditions in 2017 and 2018. The stress tolerance index (STI) determines tolerant and superior genotypes. From the principal component and cluster analyses' findings, G9, G40, G32, G36, G5, G11, G22, G30, G37, and G13 showed the highest water stress tolerance among the inbred lines. During future breeding experiments, these inbred lines may have significant potential for developing novel water stress-tolerant cultivars for pumpkin cultivation in semiarid regions.

Key words: inbred line, pumpkin, STI, water stress, yield

INTRODUCTION

The family Cucurbitaceae comprises approximately 130 genera and 800 species, and some of them, such as pumpkin, melon, watermelon, and cucumber, are commercially important [Perez Gutierrez 2016]. Among these species, pumpkin is an ancient species cultivated for several decades. While unripe fruits of pumpkin are used as vegetables, ripe fruits are used for making sweets and confectionery, and the seeds, which are rich sources of oil, are used directly or indirectly for human nutrition. Pumpkin seeds are also consumed in roasted or uncooked form, as well as in the form of additives in bread, cakes, and salads.

Pumpkin seeds are a rich source of natural phytosterols due to their high fat content. They are also rich in protein [Achu et al. 2005], unsaturated fatty acids [Seymen et al. 2016], antioxidants and vitamins, carotenoids, and tocopherols [Stevenson et al. 2007]. In addition, squalene, found in high levels in pumpkin

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seeds, is the precursor of steroid hormones, cholesterol, and vitamin D produced by humans, animals, and plants and has positive effects on treating certain types of cancer [Yang et al. 2020].

Pumpkins can be grown without irrigation in areas with good precipitation, and the consumer demand for the crop is continuously increasing in Turkey. However, drought, especially during the vegetation period, can adversely affect plant growth and yield [Seymen et al. 2019, Yavuz et al. 2021], and a decrease of approximately 75% in the yield of pumpkin has been reported [Yavuz et al. 2015]. Therefore, supplementary irrigation is essential to obtain commercially sustainable yields in pumpkin cropping periods in arid and semiarid regions. Apart from irrigation, it is also essential that plants can adapt to these environmental conditions or water stress to obtain a commercially viable yield from plants under drought stress [Shubha and Tyagi 2007]. Therefore, using highly adaptable genotypes to arid conditions or developing drought-tolerant varieties is an essential area of research [Karipcin et al. 2009]. The levels of drought tolerance of various genotypes have been previously determined in arid and irrigated conditions [Kumar et al. 2015]. The yield performance of the genotypes in irrigated and drought conditions is another indicator of drought tolerance [Mohammadi 2016].

Fernandez [1992] categorized the genotypes into the following four groups based on their yield performance in irrigated and water stress conditions: 'A' – highly productive in irrigated and water stress conditions, 'B' – highly productive in irrigated conditions but low productivity in water stress conditions, 'C' – highly productive in water stress conditions but low productivity in irrigated conditions, and 'D' low productivity in both irrigated and nonirrigated conditions. To evaluate the tolerance of the genotypes to drought conditions, some mathematical indices were calculated from the yields derived from irrigated and drought conditions. Rosielle and Hamblin [1981] defined drought stress tolerance (TOL) as the difference between the yields of genotypes in irrigated and drought conditions, and the average productivity in both conditions was considered the mean productivity (MP). The sensitivity of the genotypes to stress is closely related to the TOL and MP values. High TOL and low MP indices indicate lower tolerance for stress. Fernandez [1992] reported that high geometric mean productivity (GMP) indicated good tolerance, while the stress tolerance index (STI) was an important index for the determination of productivity and drought tolerance. On the other hand, the stress sensitivity index (SSI) indicated the performance of genotypes under irrigated and drought conditions [Fischer and Maurer 1978]. SSI values above 1.0 indicated that the genotypes were sensitive to water stress, whereas values below 1.0 indicated tolerance. The researchers also reported that the tolerance increased as the relative drought index (RDI) increased beyond 1.0. The drought resistance index (DI) was also used to identify highly productive genotypes under irrigated and drought conditions [Bidinger et al. 1987]. Moreover, the harmonic mean (HAM) has been reported to be a valuable index for determining the genotype's tolerance to stress [Kristin et al. 1997], and the yield index (YI) is a measure of the stability of genotypes in irrigated conditions [Gavuzzi et al. 1997]. Sensitivity drought index (SDI) values approaching 1.0 indicate that the genotypes are susceptible to drought [Farshadfar and Javadinia 2011]. Identifying water stress-tolerant and superior genotypes from the calculated indices may be discussed in a breeding program. On the other hand, knowledge of the genetic relationships between drought indices and genotypes can help select tolerant genotypes. However, screening the genotypes according to yield performance in arid or irrigated conditions is an effective method to achieve high-performing and productive genotypes in arid conditions [Kirigwi et al. 2004]. Researchers working on this subject report that genotypes should be evaluated in both irrigated and drought conditions to determine their tolerance [Fernandez 1992]. Pumpkin is grown in arid and semiarid regions, and its cultivation is increasing daily. However, few hybrid varieties are available, and large-scale cultivation with standard varieties adversely affects pumpkin yield. Therefore, screening gene pools for developing water stress-tolerant varieties of pumpkin is essential to maximize yield.

