Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 11(4): 2498-2505, 2021
Journal of the Institute of Science and Technology, 11(4): 2498-2505, 2021

ISSN: 2146-0574, eISSN: 2536-4618

Bitki Koruma / *Plant Protection* **DOI: 10.21597/jist.959880**

Araştırma Makalesi / *Research Article*

Geliş tarihi / Received: 30.06.2021 **Kabul tarihi / Accepted: 17.08.2021**

Atıf İçin: Yörük E, Keleş EN, Erlik S, Yıldız S, Özsoy E, Kocabaş G, 2021. Physiological and Genetic Variation of *Hordeum vulgare* L. and *Triticum aestivum* L. Lines Planted in Turkey. Iğdır Fen Bilimleri Enstitüsü Dergisi, 11(4): 2498-2505.

To Cite: Yörük E, Keleş EN, Erlik S, Yıldız S, Özsoy E, Kocabaş G, 2021. Physiological and Genetic Variation of *Hordeum vulgare* L. and *Triticum aestivum* L. Lines Planted in Turkey. Journal of the Institute of Science and Technology, 11(4): 2498-2505, 2021.

Physiological and Genetic Variation of *Hordeum vulgare* L. and *Triticum aestivum* L. Lines Planted in Turkey

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ABSTRACT: *Hordeum vulgare* L. (barley) and *Triticum aestivum* L. (wheat) are among the most valuable crops cultivated and planted in many regions including Turkey. These plants have wide range of adaptation ability and capacity; they represent high level of variation in terms of physiological, genetics and epigenetics parameters and characteristics. Physiological and genetic variations were investigated by relative water content (RWC) assays, measuring electroconductivity (EC) levels and amplification of microsatellite markers in 21 barley and 43 wheat lines. At least three drought sensitive and three drought resistant lines were detected in barley and wheat lines via RWC assays. RWC values were recorded between 0.05±0.013 and 0.55±0.003%. Similarly great variation was detected for EC values of both barley and also wheat lines. Minimum and maximum EC values were ranged from 4.00±0.06 µS cm-1 to 59.88±3.209 µS cm-1. Three microsatellite markers, Bmag0120, Bmag0306 and Bmag375, were targeted in barley genome. Similarly, Han18, Wmc506 and Wmc623 microsatellite markers were targeted in wheat genome. Among these markers only Bmag0120 and Han18 were amplified from each line's genome by PCR and qPCR assays. In PCR and qPCR analysis homozygous and heterozygous lines were detected for Bmag0120 while each line was homozygous for Han18. Idiomorphic band size as 300 bp was detected in Han18 while it was ranged from 224 to 279 bp for Bmac0120 marker. Results showed that homozygous lines were drought resistant ones in barley lines whereas no correlation was found for wheat lines investigated in this study.

Keywords: Drought, *Hordeum vulgare* L., *Triticum aestivum* L., PCR

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INTRODUCTION

Barley is an annual plant and has great adaptability for plenty of stress factors. By this way, it has a wide geographical area to grow and be distributed in the world and in Turkey (Baik and Ullrich, 2008; Kaya and Ayranci, 2016). It is among the most produced cereals in Turkey following wheat. According to the FAO data, production quantity for the year 2019 was approximately7.600.000 tonnes. It is generally preferred as feed for animal nutrition and also used in malt beer production (Blake et al., 2011; Nevo et al., 2012)

Wheat, although it offers more food sources compared to other small grain cereals, it is in the first place as a carbohydrate source in countries with a temperate climate (Shewry, 2009). Wheat has a wide range of adaptability for different environmental conditions, but it is a grain that does not need much heat/temperature and moisture and can be grown in cool climate conditions. In the early stages of its development, it is sufficient for the temperature to be 8-10 °C and the relative humidity is about to be above 60% (Nevo et al., 2012; Atar, 2017).

