Turkish Journal of Agriculture and Forestry

Volume 39 | Number 5 Article 17

1-1-2015

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Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2015) 39: 807-816
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doi:10.3906/tar-1407-124

Response of bread-wheat seedlings to waterlogging stress

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Received: 22.07.2014 • **Accepted/Published Online:** 29.10.2014 • **Printed:** 30.09.2015

Abstract: Waterlogging is a widespread problem and one of the major yield-limiting factors of wheat in some parts of the coastal plains of Turkey. The object of this study was to identify waterlogging-tolerant cultivars in bread wheat. Twenty-four bread-wheat genotypes were tested under aerobic and anaerobic conditions to determine shoot dry weight gain, root dry weight gain, total dry weight gain, dry leaf weight, specific dry leaf weight, chlorophyll content, carotenoid content, transpiration and photosynthesis rates, and their tolerance indices. A complete randomized block design with three replications was conducted for both aerobic and anaerobic conditions. The current study clearly demonstrated that wheat seedlings growing under anaerobic conditions had significantly lower shoot dry weight, root dry weight, total dry biomass weight, leaf dry weight, specific leaf dry weight, chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, and carotenoid content, and photosynthesis and transpiration rates. There were significant correlations between total dry weight gain and shoot dry weight gain and between chlorophyll *a* and specific leaf dry weight. Wheat cultivars had different tolerance indices for the investigated plant parameters. Further studies are needed to confirm which tolerance index is significantly correlated with seed yield and which one could be used as a selection criterion under field conditions.

Key words: Bread wheat, dry matter, genotype, photosynthesis, seedling, waterlogging

1. Introduction

Wheat, a cool season staple crop widely cultivated under different climatic conditions and cropping systems, is one of the most valuable crops and a staple food for the vast majority of the human population. Therefore, it occupies a central position in agricultural policy and dominates all other crops in planting area and production. Wheat is cultivated on more than 240 million ha, more than any other crop, and world trade is greater than for all other crops combined.

Wheat is cultivated under a wide range of moisture conditions from xerophytic to littoral where precipitation ranges from 250 to 1750 mm. Most wheat cultivation areas receive an average annual precipitation between 375 and 875 mm (Leonard and Martin, 1963). Optimal wheat production requires an adequate source of moisture availability during the growing season; however, too much precipitation or irrigation followed by excess rain causes waterlogging. It is estimated that 10–15 million ha of wheat production areas worldwide are under threat of waterlogging (Sayre et al., 1994). Waterlogging is one of the major restrictions for wheat production in many wheat-growing regions throughout the world, especially in high-rainfall environments. Wheat is mostly grown under rainfed conditions in the rainy season in arid and semiarid regions in the Mediterranean climate; therefore, it usually encounters waterlogging at the vegetative growth stage during the peak rainy days, hampering its productivity in these areas.

Wheat growth and development are adversely affected under anaerobic flooded conditions by chlorosis, adverse effects on mineral uptake, altered growth regulator relationships, disruption of cell membranes, stomatal closure, leaf wilting and epinasty, reduced photosynthesis and respiration, and altered carbohydrate partitioning. Some wheat genotypes can morphologically or physiologically adjust themselves to these alterations to avoid flooding injury.

Among cultivated crops, wheat is highly sensitive to waterlogging (Chauhan et al., 1997; Perera et al., 2001), as are soybeans (Vantoai et al., 1994). However, genetic tolerance, if available, could help to increase the stability in wheat production and productivity under waterlogging episodes, which are expected to become increasingly unpredictable with global warming.

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Waterlogging causes chlorophyll, protein, and RNA degradation and also reduces the concentration of nutrients like nitrogen, phosphorus, metal ions, and minerals in the shoots. Under waterlogging conditions, leaf chlorosis, inhibition in root and shoot growth, and reduction in dry matter accumulation occur, and this negatively affects final yield (Kozlowski, 1984; Bingru et al., 1994; Malik et al., 2011). To avoid waterlogging damage, planting waterlogging-tolerant wheat cultivars that can resume growth after complete submergence or flooding is one of the practical ways to obtain adequate seed yield.

Up to 50% yield loss can occur depending on the depth and duration of flooding, temperature, and the development stage of the waterlogged plant (Setter et al., 1999). The genetic improvement of waterlogging tolerance is critical for increasing and stabilizing wheat yield in waterlogging regions. Wheat genotypes could be used as a source of genetic material to improve the tolerance of wheat to waterlogging. The purpose of the current study is to find waterlogging wheat genotype(s) that could be used as a gene source for breeding waterlogging-tolerant cultivars.