In this study, for the determination of highly productive and water stress-tolerant pumpkin genotypes, the yield performances of 44 inbred lines of pumpkin in open-field conditions were compared with two hybrids (G1-Mert Bey F1 and G2-Sena Hanım F1) and two local cultivars (G3-Hatuntırnagı and G4-Cercevelik) that were commercially grown in the region. None of the previous studies have used drought stress indices to determine drought-tolerant genotypes of pumpkins. Therefore, this study aimed to evaluate the water stress tolerance in breeding lines using drought stress indices and to determine the relationships between various drought stress indices. The tolerant genotypes obtained from this study are believed to contribute to future breeding efforts of water stress-tolerant hybrid cultivars.

MATERIAL AND METHODS

Experimental design, plant material, soil, and climate characteristics

This study was carried out at the Faculty of Agriculture at Selcuk University, Konya, Turkey, between May and September 2017 and 2018. The research area was 1006 m at 38°05'N and 32°36'E (Fig. 1). In the research area, some climatic parameters, such as temperature, relative humidity, wind speed, and precipitation, were measured and recorded every hour from an automated weather station (Davis Vantage Pro-2-6322, USA). The total amount of rainfall measured from the planting of pumpkin seeds to harvest was approximately 91 mm and 70.4 mm in 2017 and 2018, respectively. The average temperature was 23–24°C, and the relative humidity was as low as 35%, especially in July and August. The average wind speed was between 2.5–3 m s^{-1}. The climatic data for the study period (2017–2018) agreed with the region's long-term average climate data (Tab. 1). According to the long-term climate data, the Konya Plains has a semiarid climate, and the total amount of rainfall is 320 mm, of which only 90–100 mm falls during the vegetation season (Tab. 1). Therefore, irrigation is an indispensable necessity for crop production in this area.

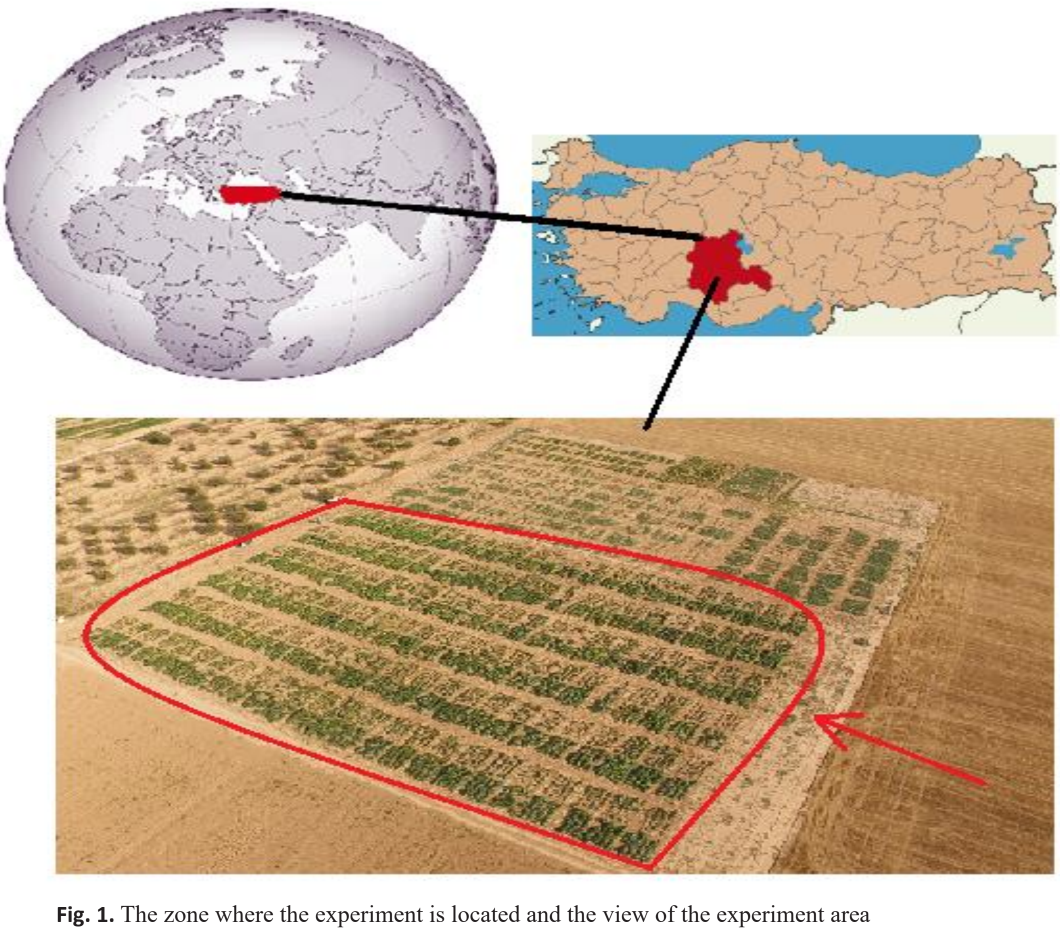

Fig. 1. The zone where the experiment is located and the view of the experiment area

Table 1. Some climatic parameters of experimental years and averages of long years

