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GENOTYPE BY ENVIRONMENT INTERACTION AND GGE BIPLOT ANALYSES IN DURUM WHEAT UNDER WATERLOGGING STRESS

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ABSTRACT

Waterlogging is one of the major limitations that reduce productivity in wheat all over the world. The present study aimed to identify waterlogging-tolerance in durum wheat genotypes. Thirty-two durum wheat genotypes were screened under aerobic and anaerobic conditions using randomized complete block design with three replications. High-purity nitrogen gas was used to provide and maintain waterlogging stress, while the control group was aerated with the air. Growth and physiological parameters i.e., shoot dry weight gain, root dry weight gain, total dry biomass weight gain, plant leaf area, dry leaf weight, specific leaf weight, chlorophyll content, carotenoid content, and their tolerance indices were determined. Durum wheat seedlings grown under anaerobic conditions had significantly lower shoot dry weight, total dry biomass weight, specific leaf weight, chlorophyll a, and chlorophyll a + b content. Results further revealed that the tolerance indices varied depending on wheat cultivars for the investigated parameters. Harran 95 under aerobic condition and Eminbey under anaerobic condition had the highest tolerance indices for shoot dry weight gain and total dry biomass weight gain. However, Kızıltan 91 had the highest tolerance indices for root dry weight gain under these two conditions. For these reasons, Kızıltan 91 came to forefront position with its stability and could be used in durum wheat breeding. For identifying the correlation of tolerance index with seed yield and potential genotypes to be used as a selection criterion, further studies are needed under field conditions.

Key words: Waterlogging, aerobic and anaerobic conditions, genotype by environment interaction, GGE biplot analysis, durum wheat.

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INTRODUCTION

Wheat is one of the main cereal crops, produced approximately 766 million tonnes on an area of approximately 215 million hectares in 2019 (FAO, 2021) and provides a substantial proportion of dietary calories for four billion people (Du *et al*., 2021). Wheat is cultivated under a wide range of moisture conditions from xerophytic to littoral where precipitation ranges from 250 to 1750 mm. Sufficient moisture availability is required during the growing season for optimum wheat production, however, excessive rains after irrigation causes waterlogging (Tiryakioğlu *et al*., 2015). When water stands on the soil surface for a prolonged period of time or when the available water fraction in the soil surface layer is at least 20% higher than the field water capacity, the soil is defined as waterlogged soil (Aggarwal *et al*., 2006). Waterlogging occurs from time to time in areas with heavy rainfall in wheat cultivation areas and in the bottom lands in streams, rivers and river deltas or in the form of closed basins, so that plant roots remain without oxygen for a certain period of time (Tiryakioğlu *et al.,* 2014). Approximately 15-20% of annual global wheat production is affected by waterlogging, and this rate is increasing due to extreme weather conditions due to ongoing global climate change (Gao *et al.,* 2021).

In wheat-growing regions around the world, especially in the areas with high rainfall, waterlogging is one of the major restrictions for wheat production. Since wheat is grown mostly under rainfed conditions in arid and semi-arid regions in the Mediterranean Climate Zone, however, it usually encounters waterlogging during the vegetative growth stage during the rainy days and the productivity is hampered in these areas (Tiryakioğlu *et al.,* 2015).Studies showed that waterlogging cause reducing approximately 20-50% in the grain yield of wheat in the United Kingdom (Belford and Cannell, 1979; Cannell *et al*., 1984), in North America (Musgrave, 1994; Musgrave and Ding, 1998), in Australia (Dennis *et* *al.*, 2000; Zhang *et al.*, 2006) and in China (Ding *et al.*, 2017; Chen *et al.*, 2018).

Wheat is a highly sensitive species to waterlogging (Tiryakioğlu *et al.,* 2015; Gao *et al.,* 2021; Katerova *et al.,* 2021). Gas exchange decreases between soil and air during waterlogging and oxygen in the soil is depleted rapidly, and the soil may become hypoxic (low oxygen) or anoxic (no oxygen) within a day (Armstrong *et al.,* 2009; Araki *et al.,* 2012). Waterlogging generally restricts root growth, inhibits photosynthesis and carbohydrate synthesis, and accelerates leaf senescence (Du *et al.,* 2021), adversely affects plant growth by causing hypoxia and excessive accumulation of reactive oxygen species in the plant roots, and causes a decrease in productivity (Zhou *et al.,* 2021). Lack of soil oxygen can limit plant yield directly by altering root metabolism or indirectly by changing plant nutrient availability (Setter *et al.*, 2009; Zhang *et al.*, 2011; Sharma *et al.*, 2018; Bailey-Serres *et al.*, 2012). Waterlogging during the stem elongation period accelerates the decrease in photosynthesis rate during post-anthesis, slow down the grain filling, and reduces thousand grain weight (Gao *et al.*, 2021).

Although waterlogging is not a preventable stress factor, and its damage can be minimized. This will be possible with the development of new wheat varieties that are resistant to waterlogging. Revealing waterlogging-tolerant varieties is critical to stabilizing and even increasing wheat yields in waterlogged areas. Different wheat genotypes can be used as a source of genetic material to improve wheat's tolerance to waterlogging. The majority of genetic materials used in durum wheat breeding in Turkiye have been obtained from CIMMYT origin lines or from crossing existing varieties with each other. The origin of the cultivars in question is largely of CIMMYT origin. There are not enough studies on the response of these cultivars to waterlogging stress.

The aim of this study is to reveal the response of commercial durum wheat cultivars that are registered in Turkiye and have different cultivation areas against waterlogging stress in the seedling stage, thus determining the genotypes that can be used as a gene source in the breeding of waterlogging stress tolerant cultivars.

MATERIALS AND METHODS

Study materials and site: In the present study, 32 widely grown durum wheat cultivars (Artuklu, Amanos-97, Kunduru-1149, Güneyyıldızı, Yelken-2000, Şahinbey, Ç-1252, Harran-95, Eminbey, Altın-40/98, Diyarbakır-81, Altıntoprak-98, Claudio, Tüten-2002, Akçakale-2000, Dumlupınar, Sarıçanak-98, Çakmak-79, Zenit, Zühre, Altıntaş-95, Kümbet-2000, Kızıltan-91, Levante, Svevo, Saragolla, Fuatbey-2000, Aydın-93, Eyyubi, İmren, Fırat-93, and Yılmaz-98) were tested in the Laboratory of Crop Science, Hatay Mustafa Kemal University in 2014, Turkiye. The pedigree information about the cultivars used in the study are provided in Table 1.