Grains such as barley and wheat are important plants in terms of nutrition as well as having economic values. Barley and wheat constitute carbohydrate sources for consuming organisms directly or indirectly (Öztürk, 1999). Drought stress has adverse effects on physiological development and yield status of all small grain cereals including wheat and barley (Sallam et al., 2019). Decreased grain number, reduced plant height and weight are some examples of effects of drought on wheat and barley. If this stress is experienced after flowering, the weight gain in the grain is negatively affected and limited (Bartels and Sunkar, 2005) In cereal production, it is necessary to produce by choosing seeds of the lines that can adapt ecologically. Barley and wheat are more resistant to factors such as drought than other small grain cereals. Moreover, it is extremely important to select barley and wheat varieties that are resistant to drought and other stress factors in order to avoid yield losses. Within the scope of this work, physiological and genotypic differences of barley and wheat lines were investigated. The relationship between the lines and their drought stress response, ion leakage measurements and genetic characteristics were determined.

MATERIALS AND METHODS

Plant Material

21 *H. vulgare* L. and 43 wheat *T. aestivum* L. lines were used in this study. The lines were obtained from the culture collections of İstanbul Yeni Yüzyıl University Phytopathology Laboratory.

Ion Leakage Tests

Seeds of barley and wheat lines were surface sterilized in triplicate and each replicate contained 7 seeds per set. After the seeds were treated with 0.64% NaOCl for 20 minutes, the NaOCl was removed. The seeds were washed with ddH2O three times and were then treated with absolute ethanol for 10 seconds after the water was removed. The seeds were dried with sterile filter paper and seeds were replaced between two filter papers on sterile petri dishes. The samples were soaked with 3 mL of ddH2O and germinated at dark for seven days at room temperature. First electroconductivity value, EC1, were recorded after the seeds were washed 3 times with ddH2O after kept in the dark for 16 hours. The samples were autoclaved for 20 minutes and then EC2 values were recorded. Ion leakage values were calculated according to the following formula: EC ($\mu S\ cm^{-1}$) = E1/E2 x 100. EC values were recorded using a conductivitymeter (Hanna, U.S.A.- Gürel et al., 2016).

Relative Water Content (RWC) Assays

Relative water content of leaves were recorded. 7 seeds per petris dish were germinated via placing them between sterile filter papers. At the end of the 7th day, plantlets were transferred to pots of 6 cm diameter including soil. Control set was well watered for ten days while the experiment set was not watered. 17-day-old plants were taken from soil and the FW was recorded. The leaves were put onto ddH2O for 24 hours at +4ºC at dark and TW was measured. Finally, leaves were incubated at 80ºC for 24 hours and DW was recorded. RWC value was calculated according to the formula following: RWC (%) = [(FW- DW)/[TW-DW)]x100 (Teulat et al. 1997).

Genomic DNA (gDNA) Isolation

SDS (sodium dodecyl sulfate)-based genomic DNA (gDNA) isolation was performed from 7-day-old shoots grown from barley and wheat. 50 mg of fresh leaves were taken from plantlets and homogenized via liquid nitrogen and sterile mortar/pestle. The protocol provided by Niu et al. (2008) were followed in gDNA extraction with slight modifications. The amount and quality of gDNA was determined by spectrophotometer according to the absorbance values at 260 nm and 280 nm wavelengths. Calculated gDNA samples were diluted for PCR to contain 10 ng μL^{-1} of DNA. Qualitative analysis of gDNAs were carried out with 1% agarose gel run at 70V for 1 hour. gDNAs were photographed under UV light using a transilluminator.

Microsatellite Genotyping Assays

gDNAs of each barley and wheat lines were used in microsatellite genotyping assays. Bmag0120, Bmag0306 and Bmag375 microsatellite markers were targeted in the barley genome (Table 1). Similarly, Han18, Wmc506 and Wmc623 markers were subjected to PCR assays for wheat. PCR (polymerase chain reaction) components, 5 pmol forward primer, 5pmol reverse primer, 50 ng gDNA and 1X PCR master mix (Takara, Japan) and ddH_2O, were combined in microtubes with a volume of 25 µL. Tubes were transferred to the thermal cycler. PCR conditions were performed at 95°C for 3 minutes, followed by a 35-rep cycle consisting of 30 seconds at 94°C, 30 seconds at 55°C, 30 seconds at 72°C, plus 3 minutes at 72°C was also performed as the final extension. Analysis of the microsatellite bands was performed with 2% agarose gel electrophoresis and UV transilluminator. PCR band sizes were measured by using GelAnalyzer ® software in comparison to 100 bp DNA size marker (Genmark Bio, Turkey).