Months		Average temperature (°C)	Average wind speed (m s^{-1})	Average relative humidity (%)	Precipitation (mm)
May	2017[a]	13.0	2.8	69.1	29.4
	2018[b]	18.1	1.9	68.6	40.4
	Multiyear[e]	15.7	2.2	55.9	44.3
June	2017	19.6	3.0	61.2	43.8
	2018	21.0	1.8	55.8	17.2
	Multiyear	20.1	2.5	48.4	23.9
July	2017	24.4	3.6	39.2	0.0
	2018	24.9	3.6	43.8	3.2
	Multiyear	23.4	2.8	42.1	6.5
August	2017	23.5	3.8	51.2	13.9
	2018	24.4	2.9	40.2	2.4
	Multiyear	22.8	2.6	42.9	5.4
September	2017[c]	20.7	3.0	43.8	3.9
	2018[d]	20.0	2.8	45.3	7.2
	Multiyear	18.4	2.1	48.0	12.9

[a] Calculated from data between 8 and 31 May (2017 seed sowing: 8 May)
[b] Calculated from data between 11 and 31 May (2018 seed sowing: 11 May)
[c] Calculated from data between 1 and 27 September (2017 fruit harvest: 27 September)
[d] Calculated from data between 1 and 25 September (2018 fruit harvest: 25 September)
[e] Multiyears: 56-year average between 1960 and 2016

The pumpkin genotypes collected from different regions (different cities of Turkey such as Konya, Eskisehir, Ankara, Nevsehir, and Aksaray) for use in this study had been self-pollinated to the S7 level for several years. Subsequently, 44 inbred lines with superior agronomic traits were selected from the gene pool, and two hybrids (G1-Mert Bey F1 and G2-Sena Hanım F1) and two local cultivars (G3-Hatun Tırnağı and G4-Cercevelik) with high commercial value were used as the plant material [Seymen et al. 2019].

The soil in the study area had a silty-clayey-loamy texture, and the organic matter content in the 0–90 cm soil profile, pH, and bulk density varied from 0.93% to 1.55%, 7.70 to 7.98, and 1.25 to 1.35 g cm^{-3}, respectively. The total available water (TAW) in the upper 90 cm of the soil profile was 148.8 mm. The soil of the research area did not hinder pumpkin cultivation in terms of its physical and chemical properties.

The study was conducted in a randomized block design with three replicates under irrigated and nonirrigated conditions. Each parcel was placed in 4 × 5 m plots spaced 2 m from each other and 2.5 m from the blocks. For each parcel, 40 pumpkin seeds were sown evenly by hand in 1 m rows spaced 0.5 m apart. The seeds were sown on 8 May 2017 and 11 May 2018. After sowing the seeds, approximately 25 mm of irrigation water was applied to all the plots (irrigated and nonirrigated treatments), obtaining uniform seed germination and emergence. Then, irrigation water was not applied to nonirrigated treatments. Most precipitation (73.2 mm in 2017 and 57.2 mm in 2018) occurred during the vegetation period in both years before starting scheduled irrigation (Tab. 1). Irrigation water was applied to the irrigated treatments ten times after the scheduled irrigation was initiated (Tab. 2). The Class-A type evaporation pan was used to calculate irrigation water, and irrigation was applied at 7-day intervals. One drip-irrigation lateral pipe was placed in each plant row. The hoeing and earthing-up process was performed when the plants reached the 3 to 4 true-leaf stage. Diammonium phosphate (DAP) fertilizer (20 kg da^{-1}) was applied by a spreader before sowing. At the beginning of June, before the induction of water stress, 10 kg da^{-1} nitrogen (N), 10 kg da^{-1} phosphorus (P), and 12 kg da^{-1} potassium (K) fertilizer were applied by drip irrigation in pure form. During the study period, no disease or pest effects were observed, and only copper (at a 5% dose) was applied for protection against fungal diseases at 30-day intervals.

When the fruits reached harvest maturation, the first harvest from each parcel was made on 27 September 2017 and 25 September 2018. After harvesting the fru-

Table 2. The amount of irrigation water and irrigation date in 2017 and 2018

2017		2018	
irrigation date	irrigation water (mm)	irrigation date	irrigation water (mm)
12 May*	25.0	14 May*	25.0
26 June	30.1	28 June	28.3
3 July	39.0	4 July	34.8
11 July	39.6	11 July	42.5
18 July	55.3	19 July	65.4
25 July	52.2	26 July	48.3
1 August	53.0	1 August	42.1
7 August	19.5	8 August	48.3
15 August	38.4	15 August	30.4
22 August	24.0	21 August	27.7
28 August	26.7	29 August	32.5
total	402.8	total	425.3

* While the first irrigation was applied to all experimental treatments (non-stress and stress), subsequent

its, the seeds were extracted and dried in cloth bags under shaded conditions. The seeds were then weighed, and the seed yield for each parcel was determined.

Calculation of drought indices

After harvesting the fruits, the water stress parameters were calculated using the following equations using the yield values obtained under irrigated and nonirrigated conditions. Drought stress tolerance (TOL), mean productivity (MP) [Rosielle and Hamblin 1981], stress sensitivity index (SSI), relative drought index (RDI) [Fischer and Maurer 1978], mean geometric productivity (GMP), stress tolerance index (STI) [Fernandez 1992], yield index (YI) [Gavuzzi et al. 1997], harmonic mean (HAM) [Kristin et al. 1997], sensitivity drought index (SDI) [Farshadfar and Javadinia 2011], and drought resistance index (DI) [Bidinger et al. 1987] were calculated.

