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EVALUATION OF QUANTITATIVE TRAIT LOCI FOR DROUGHT TOLERANCE, LOCATED ON THE BARLEY (*HORDEUM VULGARE* L.) CHROMOSOMES 1, 2, AND 6, IN THE GENOMIC BACKGROUND OF THREE CULTIVARS

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Abstract

In this study, some barley genotypes, to which three quantitative character loci (QTLs) were transferred and found as drought tolerant, were used as the research materials and evaluated for their performance in arid conditions in the province of Konya located in the Central Anatolia Region in Turkey. This study was carried out in two separate trials, including in field and controlled conditions. The experiments were laid out in a randomized complete block design (RCBD) with three replications. The parameters, including the leaf relative water content, plant height, spike length, number of spikes per square meter, number of grains per spike, thousand-grain weight, hectoliter weight, harvest index, and grain yield, were investigated. The results of the principal component analysis (PCA) showed that agronomic and physiological measurements explained 84.45% of the first three components for the experiment conducted under field conditions, while 72.89% of the first three components were explained for the controlled experiment. In the controlled trial, where the degree of water stress was more pronounced, the transfer of QTL1 and QTL6 regions into the Bolayır variety and the QTL2 region was transferred to the Baronesse variety resulting into higher grain yield. The Baronesse line (R1), developed by the transfer of the QTL1 region to the Baronesse variety with 819.9 kg da^{-1} in the field trial, and the Bolayır line (B6), obtained from the transfer of the QTL6 region to Bolayır, with 588.0 kg da^{-1} in the controlled trial gave the highest grain yields. The lowest grain yields, however, were recorded in the Tadmor variety under the field conditions (418.5 kg da^{-1}) and in the Aydanhanım (A2) line (363.1 kg da^{-1}), in which the QTL2 region was transferred, in the controlled conditions. The developed lines, including Aydanhanım QTL1, Aydanhanım QTL2, Aydanhanım QTL6, Bolayır QTL1, Bolayır QTL2, Bolayır QTL6, Baronesse QTL1, Baronesse QTL2, and Baronesse QTL6 may harbor new alleles associated with drought tolerance traits for breeding drought-tolerant barley cultivars.

Key words: Crops, Genetic structure, Quantitative trait loci**,** Relative water content.

Abbreviations used: U.S (united states), ABA (abscisic acid), QTL (quantitative trait loci), DAP (diammonium phosphate), A (Aydanhanım), A1 (Aydanhanım QTL1), A2 (Aydanhanım QTL2), A6 (Aydanhanım QTL6), B (Bolayır), B1 (Bolayır QTL1), B2 (Bolayır QTL2), B6 (Bolayır QTL6), R (Baronesse), R1 (Baronesse QTL1), R2 (Baronesse QTL2), and R6 (Baronesse QTL6).

Introduction

Barley (*Hordeum vulgare*) is widely cultivated in arid and semi-arid regions worldwide, including Turkey. Drought, occurs in these regions due to the combined effects of irregular and inadequate rainfall and high temperatures, negatively affects the growth and development of barley and thus reduces its yield and quality (Turan, 2018; Marwat *et al.*, 2011). The impact of drought caused by the irregular distribution of rainfall throughout the year on crops grown in arid regions, especially barley, is expected to increase in the future due to global climate change (Lakew *et al.*, 2011). Therefore, the most effective way to satisfactorily produce crops under the dryland agriculture system in rainfed areas is to use genetic mechanisms conferring tolerance.

Traits that are relevant with tolerance to plants under drought conditions could be transfer to some other crops through breeding methods. However, breeding for drought tolerance is a difficult and slow process because the mechanisms of drought tolerance are controlled by many small-effect genes and QTLs (Fleury *et al.*, 2010), as well as by differences in plant phenology (Mir *et al.*, 2012; Arshad *et al.*, 2022), which are affected by high temperature and other environmental factors (Fleury *et al.*, 2010), and therefore, they are difficult to measure or observe (Lakew *et al.*, 2011; Saygılı, 2019).

Varieties that maintain grain yield or have low yield reduction under dry conditions are considered tolerant (Turner, 1979). Barley can employ the mechanism of drought escape, which involves rapid growth and development (earliness). However, irregular rainfall in regions under dryland farming systems and drought stress during fertilization and grain-filling periods, when barley is most sensitive to adverse environmental factors, negatively affect barley production. The development and production of varieties which are less affected by drought stress in these regions will reduce the drought risks posing threats to barley production.

With increasing drought severity, the water content and the rate of photosynthesis in the plant was decreased, thus shortening the grain-filling period (Samarah *et al.*, 2009). Drought occurring after flowering limited the leaf area expansion and caused a decrease in grain weight (Fischer & Wood, 1979). Under drought, especially during flowering and milking periods, grains remained small and thousand-grain weight was decreased (Beigzadeh *et al.*, 2013).

Cooper *et al.*, (1994) reported that drought occurring in early developmental stages shortened flowering time and reduced plant height, leaf area, and the number of fertile siblings in wheat, while just before spike formation or close to flowering, it affected grain yield more negatively than in the other developmental stages.

Although there are many studies on the identification of gene regions that confer drought tolerance in barley (Teulat *et al*., 1998; Baum *et al*., 2003; Tondelli *et al*., 2006; Chen *et al*., 2010; Zhang *et al.*, 2022), studies on the use of QTLs in plant breeding are limited.

In some studies, on drought tolerance in barley, the identified QTL1 region was found not to be associated with grain yield (Teulat *et al*., 2001; von Korff *et al*., 2008). Lakew *et al*., (2013) stated that the QTL2 region increased grain yield due to the prolongation of photosynthesis under drought conditions, and therefore, the corresponding QTL region may be useful, especially for drought tolerance. However, there are studies suggesting that the effect of the QTL2 region on grain yield varies according to the environment (Pillen *et al*., 2004; von Korff *et al*., 2008). Talame *et al*., (2004) reported that the QTL2 region affected grain yield in all environments.

A study (Saygılı, 2019) using 6 barley lines carrying the QTL1, QTL2, and QTL6 regions reported that the effects of QTL regions for many traits examined were more pronounced in the controlled experiment where terminal drought stress took place. While the effects of these QTL regions were decreased in trials did not experienced less drought stress. Moreover, the effect of each of these three QTL regions evaluated also varied depending on the genetic structure and also the ecological conditions.

Present research realized under dry conditions to determine the performance of barley genotypes, to which three quantitative trait loci, which are located on chromosomes 1, 2, and 6 and associated with traits conferring drought tolerance, were transferred.