Table 1. PCR Primers used in this study

Primer Set	Forward sequence (5'-3')	Reverse sequence (5'-3')	Band size in bp	Reference
Bmag375F/R	ttcagcaatggcactag	cttcaaggaaggctaggg	640	Oliver et al. 2010
Bmac0306F/R	acttcctcaacatgccaga	ttcaatgtggaaggcgtc	405-430	Oliver et al. 2010
Bmag0120F/R	atttcatcccaaaggagac	gtcacatagacagttgtcttcc	226-260	Abderrazek 2020
Wmc623F/R	acatacccgaagccttgga	gtgctcggcaggattgtc	400-410	Oliver et al. 2010
Wmc506F/R	acttcctcaacatgccaga	gtcgccttccacattgaaa	387	Oliver et al. 2010
Han18F/R	atcacatgttcgtacaacgc	tggacccctagttgtgagt	300	Han et al. 2015

Real-Time PCR (qPCR) Assays

Validation of homozygous and heterozygous individuals was carried out by qPCR assays. 1X Eva Green mixture, 5 pmol forward primer, 5 pmol reverse primer and 25 ng gDNA were combined in microtubes in 20 µL reaction volume and transferred to qPCR thermal cycler. Real-time PCR conditions; were as follows; the first denaturation phase at 95 °C for 5 minutes followed by 20 seconds at 95 °C, 30 seconds at 55 °C, and 30 seconds at 72 °C with 45 repetitions were carried out. A common temperature

scanning step was carried out for melting curve analysis. Ct values and signal pics for individuals were recorded.

RESULTS AND DISCUSSION

Ion Leakage Analysis

EC values for each line were recorded using a portable conductivitymeter. EC values were ranged from 10.87±2.04 to 45.58±22.44 for barley lines (Figure 1.A). Minimum and maximum EC values were changed between 4.00±0.06 µS cm^{-1} to 59.88±3.209 µS cm^{-1} for wheat lines (Figure 1.B). Significant differences were detected ($p<0.05$) between relatively contrasting lines such as *H. vulgare* L. cv. Oliver and *H. vulgare* L. Lord and *T. aestivum* L. cv. Basribey and *T. aestivum* L. cv. Güneyyıldız.