Explanation: Ys – yield in stress plot, Yp – yield in the fully irrigated plot, ($\overline{\text{Ys}}$) – mean yield in stress plot, ($\overline{\text{Yp}}$) – mean yield in fully irrigated plot.

$$TOL = Yp - Ys$$

$$SSI = \frac{1 - (Ys \div Yp)}{\overline{Ys} \div \overline{Yp}}$$

$$MP = \frac{Ys + Yp}{2}$$

$$GMP = \sqrt{Ys \times Yp}$$

$$STI = \frac{(Ys \times Yp)}{\overline{Ys}^2}$$

$$YI = \frac{Ys}{\overline{Ys}}$$

$$HAM = \frac{2 \times Ys \times Yp}{(Ys + Yp)}$$

$$SDI = \frac{Yp - Ys}{Yp}$$

$$DI = \left[Ys \times \frac{(Ys \div Yp)}{\overline{Ys}} \right]$$

$$RDI = \frac{(Ys \div Yp)}{(\overline{Ys} \div \overline{Ys})}$$

Evaluation of data

In this study, we aimed to interpret the yield under irrigated (Yp) and nonirrigated (Ys) conditions and the water stress indices obtained together. The combined variance analysis performed for the Ys and Yp components obtained for both trial years (2017 and 2018) was examined using homogeneity tests. According to the results of homogeneity tests, Ys and Yp values were evaluated together since they were homogeneous regarding the error variance of the years. Ys and Yp were subjected to analysis of variance, and the results were considered statistically significant at 5% significance levels according to Duncan's test. The analysis of variance and correlation tests was performed using SPSS statistics 22.0 packaged software. Correlations between drought indices were interpreted as a result of the correlation analysis. Principal component analysis (PCA) was performed on the TOL, SSI, MP, STI, YI, and DI indices that were weakly correlated. The score-plot and loading-plot graphics were drawn according to the two components obtained from PCA. In addition, according to Ward's method, similarity dendrograms for the genotypes were drawn from the drought indices using a hierarchical grouping method. The analyses were performed using the statistical program JMP 10.

RESULTS AND DISCUSSION

The two-year averages of seed yield and water stress parameters in irrigated and nonirrigated conditions are presented in Table 3. The seed yield varied between years and across genotypes. When the averages of all the genotypes in both conditions were compared, an approximately 80% reduction in yield was observed under water stress conditions. Under irrigation conditions, the commercial cultivars G1 and G2 produced a high yield, while the inbred lines G9, G11, G13, G22, G28, G30, G31, and G40 (181–220 kg da^{-1}) were the most productive. Overall, inbred lines G7, G9, G32, G34, and G40 were the genotypes with the highest yield (49–68 kg da^{-1}) under water stress conditions.

The G28 and G31 genotypes had high yields under irrigated conditions and had the highest TOL and SSI values. In addition, these two genotypes showed maximum yield loss under water stress conditions. The highest MP (144.4), GMP (122.9), STI (0.62), YI (2.13), and HAM (104.6) values were obtained from the G9 genotype. The highest SDI (0.94) value was obtained from the G19 and G28 genotypes. The highest RDI (2.39) value was obtained from the G7 genotype (Tab. 3). Following our findings, it has been previously reported that the selection of genotypes based only on low TOL values might lead to inefficient genotypes under nonirrigated conditions [Kamrani et al. 2018]. The SSI index was a better index than TOL for determining high-yielding genotypes in both cases [Kamrani et al. 2018]. The most effective way to identify stress tolerance is to evaluate the correlation between the yields obtained under irrigated and nonirrigated conditions and the drought index parameters [Kamrani et al. 2018].

When the correlation table was examined, Ys showed a high positive correlation with the indices HAM ($r = 0.992^{**}$), DI ($r = 0.934^{**}$), GMP ($r = 0.942^{**}$), STI ($r = 0.927^{**}$), RDI ($r = 0.861^{**}$) and YI ($r = 1.00^{**}$). On the other hand, a high negative correlation was observed with the indices SSI ($r = –0.859^{**}$) and SDI ($r = -0.859^{**}$). YP showed a high positive correlation with the indices TOL ($r = 0.876^{**}$) and MP ($r = 0.927^{**}$). MP could be an essential index due to its high positive correlation with GMP and STI (Table 4). GMP had a highly positive relationship with STI, YI, and HAM. Similarly, the SSI was negatively correlated with the YS in wheat [Mohammadi 2016] and maize [Kumar et al. 2015]. Generally, MP has been reported to be an effective index that exhibits a high positive correlation with yields in irrigated and nonirrigated conditions [Naghavi et al. 2013]. In addition, a significant positive correlation was observed between seed yield and MP ($r = 0.839$), GMP ($r = 0.934$), and STI ($r = 0.950$) under stress conditions. These have been reported to be essential indices for selecting drought-tolerant wheat genotypes in both cases [Kamrani et al. 2018]. In maize, the indices YI and DI were positively correlated with the yield obtained under stress conditions, while a negative correlation was observed with RDI [Naghavi et al. 2013].