Experimental design andimplementation: The study was carried out in 3 replications according to the completely randomized design for a split plot. The anoxic factor was planned as the main (control=continuous oxygen, flooding=oxygen-free environment) and the cultivar (32 genotypes) factor as the sub-factor, each combination being in a pot and all of the combinations are 144 pots.

The durum cultivar seeds were germinated in perlite moisturized with saturated $CaSO_4$ solution for five days at room temperature before being transferred to solution culture. Seedlings of equal length were transferred to 5 L polyethylene pots containing Hoagland solution. Five seedlings were fixed in each of the 5 small apertures at the top of each container (25 plants in total per pot). The composition of the Hoagland nutrient solution was 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.9 mM K_2SO_4, 0.2 mM KH_2PO_4 , 10^{-6} M H_3BO_3, 2 x 10^{-7} M $MnSO_4$, 2 x 10^{-6} M $ZnSO_4$, 2 x 10^{-7} M $CuSO_4$, 2 x 10^{-8} M $(NH_4)_6$ Mo_7O_{24} and 10^{-4} M $C_{10}H_{12}FeN_2NaO_8$ (FeEDTA).

The containers were kept in a growth chamber under controlled conditions (12 h light/12 h dark cycle at 20/15 °C day/night temperature, relative humidity 60%, and light intensity 25 klux or 300 µmol m^{-2} s^{-1}) until the 3 to 4 leaf stage, according to the Zadoks growth scale (ZGS 13) (Zadoks *et al.,* 1974). Solutions in the containers were aerated with an air pump to supply oxygen for root respiration. When the seedlings were at the tillering stage (ZGS 20), nitrogen gas (99.99% pure) was pumped into the waterlogging group (Biemelt *et al*., 1998), and the other group was aerated with the air (control group) during 15 days. After 15 days, the samples of plants were collected. Dry weight gain for shoot and root were calculated as the difference between the shoot or root dry weight at the beginning of waterlogging and at the end of the waterlogging treatments.

Data collection: From each container, five plants were randomly selected and oven-dried at 70 °C for 48 h to determine the dry weight. To measure the plant leaf area, shoot and root dry weight, 10 randomly selected plant samples from each pot were used. Specific leaf weight was determined by using 5 newly developed leaf samples of these plants. The chlorophyll was determined according to Arnon (1949) by taken on the last completed leaves of 5 randomly selected plants. Leaf material (0.5 g) was homogenized in acetone and centrifuged in a table centrifuge for 15 min. After that, the supernatant was treated with acetone to 15 mL. The absorbance value of the sample was read at 645–663 nm, spectrophotometrically. Data were assessed through below equations, and chlorophyll amount was calculated as mg chlorophyll/g fresh leaf:

Chlorophyll *a* (mg/L) = 12:7 A_{663} – 2:69 A_{645}

Chlorophyll *b* (mg/L) = 22:9 A_{645} – 4:68 $_{A663}$.

Waterlogging tolerance/susceptibility indices (TI) were calculated for each cultivar using the following equations:

TI = (measured plant parameter under anaerobic conditions/measured plant parameter under anaerobic conditions) × 100.

Data analysis: The data obtained from the experiments were subjected to analysis of variance using the completely randomized design for a split plot in the Statistical Analysis System (SAS Institute, 1996). Differences among means were tested through Duncan and values of P<0.05 were considered significantly different.

RESULTS

Based on plant leaf area, shoot dry weight gain, root dry weight gain, total dry biomass weight gain, dry leaf weight, specific leaf weight, chlorophyll *a*, *b*, chlorophyll *a* + *b*, and carotenoid content, the durum wheat cultivars seedling response differed significantly under aerobic and anaerobic conditions (Table 2).

Leaf area: Durum wheat cultivar Eminbey had the highest plant leaf area (78.0 cm^2 $plant^{-1}$) among all the cultivars under aerobic condition, followed by cultivar Kızıltan-91 (76.0 cm^2 $plant^{-1}$). However, under anaerobic condition, the cultivar Akçakale-2000 had the highest plant leaf area (67.1 cm^2 $plant^{-1}$). Under anaerobic condition, the durum wheat cultivar Eminbey's plant leaf area was reduced dramatically about to half compared to aerobic condition (Table 3).

Shoot, root and total dry weight gain: Under aerobic condition, durum wheat cultivar Harran-95 came to the forefront with the value of 335.7 mg $plant^{-1}$ shoot dry weight gain, followed by cultivar Kızıltan-91 (232.6 mg $plant^{-1}$). Under anaerobic condition, cultivar Eminbey had the highest value (242.0 mg $plant^{-1}$) among all the cultivars. Durum wheat cultivar Kızıltan-91 had the remarkable stability (232.6 and 199.4 mg $plant^{-1}$), under aerobic and anaerobic conditions, respectively.

Under anaerobic condition, the waterlogging-tolerant cultivars can continue their root growth up to some extent. Cultivar Kızıltan-91, grabbed attention with stability in shoot dry weight gain, also had the greatest root dry weight gain under aerobic and anaerobic conditions with the values of 52.9 and 57.8 mg $plant^{-1}$, respectively. Durum wheat cultivar Altıntoprak-98 was at the second position with 51.8 mg $plant^{-1}$ under aerobic condition while cultivar Dumlupınar obtained the second position with 50.2 mg $plant^{-1}$ under anaerobic condition.

The highest total dry biomass weight gain was observed in cultivar Harran-95, which doubled the last cultivar, with value of 378.5 mg $plant^{-1}$ under aerobic condition. Durum wheat cultivar Eminbey had the highest total dry biomass weight (291.8 mg $plant^{-1}$) under anaerobic condition. Besides, cultivar Kızıltan-91 took the second place (285.5 mg $plant^{-1}$) under aerobic condition, and the third place (257.2 mg $plant^{-1}$) under anaerobic condition. Although durum wheat cultivars Harran-95 under aerobic condition and cultivar Eminbey with anaerobic condition produced the highest total dry biomass weight. Cultivar Kızıltan-91 was located in a special place with its stability under both conditions.

Dry and specific leaf weight: Dry leaf weight varied between 25.2 to 53.4 g under aerobic condition and durum wheat cultivar Altıntaş-95 had the highest dry leaf weight (53.4 g), followed by the cultivars Şahinbey (43.3 g) and Zühre (43.1 g). The dry leaf weight varied between 27.5 to 47.3 g under anaerobic condition, and the highest dry leaf weight was recorded in cultivar Harran-95 (47.3 g), followed by two other cultivars Diyarbakır-81 (47.2 g) and Eminbey (46.6 g). The highest specific leaf weight value was achieved in cultivar Svevo (96.93 g m^{-2}) while the lowest in cultivar Altıntaş-95 (47.86 g m^{-2})under aerobic condition. Durum wheat cultivar Zenit took the first place with the highest specific leaf weight (104.43 g m^{-2}) under anaerobic condition, while it was the fourth-ranked cultivar(93.71 g m^{-2}) under aerobic condition (Table 4). The reduction in specific leaf dry weight was probably mediated by failure of the leaf tissue to expand fully. Observations further showed that oxygen deficiency reduced the specific leaf dry weight of the durum wheat cultivars. However, the cultivar Zenit was not affected by this situation, and in contrary, the specific leaf weight enhanced under anaerobic condition.