2. Materials and methods

Two waterlogging-tolerant wheat cultivars (Ceyhan-99 and Karasu-90) and 22 widely grown bread-wheat cultivars (Bezostaja 1, Cumhuriyet 75, İzmir 85, Kasif Bey 95, Ziyabey 98, Gonen, Pehlivan, Adana-99, Genç-99, Balattila, Pandas, Sagittario, META 2002, Sakin, Canik 2003, Ozcan, Tekirdag, Gelibolu, Osmaniyem, Selimiye, and Vittorio) were tested in the crop science laboratory of Mustafa Kemal University.

The seeds of the cultivars were germinated in perlite moisturized with saturated $CaSO_4$ solution for 5 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. The composition of the nutrient solution (Hoagland) 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, 2×10^{-7} M $MnSO_4$, 2×10^{-6} M $ZnSO_4$, 2×10^{-7} M $CuSO_4$, 2×10^{-8} M $(NH_4)_6Mo_7O_{24}$, 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. 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.

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). In each container, 5 plants were randomly sampled and oven-dried at 70 °C for 48 h to determine dry weight. A newly developed leaf was taken from each container to measure shoot and root dry weight and specific leaf dry weight.

Photosynthesis and transpiration rates were measured at 5-day intervals following waterlogging. However, the measures taken on the 15th day were used for statistical analysis.

The amount of chlorophyll was determined according to Arnon (1949). Leaf material (0.5 g) was homogenized in acetone and centrifuged in a table centrifuge for 15 min. Then 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 in formulae (1) and (2) below, and amounts of chlorophyll were calculated as mg chlorophyll/g fresh leaf:

(1) Chlorophyll *a* (mg/L) = $12{:}7\ A_{663} - 2{:}69\ A_{645}$

(2) Chlorophyll *b* (mg/L) = $22{:}9\ A_{645} - 4{:}68\ A_{663}$.

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.

The data obtained from the experiments were subjected to analysis of variance using the general linear models procedure in the Statistical Analysis System (SAS Institute, 1996). Differences among means were tested through LSD and values of $P < 0.05$ were considered significantly different.

3. Results

Based on shoot dry weight, rood dry weight, total dry biomass weight, leaf dry weight, specific leaf dry weight, chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, carotenoid content, photosynthesis rate, and transpiration rate, the response of seedling wheat cultivars under aerobic and anaerobic conditions differed significantly (Table 1). In the case of shoot dry weight gain under anaerobic condition, cultivar Bezostaja had significantly higher dry weight gain than the control cultivar Ceyhan 99. Gonen and İzmir 85 had higher shoot dry weight gain than the control cultivar Ceyhan-99, but the difference was not significant (Table 2). Seven cultivars, Bezostaja 1, İzmir 85, Gonen, Ceyhan-99, Canik 2003, Gelibolu, and Osmaniyem had significantly higher shoot dry weight gain than the control cultivar Karasu-90. However, under aerated conditions, 4 cultivars, İzmir 85, Bezostaja 1 Gonen, and Adana-99 had significantly higher shoot dry weight gain than the control

Table 1. Mean plant parameters measured under aerobic and anaerobic conditions.

Plant parameters	Growth condition		LSD
	Aerobic	Anaerobic	–0.05
Shoot dry weight gain (mg $plant^{-1}$)	61.6	44.47	1.12
Root dry weight gain (mg $plant^{-1}$)	12.9	11.09	0.2
Total dry biomass weight gain (mg $plant^{-1}$)	72.79	57.25	1.24
Dry leaf weight (mg)	248.29	189.2	2.37
Specific leaf weight (g m^{-2})	127.81	101.11	1.46
Chlorophyll *a* (mg g^{-1})	2.15	1.87	0.04
Chlorophyll *b* (mg g^{-1})	0.57	0.41	0.01
Chlorophyll *a* + *b* (mg g^{-1})	2.15	1.61	0.05
Carotenoid content (mg g^{-1})	2.09	1.48	0.04
Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})	9.42	1.53	0.06
Transpiration rate (mmol H_2O m^{-2} s^{-1})	2.57	1.53	0.18

Table 2. Mean comparison of shoot dry weight gain (SDWG), root dry weight gain (RDWG), total dry weight gain (TDWG), dry leaf weight (RLW), and specific leaf weight (SLW) under aerobic (+O), and anaerobic (–O) conditions.