As a result of correlation analysis, one of the indices showing high correlation was considered, and PCA was performed from the TOL, SSI, MP, STI, YI, and DI indices (Tab. 5). From the findings of the PCA, components were generated considering eigenvalues of 1.0 and above [Kamrani et al. 2018]. The first two

Table 3. Drought tolerance indices and yield of pumpkin genotypes under non-stress and stress conditions (in combined years)

Genotype	Ys*	Yp*	TOL	SSI	MP	GMP	STI	YI	HAM	SDI	DI	RDI
G1	46.9^{b-g}	184.9^{a-g}	138	0.94	115.9	93.1	0.36	1.46	74.82	0.75	0.37	1.23
G2	42.7^{b-i}	206.7^{abc}	164	1.00	124.7	93.9	0.36	1.33	70.78	0.79	0.27	1.00
G3	18.6^{k-n}	176.6^{b-k}	158	1.13	97.6	57.3	0.13	0.58	33.66	0.89	0.06	0.51
G4	20.5^{k-n}	150.1^{g-s}	129.6	1.09	85.3	55.5	0.13	0.64	36.07	0.86	0.09	0.66
G5	34.3^{c-l}	162.8^{e-o}	128.5	0.99	98.6	74.7	0.23	1.07	56.66	0.79	0.22	1.02
G6	10.9^{mn}	140.9^{j-t}	130	1.16	75.9	39.2	0.06	0.34	20.23	0.92	0.03	0.38
G7	55.5^{ab}	112.4^{r-u}	56.9	0.64	84.0	79.0	0.26	1.72	74.31	0.51	0.85	2.39
G8	17.1^{k-n}	124.0^{o-u}	106.9	1.09	70.6	46.0	0.09	0.53	30.06	0.86	0.07	0.67
G9	68.6^{a}	220.2^{a}	151.6	0.87	144.4	122.9	0.62	2.13	104.61	0.69	0.66	1.51
G10	15.9^{k-n}	129.1^{m-u}	113.2	1.10	72.5	45.3	0.08	0.49	28.31	0.88	0.06	0.60
G11	41.4^{b-j}	183.7^{a-h}	142.3	0.98	112.6	87.2	0.31	1.29	67.57	0.77	0.29	1.09
G12	27.3^{g-n}	169.1^{c-m}	141.8	1.06	98.2	67.9	0.19	0.85	47.01	0.84	0.14	0.78
G13	46.4^{b-g}	200.8^{a-e}	154.4	0.97	123.6	96.5	0.38	1.44	75.38	0.77	0.33	1.12
G14	51.3^{abc}	143.1^{i-s}	91.8	0.81	97.2	85.7	0.30	1.59	75.53	0.64	0.57	1.74
G15	30.4^{d-m}	165.2^{d-n}	134.8	1.03	97.8	70.9	0.21	0.94	51.35	0.82	0.17	0.89
G16	42.3^{b-i}	111.4^{stu}	69.1	0.78	76.9	68.6	0.19	1.31	61.32	0.62	0.50	1.84
G17	15.7^{k-n}	144.0^{h-s}	128.3	1.12	79.9	47.5	0.09	0.49	28.31	0.89	0.05	0.53
G18	21.9^{j-n}	166.6^{d-n}	144.7	1.09	94.3	60.4	0.15	0.68	38.71	0.87	0.09	0.64
G19	7.9^{n}	139.4^{j-t}	131.5	1.19	73.7	33.2	0.05	0.25	14.95	0.94	0.01	0.27
G20	48.0^{b-e}	156.5^{f-g}	108.5	0.87	102.3	86.7	0.31	1.49	73.47	0.69	0.46	1.49
G21	29.9^{d-m}	178.0^{b-j}	148.1	1.05	104.0	73.0	0.22	0.93	51.20	0.83	0.16	0.81
G22	41.9^{b-j}	193.4^{a-f}	151.5	0.99	117.7	90.0	0.33	1.30	68.88	0.78	0.28	1.05
G23	47.0^{b-g}	160.3^{f-p}	113.3	0.89	103.7	86.8	0.31	1.46	72.69	0.71	0.43	1.42
G24	22.8^{i-n}	120.1^{g-u}	97.3	1.02	71.5	52.3	0.11	0.71	38.32	0.81	0.13	0.92
G25	18.8^{k-n}	126.8^{n-u}	108	1.07	72.8	48.8	0.10	0.58	32.75	0.85	0.09	0.72
G26	28.6^{e-m}	101.9^{tu}	73.3	0.91	65.3	54.0	0.12	0.89	44.66	0.72	0.25	1.36
G27	28.9^{e-m}	153.3^{g-q}	124.4	1.02	91.1	66.6	0.18	0.90	48.63	0.81	0.17	0.91
G28	11.8^{mn}	183.3^{a-h}	171.5	1.18	97.6	46.5	0.09	0.37	22.17	0.94	0.02	0.31
G29	22.1^{j-n}	152.4^{g-r}	130.3	1.08	87.3	58.0	0.14	0.69	38.60	0.85	0.10	0.70
G30	47.3^{b-f}	205.0^{a-d}	157.7	0.97	126.2	98.5	0.40	1.47	76.86	0.77	0.34	1.12
G31	14.4^{lmn}	211.4^{ab}	197	1.17	112.9	55.2	0.12	0.45	26.96	0.93	0.03	0.33
G32	49.3^{a-d}	168.9^{c-m}	119.6	0.89	109.1	91.3	0.34	1.53	76.32	0.71	0.45	1.42
G33	32.5^{c-l}	122.1^{p-u}	89.6	0.92	77.3	63.0	0.16	1.01	51.34	0.73	0.27	1.29
G34	58.9^{ab}	159.9^{f-q}	101	0.80	109.4	97.0	0.39	1.83	86.09	0.63	0.67	1.79
G35	20.7^{k-n}	151.8^{g-r}	131.1	1.09	86.3	56.1	0.13	0.64	36.43	0.86	0.09	0.66
G36	47.7^{b-e}	170.9^{c-l}	123.2	0.91	109.3	90.3	0.33	1.48	74.58	0.72	0.41	1.35
G37	34.8^{c-k}	176.1^{b-k}	141.3	1.01	105.5	78.3	0.25	1.08	58.12	0.80	0.21	0.96
G38	30.3^{d-m}	164.2^{e-n}	133.9	1.03	97.3	70.5	0.20	0.94	51.16	0.82	0.17	0.89
G39	24.7^{h-n}	167.6^{c-m}	142.9	1.07	96.2	64.3	0.17	0.77	43.05	0.85	0.11	0.71
G40	51.5^{abc}	181.1^{a-i}	129.6	0.90	116.3	96.6	0.38	1.60	80.19	0.72	0.45	1.38
G41	10.5^{mn}	155.6^{f-q}	145.1	1.17	83.1	40.4	0.07	0.33	19.67	0.93	0.02	0.33
G42	24.0^{i-n}	121.0^{p-u}	97	1.01	72.5	53.9	0.12	0.75	40.06	0.80	0.15	0.96
G43	27.5^{f-n}	92.6^{u}	65.1	0.89	60.1	50.5	0.10	0.85	42.41	0.70	0.25	1.44
G44	21.3^{k-n}	122.1^{p-u}	100.8	1.04	71.7	51.0	0.11	0.66	36.27	0.83	0.12	0.85
G45	28.3^{e-m}	137.4^{k-t}	109.1	1.00	82.9	62.4	0.16	0.88	46.93	0.79	0.18	1.00
G46	24.4^{i-n}	140.0^{j-t}	115.6	1.04	82.2	58.4	0.14	0.76	41.56	0.83	0.13	0.84
G47	44.4^{b-h}	131.5^{l-u}	87.1	0.83	88.0	76.4	0.24	1.38	66.39	0.66	0.47	1.64
G48	35.4^{c-k}	156.2^{f-q}	120.8	0.97	95.8	74.4	0.23	1.10	57.72	0.77	0.25	1.10
Mean	32.2	156.1	123.94	1.00	94.12	69.08	0.21	1.00	51.94	0.79	0.24	1.01