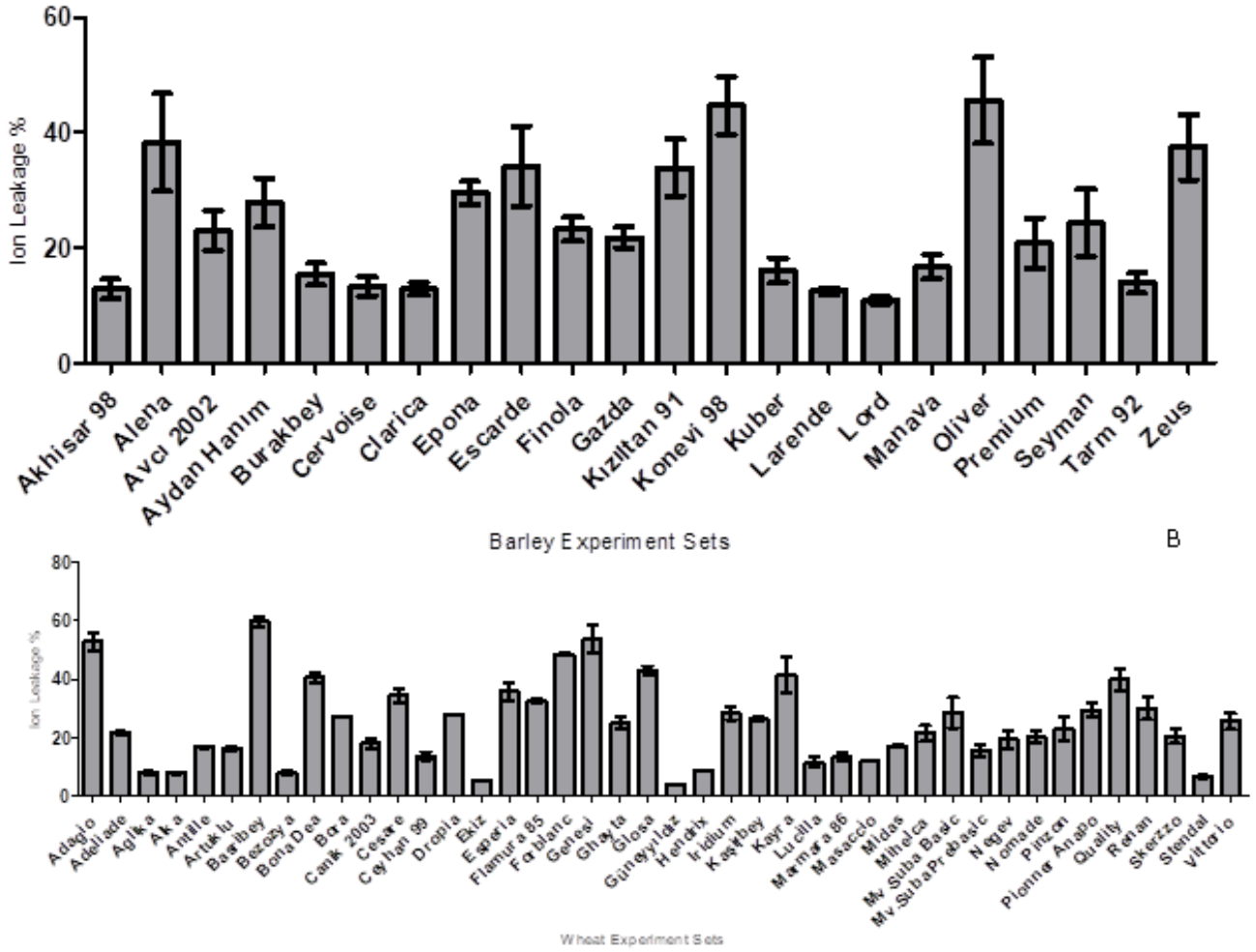

Figure 1. EC values for barley (A) and wheat (B) lines used in this study

Relative Water Content Analysis

RWC values were calculated for each barley and wheat lines in control and experiment sets. Minimum and maximum RWC values were ranged from 0.05±0.013 to 0.55±0.03 for EC barley lines (Figure 2.A). RWC values for wheat lines were detected between 0.07±0.01 to 0.48±0.1 (Figure 2.B). Significant differences were detected ($p<0.05$) between contrasting lines. Relatively drought resistant barley and wheat lines were as *H. vulgare* L. cv. Burakbey and *T. aestivum* L. cv. Midas, respectively. Contrasting lines were *H. vulgare* L. cv. Finola for barley and *T. aestivum* L. cv. Negev for wheat.