	SDWG (mg $plant^{-1}$)		RDWG (mg $plant^{-1}$)		TDWG (mg $plant^{-1}$)		DLW (mg)		SLW (g m^{-2})	
Cultivar	+O	–O	+O	–O	+O	–O	+O	–O	+O	–O
Adana-99	76.3	38.2	12.0	11.8	88.3	50.0	298.4	274.8	86.2	71.8
Sakin	40.0	39.5	10.5	10.4	50.6	49.8	212.5	195.3	134.5	119.3
Osmaniyem	67.0	52.1	15.1	13.1	84.1	65.2	157.3	142.4	167.5	160.5
Selimiye	49.5	38.7	11.3	9.8	60.7	48.5	302.3	162.6	160.0	87.4
Cumhuriyet 75	67.6	36.0	20.9	9.6	88.5	45.6	298.4	221.6	59.5	51.4
Balatilla	51.7	44.4	10.1	8.1	61.8	52.5	137.5	113.2	141.4	130.9
Meta 2002	50.7	36.1	10.5	8.8	61.2	44.8	175.2	166.0	134.6	128.8
Pehlivan	41.7	31.5	9.2	7.7	50.9	39.2	280.5	266.7	97.5	69.8
Kasif Bey 95	38.1	20.1	7.4	7.3	45.5	27.4	209.1	110.9	203.6	110.9
Genç-99	74.5	22.8	15.8	10.6	90.3	33.4	262.4	235.9	90.3	80.9
Sagittario	73.5	46.8	14.1	14.1	87.6	60.8	182.6	163.6	157.7	123.2
Tekirdag	39.8	36.0	15.1	8.7	54.9	44.7	223.9	117.9	166.1	125.9
Gonen 98	83.7	71.2	21.5	12.5	105.2	83.7	213.6	210.6	88.2	65.6
Bezostaja	89.9	79.1	18.0	14.8	107.9	93.9	316.5	203.3	104.8	82.1
Ceyhan-99	75.6	67.8	12.9	12.5	88.5	80.3	302.3	206.4	120.8	70.5
Claudio	62.6	48.0	12.3	11.8	74.9	59.9	172.9	156.1	74.3	72.7
Gelibolu	69.8	55.2	8.2	6.5	77.9	61.7	301.1	177.9	100.0	78.2
Canik 2003	73.1	55.3	12.2	17.4	85.3	72.7	176.9	138.1	169.6	148.8
Ziyabey 98	46.3	35.5	10.7	10.5	57.0	46.0	372.3	363.4	68.8	50.8
Karasu-90	61.9	45.8	18.6	13.9	80.5	59.7	229.3	201.5	110.7	102.3
Vittorio	48.5	39.1	7.3	8.6	55.9	47.7	361.8	149.4	126.5	122.0
İzmir 85	91.2	71.9	16.9	15.1	108.1	87.0	242.2	209.9	97.4	59.1
Pandas	45.1	25.8	10.3	10.3	55.4	36.0	225.7	221.2	229.8	209.7
Ozcan	55.2	30.4	11.3	8.9	66.5	64.1	304.3	132.3	177.5	104.0
LSD (0.05)	7.6	6.0	1.1	1.2	2.1	6.7	14.7	10.6	7.3	8.7

cultivar Ceyhan 99. Control cultivar Karasu-90 ranked 13th among 24 cultivars.

Tolerant cultivars have the ability to continue their root growth under anaerobic conditions to some extent. Dry root weight gain declined for all cultivars under anaerobic conditions. Gonen and Cumhuriyet 75 had the highest root growth under anaerobic conditions. Control cultivar Karasu-90 had the third highest root dry weight (18.63 mg $plant^{-1}$) gain followed by Bezostaja 1.

Total dry biomass gain under anaerobic conditions varied between 27.40 and 93.93 mg $plant^{-1}$. The highest and the lowest total dry biomasses were obtained from Bezostaja 1 and Kasif Bey 95, respectively. Bezostaja 1 and Gonen were superior to the check cultivar Ceyhan-99. Cultivars Canik 2003 and Osmaniyem produced significantly higher total dry biomass gains than the control cultivar, Karasu-90, but lower gains than control cultivar Ceyhan-99. When aerobic conditions were considered İzmir 85, Bezostaja 1, and Gonen had significantly higher total dry weight gain than the control cultivar Ceyhan-99.

The highest dry leaf weight was observed in Ziyabey 98 followed by Vittorio, Bezostaja 1, and Ozcan under aerated conditions and for Ziyabey 98 followed by Adana-99, Pehlivan, and Genç-99 under anaerobic conditions. Among the tested cultivars, standard cultivars Ceyhan-99 and Karasu-90 had moderately lower dry leaf weight gains. Specific leaf dry weight varied between 59.53 and 229.77 g m^{-2} under aerobic conditions; the highest and the lowest specific leaf dry weight was obtained from Pandas and Cumhuriyet 75, respectively. Under anaerobic conditions, however, cultivar Pandas had the highest specific leaf dry weight followed by Osmaniyem and Canik 2003. Standard cultivars Karasu-90 and Ceyhan-99 had lower specific leaf dry weight among the 24 wheat cultivars tested. The reduction in specific leaf dry weight was probably mediated by failure of the leaf tissue to expand fully.