* Ys and Yp – statistically significant at $p \leq 0.05$.

Ys – stress yield kg da^{-1}; Yp – non-stress yield kg da^{-1}; TOL – drought tolerance index; SSI – stress susceptibility index; MP – mean productivity; GMP – geometric mean productivity; STI – stress tolerance index; YI – yield index; HAM – harmonic mean; SDI – sensitivity drought index; DI – drought resistance index; RDI – relative drought index

Table 4. Correlation coefficients between the seed yield of pumpkin genotypes under non-stress (Yp) and stress (Ys) conditions and drought tolerance indices

	Ys	Yp	TOL	SSI	MP	GMP	STI	YI	HAM	SDI	DI	RDI
Ys	1.000	–	–	–	–	–	–	–	–	–	–	–
Yp	0.315	1.000	–	–	–	–	–	–	–	–	–	–
TOL	–0.179	0.876**	–	–	–	–	–	–	–	–	–	–
SSI	–0.859**	0.175	0.617*	1.000	–	–	–	–	–	–	–	–
MP	0.646*	0.927**	0.633*	–0.197	1.000	–	–	–	–	–	–	–
GMP	0.942**	0.595*	0.139	–0.650*	0.849**	1.000	–	–	–	–	–	–
STI	0.927**	0.614*	0.166	–0.611*	0.858**	0.987**	1.000	–	–	–	–	–
YI	1.000**	0.317	–0.177	–0.858**	0.648*	0.943**	0.928**	1.000	–	–	–	–
HAM	0.992**	0.396	–0.091	–0.802**	0.709*	0.972**	0.955**	0.992**	1.000	–	–	–
SDI	–0.859**	0.175	0.618*	0.999**	–0.196	–0.650*	–0.611*	–0.858**	–0.802**	1.000	–	–
DI	0.934**	0.065	–0.406	–0.946**	0.419*	0.775*	0.765*	0.933**	0.883**	–0.945**	1.000	–
RDI	0.861**	–0.173	–0.616*	–0.999**	0.199	0.652*	0.614*	0.859**	0.803**	–0.999**	0.947*	1.000

Ys – stress yield; Yp – non-stress yield; TOL – drought tolerance index; SSI – stress susceptibility index; MP – mean productivity; GMP – geometric mean productivity; STI – stress tolerance index; YI – yield index; HAM – harmonic mean; SDI – sensitivity drought index; DI – drought resistance index; RDI – relative drought index.
* Statistically significant according to $P < 0.05$
** Statistically significant according to $P < 0.01$.