Material and Methods

This study was carried out in Bahri Dağdaş International Agricultural Research Institute test fields in Konya, Turkey, during the 2019–2020 vegetation period.

Table 1 shows no significant difference between the trial year and the long-term evaluation in terms of average temperatures. However, during the nine-month period of the experiment around 1.4°C higher than those for the long-term monitoring. In the trial year, the total precipitation rate for nine months was 305.2 kg, approximately 4mm higher than that recorded for the long-term trial. The average relative humidity values were higher during the vegetation period than the long-term average annual values in all months, except for October.

The field trial study was carried out using neutral and slightly saline and slightly alkaline and unsalted soils.

In the present study from project 1130934 supported by TUBITAK, materials used were three QTLs, associated with drought tolerance. QTLs were located in the Dhn4-BCD276 interval on chromosome 6, centered around the Acl3 marker on chromosome 1 (7H) and around the EbMac0684 marker on chromosome 2. They transferred from the Syrian Tadmor variety to three barley varieties. The transferred varieties were consisted from one malting variety (Aydanhanım) and two forage varieties (Bolayır and Baronesse). The Baronesse is a foreign variety, while the other two are local. A total of 15 genotypes, including 9 lines belonging to the Aydanhanım and Bolayır cultivars developed in Turkey, four parents (Baronesse, Aydanhanım, Bolayır, and Tadmor), as well as the Tokak 157/37 and Arta cultivars, were used.

This study was carried out in two separate trials, under the field and controlled (rain shelter) conditions. Trials were arranged in a randomized block design (RCBD) with three replications. In the field trial, each plot consisted of six rows of 4 m and plants were sown with a seeder on 13 November 2019, whereas in the controlled trial, there were two rows of 1.5 m, and plants were sown by hand on 8 November 2019. In both trials, planting was done with a row spacing of 20 cm and 500 plants per m^2.

In the controlled experiment, in the study area rainout shelters were used during the experimental period (from the rooting phase to harvest) to prevent the rainwater. Weeds were controlled using herbicide products containing 2,4-D as the active ingredient (Namlı *et al*., 2017). Fertilizers were applied to plots with the calculated utilization rates of approximately 7.0 kg of phosphorus as P_2O_5 and 8 kg of pure nitrogen (N) per decare. In this context, the diammonium phosphate (DAP) fertilizer was given at the rate of 15 kg per decare before planting. Phosphorus fertilizer was applied at the time of planting, and the remaining part of the nitrogen fertilizer was applied before the rooting phase. Harvesting was done by hand in the controlled trial, but in the field trial, it was carried out by a plot harvester after the moisture content of the grain fell below 12%.

The data obtained were subjected to statistical analysis by the computer package program "MSTAT-C". Accordingly, the comparison between mean values, which were significant according to the F test, was carried out using Duncan's multiple range test.

Results

According to the statistical analysis, significant differences were found between genotypes in terms of all traits examined in both trials. In the field trial, the performance of genotypes for many traits was observed to be better, and the difference between genotypes was more pronounced. On the other hand, in the controlled trial, genotypes could not perform sufficiently well, and the differences between genotypes were decreased. The average values of the parameters examined in the study and the differences between the treatment groups were determined using Duncan's test (Tables 2 & 3), and the biplot graphs generated from PCA for the values of these parameters (Figs. 1 and 2).

Relative water content: In terms of the relative water content, the difference between genotypes was found to be significant at the 1% significance level in both trials. Although the Tadmor cultivar had the highest relative water content (65.07%) in the field trial but the Bolayır genotype (B1), to which the QTL1 region was transferred, had the highest relative water content (62.40%) in the controlled trial. Considering the average relative water content in both trials, the highest value was observed in the Bolayır genotype (B1). However, the Aydanhanım genotype (A1) carrying the QTL1 region showed the lowest relative water content. The Baronesse genotype (R2) with the transferred QTL2 region was found to be one of the evaluated genotypes with the lowest relative water content in both trials. The B1 genotype in the controlled trial was in the first group, along with the Tadmor variety in the field trial based on Duncan's test, as both showed the highest relative water content. In the controlled trial, in which the degree of drought stress was higher than in the field trial, genotypes had an average of around 8.4% lower relative water content (Table 2).

Table 1. Meteorological data for the 2019–2020 vegetation period and long-term monitoring (1929–2019).

	2019–2020 Vegetation period					Long-term evaluation (1929–2019)				
Months	**Mean**	**Max.**	**Min.**	**Precip. [mm]**	**Rel. hum. (%)**	**Mean**	**Max.**	**Min.**	**Precip. [mm]**	**Rel. Hum. (%)**
Oct.	16,3	28,7	3,6	13	56	12,6	31,6	-7,6	29,9	58
Nov.	7,9	22,4	-4,1	45,8	81	6,5	25,2	-20,0	32,2	69
Dec.	2,9	13,6	-3,4	112,4	98	1,6	20,0	-22,4	42,8	77
Jan.	0,4	8,9	-9,2	36	94	-0,1	17,6	-25,8	37,9	76
Feb.	4,4	18,0	-6,3	29	81	1,4	21,2	-25,0	28,5	70
March	6,5	21,4	-7,2	6,4	76	5,5	28,9	-15,8	28,7	62
April	12,1	24,3	-0,7	3,4	63	11	31,5	-8,6	31,9	58
May	16,2	34,5	0,3	23,4	56	15,8	33,4	-1,2	43,3	55
June	20,3	34,4	5,8	35,8	51	20,1	37,2	3,2	25,7	47
Mean/Total	9,7	-	-	305,2	73	8.3	-	-	300,9	64

Source: Bahri Dağdaş International Agricultural Research Institute (BDIARI)

Table 2. Mean values and Duncan's results of the mean values of the characteristics examined in the present study.