Observations showed that oxygen deficiency reduced the specific leaf dry weight of cultivars. Under anaerobic conditions cultivar Pandas had the highest specific leaf dry weight with 209.70 g m^{-2}, and cultivar Ziyabey 98 had the lowest specific leaf dry weight with 50.77 g m^{-2}. Specific leaf dry weight of cultivars grown under aerobic conditions were significantly greater than those of cultivars under anaerobic conditions. The lowest and the highest specific leaf dry weight of cultivars grown under aerobic conditions varied between 59.53 and 229.77 g m^{-2}.

As shown in Table 3, a greater chlorophyll *a* value was obtained from cultivar Genç-99 followed by Cumhuriyet 75, Ziyabey 98, and Claudio under aerobic conditions. However, under anaerobic conditions the tested wheat cultivars showed different chlorophyll *a* contents. Cultivar Sagittario had the highest with 1.67 mg g^{-1}, while cultivar Pehlivan had the lowest value with 1.15 mg m^{-2}. The chlorophyll *a* content of six cultivars, Sagittario, Pandas, Vittorio, and Gonen was significantly greater than that of control cultivar Ceyhan-99 under anaerobic conditions. Control cultivar Karasu-90 had much lower chlorophyll *a* content than the 22 tested wheat cultivars under anaerobic conditions. When chlorophyll *b* content was under consideration, Ozcan, Sakin, and Balattila had the highest values under aerobic conditions, while Gonen, Claudio, and Ceyhan-99 had the highest values under anaerobic conditions.

Chlorophll *a* + *b* content of cultivars tested under anaerobic conditions varied between 1.28 and 1.91 mg g^{-1}. The highest and the lowest values were obtained from cultivars Gonen and Karasu-90, respectively. Control cultivar Ceyhan-99 was among the highest in chlorophyll *a* + *b* content-containing groups, while the other control cultivar, Karasu-99, was among the lowest chlorophyll *a* + *b* content-containing groups. Under aerobic conditions, however, Ceyhan-99 was among the lowest chlorophyll *a* + *b* content-containing groups, with 2.22 mg g^{-1}.

Observations showed that carotenoid content varied between 0.64 and 3.54 mg g^{-1} among the tested cultivars under aerobic conditions. Under anaerobic conditions, the highest and the lowest carotenoid content was obtained from Gelibolu and Kasif Bey 95, respectively. The control cultivars Ceyhan-99 and Karasu-90 had much lower carotenoid content than most of the cultivars. When anaerobic conditions were considered, cultivar Vittorio had the highest carotenoid content with 3.79 mg g^{-1} followed by cultivars Ozcan and Meta 2002. The lowest carotenoid content was obtained from Claudio with 0.85 mg g^{-1} followed by cultivar Bezostoja 1 with 0.89 mg g^{-1}. Control cultivars Ceyhan-99 and Karasu-90 had moderate carotenoid content under anaerobic conditions; however, control cultivar Ceyhan-99 had very low carotenoid content. Under anaerobic conditions only Adana-99, Balattila, Meta 2002, Kasif Bey 95, and Karasu-90 had carotenoid content higher than the content produced under aerobic conditions, and the rest of the cultivars had lower carotenoid content when grown under anaerobic conditions.

Significant variation ($P < 0.05$) among cultivars was observed for photosynthesis rates under aerobic and anaerobic conditions (Table 3). The rate of photosynthesis under aerobic conditions varied between 5.96 and 14.48 µmol CO_2 m^{-2} s^{-1}. Control cultivars Karasu-90 and Ceyhan-99 had the lowest values with 7.66 and 6.40 µmol CO_2 m^{-2} s^{-1} under aerobic conditions, respectively. When anaerobic conditions were under consideration cultivar Selimiye had the highest photosynthesis rate with 12.30 µmol CO_2 m^{-2} s^{-1}, while cultivar İzmir 85 had the lowest photosynthesis rate with 0.15 µmol CO_2 m^{-2} s^{-1}.

Table 3. Mean comparison of chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*), chlorophyll *a* + *b* (Cl *a* + *b*), carotenoid content (CarC), photosynthesis rate (PnR), and transpiration rate (TrR) under aerobic (+O) and anaerobic (-O) conditions.