Table 5. The results of principal component analysis for seed yield of pumpkin genotypes under non-stress and stress conditions and drought tolerance indices

Component	PV	CP	TOL	SSI	MP	STI	YI	DI
PC1	65.74	65.79	– 0.092	– 0.437	0.323	0.462	0.502	0.479
PC2	32.69	98.44	0.697	0.323	0.546	0.256	0.008	– 0.179

PV – percent of variance; CP – cumulative percentage; TOL – drought tolerance index; SSI – stress susceptibility index; MP – mean productivity; STI – stress tolerance index; YI – yield index; DI – drought resistance index.

components accounted for 98.44% of the total variance considering the eigenvalues. Mohammadi and Prasanna [2003] suggested that to effectively use and correctly interpret the PCA, the ratio of the first two or three components must be greater than 25% of the total variation. Thus, the high variance shown by the first two components indicated that the PCA could firmly explain drought indices. The first two components accounted for 98.2% of the total variance in wheat [Mohammadi and Abdulahi 2017], 99.4% in safflower [Bahrami et al. 2014], and 99.4% in wheat [Kamrani et al. 2018] when determining drought-tolerant genotypes, and it was reported that drought index parameters could also be explained in this manner. In our study, the first component accounted for 65.74% of the total variance, highly correlated with SSI, STI, YI, and DI. Therefore, this component is the most critical in determining water stress tolerance. The second component, the stress susceptibility component, accounted for 32.69% of the total variance and was positively correlated with TOL and MP. Similar approaches were also used in PC1 and PC2 studies on other species [Bahrami et al. 2014, Kamrani et al. 2018].

A graph was generated using PC1 and PC2 to assess the relationships between indices of water stress tolerance (Fig. 2). It has been reported that if the angle between the vectors in the figure is <90°, there is a positive relationship; if it is >90°, there is a negative

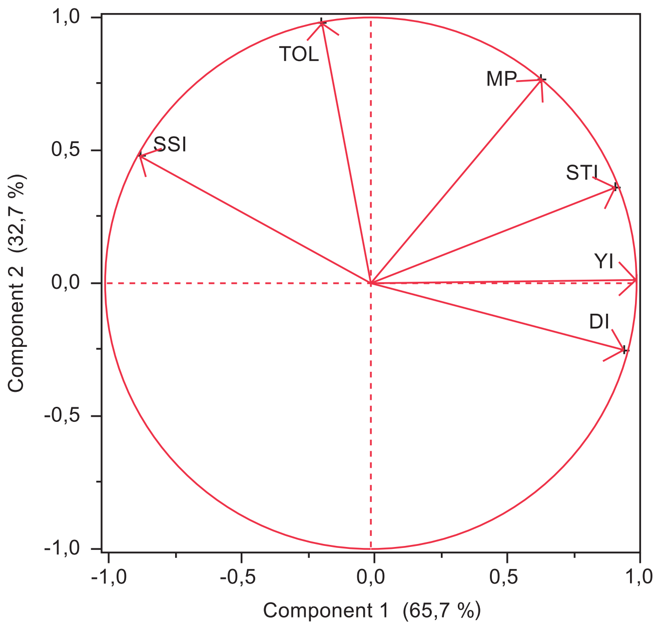

Fig. 2. Loading plot based on components 1 and 2 obtained from principal component analysis using drought tolerance indices for 48 pumpkin genotypes. Drought tolerance index (TOL), stress susceptibility index (SSI), mean productivity (MP), stress tolerance index (STI), yield index (YI), drought resistance index (DI).

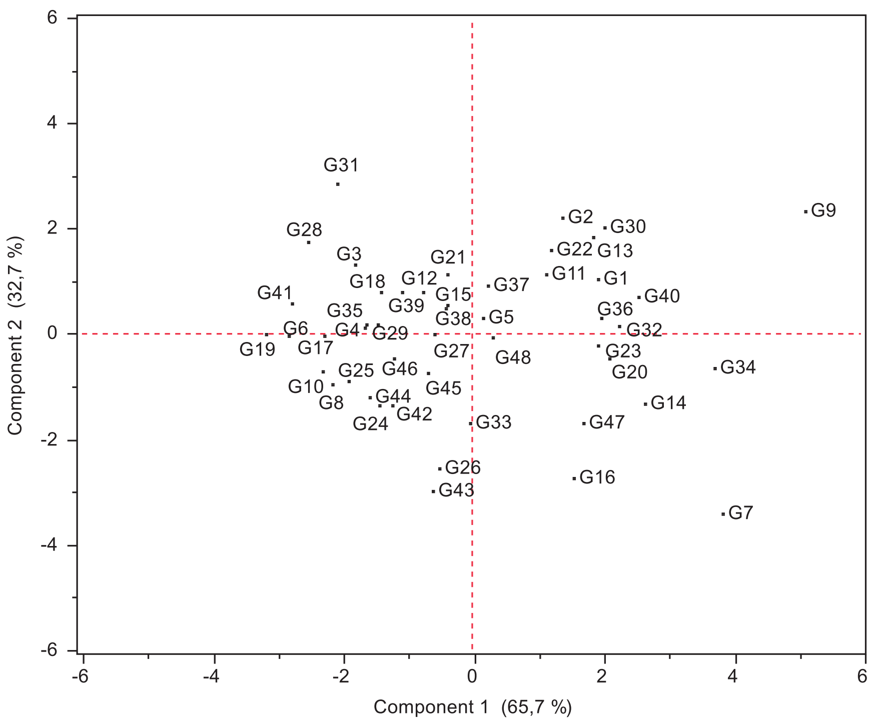

Fig. 3. Score plot based on components 1 and 2 obtained from principal component analysis using the drought tolerance indices for 48 pumpkin genotypes