Genotypes	**Relative water content**		**Plant height**		**Number of spikes per square meter**		**Number of grains per spike**	
	Field	**Controlled**	**Field**	**Controlled**	**Field**	**Controlled**	**Field**	**Controlled**
Tadmor	65.07 a**	45.83 de**	82.3 efg**	90.6 ab**	1467 a**	1374 a**	14.6 f**	16.7 c**
Arta	59.63 abcd	50.10 abcde	75.3 g	73.6 c	1210 ab	1073 bcd	15.6 f	18.8 b
Tokak 157/37	59.00 abcd	45.23 de	95.1 bc	94.4 a	898 bcdef	770 efg	20.9 de	21.1 a
Aydanhanım	63.73 abc	51.33 abcde	102.9 a	90.2 ab	650 f	680 fg	27.3 a	20.1 ab
Aydanhanım QTL 1	56.67 cd	43.70 e	103.4 a	89.6 ab	865 cdef	837 cdef	25.1 ab	20.1 ab
Aydanhanım QTL 2	59.70 abcd	56.57 abcd	106.4 a	91.3 ab	757 def	563 g	24.8 abc	20.5 ab
Aydanhanım QTL 6	57.80 bcd	58.43 abc	99.3 ab	78.8 abc	673 ef	805 defg	26.7 a	18.6 b
Bolayır	58.47 abcd	60.97 ab	88.8 cde	77.1 bc	900 bcdef	818 defg	20.5 de	20.0 ab
Bolayır QTL 1	64.73 ab	62.40 a	91.4 cd	81.4 abc	755 def	825 defg	20.9 de	20.1 ab
Bolayır QTL 2	61.10 abcd	57.87 abcd	85.9 def	75.7 bc	827 def	727 fg	22.4 bcd	20.0 ab
Bolayır QTL 6	62.23 abcd	46.03 cde	91.6 cd	79.5 abc	948 bcdef	1053 bcd	20.1 de	21.3 a
Baronesse	60.30 abcd	49.57 bcde	80.3 fg	79.1 abc	850 cdef	1037 bcde	21.5 cde	19.8 ab
Baronesse QTL 1	60.90 abcd	50.30 abcde	78.1 g	69.9 c	1095 bcd	1202 ab	21.8 cde	20.1 ab
Baronesse QTL 2	58.37 abcd	45.83 cde	77.0 g	72.7 c	1173 abc	940 bcdef	18.8 e	19.3 ab
Baronesse QTL 6	55.97 d	52.83 abcde	80.1 fg	78.2 bc	1003 bcde	1100 bc	21.0 de	19.8 ab

**: The 0.01 significance level (p<0.01)

Table 3. Average values and Duncan's results of the mean values of the characteristics examined in the present study.

Genotipler	**Thousand-grain weight**		**Hectoliter weight**		**Grain yield**		**Harvest index**	
	Field	**Controlled**	**Field**	**Controlled**	**Field**	**Controlled**	**Field**	**Controlled**
Tadmor	32.00 e**	32.00 def**	73.6 bcd**	75.4 a**	418.5 d**	527.0 abc**	32.8 d**	23.8 de**
Arta	43.00 a	34.00 cd	67.1 e	66.8 ghı	610.6 c	515.9 abcd	44.7 ab	23.2 e
Tokak 157/37	43.33 a	36.00 bc	72.1 cd	66.0 hı	757.5 ab	452.3 cde	45.5 ab	28.5 bcd
Aydanhanım	42.67 a	35.33 c	73.9 abc	70.1 cdef	749.6 ab	404.9 de	42.5 abc	23.9 de
Aydanhanım QTL 1	40.34 ab	36.00 bc	74.6 ab	72.7 bc	675.6 bc	408.5 de	36.8 cd	24.0 de
Aydanhanım QTL 2	41.00 a	39.00 ab	75.9 a	73.7 ab	752.9 ab	363.1 e	39.9 bcd	23.6 de
Aydanhanım QTL 6	40.33 ab	40.00 a	73.9 abc	70.9 cde	738.9 ab	468.3 bcde	41.5 abc	27.3 cde
Bolayır	35.33 cde	30.67 efgh	72.2 cd	65.9 ı	760.9 ab	453.0 cde	43.9 abc	32.7 ab
Bolayır QTL 1	37.00 bc	31.67 defg	71.9 cd	71.8 bcd	778.4 ab	577.4 ab	47.4 ab	33.7 a
Bolayır QTL 2	36.33 cd	33.00 cde	72.9 bcd	72.0 bcd	774.1 ab	508.9 abcd	47.3 ab	27.2 cde
Bolayır QTL 6	37.00 c	29.33 fgh	73.2 bcd	71.3 bcd	817.2 ab	588.0 a	47.0 ab	31.5 abc
Baronesse	34.33 cde	28.33 h	73.1 bcd	68.5 efgh	781.9 ab	431.0 cde	43.7 abc	25.2 de
Baronesse QTL 1	34.33 cde	28.33 gh	72.5 bcd	69.6 def	819.9 a	439.4 cde	45.7 ab	27.7 cde
Baronesse QTL 2	34.33 cde	28.67 gh	71.6 d	69.3 defg	796.2 ab	534.9 abc	47.1 ab	32.5 ab
Baronesse QTL 6	33.33 de	27.67 h	72.8 bcd	67.6 fghı	782.4 ab	458.9 cde	48.5 a	27.0 cde
Mean	**37.64**	**32.67**	**72.7**	**70.1**	**734.3**	**475.4**	**43.6**	**27.5**
LSD	3.18	3.03	1.96	2.43	122.6	98.64	6.95	4.32