	Cl *a* (mg g^{-1})		Cl *b* (mg g^{-1})		Cl *a* + *b* (mg g^{-1})		CarC (mg g^{-1})		PnR (μmol CO_2 m^{-2} s^{-1})		TrR (mmol H_2O m^{-2} s^{-1})	
Cultivar	+O	–O	+O	–O	+O	–O	+O	–O	+O	–O	+O	–O
Adana-99	1.44	1.30	0.37	0.37	1.81	1.67	1.28	0.78	7.43	6.41	3.60	1.84
Sakin	1.93	1.48	0.78	0.39	2.71	1.87	1.45	1.30	11.77	8.30	2.31	2.06
Osmaniyem	1.64	1.43	0.58	0.37	2.22	1.80	2.92	2.12	11.48	9.74	2.65	1.93
Selimiye	1.98	1.44	0.65	0.35	2.63	1.79	3.17	1.79	14.48	12.30	2.66	1.37
Cumhuriyet 75	2.17	1.35	0.60	0.36	2.77	1.71	1.72	0.98	8.52	7.64	2.10	1.83
Balatilla	2.07	1.46	0.69	0.37	2.76	1.83	2.35	1.86	10.44	6.08	1.93	1.15
Meta 2002	1.42	1.20	0.40	0.34	1.82	1.54	2.71	1.84	6.30	6.05	1.87	0.64
Pehlivan	2.03	1.15	0.54	0.41	2.57	1.56	1.14	1.08	6.09	4.56	3.25	1.96
Kasif Bey 95	1.78	1.42	0.53	0.44	2.31	1.86	1.37	0.64	5.96	4.08	2.89	1.18
Genç-99	2.33	1.48	0.60	0.45	2.93	1.93	1.76	1.48	10.84	8.32	2.08	1.51
Sagittario	1.80	1.67	0.62	0.43	2.42	2.10	2.84	2.38	7.97	5.45	1.70	1.12
Tekirdag	1.57	1.32	0.55	0.33	2.12	1.65	2.70	1.43	8.90	8.69	2.26	1.49
Gonen 98	2.02	1.61	0.56	0.52	2.58	2.13	1.76	1.48	13.77	6.31	3.42	1.14
Bezostaja	1.65	1.40	0.50	0.47	2.15	1.87	1.59	0.89	14.29	6.41	4.52	1.48
Ceyhan-99	1.71	1.55	0.51	0.48	2.22	2.03	0.85	1.03	6.40	5.52	4.25	1.19
Claudio	2.12	1.51	0.57	0.50	2.69	2.01	1.45	0.85	11.45	5.65	3.33	2.43
Gelibolu	1.96	1.46	0.64	0.36	2.60	1.82	3.54	1.28	8.23	5.42	1.39	0.78
Canik 2003	1.64	1.59	0.62	0.44	2.21	2.08	2.10	1.98	8.64	6.71	1.56	1.40
Ziyabey 98	2.16	1.28	0.54	0.39	2.70	1.67	0.66	1.16	6.24	5.62	2.48	1.43
Karasu-90	1.90	1.17	0.50	0.30	2.40	1.47	0.79	1.30	7.66	6.65	2.72	1.79
Vittorio	1.88	1.64	0.61	0.46	2.49	2.10	3.79	3.43	12.65	6.23	2.10	2.02
İzmir 85	2.01	1.22	0.52	0.34	2.53	1.56	1.67	1.23	8.65	0.15	2.71	2.57
Pandas	1.72	1.47	0.50	0.44	2.22	1.91	2.56	2.16	8.15	5.82	2.14	1.60
Ozcan	1.80	1.60	0.70	0.45	2.50	2.05	3.22	2.82	9.87	9.04	1.77	1.01
LSD (0.05)	0.24	0.45	0.10	0.05	0.28	0.11	0.22	0.18	1.00	1.24	0.40	0.20

The transpiration rate of cultivars under each condition differed. Anaerobic conditions reduced transpiration rates of the tested cultivars in comparison to aerobic conditions. However, the percent reduction varied by cultivar. Under aerobic conditions the highest transpiration rate value was observed for Bezostaja 1 and the lowest value for Gelibolu, while under anaerobic conditions the highest transpiration rate was assigned to İzmir 85, and the lowest transpiration rate was observed in Meta 2002.

To evaluate the 24 bread-wheat cultivars for waterlogging tolerance, 9 tolerance indices (tolerance index for total dry weight gain, tolerance leaf dry weight, specific leaf dry weight, chlorophyll *a* content, chlorophyll *b* content, chlorophyll *a* + *b* content, carotenoid content, photosynthesis rate, and transpiration rate) were used (Table 4). As shown in Table 5, a greater tolerance index value for specific dry leaf weight was related to Claudio, indicating that this cultivar had a lower specific leaf

Table 4. Mean comparison of tolerance indices of total dry weight, dry leaf weight, specific leaf dry weight, chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, carotenoid content, photosynthesis rate, and transpiration rate.