Chlorophyll a, b and a + b, and carotenoid content: Chlorophyll *a* content varied from 0.64 to 3.67 mg g^{-1} under aerobic condition. However, the highest Chlorophyll *a* content was obtained in durum wheat cultivar Levante (3.67 mg g^{-1}), followed by cultivar Akçakale-2000 (2.74 mg g^{-1}) (Table 4). The rest of cultivars were under the value of 2 mg g^{-1}. However, under anaerobic condition, the highest chlorophyll *a* content was obtained in cultivar Fuatbey-2000 (1.71 mg g^{-1}), was half of the greatest value under aerobic condition. Durum wheat cultivar Amanos-97 secured the second position with value of 1.64 mg g^{-1}. Only three out all the tested cultivars were higher than 1 mg g^{-1} for chlorophyll *b* content under aerobic condition i.e., cultivars Levante, Akçakale-2000, and Ç-1252 with values of 1.92, 1.25, and 1.01 mg g^{-1}, respectively. Durum wheat cultivars Levante and Akçakale-2000 came to the forefront same as in chlorophyll *a* content. The highest chlorophyll *b* content under anaerobic condition was obtained by cultivar Amanos-97 (1.24 mg g^{-1}), followed by Fuatbey-2000 (1.22 mg g^{-1}).

Under aerobic condition, durum wheat cultivar Akçakale-2000 had the highest chlorophyll *a* + *b* content (3.63 mg g^{-1}), followed by two other cultivars Ç-1252 (2.33 mg g^{-1}) and Amanos-97 (2.26 mg g^{-1}). Cultivar Fuatbey-2000 had the greatest chlorophyll *a* + *b* content (2.62 mg g^{-1}) under anaerobic condition as like chlorophyll *a* and *b* contents. Durum wheat cultivar Amanos-97 had the remarkable stability under both aerobic and anaerobic conditions with values of 2.26 and 2.54 mg g^{-1}, respectively.

Table 1. Pedigree information about the cultivars.

	Cultivars	Habit	Pedigree
C1	Artuklu	S	LAHN//GANSO/STORK
C2	Amanos-97	S	OSTRERO//CELTA/YAVAROS,AUS
C3	Kunduru-1149	I	(S)LV-TUR
C4	Güneyyıldızı	S	RASCON-39/TILD-1
C5	Yelken-2000	W	ZF/LEEDS//FORAT/3/ND-61-130/LEEDS/4/(TR.SE)AU-107/5/GERARDO
C6	Şahinbey	S	---
C7	Ç-1252	W	ND-61-130//414-44/377-2
C8	Harran-95	S	KORIFLA//DS-15/GEIGER; DURUM-DWARF-S-15/CRANE//GEIER
C9	Eminbey	W	CMK79//14-44/OVIACHIC-65/3/BERKMEN/OVIACHIC-65/4/KUNDURU-1149/5/LEEDS//DWARF-MUTANT/SARIBASAK
C10	Altın-40/98	I	BARRIGON-YAQUI-ENANO/2*TEHUACAN-60//2B//LONGSHANKS/3/BERKMEN-469
C11	Diyarbakır-81	S	LD-393//BELADI-116-E/2*TEHUACAN-60/3/COCORIT-71
C12	Altıntoprak-98	S	ALTAR-84/ARAOS
C13	Claudio	S	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
C14	Tüten-2002	S	ALTAR-84/(ALD)AVETORO/3/GANSO/FLAMINGO,MEX//CANDO
C15	Akçakale-2000	S	SCHELLENTE//CORMORANT/RUFFOUS/3/AJAIA
C16	Dumlupınar	W	BERKMEN/G-75-T-181
C17	Sarıçanak-98	S	DACKIYE/GEDIZ-75//USDA-575
C18	Çakmak-79	I	UVEYIK-162/ND-61-130
C19	Zenit	S	VALRICCARDO/VIC
C20	Zühre	S	SN-TURK-M-183-84-375/(SIB)NIGRIS-5//TANTLO-1
C21	Altıntaş 95	W	KUNDURU//D-68111/WARD
C22	Kümbet 2000	I	ND-61-130//414-44/377-2/3/DF-15-72
C23	Kızıltan 91	I	UVEYIK-162/61-130//BARRIGON-YAQUI-ENANO*2/TE
C24	Levante	S	G-80/PICENO//IONIO
C25	Svevo	S	CIMMYT-SELECTION/ZENIT
C26	Saragolla	W	LV-FROSINONE
C27	Fuatbey-2000	S	---
C28	Aydın 93	S	JORI-C-69/HAURANI
C29	Eyyubi	S	MORUS//ALTAR-84/ALONDRA
C30	İmren	W	DF-21-72/GERARDO-VZ-466//ND-61-130/414-44/3/ERGENE/4/DF-21-72//ND-61-130/UVEYIK-162/3/128-3
C31	Fırat-93	S	SNIPE/3/JORI-C-69/CRANE/GANSO/ANHINGA; ANHINGA(SIB)/(SIB)VOL//(SIB)FLAMINGO,MEX/3/SHAW
C32	Yılmaz-98	W	DF-9-71/3/V-2466//ND-61-130/414-44/4/ERGENE

S: Spring, W: Winter, I: Intermediate

The highest and same carotenoid content (3.78 mg g^{-1}) were recorded in cultivar Harran-95 under aerobic condition and cultivar Saragolla under anaerobic condition. Also, the cultivar Saragolla was at the second position (3.67 mg g^{-1}) under aerobic condition. Results further revealed that cultivar durum wheat Saragolla was leading genotype with its stability for the carotenoid content under both aerobic and anaerobic conditions. The lowest carotenoid contents were exhibited by the cultivar Çakmak-79 (1.62 mg g^{-1}) under aerobic condition and cultivar Yılmaz-98 (2.04 mg g^{-1}) under anaerobic condition.