**: the 0.01 significance level (p<0.01)

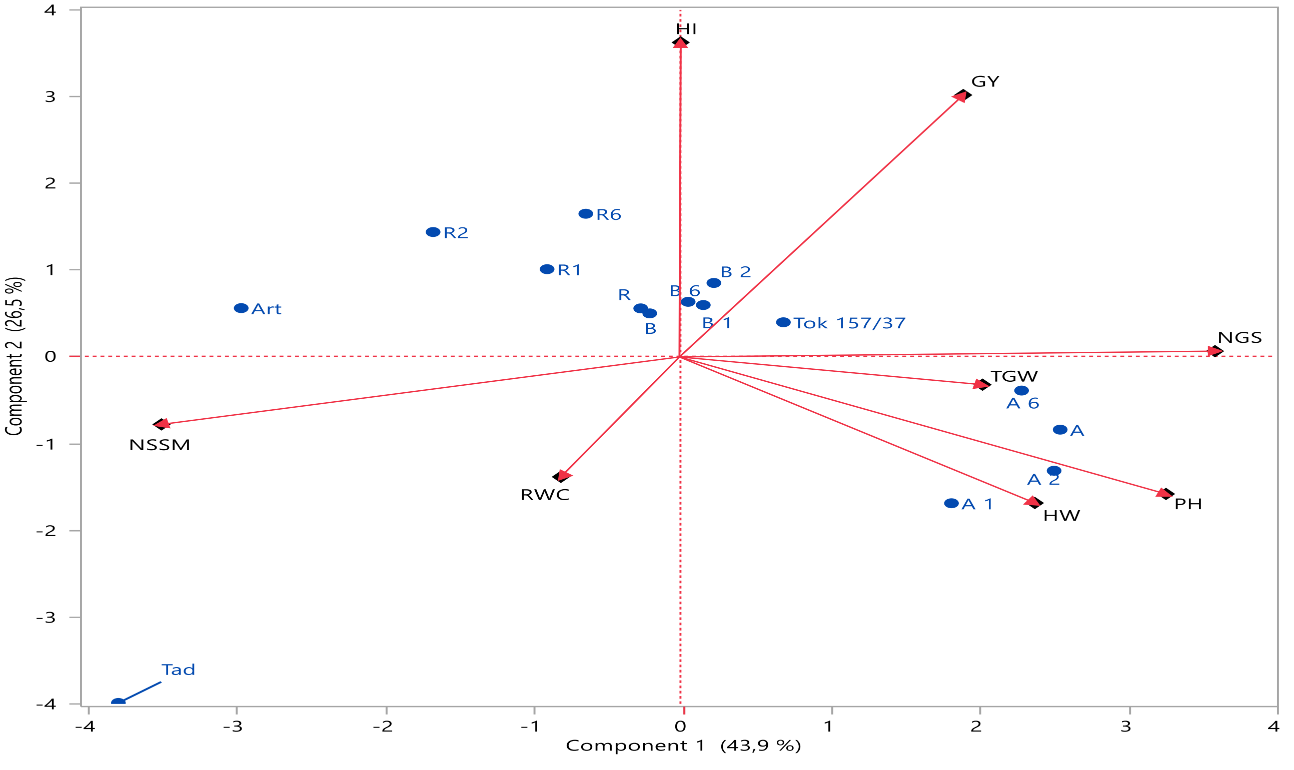

Fig. 1. The biplot graph generated from PCA with the two main components (PC1 and PC2) showing PCA score and loadings of different parameters, including RWC, Relative water content; TGW, Thousand-grain weight; HI, Harvest index; GY, Grain yield; HW, Hectoliter weight; PH, Plant height; NGS, Number of grains per spike; and NSSM, Number of spikes per square meter) in the field trial, Cultivars included Tad (Tadmor), Art (Arta), Tok 157/37 (Tokak 157/37), A (Aydanhanım), A1 (Aydanhanım QTL1), A2 (Aydanhanım QTL2), A6 (Aydanhanım QTL6), R (Baronesse), R2 (Baronesse QTL2), and R6 (Baronesse QTL6).

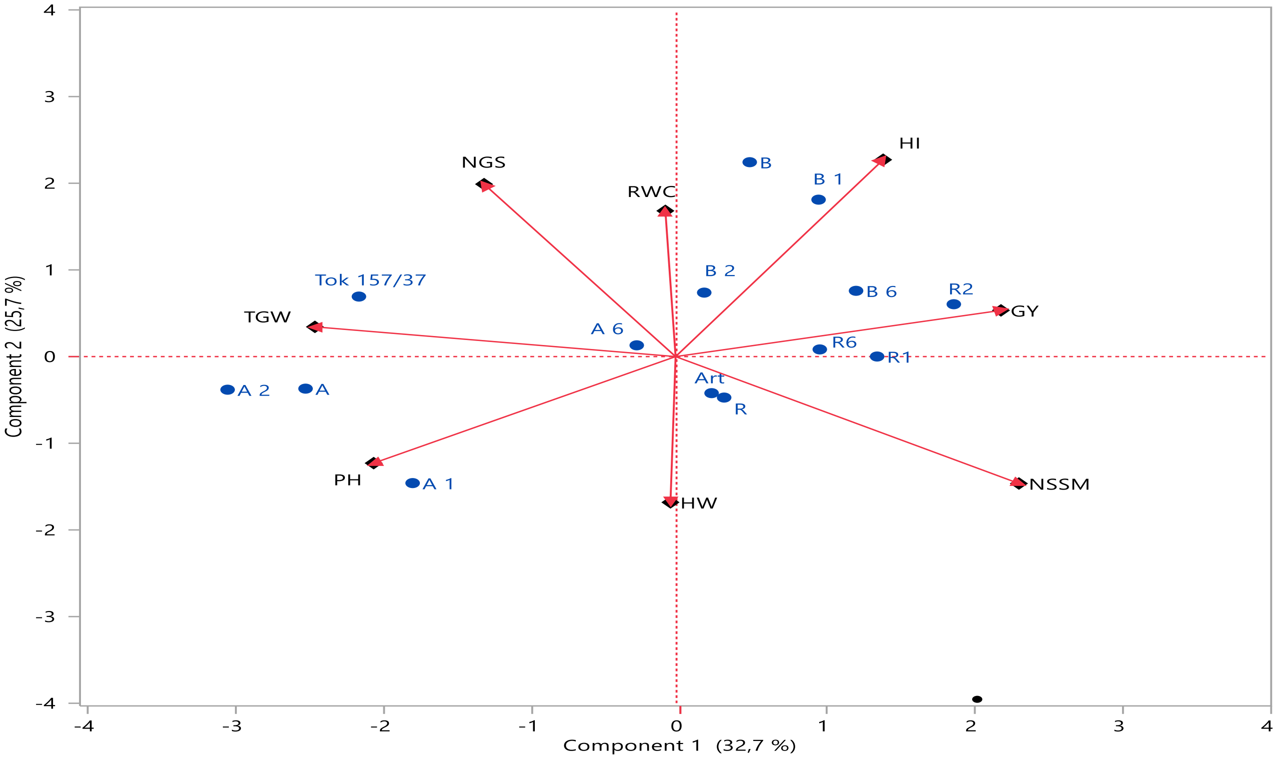

Fig. 2. The biplot graph generated from PCA with the two main components (PC1 and PC2) showing PCA score and loadings of different parameters, including RWC, Relative water content; TGW, Thousand-grain weight; HI, Harvest index; GY, Grain yield; HW, Hectoliter weight; PH, Plant height; NGS, Number of grains per spike; and NSSM, Number of spikes per square meter in the controlled trial; Cultivars included Tad (Tadmor), Art (Arta), Tok 157/37 (Tokak 157/37), A (Aydanhanım), A1 (Aydanhanım QTL1), A2 (Aydanhanım QTL2), A6 (Aydanhanım QTL6), R (Baronesse), R2 (Baronesse QTL2), and R6 (Baronesse QTL6).