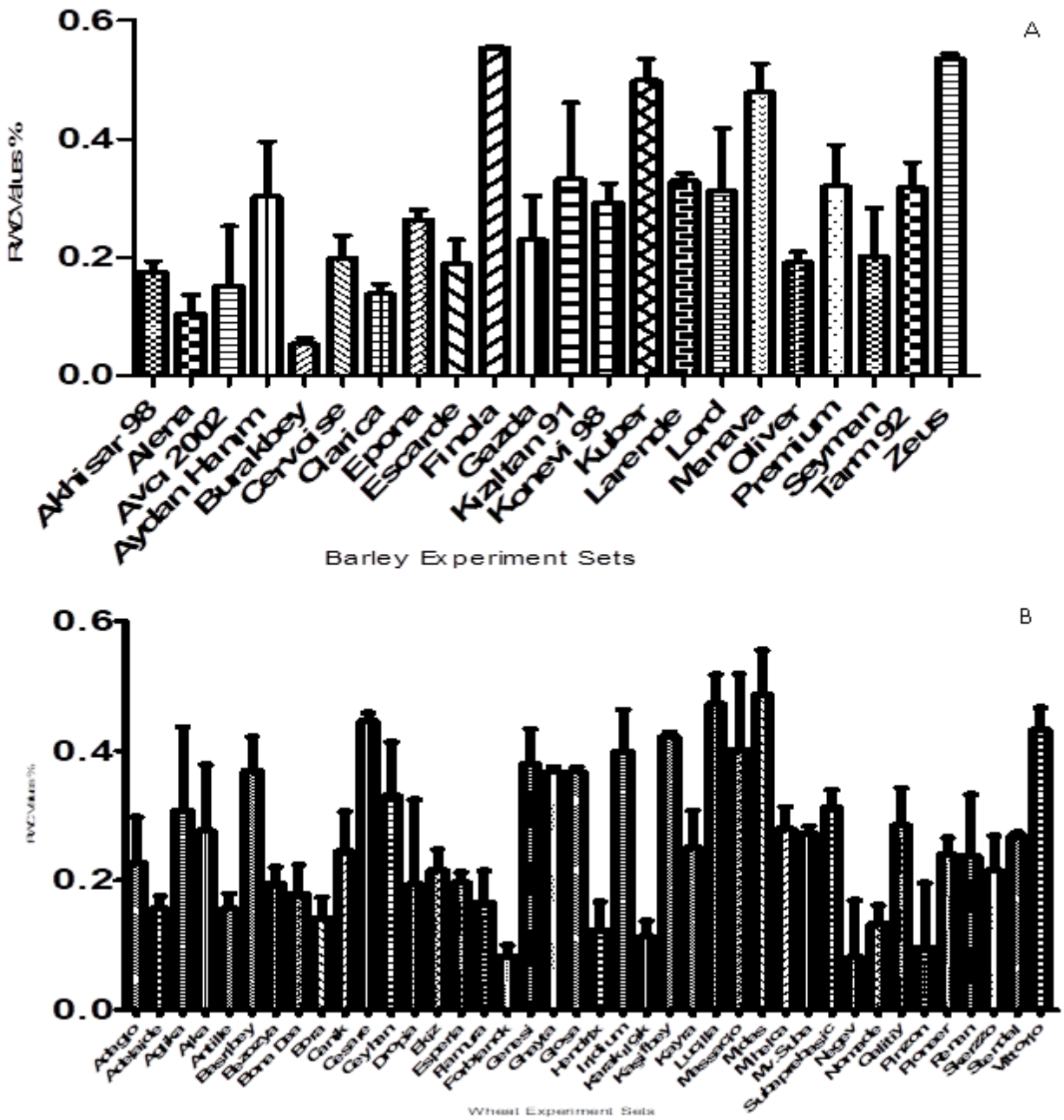

Figure 2. RWC graphic for barley (A) and wheat (B) lines used in this study

Microsatellite PCR Genotyping Analysis

In microsatellite PCR assays, only Bmag0120 and Han18 markers were amplified. In Bmag0120 amplification assays, each line yielded a single amplicon except *H. vulgare* L. cv. Alena. *H. vulgare* L. cv. Akhisar yielded two bands with different sizes of 236 bp and 259 bp (Figure 3). The amplicon sizes were ranged from 224 bp (*H. vulgare* L. cv. Akhisar) to 279 bp (*H. vulgare* L. cv. Kuber). In Han18, idiomorphic banding profile was obtained. Each wheat line produced a band with the size of 300 bp.

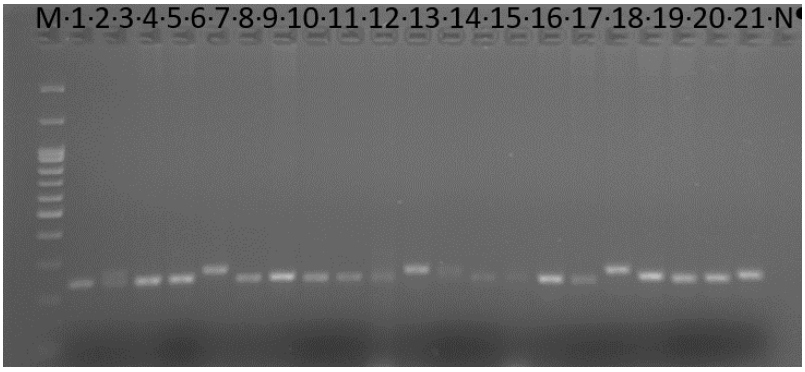

Figure 3. Bmag0120 microsatellite PCR profiling of 21 barley lines. M: 100 bp ladder, 1- Akhisar 98, 2- Alena, 3- Avcı 2002, 4- Aydan Hanım, 5- Burakbey, 6- Cervoise, 7- Clarica, 8- Epona, 9- Escarde, 10- Kızıltan 91, 11- Kuber, 12-Larende, 13-Lord,14- Manava,15- Oliver, 16- Premium,17- Seyman,18- Tarm 92, 19- Finola, 20- Zeus, 21- Konevi 98, N: no template control