Tolerance indices: To evaluate the 32 durum wheat cultivars for waterlogging tolerance, ten tolerance indices i.e., tolerance index for plant leaf area, shoot dry weight gain, root dry weight gain, total dry weight, dry leaf weight, specific leaf weight, chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoid content were used (Table 5). Six durum-wheat cultivars, Akçakale-2000, Kızıltan-91, Tüten-2002, Dumlupınar, Altıntaş-95, and Çakmak-79 have the values above unity (1.00) for tolerance index of plant leaf area. Cultivar Akçakale-2000 had the greatest value (1.34) among all tested durum wheat cultivars.

Durum wheat cultivars Harran-95 and Eminbey had the highest tolerance index for shoot dry weight (1.64, 1.58) and total dry weight (1.61, 1.61), while cultivar Kızıltan-91 had the highest tolerance index for root dry weight (2.25). Tolerance index of dry leaf weight varied between 0.60 to 1.75, and the highest value was obtained by cultivar Altıntaş-95, while the lowest by cultivar Amanos-97. The highest tolerance index of specific leaf dry weight was obtained in cultivar Zenit (1.62), followed by three other cultivars Çakmak-79 (1.47), Svevo (1.43), and Kümbet-2000 (1.40).

Cultivar Levante had the greatest tolerance indexes for chlorophyll *a* and *b*(2.59 and 3.37), followed by cultivar Amanos-97 (1.48 and 2.24). Also, durum wheat cultivar Amanos-97 had the greatest tolerance index of chlorophyll *a* + *b*(1.91). The tolerance index for carotenoid content varied from 0.62 to 2.04, and the highest value was recorded in cultivar Saragolla (2.04).

Table 2. Mean plant parameters measured under aerobic and anaerobic conditions.

Plant parameters	Growth condition		LSD
	Aerobic	Anaerobic	–0.05
Plant leaf area (cm^2 plant^{-1})	59.3	51.0	6.97
Shoot dry weight gain (mg plant^{-1})	177.3	170.1	1.61
Root dry weight gain (mg plant^{-1})	36.8	40.9	1.60
Total dry biomass weight gain (mg plant^{-1})	214.1	211.0	0.66
Dry leaf weight (g)	36.0	38.8	1.04
Specific leaf weight (g m^{-2})	77.7	76.3	1.04
Chlorophyll *a* (mg g^{-1})	1.420	1.086	0.05
Chlorophyll *b* (mg g^{-1})	0.653	0.684	0.08
Chlorophyll *a* + *b* (mg g^{-1})	1.737	1.577	0.02
Carotenoid content (mg g^{-1})	2.605	2.869	0.04

Table 3. Mean comparison of plant leaf area (PLA), shoot dry weight gain (SDWG), root dry weight gain (RDWG), total dry biomass weight gain (TDWG), and dry leaf weight (DLW) under aerobic (+O), and anaerobic (–O) conditions.

Cultivar	PLA (cm^2 plant^{-1})		SDWG (mg plant^{-1})		RDWG (mg plant^{-1})		TDWG (mg plant^{-1})		DLW (g)	
	+O	–O	+O	–O	+O	–O	+O	–O	+O	–O
Artuklu	55.4	39.9	191.2	151.7	34.7	36.9	225.9	188.5	36.0	33.2
Amanos-97	54.1	32.6	141.4	147.2	34.9	43.4	176.3	190.6	25.2	30.9
Kunduru-1149	52.4	40.8	178.0	167.0	38.0	45.8	216.0	212.8	32.9	43.5
Güneyyıldızı	47.5	32.2	145.3	137.3	29.1	34.3	174.4	171.6	33.3	39.8
Yelken-2000	45.6	40.7	126.0	162.7	29.7	36.4	155.7	199.1	31.4	39.8
Şahinbey	59.5	46.3	172.0	173.0	35.7	36.0	207.7	209.0	43.3	37.4
Ç-1252	56.4	42.4	156.7	145.7	36.7	40.8	193.4	186.4	38.2	27.5
Harran-95	65.3	51.7	335.7	153.7	42.8	41.0	378.5	194.7	36.6	47.3
Eminbey	78.0	37.4	205.3	242.0	47.4	49.8	252.7	291.8	39.5	46.6
Altın-40/98	62.6	36.7	176.5	169.8	38.4	38.7	215.0	208.5	38.2	38.6
Diyarbakır-81	57.6	50.6	170.5	198.7	36.9	43.4	207.4	242.0	36.9	47.2
Altıntoprak-98	69.2	47.7	230.7	181.3	51.8	48.6	282.5	230.0	41.8	44.4
Claudio	66.0	48.4	160.0	176.5	34.2	45.0	194.3	221.5	33.5	44.1
Tüten-2002	66.3	65.6	215.8	186.4	40.1	43.8	255.8	230.2	37.8	42.3
Akçakale-2000	70.0	67.1	201.7	188.2	42.5	42.5	244.1	230.7	36.7	45.4
Dumlupınar	70.6	59.1	201.4	171.7	48.8	50.2	250.1	221.9	36.5	39.2
Sarıçanak-98	55.6	58.6	178.0	215.0	37.6	45.4	215.6	260.4	32.8	36.9
Çakmak-79	67.7	59.2	142.9	141.7	39.0	39.5	181.9	181.2	28.9	30.0
Zenit	53.6	48.7	155.0	174.7	31.4	31.3	186.4	206.0	38.3	29.3
Zühre	51.8	65.1	147.7	160.0	36.0	40.1	183.7	200.1	43.1	42.4
Altıntaş-95	62.2	65.9	170.9	205.1	41.5	49.6	212.4	254.7	53.4	42.4
Kümbet-2000	60.5	57.6	137.3	179.5	29.0	37.2	166.3	216.7	30.5	36.5
Kızıltan-91	76.0	58.0	232.6	199.4	52.9	57.8	285.5	257.2	31.2	42.3
Levante	58.1	48.6	160.2	148.8	34.0	38.6	194.2	187.4	34.5	35.8

Svevo	60.0	47.1	221.8	188.1	37.6	44.4	259.4	232.5	34.7	37.2
Saragolla	57.5	55.5	174.3	145.5	30.1	29.3	204.5	174.8	40.1	33.2
Fuatbey-2000	61.7	52.5	138.2	186.6	37.3	40.5	175.5	227.2	39.5	33.9
Aydın-93	39.3	46.0	117.2	149.0	27.5	31.7	144.7	180.7	26.1	32.0
Eyyubi	50.1	63.6	189.5	139.7	25.5	29.7	215.0	169.4	33.9	36.2
İmren	53.8	52.1	170.7	179.7	34.3	39.6	205.0	219.3	35.3	39.7
Fırat-93	48.0	48.6	158.3	146.9	24.7	35.8	183.0	182.7	36.0	43.9
Yılmaz-98	64.6	53.3	170.9	173.3	37.8	39.6	208.7	212.9	35.5	39.8
LSD (0.05)	6.94	1.79	4.23	2.35	23.29	5.03	3.88	2.15	26.45	5.99

Table 4. Mean comparison of specific leaf weight (SLW), chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*), chlorophyll *a* + *b* (Cl *a* + *b*), and carotenoid content (CarC) under aerobic (+O) and anaerobic (-O) conditions.