Plant height: The difference between genotypes in terms of plant height was found to be statistically significant at the 1% level (P = 0.01) in both trials. The Arta cultivar had the lowest plant height among all tested cultivars in both trials, except for the Baronesse QTL1 (R1) and Baronesse QTL2 (R2) genotypes in the controlled trial. The Aydanhanım variety and its genotypes carrying three different QTL regions (A1, A2, and A6) in the field trial showed the highest plant height, whereas Tokak 157/37, the Aydanhanım cultivar, along with its three genotypes, and the Tadmor cultivar had the highest plant height in the controlled trial. The QTL6 region caused a reduction in the plant height by about 4 cm in the Aydanhanım cultivar, and similarly, a reduction of around 3 cm was found by the transfer of the QTL2 region to the Bolayır cultivar, both in the field experiment. QTL1, QTL2, and QTL6 regions also caused a statistically insignificant decrease in the plant height of Baronesse in both trials (Table 2). In this study, the plant height values of all cultivars and lines, except for the Tadmor cultivar, were reduced within the range from 0.7 cm (for Tokak 157/37) to 20.5 cm (for A6) depending on the cultivar and genotypes, in the controlled trial compared to those in the field trial (Table 2 and Fig. 1). As the average of 15 genotypes, the plants were 7.7 cm shorter in the controlled trial.

Number of spikes per square meter: Significant differences were determined between the genotypes in terms of the number of spikes (ears) per square meter in both trials. The highest number of ears per square meter was obtained in the Tadmor variety, while the lowest was recorded in the Aydanhanım variety, both in the field test, whereas the Aydanhanım QTL2 line (A2) exhibited the lowest number of ears in the controlled experiment, with significant differences between the two experiments carried out in the field and controlled conditions. The average number of ears per square meter was found to be 18 units lower in the controlled trial than in the field trial (Table 2 and Fig. 1).

Number of grains per spike: In this study, the difference between genotypes was found to be significant at (P = 0.01) in terms of the number of grains per spike in both trials conducted in the field and controlled conditions. Although the Aydanhanım variety in the field trial and the Bolayır QTL6 line (B6) and the Tokak 157/37 cultivar in the controlled trial had the highest number of grains per spike. Tadmor, known to be the drought-resistant variety, had the lowest number of grains per spike in both trials, followed by the Arta variety, which was also considered drought-tolerant and had an average number of 17.2 grains in both trials. The Aydanhanım, Bolayır, and Baronesse varieties and their lines carrying different QTL regions. The regions more negatively affected by the increased degree of drought stress in the controlled trial. Therefore, the grain number per spike except for the Bolayır QTL6 and Baronesse QTL2 lines, the number of grains per year decreased significantly in the controlled trial. In none of the genotypes of the three cultivars carrying QTL regions, except for the Bolayır QTL2 (B2) line, the number of grains per ear increased. On the other hand, the Aydanhanım QTL2 (A2) line exhibited 2.5 units decrease compared to its parent in the field trial. Furthermore, the average performance of the genotypes was found to be better in the field trial compared to the controlled trial in terms of the number of grains per spike (Table 2 and Fig.1).

Thousand-grain weight: In this study, the difference between genotypes in terms of thousand-grain weight was found to be statistically significant at the 1% level in both trials. The Tokak 157/37 cultivar had the maximum 1000 grain weight in the field experiment, followed by Arta and Aydanhanım cultivars and the Aydanhanım genotypes (A2, A1, and A6), which carried QTL2, QTL1, and QTL6 regions, respectively. According to the multiple comparison range test, all the aforementioned genotypes formed the first group. In the controlled trial, the Aydanhanım genotype (A6) carrying the QTL6 region exhibited a maximum 1000-grain weight (40 g), while the A2 genotype belonging to the same group as A6 had a thousand-grain weight of 39.0 g. Tadmor in the field trial and the R6 genotype in the controlled trial the lowest thousand-grain weight. In the controlled trial with more severe drought stress than in the field trial, the genotypes had an average of 5 g lower thousand-grain weight (Table 3, Fig. 1).

Hectoliter weight: In this study, the differences between genotypes were found to be significant in terms of hectoliter weight in both trials. As shown in Table 3, the Aydanhanım genotype (A2), carrying the QTL2 region, in the field experiment and the Tadmor variety in the controlled trial had the highest hectoliter weight (75.9 and 75.4 kg, respectively). The Arta cultivar, with a hectoliter weight of 67.1 kg in the field trial, and the Bolayır cultivar, with 65.9 kg in the controlled trial, had the lowest hectoliter weight; Bolayır one of the investigated cultivars had the lowest hectoliter weight in both trials. The Aydanhanım genotype (A2), to which the QTL2 region was transferred, was one of the evaluated genotypes that exhibited the highest hectoliter weight in both the field and controlled trials, and it was in the first group according to the multiple comparison test (LSD). Considering the average values for the two trials, it was observed that the Aydanhanım genotype (A2) displayed the highest hectoliter weight the Arta variety showed the lowest hectoliter weight. In this study, hectoliter weights of all cultivars and their genotypes, except for the Tadmor and Arta cultivars, (known to be drought resistant) were decreased by the ranges from 0.1 to –6.3 kg in the controlled trial compared to those in the field trial (Table 3, Fig. 1).

Grain yield: In terms of grain yield, the difference between genotypes in both trials was statistically significant at 1% level. As shown in Table 3, although the Baronesse genotype (R1) carrying the QTL1 region had the highest grain yield in the field trial (819.9 kg da^{-1}), according to Duncan's test, it was placed in the same group along with the other 11 genotypes except for the Tadmor and Arta cultivars and the A1 line. In the controlled trial, the Bolayır genotype (B6), to which the QTL6 region was transferred, achieved the highest grain yield (588.0 kg da^{-}

^{1}), and according to the multiple comparison test, together with B1, B2, and R2 lines, as well as the Tadmor and Arta varieties. These varieties were known to be drought resistant and formed the first group. The lowest grain yield was recorded in Tadmor (418.5 kg da^{-1}) and Arta (610.6 kg da^{-1}) cultivars in the field trial, while the A2 line with the grain yield of 363.1 kg da^{-1} showed the lowest value in the controlled trial.

The genotypes exhibited better average grain yield performance in the field trial in which plants did not experience or experienced less drought stress compared to the controlled trial, and as a result, in the former, the difference between the genotypes with the lowest (Tadmor) and the highest (R1) grain yield was 401.4 kg da^{-1}. In the controlled trial, however, this difference was approximately 225 kg da^{-1}. Although the Tadmor and Arta cultivars were placed in the last group (with the lowest values) in terms of grain yield in the field trial, they belonged to the first group (among the genotypes with the highest values) in the controlled trial.