Cultivar	TITDW	TIDLW	TISDLW	TICl *a*	TICl *b*	TICl *a* + *b*	TICar	TITR	TIPR
Adana-99	56.9	92.1	90.5	91.7	99.0	93.9	61.0	51.3	86.3
Sakin	93.2	92.0	81.7	76.5	50.9	65.7	89.7	89.6	70.8
Osmaniyem	82.2	90.6	96.1	88.5	64.0	78.7	72.4	73.2	84.7
Selimiye	84.4	53.8	98.5	72.9	54.0	61.9	56.4	51.9	84.9
Cumhuriyet 75	74.3	74.3	85.9	62.5	60.8	62.9	57.1	87.0	89.8
Balatilla	91.3	82.3	89.1	70.4	54.1	65.9	79.4	60.0	58.6
Meta 2002	73.6	94.7	90.6	95.1	85.7	97.8	68.1	34.3	95.8
Pehlivan	82.1	95.1	68.1	57.3	76.8	59.4	96.4	60.4	75.3
Kasif Bey 95	61.4	53.1	94.2	80.5	82.5	80.5	47.3	42.5	68.6
Genç-99	37.2	89.9	98.7	63.3	76.9	68.0	84.0	72.6	76.8
Sagittario	71.2	89.8	69.9	92.5	70.2	83.8	83.7	66.0	68.7
Tekirdag	95.0	52.6	70.0	85.1	62.7	76.1	53.2	67.9	97.7
Gonen 98	96.4	98.6	75.6	80.4	94.4	84.8	72.4	33.4	46.1
Bezostaja	92.8	64.2	50.3	85.8	94.7	88.5	56.4	32.8	45.0
Ceyhan-99	90.8	68.3	85.7	90.6	93.7	91.6	82.5	28.1	86.4
Claudio	81.0	90.6	88.5	71.8	88.8	77.5	58.4	73.2	49.5
Gelibolu	83.1	59.1	75.8	74.8	56.7	67.5	36.3	56.2	66.5
Canik 2003	85.2	78.1	89.1	94.7	71.9	90.9	94.3	89.6	77.5
Ziyabey 98	80.9	97.6	74.3	59.5	73.2	65.2	50.7	57.5	90.1
Karasu-90	85.7	87.9	81.2	61.6	60.9	61.2	60.8	66.2	86.9
Vittorio	85.3	41.5	39.9	87.5	76.5	83.2	90.6	96.5	49.3
İzmir 85	83.6	86.7	70.2	60.8	66.8	62.6	74.1	95.2	–4.3
Pandas	65.3	98.0	73.3	94.2	85.1	73.2	85.1	74.8	71.5
Ozcan	59.1	43.5	74.2	89.0	65.6	79.5	87.7	58.2	91.8
LSD (0.05)	11.7	4.7	7.2	14.1	16.0	17.8	10.8	11.5	14.5

TITDWG = 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, TIPR = tolerance index for photosynthesis rate, TITR = tolerance index for transpiration rate.

weight reduction under anaerobic conditions, and a lower tolerance index for specific leaf weight was found in Kasif Bey 95, followed by Selimiye, Ceyhan-99, Ozcan, and İzmir 85. The specific leaf weight lost was greater than 39% under anaerobic conditions. Seven of the tested cultivars, Claudio, Vittorio, Osmaniyem, Meta 2002, Balattila, Karasu-90, and Pandas, had a higher tolerance index for specific leaf weight, and their weight lost was lower than 8.7% under anaerobic conditions. The control cultivar Karasu-90 was among the most tolerant cultivars in terms of specific leaf weight tolerance index.

When tolerance index of specific weight was under consideration, the tolerance index of the tested cultivars varied between 39.91 and 98.73. The highest tolerance index was recorded for Genç-99 followed by Selimiye, Osmaniyem, and Kasif Bey 95. The control cultivar

Table 5. Correlation coefficient between investigated plant parameters.

	SDWG	RDWG	TDWG	DLW	SLDW	Cl *a*	Cl *b*	Cl *a* + *b*	Car
RDWG	0.62 **								
TDWG	0.95 **	0.67 **							
DLW	0.08	0.15	0.03						
SLW	−0.31	0	−0.28	−0.42					
Cl *a*	0.11	0.11	0.19	−0.37	0.2				
Cl *b*	0.18	0.15	0.23	0.02	−0.06	0.68 **			
Cl *a* + *b*	0.14	0.16	0.22	−0.28	0.15	0.97 **	0.82 **		
Car	−0.17	−0.15	−0.05	−0.39	0.54 **	0.5 *	0.09	0.41 *	
PR	−0.29	−0.14	−0.22	−0.2	0.19	0.22	−0.1	0.14	0.27
TR	0.07	0.29	0.04	0.31	−0.16	−0.24	−0.05	−0.2	−0.17
TITDWG	0.63 **	0.17	0.51 *	−0.22	−0.03	−0.01	−0.08	−0.03	−0.04
TIDLW	0.09	0.34	0.02	0.52	0.07	−0.27	−0.02	−0.21	−0.26
TISLW	−0.31	−0.1	−0.32	−0.03	−0.03	−0.16	−0.24	−0.2	−0.36
TICl *a*	0.01	−0.01	0.05	−0.23	0.47 *	0.38	0.24	0.36	−0.22
TICl *b*	0.23	0.35	0.23	0.3	−0.07	0.12	0.62 **	0.29	0.37
TICl *a* + *b*	0.23	0.3	0.27	−0.15	0.18	0.33	0.41 *	0.39	−0.2
TICar	−0.13	0.12	−0.03	0.18	0.26	0.25	0.32	0.29	0.13
TIPR	−0.51	−0.23	−0.44 *	−0.09	0.27	−0.05	−0.23	−0.1	0.51 *
TITR	−0.27	−0.12	−0.28	0.06	0.07	−0.06	−0.27	−0.14	0.08