Cultivar	SLW (g m^{-2})		Cl *a* (mg g^{-1})		Cl *b* (mg g^{-1})		Cl *a* + *b* (mg g^{-1})		CarC (mg g^{-1})	
	+O	–O	+O	–O	+O	–O	+O	–O	+O	–O
Artuklu	66.16	56.48	1.56	1.30	0.68	0.83	1.97	1.91	2.95	2.94
Amanos-97	65.65	63.31	1.83	1.64	0.77	1.24	2.26	2.54	3.02	2.84
Kunduru-1149	68.61	46.84	1.22	0.93	0.63	0.61	1.66	1.38	2.52	2.20
Güneyyıldızı	70.46	52.13	1.29	1.08	0.63	0.70	1.70	1.59	2.66	2.40
Yelken-2000	57.56	64.46	1.39	0.64	0.63	0.25	1.80	0.78	2.54	2.45
Şahinbey	61.10	56.70	1.12	1.32	0.52	0.70	1.45	1.81	2.41	2.56
Ç-1252	63.47	50.37	1.60	0.52	1.01	0.24	2.33	0.67	2.88	2.42
Harran-95	74.63	58.88	1.64	1.49	0.71	1.05	2.13	1.97	3.78	3.07
Eminbey	61.13	47.25	1.34	1.05	0.47	0.78	1.61	1.66	1.86	2.68
Altın-40/98	65.84	61.99	1.53	1.12	0.69	0.76	2.01	1.72	2.52	2.69
Diyarbakır-81	81.94	67.01	1.21	1.25	0.56	0.87	1.55	1.91	2.68	3.39
Altıntoprak-98	85.63	72.48	1.26	1.08	0.60	0.88	1.66	1.77	3.02	2.36
Claudio	76.69	67.64	1.15	1.15	0.58	0.83	1.54	1.80	2.14	2.86
Tüten-2002	58.68	87.60	1.51	0.51	0.63	0.25	1.75	0.68	3.56	3.02
Akçakale-2000	85.96	88.44	2.74	1.01	1.25	0.62	3.63	1.47	2.44	2.98
Dumlupınar	94.41	87.36	1.04	1.24	0.41	0.67	1.27	1.69	1.98	2.58
Sarıçanak-98	84.53	81.95	1.24	1.00	0.60	0.63	1.63	1.47	2.32	2.62
Çakmak-79	90.56	97.71	1.06	0.59	0.61	0.23	1.47	0.68	1.62	3.50
Zenit	93.71	104.43	1.55	0.58	0.65	0.47	1.96	0.95	2.69	3.09
Zühre	77.58	84.96	1.47	1.02	0.62	0.69	1.85	1.54	3.03	3.04
Altıntaş-95	47.86	93.62	1.12	0.56	0.28	0.29	1.20	0.78	2.29	2.70
Kümbet-2000	87.36	96.67	0.88	1.13	0.48	0.55	1.20	1.48	1.73	3.14
Kızıltan-91	82.17	74.35	1.27	0.67	0.53	0.30	1.62	0.88	2.23	3.12
Levante	92.80	83.27	3.67	1.42	1.92	0.75	1.73	1.94	2.59	2.92
Svevo	96.93	89.25	1.51	1.45	0.73	0.93	1.96	2.14	2.66	3.32
Saragolla	79.83	89.22	1.35	1.36	0.62	0.96	1.76	2.10	3.67	3.78
Fuatbey-2000	87.31	92.98	1.10	1.71	0.55	1.22	1.48	2.62	2.75	3.61
Aydın-93	93.38	89.10	0.64	1.25	0.24	0.80	0.78	1.85	2.41	2.69
Eyyubi	76.26	76.47	1.26	1.42	0.60	0.69	1.68	1.88	3.38	3.16
İmren	83.75	85.32	1.50	1.07	0.74	0.70	1.96	1.56	2.84	2.30
Fırat-93	94.20	80.35	1.09	1.30	0.42	0.80	1.33	1.87	2.15	3.16
Yılmaz-98	80.79	84.24	1.32	0.86	0.55	0.61	1.66	1.32	2.05	2.04
LSD (0.05)	1.63	1.63	0.071	0.052	0.016	0.052	0.016	0.052	0.19	0.46

Table 5. Mean comparison of tolerance indices of plant leaf area, shoot dry weight gain, root dry weight gain, total dry weight, dry leaf weight, specific leaf dry weight, chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, carotenoid content.