Harvest index: In this study, the difference in terms of harvest index among the genotypes was found to be significant at the 1% level in both trials. As shown in Table 3, the Baronesse genotype (R6) carrying the QTL6 region in the field trial had the highest harvest index (48.5%), and the Tadmor cultivar, followed by the Aydanhanım QTL1 (A1) and Aydanhanım QTL2 (A2) genotypes had the lowest values under field conditions, they all were in the same group as the R6 genotype under the controlled conditions according to the multiple comparison test. In the controlled trial, the Bolayır genotype (B1), to which the QTL1 region was transferred, had the highest harvest index (33.7%). In both trials, the Tadmor cultivar among all the genotypes was with the lowest harvest index.

Moreover, the Aydanhanım genotype A6 carrying the QTL6 region had a 3.4% higher harvest index than the Aydanhanım variety in the controlled trial, while they had similar values with no significant difference in the field trial. Similarly, the Bolayır genotype (B1) having QTL1 region had a 3.5% higher harvest index in the field trial and a 1% higher value in the controlled trial than the Bolayır cultivar. The R6 genotype carrying the QTL6 region in the field trial had a 4.8% higher harvest index than the Baronesse variety, and the R2 genotype obtained from the Baronesse variety and the QTL2 region had a 7.3% higher harvest index than the Baronesse variety in the controlled trial (Table 3, Fig. 3).

PCA analysis: In this study, to comprehensively evaluate the obtained data, principal component analysis (PCA) was performed to interpret the investigated parameters and varieties. Many researchers have reported that the number of components explaining at least 2/3 of the total variation in PCA should be considered when applying PCA to the dataset (Rencher, 2002; Pierce *et al*., 2006). Agronomic and physiological measurements of the trials carried out under field and controlled conditions were subjected to principal component analysis (PCA) in some barley genotypes to which the QTLs were transferred for drought tolerance traits (Table 4). Although the first three components explained 84.45% of the total variance in the study as a result of the PCA analysis of the parameters investigated under field conditions, these components explained 72.89% of the variance in measurements done under controlled conditions. Some related studies have reported that the first component should have explained more than 25% of the total variance in PCA analysis (Seymen *et al*., 2019). The results of the analysis revealed that over 25% of the variance was explained by PCA, and therefore, its applicability to the obtained data in the study was verified. In some studies, PCA analysis was used to determine the effects of drought stress (Seymen *et al*., 2019). Using the PCA analysis, the first component explained 32.69% of the total variance under controlled conditions, and the parameters GY and NSSM had the highest positive loadings (vectors); in contrast, PH and TGW had the highest negative loadings. The second component explained 25.67% of the total variance, and it was strongly and positively correlated with NGS and HI parameters but strongly and negatively correlated with HW. The third component explained 14.53% of the study variance, and it was positively correlated with RWC and HW parameters.

Table 4. RWC (Relative water content), TGW (Thousand-grain weight), HI (Harvest index), GY (Grain yield), HW (Hectoliter weight), PH (Plant height), NGS (Number of grains per spike), and NSSM (Number of spikes per square meter).

Items	Controlled			Field		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	2.61	2.05	1.16	3.50	2.11	1.12
Percentage of variance	32.69	25.67	14.53	43.85	26.49	14.10
Cumulative variance	32.69	58.36	72.89	43.85	70.35	84.45
Eigenvectors						
RWC	–0.01409	0.38748	0.62941	–0.11287	–0.25154	0.11309
PH	–0.41673	–0.28333	0.12106	0.46291	–0.28739	–0.11629
NSSM	0.47392	–0.33747	–0.30035	–0.49287	–0.14153	0.02063
NGS	–0.26433	0.45850	–0.24986	0.50955	0.01215	0.09556
TGW	–0.49782	0.07904	–0.13454	0.28837	–0.05822	–0.77252
HW	–0.00708	–0.38756	0.63193	0.33815	–0.30637	0.56341
GY	0.44912	0.12284	0.10664	0.27002	0.54926	0.22339
HI	0.28649	0.52339	0.08801	0.00117	0.65957	0.00261