SDWG = shoot dry weight gain, RDWG = root dry weight gain, TDWG = total dry weight gain, DLW = dry leaf weight, SLDW = specific dry leaf weight, Cl *a* = chlorophyll *a*, Cl *b* = chlorophyll *b*, Cl *a* + *b* = chlorophyll *a* + *b*, Car = carotenoid content, TR = transpiration rate, TITDWG = 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, TIPR = tolerance index for photosynthesis rate, TITR = tolerance index for transpiration rate.

Table 5. Continued.

	TS	TITDWG	TIDLW	TISDLW	TICl *a*	TICl *b*	TICl *a* + *b*	TICar	TIPR
TITDWG	0.10								
TIDLW	0.24	−0.07							
TISLW	−0.15	−0.36	0.30						
TICl *a*	−0.41 **	−0.06	−0.15	−0.15					
TICl *b*	−0.04	−0.17	0.21	−0.13	0.36				
TICl *a* + *b*	−0.36	−0.05	−0.04	−0.11	0.75 **	0.74 **			
TICar	0.18	−0.09	0.28	−0.23	0.04	0.05	0.01		
TIPR	−0.43 *	−0.22	−0.11	0.41	0.24	−0.07	0.18	−0.05	
TITR	0.68 **	−0.09	0.04	−0.19	0.31	0.54 **	0.59 **	0.24	0.29

TR = transpiration rate, TITDWG = tolerance index for dry weight gain, TIDLW = tolerance index for dry leaf weight, TISLW = tolerance index for specific dry 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, TIPR = tolerance index for photosynthesis rate, TITR = tolerance index for transpiration rate.

Ceyhan-99 had a specific leaf area tolerance index of 85.65, and Karasu-90 had a specific leaf area tolerance index of 81.25. Both control cultivars had moderate specific leaf area tolerance indices. The specific leaf area reductions of Cehyan-99 and Karasu-90 were 14.35% and 18.76%, respectively. The cultivar Vittorio had the highest specific leaf area reduction with 60%.

The tolerance index for total dry weight gain had the highest correlation with shoot dry weight gain ($r = 0.63^{**}$), tolerance index for chlorophyll *a* content had the highest correlation with specific dry leaf weight ($r = 0.47^{*}$), tolerance index for photosynthesis rate significantly and negatively correlated with total dry weight gain ($r = -0.44^{*}$), and tolerance index for photosynthesis speed significantly and positively correlated with carotenoid content ($r = 0.51^{*}$) (Table 5).

There was a positive and a significant correlation between shoot dry weight gain and root dry weight gain and between shoot dry weight gain and total dry weight gain. Specific dry leaf weight gain was positively and significantly correlated with carotenoid content. Chlorophyll *a* was positively and significantly correlated with chlorophyll *b*, chlorophyll $a + b$, and carotenoid content (Table 5).

4. Discussion

Waterlogging tolerance can be defined as biomass production or seed yield under conditions ranging from waterlogged to nonwaterlogged (Setter and Waters, 2003). Hence, dry shoot and root weight gains and total biomass weight gain, leaf dry weight, specific leaf weight, and specific leaf area were examined as growth parameters under waterlogged conditions to determine whether some of them could be used for selection criteria while determining waterlogging-tolerant cultivars at the seedling stage. In addition to growth parameters, chlorophyll *a*, chlorophyll *b*, total chlorophyll content, carotenoid content, photosynthesis rate, and transpiration rate were investigated. Considering all investigated plant parameters, great variation occurred among tested wheat cultivars. Cultivar Bezostoja 1 had significantly higher shoot dry weight gain and total dry biomass weight gain under anaerobic conditions. When growth parameters were considered this cultivar seems to be one of the cultivars with the best tolerance, since its tolerance index for total dry biomass was greater than 92 (Table 4) above the standard cultivars Ceyhan-99 and Karasu-90. The mean comparison of investigated parameters under anaerobic conditions showed that Bezostoja 1 had the highest shoot dry weight and the highest total dry biomass weight values. This cultivar is also among the cultivars having the highest total dry biomass tolerance index. Yaduvanshi et al. (2010) stated that this differential response of cultivars might be due to the operation of different tolerance mechanisms for waterlogging.