Cultivar	TIPLA	TISDW	TIRDW	TITDW	TIDLW	TISLW	TICl*a*	TICl*b*	TICl*a* + *b*	TICar
Artuklu	0.63	0.92	0.94	0.93	0.92	0.62	1.01	1.34	1.25	1.28
Amanos-97	0.50	0.66	1.12	0.73	0.60	0.69	1.48	2.24	1.91	1.26
Kunduru-1149	0.61	0.95	1.28	1.00	1.10	0.53	0.56	0.90	0.76	0.82
Güneyyıldızı	0.44	0.63	0.74	0.65	1.02	0.61	0.69	1.03	0.90	0.94
Yelken-2000	0.53	0.65	0.80	0.68	0.97	0.61	0.44	0.37	0.47	0.92
Şahinbey	0.78	0.95	0.95	0.95	1.25	0.57	0.73	0.86	0.87	0.91
Ç-1252	0.68	0.73	1.11	0.79	0.81	0.53	0.41	0.56	0.52	1.02
Harran-95	0.96	1.64	1.30	1.61	1.34	0.73	1.21	1.73	1.39	1.71
Eminbey	0.83	1.58	1.74	1.61	1.42	0.48	0.69	0.86	0.89	0.74
Altın-40/98	0.65	0.95	1.10	0.98	1.14	0.68	0.84	1.23	1.15	1.00
Diyarbakır-81	0.83	1.08	1.18	1.10	1.35	0.91	0.75	1.14	0.98	1.34
Altıntoprak-98	0.94	1.33	1.86	1.42	1.44	1.03	0.68	1.24	0.98	1.05
Claudio	0.91	0.90	1.14	0.94	1.14	0.86	0.66	1.13	0.92	0.90
Tüten-2002	1.24	1.28	1.30	1.28	1.23	0.85	0.38	0.37	0.39	1.59
Akçakale-2000	1.34	1.21	1.33	1.23	1.29	1.26	1.37	1.81	1.77	1.07
Dumlupınar	1.19	1.10	1.81	1.21	1.11	1.37	0.64	0.64	0.71	0.75
Sarıçanak-98	0.93	1.22	1.26	1.22	0.93	1.15	0.61	0.89	0.79	0.89
Çakmak-79	1.14	0.64	1.14	0.72	0.67	1.47	0.31	0.33	0.33	0.83
Zenit	0.74	0.86	0.72	0.84	0.87	1.62	0.44	0.72	0.62	1.22
Zühre	0.96	0.75	1.07	0.80	1.41	1.09	0.74	0.99	0.94	1.36
Altıntaş-95	1.17	1.11	1.52	1.18	1.75	0.74	0.31	0.19	0.31	0.91
Kümbet-2000	0.99	0.78	0.80	0.79	0.86	1.40	0.49	0.62	0.59	0.80
Kızıltan-91	1.25	1.48	2.25	1.60	1.02	1.01	0.42	0.37	0.47	1.03
Levante	0.80	0.76	0.97	0.79	0.95	1.28	2.59	3.37	1.11	1.12
Svevo	0.80	1.33	1.23	1.32	1.00	1.43	1.09	1.59	1.39	1.30
Saragolla	0.91	0.81	0.65	0.78	1.03	1.18	0.91	1.40	1.23	2.04
Fuatbey-2000	0.92	0.82	1.12	0.87	1.03	1.34	0.93	1.58	1.29	1.47
Aydın-93	0.51	0.56	0.64	0.57	0.64	1.38	0.40	0.46	0.48	0.95
Eyyubi	0.91	0.84	0.56	0.79	0.95	0.97	0.89	0.98	1.05	1.58
İmren	0.80	0.98	1.00	0.98	1.08	1.18	0.80	1.22	1.01	0.96
Fırat-93	0.66	0.74	0.65	0.73	1.22	1.25	0.70	0.79	0.82	1.00
Yılmaz-98	0.98	0.94	1.10	0.97	1.09	1.13	0.56	0.78	0.72	0.62

TIPLA = tolerance index for plant leaf area, TISDW = tolerance index for shoot dry weight gain, TIRDW = tolerance index for root dry weight gain, TITDW = tolerance index for total dry weight, TIDLW = tolerance index for dry leaf weight, TISLW = tolerance index for specific leaf weight, TICl*a* = tolerance index for chlorophyll *a*, TICl*b* = tolerance index for chlorophyll *b*, TICl*a* + *b* = tolerance index for chlorophyll *a* + *b*, TICar = tolerance index for carotenoid content.

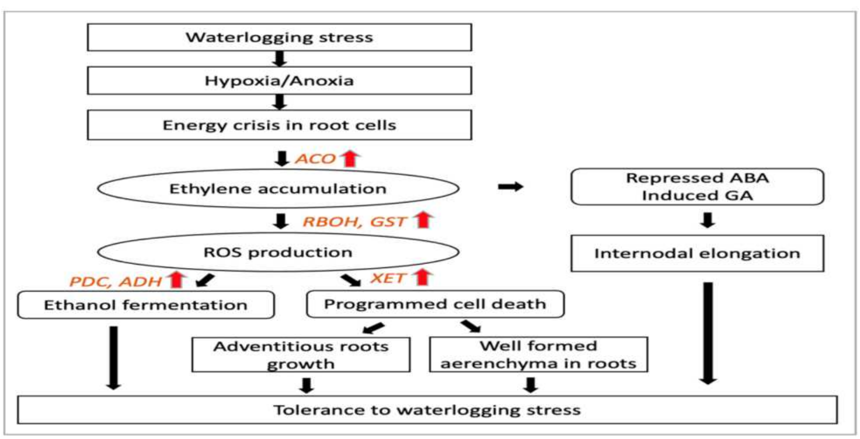

Figure 1. Schematic diagram of the main waterlogging stress responses and metabolic adaptive traits for waterlogging tolerance in plants.

PDC: Pyruvate decarboxylase, ADH: Alcohol dehydrogenase, RBOH: Respiratory burst oxidase homolog, GST: Glutathione S transferase, XET: Xyloglucan endo-transglucosylase, ACO: 1-amino-cyclopropane-1-carboxylic acid oxidase (Tong et al., 2021).