*: Statistically significant at $p<0.05$ and 0.001

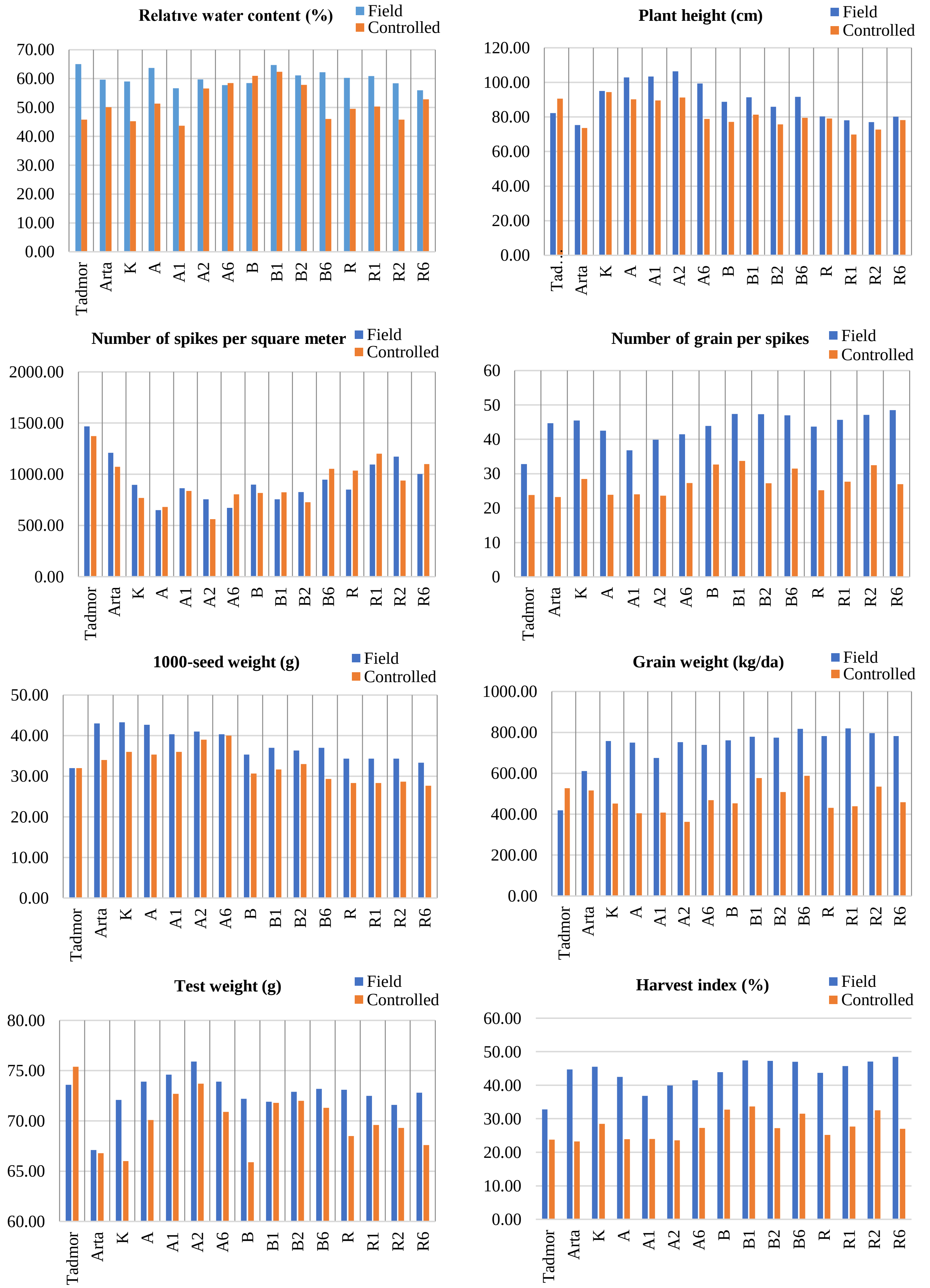

Fig. 3. K: Tokak 157/37, A: Aydanhanım, A1: Aydanhanım QTL1, A2: Aydanhanım QTL2, A6: Aydanhanım QTL6, B: Bolayır, B1: Bolayır QTL1, B2: Bolayır QTL2, B6: Bolayır QTL6, R: Baronesse, R1: Baronesse QTL1, R2: Baronesse QTL2, and R6: Baronesse QTL.

In the present study, the results of the biplot provide a useful way to determine the effects of QTL regions. This graph shows a positive correlation between the parameters. Similar findings reported in the previous researches (Kyriacou *et al*., 2016; Yavuz *et al*., 2021). Using the PCA analysis, significant relationships between the parameters were determined by the biplot graph generated from the first two highest components for both the experiments under field and controlled conditions (Figs. 1, 2). Figure 1 shows a positive relationship between NGS, TGW, PH, and HW, while these parameters exhibited high negative relationships with NSSM. The highest negative correlations were found between NGS and NSSM and between GY and RWC. The varieties with the most strongly explained variance in terms of parameters, including NGS, TGW, PH, and HW, in the positive region of the first component and the negative region of the second component were A, A 1, A 2, and A 6. (Fig. 2) shows a positive relationship between HI and GY in the positive region of both components, and B, B 1, B 2, B 6, R 1, R 2, and R 6 in this region were the varieties whose variance was explained by these parameters. The strongest negative correlation was observed between the parameters GY and PH, RWC and HW, and NGS and NSSM. The genotypes of A varieties gave the best results in terms of plant height in both environmental conditions. On the other hand, varieties B and R gave the best results in terms of harvest index and GY in both trials.

Principal component analysis (PCA) to determine the performance of barley genotypes carrying QTLs associated with drought tolerance traits under dry conditions.

Discussion

In terms of many characteristics examined in the present study, the effects of QTL regions were more pronounced in the controlled trial in which plants experienced terminal drought stress, while they were less pronounced in the field trial where drought stress was not severe.

When the results obtained from other related studies and the data from the field study and controlled conditions were evaluated together, the effect of each of the three QTL regions on the characteristics evaluated was found to vary depending on the genetic structure of the varieties to which these QTLs were transferred and ecological conditions under which they were grown. In other words, although a QTL region for a trait was effective in different genetic backgrounds and some environments.

As a result of the activation of rainout shelters and the exposure of plants to a certain degree of drought stress during the experimental period from ear emergence to harvest, a decrease was found in the values of all traits examined in the controlled trial compared to that in the field trial, except for Tadmor and Arta cultivars.

Leaf relative water content, one of the most important physiological indicators of drought tolerance (May & Milthorpe 1962), was found to vary significantly among genotypes in a study using different barley lines carrying QTL1, QTL2, and QTL6 regions (Saygılı, 2019).

In some studies, carried out on barley plants in Turkey and other parts of the world, significant differences were found in the leaf relative water content between drought-tolerant and sensitive varieties, with the former having higher values (Matin *et al*., 1989; Budaklı, 2003). Adjei & Kirkham (1980) and Shahram (2007) stated that drought-tolerant wheat varieties had higher leaf-relative water content than their sensitive counterparts.

The relative water content of leaves was significantly reduced in plants exposed to drought stress (Anjum *et al*., 2011). Aydin *et al*., (1999) also found that the average water content of leaves in 20 bread wheat varieties was decreased by 58.2% due to water stress under experimental conditions.

There was a positive relationship between the relative water content of leaves and the rate of photosynthesis (Anjum *et al*., 2011). It is extremely important for the plant to retain its high relative water content and sustain its metabolic activities under dry conditions. Therefore, leaf relative water content is an important indicator of plant water status under drought stress (Dhanda & Sethi, 1998).

In a study conducted in the province of Tokat, significant differences were observed between genotypes in terms of plant height (Saygılı, 2019). In the same study, the plant height values of the two lines carrying the QTL2 region were found to be 3.6–6.9 cm lower but significant compared to the Baronesse variety in four separate trials. On the other hand, QTL1 and QTL6 regions did not affect the plant height of Baronesse. QTL regions were reported to give different results in terms of plant height among the varieties to which QTLs were transferred and the traits examined. Similar to the results obtained by Teulat *et al*., (2001) with regard to the QTL1 region, Lakew *et al*., (2013) reported that the effect of many QTL regions, including QTL6, on plant height was not significant. Likewise, Samarah *et al*., (2009) also found shorter barley plants due to the increase in drought severity.

On the other hand, in a study conducted under Tokat ecological conditions on the lines carrying three different QTL regions, significant differences were found between the parent cultivars and the developed lines in terms of the number of ears per square meter and the Baronesse cultivar had a larger number of ears per square meter than the Tadmor cultivar in all trials (Saygılı, 2019).