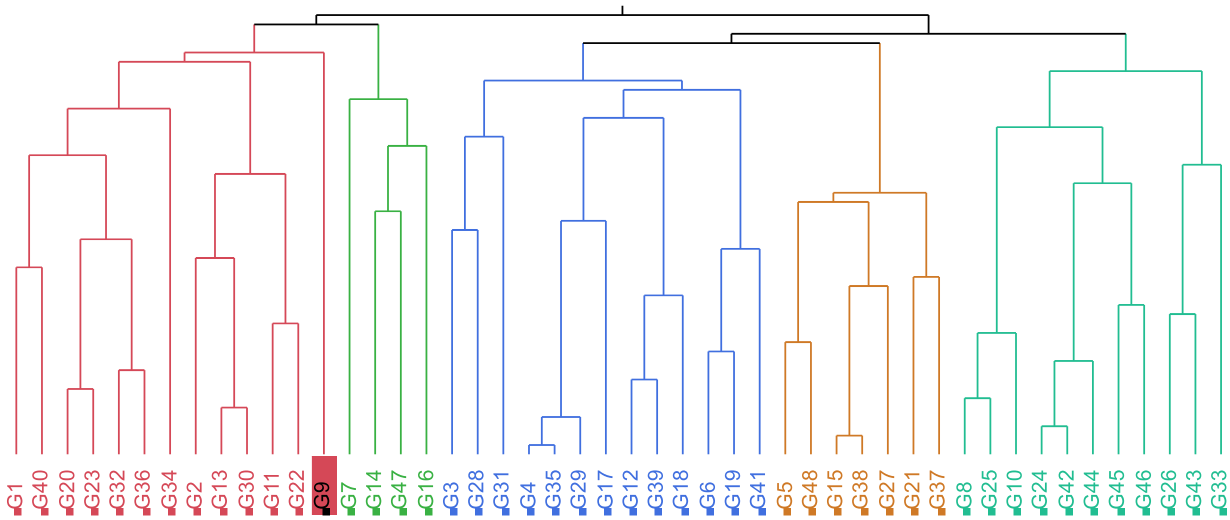

Fig. 4. Dendrogram showing the hierarchical grouping patterns of 48 pumpkin genotypes in five clusters based on drought tolerance indices

relationship; and if it is equal to 90°, there is no relationship [Yavuz et al. 2020]. Among the drought stress indices, the highest positive relationship was found between STI, YI, and DI. The drought resistance index was also used to identify highly productive genotypes in irrigated and drought conditions [Bidinger et al. 1987]. The STI is an essential index for determining productivity and water stress tolerance. A similar method was used to determine significant correlations in water stress conditions [Yavuz et al. 2020, Seymen 2021, Yavuz et al. 2021].

A graph was generated to examine the relationships between genotypes using PC1 and PC2 (Fig. 3). From the graph, the genotypes G9, G1, G40, G32, G36, G2, G5, G11, G22, G30, G37 and G13 were determined to be water stress tolerant, while G8, G10, G24, G25, G26, G42, G43, G44, G45 and G46 were determined to be water stress sensitive. When Figure 3 is examined, the STI index is seen as the index that gives the best results in irrigation and stress conditions. This index has an important role in determining the superior varieties of pumpkins. Different researchers have used similar approaches to evaluate the tolerance of genotypes to water stress conditions [Bahrami et al. 2014, Kamrani et al. 2018, Yavuz et al. 2020].

Cluster analysis was performed using the ward method based on the TOL, SSI, MP, STI, YI, and DI indices (Fig. 4). As a result of the analysis, five different clusters were formed. As seen on the dendrogram, genotypes G9, G34, G1, G40, G32, G22, G11, G30, G13, G2, G23, G20 and G36 were determined to be the water stress-tolerant genotypes. Although the G9 genotype was in the same group in the cluster analysis as in PCA, it showed a significant difference. In addition, G20, G23, and G34 were not seen as tolerant in PCA, while the G5 and G37 genotypes were not included in the tolerance cluster. On the other hand, the genotypes G8, G10, G24, G25, G26, G42, G43, G44, G45, and G46 were identified as the cluster representing the sensitive genotypes as in PCA. In addition to PCA, G33 was among the sensitive genotypes in cluster analysis. The same method was used by Naghavi et al. [2013] and Bahrami et al. [2014] to identify the water stress-tolerant genotypes of safflower, maize, and bean.

CONCLUSIONS

The present study determined the responses of 44 inbred pumpkin genotypes to water stress. As a result of the analyses, Ys showed a high positive correlation with the indices HAM, DI, GMP, STI, RDI, and YI. On the other hand, the PCA and cluster analyses showed the hybrid cultivars G1 (Mert Bey F1) and G2 (Sena Hanım F1) to be the most tolerant among the commercial cultivars. In contrast, water stress conditions negatively affected other commercial cultivars, and their yield potential decreased significantly. The analysis also led to the categorization of the inbred lines G9, G40, G32, G36, G5, G11, G22, G30, G37, and G13 in the same group as G1 and G2 under water stress conditions, and these inbred lines were also determined to be tolerant, producing higher yields than the other genotypes. STI is an essential index in determining tolerant and superior genotypes. These water stress-tolerant inbred pumpkin lines might play an important role in breeding efforts to develop novel superior pumpkin cultivars for cultivation in arid and semiarid regions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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