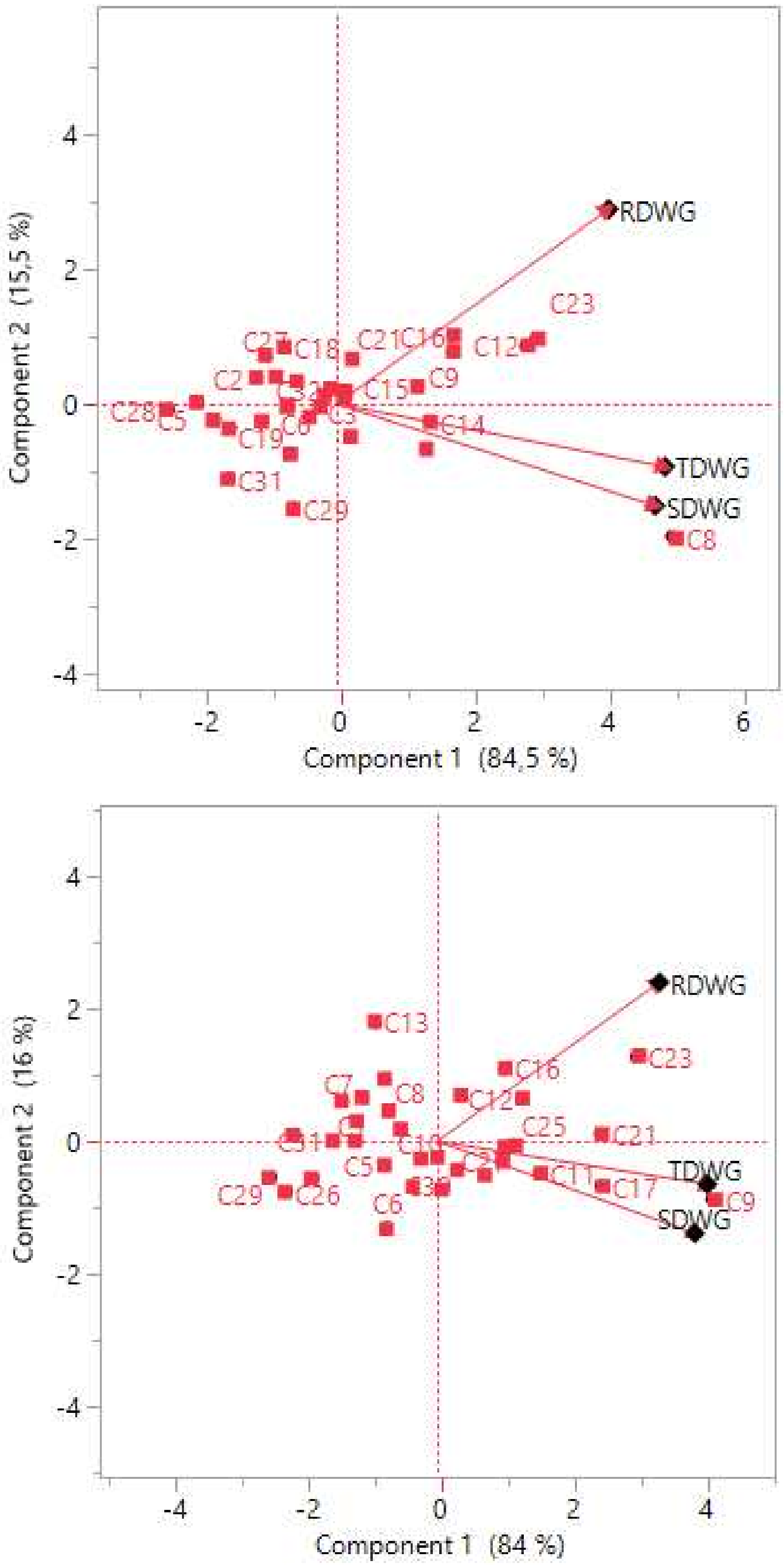

Figure 2. Results of biplot for durum-wheat cultivars on shoot dry weight gain (SDWG), root dry weight gain (RDWG), total dry weight gain (TDWG) (left: aerobic, right: anaerobic)

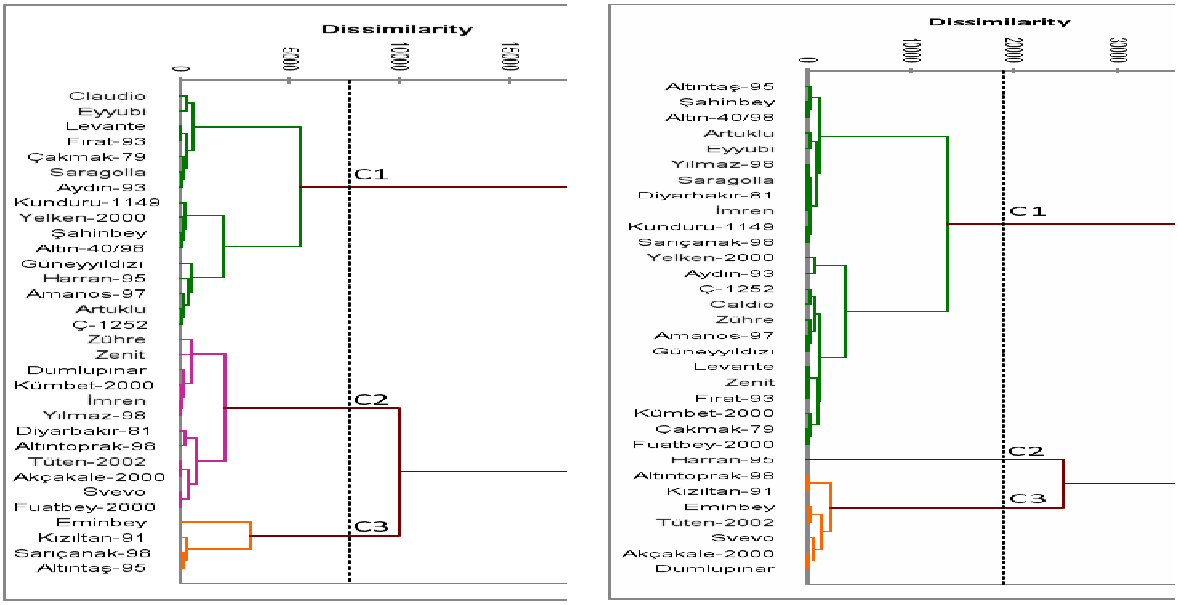

Figure 3. Dendrogram for durum-wheat cultivars on investigated parameters (left: aerobic, right: anaerobic)

DISCUSSION

Few studies have evaluated the impact of waterlogging on durum wheat (Tesemma *et al*., 1991; Pampana *et al*., 2016; Cotrozzi *et al*., 2021) and studies revealing the response of cultivars are even fewer. However, waterlogging stress is one of the most common abiotic stress factors on earth after drought and high temperature. Moreover, it is expected in the near future that there will be more risk of waterlogging due to an increase of occurrence of more intense precipitations and extreme events of high rainfall around the world, as a result of climate change (Wollenweber *et al,* 2003, Trenberth *et al,* 2007, IPCC, 2014).

A schematic summary of the basic changes in plant metabolism with stress in plants exposed to seedling period waterlogging in wheat is given in Figure 1. Depending on the duration and severity of the waterlogging stress, the most affected features are as plant green area, root dry weight, shoot dry weight and specific leaf weight.

In the present study, as a result of waterlogging, there was a significant decrease in PLA, TDGW, SDWG, SLW, Cla and Cla + b, while an increase was detected in RDGW, DLW, Clb and CarC. The cultivars in question gave different responses in terms of RDWG, except for Altıntoprak-98, Harran-95 and Saragolla, although at varying rates (Altın 40/98: 0.7%, Fırat-93: 45.0%); it was generally increased. In general, waterlogging severely inhibits gas exchange between plant roots and the atmosphere. Oxygen in the water-filled soil is rapidly depleted, causing roots to change from aerobic respiration to anaerobic fermentation, while CO_2 and ethylene concentrations increase rapidly. This causes a drastic decrease in ATP synthesis of stem cells and affects multiple metabolic processes of plants (Sairam *et al.*, 2009; Pampana *et al.*, 2016; Kaur *et al.*, 2020). Therefore, it has been reported that the negative effects begin to occur in plants exposed to waterlogging within 48 hours (Brisson *et al.*, 2002), and the oxygen level in the root zone decreases rapidly during this period (Setter and Waters, 2003). In this case, one of the hypoxic or anoxic conditions occurs in the root region, depending on the environmental conditions. In our study, RDWG was positively affected by waterlogging; possibly due to the formation of a hypoxic rather than anoxic state in the root zone. As a matter of fact, Huang *et al.,* (1997), in their study conducted with Bayles and Jackson bread wheat varieties that show different tolerance to waterlogging in hypoxic conditions, reported that root growth was adversely affected in the waterlogging-sensitive Bayles variety, while the elongation of the crown roots increased in the tolerant Jackson variety. Although RDWG results increased in the results free of individual responses of the cultivars in our study, when the results at the cultivar level were examined, it was seen that RDWG values in some cultivars were adversely affected by waterlogging (Table 3). Therefore, our findings were similar to Huang *et al.,* (1997). Huang and Johnson (1995) reported in another study that this difference between cultivars was due to the fact that the root/leaf sugar ratio in tolerant cultivars increased in favor of the root under stress, and this was due to the transport of these sugars from the leaves to the root. Again, Sauter (2013) reported that plants resistant to waterlogging have a number of adaptations that help to maintain oxygen transport to the root, they are also able to initiate organogenesis to replace their original root systems with adventitious roots if oxygen supply becomes impossible.