In this study, the lower number of ears per square meter in the controlled trial than in the field trial indicates that the siblings of some genotypes in the controlled trial experienced water scarcity while forming or developing heads because the trial area did not receive precipitation due to the activation of rainout shelters after stemming. In a study conducted on wheat, the drought that occurred before the ear formation significantly reduced the number of fertile ears (Abayomi & Wright, 1999). In another study conducted in Italy, drought decreased the number of fertile ears per unit area in wheat by 60% (Giunta *et al*., 1993), whereas, in a study carried out in Egypt, this value was decreased by 6.16–14.81% depending on genotypes (Salem *et al*., 1996). In some other studies on barley and wheat, drought significantly reduced the number of ears per square meter (Öztürk, 1999; Dickin & Wright 2008; Samarah *et al*., 2009; Kılıç & Yağbasanlar, 2010; Moayedi *et al*., 2010; Yavaş, 2010).

Another study showed that QTL1 and QTL6 regions did not affect the number of grains per spike, and only one line (QTL2A) among the lines developed with the QTL2 region exhibited a significant decrease in the closed and open trials under Tokat ecological conditions (Saygılı, 2019).

Bukhat (2005) reported the negative effect of drought stress on the number of grains per spike, which varied depending on the duration and intensity of the stress. In studies on barley and wheat in different parts of the world and Turkey, it was reported that drought had significantly reduced the number of grains per spike (Samarah *et al*., 2009; Kılıç & Yağbasanlar 2010; Moayedi *et al*., 2010; Yavaş, 2010; Geravandi *et al*., 2011; Aghanejad *et al*., 2015). On the other hand, the controlled trial, where drought stress was more common and severe than in the field trial; the genotypes had an average of 5 g lower thousand-grain weight. The decrease in the number of substances produced during photosynthesis in plants induced by drought stress (Kutlu, 2010) and the shortening of the grain-filling period (Samarah *et al*., 2009) can explain these results. Similarly, drought also reported by other studies to reduce thousand-grain weight (Kılıç & Yağbasanlar, 2010; Moayedi *et al*., 2010; Yavaş, 2010; Balkan, 2011; Kanani *et al*., 2013; Khurshid *et al*., 2022).

These results indicated that the effect of QTL regions on hectoliter weight was more evident, especially under arid conditions, and it varied depending on the genetic structure of the variety. In a study conducted by Saygılı (2019) with four separate trials under Tokat ecological conditions, hectoliter weights of the developed lines were found to be the same as those of the Baronesse variety in all trials, except for small differences in the QTL2B line in the Tokat Açık Yazlık trial and the QTL6B line in the Tokat Açık trial, and the QTL regions examined were not related to hectoliter weights.

Balkan (2011) stated that drought-resistant wheat varieties had higher hectoliter weights than the sensitive varieties under arid conditions. Our results obtained from the present study, in a study conducted on 16 different wheat varieties, Guttieri *et al*., (2001) found that hectoliter weights decreased significantly under stress.

Likewise, Yavaş (2010) also reported that the grain yields of drought-resistant genotypes were quite low but higher than those of the sensitive genotypes that experienced drought stress, especially during years and in regions that received adequate precipitation.

Jamieson *et al*., (1995) reported that grain yield in wheat and barley was decreased due to drought stress. Bauder (1985) stated that the drought stress occurring during the vegetative phase of development in barley also significantly reduced the yield. Similarly, other studies demonstrated that drought significantly reduced the grain yield in wheat (Dickin & Wright 2008; Ganbalani *et al*., 2009; Kılıç & Yağbasanlar, 2010; Moayedi *et al*., 2010; Geravandi *et al*., 2011).

In a study conducted on barley lines carrying the QTL1 region, a higher harvest index was noticed than that of the Baronesse variety in the closed trial under Tokat ecological conditions (Saygılı, 2019). In the same study, the lines carrying the QTL2 region generally had higher harvest index values. In the controlled trial, the line carrying the QTL2 region had a higher harvest index than the parent variety because the shorter plant had a higher harvest index (Jia *et al*., 2009), and the QTL2 region produced a shorter plant of the Baronesse variety. Similar results are reported by Saygılı (2019).

In a study carried out by, significant differences found between the parent varieties and the developed lines in terms of harvest index. In another study, the Tadmor variety, known to be drought resistant, had a higher harvest index in the trials where the drought stress was simulated (Saygılı, 2019). Some studies consider the high harvest index in arid conditions as an indicator of drought tolerance (Chloupek *et al*., 2010).

Barley is a major cereal grain mostly grown for feed in Turkey and around the world, and drought is the leading factor limiting its yield, especially in dry areas under dryland farming. Taking this into account, some lines, including B1, B2, B6, and R2, to which QTL regions were transferred, along with Aydanhanım, Bolayır, and Tokak 157/37 varieties, grown widely in Turkey. They are also bred in Germany and have large production areas in the USA. Meanwhile, it is very important to produce more than 100 kg of grain yields of the Baronesse variety, which is used as a valuable gene source to increase grain yield because considering that barley is grown on a total area of around 3 million hectares every year in Turkey, even an increase of 10 kg per decare indicates a total production increase of 300 thousand tons, which is very important for the country's economy.

The total precipitation falling during present study period and the monthly distribution of the precipitation could almost met the water requirements of the barley plant, especially in the field trial, improving the performance of varieties and increasing grain yields. Likewise, the amount of precipitation falling before the activation of rainout shelters in the controlled trial the plants experienced milder drought stress and the grain yield was increased above the predicted value. Due to lodging, the Tadmor and Arta cultivars, which were known to be drought-resistant in the field trial, lagged behind compared with the other cultivars in terms of yield.

The Bolayır (B1) and the Baronesse (R1) lines carrying the QTL1 region achieved higher grain yields than their parents in the rainout shelter and field trials, respectively. Although the Aydanhanım line (A2) carrying the QTL2 region had a lower grain yield than its parent in the rain-sheltered trial, the Bolayır QTL2 (B2) and Baronesse QTL2 (R2) lines gave higher grain yields than their parents in the same trial. Furthermore, Aydanhanım and Bolayır lines (A6 and B6, respectively) carrying the QTL6 region obtained higher grain yields than their parents in the rain-sheltered experiment.

For the better evaluation of drought tolerance in varieties to which the three tested QTL regions were transferred in this study, more field trials should be conducted under arid conditions or in more locations where drought conditions exist.

In this one-year study, when both trials (field and controlled) were evaluated together, Bolayır QTL6 (B6), Bolayır QTL1 (B1), and Baronesse QTL2 (R2) lines were found to be of high-performance genotypes in terms of grain yield, and thus, they should be given priority to create the breeding populations for developing drought-tolerant barley varieties.

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