Trought and Drew (1980) reported that dry matter accumulation of wheat seedlings grown under 8 days of oxygen-deficient conditions increased, due to starch accumulation, and then dramatically decreased compared with the control. Therefore, shoot dry weight in the early stages of waterlogging could not be used as a reliable selection criterion while assessing waterlogging resistant/tolerant genotypes. In a pod study, Basribey 95 and Sagittario wheat cultivars were reported as waterlogging-tolerant cultivars (Yavas et al., 2012). In a field study, waterlogging at seedling stage reduced tiller number; plant height; delayed ear emergence; and produced 8%, 17%, 27%, and 39% yield reduction when exposed to waterlogging for 1, 2, 4, and 6 days, respectively (Sharma and Swarup, 1989).

Oxygen-deficient conditions have been reported to severely reduce leaf photosynthesis rate. The reduction in photosynthesis has been attributed to stomatal closure (Yordanova et al., 2005), decreased leaf chlorophyll content (Bradford, 1983), disruption of the carbohydrate translocation (Chen et al., 2005), and increased ethylene production (Chen et al., 2002; Ahmed et al., 2006). The current study indicated that photosynthesis rate is one of the most sensitive traits in response to oxygen deficiency, which is severely decreased under anaerobic conditions. This is in good agreement with the findings of Ahmed et al. (2002). Decreases in transpiration rate and carotenoid content reductions were observed, together with the synchronized decline in the photosynthesis rate. Thus, in the present study reduction in photosynthesis rate under anaerobic conditions at the seedling stage might be ascribed to decreases in chlorophyll and carotenoid contents.

Heritability of the investigated traits is very important as selection criteria. The probability of developing a wheat cultivar tolerant to waterlogging can be increased by using related traits with a high heritability rate. Collaku and Harrison (2005) found a high heritability rate for grain weight (0.49), followed by chlorophyll content (0.37), and tiller number (0.31). Further studies are needed to discern heritability of plant parameters investigated at the seeding stage.

In previous studies germination rate and plant survival have been used as characteristics of waterlogging tolerance in barley (Li et al., 2008) and rice (Nandi et al., 1997), as they visibly respond to waterlogging. In the current study, plant parameters measured under anaerobic conditions relative to aerobic conditions were the other main parameters for investigating waterlogging tolerance. Therefore, we used total dry weight gain, leaf dry weight, specific leaf dry weight, chlorophyll *a* content, chlorophyll *b* content, chlorophyll *a* + *b* content, carotenoid content, transpiration rate, and photosynthesis rate as characteristics of waterlogging tolerance in wheat. Although there were clear differences in tolerance indices among cultivars for all investigated indices, it is necessary to conduct a waterlogged yield trial under controlled conditions in order to confirm and determine which tolerance index is most appropriate while assessing tolerance levels of cultivars at the seedling stage.

Nine tolerance indices (tolerance index for total dry weight gain, leaf dry weight, specific leaf dry weight, chlorophyll *a* content, chlorophyll *b* content, chlorophyll *a* + *b* content, carotenoid content, transpiration rate, and photosynthesis rate) were used.

In conclusion, anaerobic conditions dramatically reduced shoot, root, and total dry weight gains and dry leaf weight, specific dry leaf weight, and chlorophyll and carotenoid contents of wheat seedlings. There was great cultivar variation for all of the investigated plant parameters. Bezostoja 1, İzmir 85, and Gonen 98 provided greater shoot and total dry weight gains under anaerobic conditions. Cultivars Selimiye, Osmaniyem, and Ozcan had greater photosynthesis speeds under anaerobic conditions. Tekirdag, Meta 2002, and Ozcan had greater tolerance indices for photosynthesis speed, while Gonen, Tekirdag, and Sakin had higher tolerance indices for total dry weight gain. Meta 2002, Adana 99, and Ceyhan 99 had greater tolerance indices for total chlorophyll content, while cultivars Pehlivan, Canik 2003, and Vittorio had higher tolerance indices for carotenoid content. Further studies are needed under field conditions to confirm which of the tolerance indices is highly correlated with seed yield in order to use this parameter as a selection criterion.

Acknowledgment

This research was supported by the Scientific and Technological Council of Turkey (TÜBİTAK) (project no.: 110 O 486).

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