qPCR Analysis

The heterozygous and homozygous lines for Bmag0120 were confirmed via qPCR analysis. E value for Bmag0120 marker was in the range of 1.8-2.2 meaning that qPCR was conducted efficiently. Among the lines, totally 9 lines (*H. vulgare* L. Akhisar, Alena, Avcı 2002, Aydan Hanım, Burakbey, Epona, Larende) were detected as heterozygous while remaining were as homozygous for Bmag0120 marker (Figure 4).

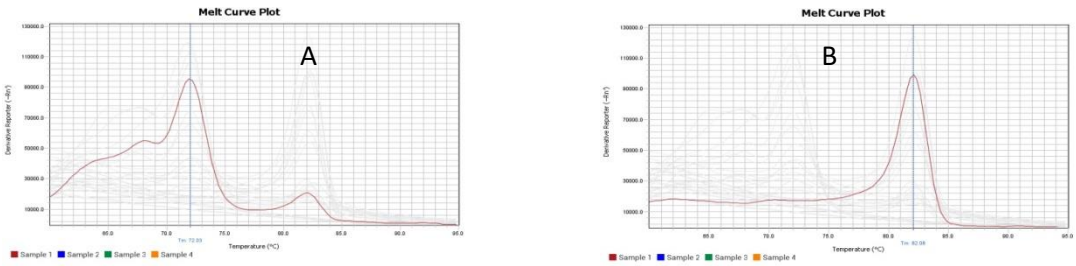

Figure 4. *H. vulgare* L. cv. Burakbey (A) as heterozygous and *H. vulgare* L. cv. Cervoise (B) as homozygous lines resulted from qPCR analysis

The cereals include world's most important domesticated plant species such as barley and wheat. Barley and wheat have a great significance in economies of many countries including Turkey. Several investigations have been conducted on drought and salinity response of barley and wheat cultivars planted in Turkey (Gürel et al., 2016; Yörük et al., 2018; Tufan et al., 2020). However, limited number of barley and wheat lines were investigated in terms of physiological and genetic differences of lines planted/cultivated in Turkey. In this study, we showed that there was great variation in EC capacity of barley and wheat lines. Similarly, in comparison to previous studies relatively drought resistant lines, which could be used in further studies, were detected (Suprunova et al., 2004; Yörük et al., 2018).

Microsatellite markers are of 2-6 nucleotide repeat motifs distributed and dispersed along the genomes. They, especially locating on coding regions of genes, could represent important physiological characteristics of cereals (Ivandic et al., 2002, 2003). In this study, Bmag0120 marker was found to be polymorphic in barley lines cultivated in Turkey. Since this study revealed the significant level of difference on EC and RWC values, this marker was evaluated in terms of association between drought responses of barley lines. According to the results it was clear that drought resistance lines were homozygous in terms of Bmag0120. In other words, Bmag0120 microsatellite region could be a specific marker to distiguish and identify the barley lines as their drought resistance and sensivity characteristics.

Thus, it could be suggested that this marker could be used in further in planta studies including water deficiency investigations.

CONCLUSION

In this study, it was shown that barley and wheat lines cultivated in Turkey possess variation in response to water deficiency. To our knowledge, this is first report showing variations in EC and RWC values of barley and wheat lines of Turkey. Moreover, it was revealed that Bmag0120 marker could be linked to drought stress response capacity of barley lines. Further studies could include more abiotic stress factors and microsatellite markers in order to get comprehensive data related to abiotic stress response of wheat and barley. Thus, these detailed data related to barley and wheat lines' genotypic and phenotypic diversities could faciliate the selection of lines which will be used in studies including transcriptomics, proteomics and/or metabolomics investigations.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Ayşe Feyza Tufan Dülger for providing plant materials.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

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

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