In the present study, PLA was the feature most negatively affected by waterlogging. Of the 32 durum wheat genotypes that were the subject of the study, a decrease between 1.0% and 52.0% occurred in all of them, except for Eyyubi, Zühre, Aydın 93, Altıntaş 95 and Sarıçanak 98. A similar situation occurred in SDWG, although not so dramatically. Studies (Cannell *et al.,* 1980; Setter and Waters 2003; San Celedonio *et al.,* 2014; Marti *et al.,* 2015; Arduini *et al.*, 2016 and Ding *et al.*, 2017) generally showed that shoot growth is the most adversely affected feature by waterlogging. Because the root function is damaged under the pond, the stoma closes. This, in addition to inhibition of photosynthesis, causes leaf wilting and senescence, inhibiting the flow of carbon dioxide to the leaf as well as transpiration, leading to lower biomass accumulation (Tian *et al*., 2021). During water accumulation, reductions in chlorophyll or other components of the photosynthetic apparatus as a result of nitrogen deficiency and/or negative feedback from carbohydrate accumulation have been reported as possible causes of low CO_2 fixation (Shabala *et al*., 2014; Herzog *et al*., 2016; Fukao *et al*., 2019). In some conditions, disruption of cation homeostasis (eg K+ and Ca 2+) and damage to leaves from ROS or phytotoxins (eg Fe 2+ or Mn 2+) may also contribute (Cotrozzi *et al*., 2021).

Shoot, root and total dry weight values are more important than the rest of the investigated parameters as directly affected from anaerobic condition. Therefore, the results of biplot analysis based on these parameters are provided in Figure 2. Total variation for shoot dry weight gain, root dry weight gain, and total dry weight gain was about 100% under aerobic and anaerobic conditions. Under aerobic condition, principle component 1 (PC1) explained 84.5% and PC2 explained 15.5% of the total variation. Similarly, PC1 explained 84% and PC2 explained 16% of the total variation under anaerobic condition. Two main groups were stood out in both aerobic and anaerobic conditions i.e., a) Shoot dry weight gain and total dry weight gain, and b) Root dry weight gain.

Durum wheat cultivar Harran-95 (C8) was closely related to shoot dry weight gain (SDWG) and total dry weight gain (TDWG) under aerobic condition while cultivar Eminbey (C9) was closely related to these parameters under anaerobic condition (Figure 2). Cultivar Harran-95 under anaerobic condition incurred loses to about 54% and decreased to 154 mg in SDWG, and about 49% and decreased to 195 mg in TDWG. In terms of root dry weight gain (RDWG), cultivar Kızıltan-91 (C23) came to the forefront with the highest values under both aerobic (52.9 mg) and anaerobic (57.8 mg) conditions. It was observed this cultivar increased the root development when faced waterlogging, as one of the most important abiotic stress factor. Although durum wheat cultivars Harran-95 and Eminbey had the highest values in TDWG under aerobic and anaerobic conditions, while cultivar Kızıltan-91 made a strong impress under both conditions with its stability.

As can be seen from dendrogram, three main clusters were obtained with investigated parameters under aerobic and anaerobic conditions (Figure 3). Also, these main clusters were divided into the many sub-clusters. Under aerobic condition, cultivar Harran-95 was belonged to a cluster by one own. The other cluster was consisted of seven durum wheat cultivars i.e., Altıntoprak-98, Kızıltan-91, Eminbey, Tüten-2002, Svevo, Akçakale-2000, and Dumlupınar. The rest of cultivars created the big cluster which divided into two sub-clusters. However, durum wheat four cultivars (Eminbey, Kızıltan-91, Sarıçanak-98, and Altıntaş-95) created the smallest cluster under anaerobic condition. One of the rest clusters was consisted of twelve cultivars Zühre, Zenit, Dumlupınar, Kümbet-2000, İmren, Yılmaz-98, Diyarbakır-81, Altıntoprak-98, Tüten-2002, Akçakale-2000, Svevo, and Fuatbey 2000. The rest of the cultivars created the biggest cluster under anaerobic condition.

The chlorophyll amount and leaf area reduced and finally decreased the root/shoot ratio under waterlogged conditions (Ghobadi *et al.*, 2017). Even short-term transient waterlogging can have considerable effects on growth and grain yield of dry land crops. Ultimately, both root and shoot dry mass production reduced (Hodge *et al.*, 2009; Sauter, 2013).

Past studies reported that chlorophyll *a* and *b* contents were gradually decreased and the chlorophyll *a* and *b* contents of the leaves drop by 19-45% after 16 days of waterlogging (Smethurst and Shabala, 2003). Pang *et al.* (2004) observed that increased waterlogging significantly decreased the chlorophyll contents and the CO_2 assimilation rate. Olgun *et al.* (2008) reported that being exposed to waterlogging for longer than ten days at the beginning of the flowering stage created an enormous decrease in chlorophyll *a* and *b* contents.

Conclusion: All the investigated parameters except root dry weight gain (RDWG) were negatively affected by anaerobic conditions. However, under anaerobic condition many cultivars was observed with increased values of RDWG because the plant strengthened its roots for defeating stress factors. The highest shoot dry weight gain (SDWG) and total dry biomass weight gain(TDWG) values were observed in cultivar Harran 95 under aerobic condition and from Eminbey under anaerobic condition. Also, these two durum wheat cultivars had the highest tolerance indices for these parameters. However, cultivar Kızıltan 91 had the highest RDWG value and tolerance indices for root dry weight gain under aerobic and anaerobic conditions. Due to that reasons, durum wheat cultivar Kızıltan 91 came to forefront position with its stability and could be used in durum wheat breeding.

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