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Assessment of some bread wheat (*Triticum aestivum* L.) genotypes for drought tolerance using SSR and ISSR markers

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Abstract

As a result of the rapid increase in the world population, the need for wheat, which is one of the main nutrition in the human diet, is also rapidly increases. However, due to yield losses caused by abiotic stress factors such as drought, wheat production is not sufficient. Therefore, genetic characterization studies performed on wheat genotypes in terms of drought tolerance are important. In this study, genetic characterization of wheat genotypes regardingdrought tolerance was carried out by using molecular markers associated with drought-tolerance genes. For this purpose, 14 polymorphic markers were used to be able to distinguish between the control groups. Genetic characterization of 27 bread wheat genotypes by using eight ISSR markers revealed a polymorphism rate of 75.8%, and the mean PIC was calculated as 0.55. Based on the results of the genetic characterization performed with six SSR markers, the mean PIC value was 0.77, the mean He was 0.79, and the mean allele number was 6.7. In this study, the characterization of drought-tolerant and sensitive genotypes was carried out, and the potentials of genotypes for breeding studies were revealed. This study also indicates that used SSRs and ISSRs markers are useful in marker-assisted breeding about drought tolerance.

Introduction

The global cereal production is estimated at 2799 million tons in FAO's 2021 forecast. Nowadays, wheat is one of the most important cereal crops cultivated worldwide with a production of approximately 777 million tons (FAOSTAT, 2021). As the world population increases, the need for wheat also increases day by day rapidly. However, current wheat production is not enough mainly due to biotic and abiotic stresses. The main abiotic stress factor causing the greatest damage in wheat production worldwide is drought (Mohammadi & Abdulahi, 2018). Drought caused by changes in climatic variables (i.e., temperature and precipitation) as a result of global warming directly affects agricultural activities such as crop growth, annual crop yield, and crop production (Ates-Sonmezoglu & Terzi, 2018; Mickelbartet al., 2015).

In order to minimize the effects of drought, genetic characterization studies are important in terms of examination of drought tolerance in wheat and determination of drought-tolerant genotypes that have potential to be used in breeding studies. Therefore, scientists have been working to develop wheat genotypes that are tolerant to drought conditions and have high yield for a long time. It is necessary to say that determining genetic diversity is very important for current and future breeding studies in terms of providing preliminary information.

Drought tolerance is a quantitative trait, and the genetic determination of it is complex. Developing superior genotypes through conventional breeding is one of the necessary steps to understand the genetic basis of drought tolerance in wheat (Khaled et al., 2018). Besides morphological analyses, biochemical and molecular techniques have also gained importance in

the determination and evaluation of genetic diversity (Hassan et al., 2020; Iqbal, 2019). Morphological studies are insufficient and not reliable because they are affected by environmental factors during the investigation of drought tolerance. Therefore, drought tolerance studies in plants should be supported by molecular characterization studies. Molecular characterization studies will increase the use of cultivars determined to be tolerant in drought-related breeding programs, and more efficient and faster results will be obtained.

The determination of genetic diversity is also very important for breeding studies. To investigate the genetic diversity of genetic resources and populations, PCR-based markers developed based on differences such as the amount of polymorphism, reproducibility, information content, and the cost are used. Of these markers, Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) based markers are used effectively in DNA fingerprinting, linkage analyses, mapping studies, and genetic diversity studies of wheat genotypes (Ates-Sonmezoglu & Terzi, 2018; Khaled et al., 2015). Compared to other markers, ISSR markers are effective DNA markers in terms of revealing genotype identification, genetic-mapping and genetic diversity of wheat due to their superior advantages, such as being highly polymorphic, repeatable, and less plant material requirement (Khaled et al., 2015; Kyrienko et al., 2018). Although they are similar to Random Amplified Polymorphic DNA (RAPD) markers, ISSR markers are more specific and reproducible than RAPD markers due to their longer oligonucleotide primers and higher annealing temperature (Isshiki et al., 2008). In addition, they are fast, easy to apply, reliable and highly informative.

On the other hand, microsatellite markers, also known as SSRs, are one of the most suitable molecular markers for genetic characterization studies of wheat due to their features such as chromosome specificity, locus specificity, co-dominant structures, high polymorphism rate, and wide distribution throughout the wheat genome (Prasad et al., 2009; Yildirim et al., 2009; Dodig et al., 2010; Yildirim et al., 2011; Ates-Sonmezoglu et al., 2012). Thanks to their mentioned advantages, SSR and ISSR markers are successfully used in drought tolerance studies of wheat.

More specifically, Gupta et al. (2017) used 18 SSR markers to understand the genetic mechanism of drought tolerance in wheat cultivation. In another study, Yadav et al. (2018) used 15 ISSR markers for six drought-tolerant and six drought-sensitive wheat varieties, and 14 of markers gave reproducible band results. As a result of the study, it was stated that the genetic diversity of drought-tolerant and drought-sensitive wheat genotypes was determined reliably and successfully by using molecular markers.

In the current study, SSR and ISSR polymorphic markers associated with drought tolerance developed by different researchers were used for the molecular characterization. In the context of the study, 27 bread wheat genotypes were tested through six SSR and eight ISSR markers to evaluate their responses to drought stress variations.

Materials and Methods

Plant materials

In this study, 27 bread wheat genotypes were used as plant material for the characterization of the drought tolerance. Selected four bread wheat varieties, including two drought-tolerant (Mufitbey and Gun 91) and two drought-sensitive (Bezostaja and Aldane), were used as control genotypes. Control varieties were obtained from Tokat Gaziosmanpasa University and the bread wheat genotypes used in the study were collected from the provinces of Amasya, Corum, and Tokat (Table 1).

Table 1. Bread wheat genotypes used in the research

No	Developing Institution	Variety / Line Name	No	Developing Institution	Variety / Line Name
1	TTAE	Aldane	15	ETAEM	TR 63501
2	GKTAE	Bezostaja	16	ETAEM	TR 63575
3	GKTAE	Mufitbey	17	ETAEM	TR 63581
4	TAGEM	Gun 91	18	TAGEM	TGB 000521
5	TIGTHM	Dimenit	19	TAGEM	TGB 000526
6	TIGTHM	Aksunteri	20	TAGEM	TGB 000534
7	TIGTHM	Calibasiran	21	TAGEM	TGB 000543
8	TIGTHM	Ormece	22	TAGEM	TGB 003232
9	TIGTHM	Cambugdayi	23	TAGEM	TGB 003246
10	TIGTHM	Zerun	24	TAGEM	TGB 003247
11	ETAEM	TR 37373	25	TAGEM	TGB 003248
12	ETAEM	TR 44433	26	TAGEM	TGB 003249
13	ETAEM	TR 48371	27	BDUTAE	Dagdas94
14	ETAEM	TR 63497			

BDUTAE: Bahri Dagdas International Agricultural Research Institute
TIGTH: Tokat Directorate of Provincial Agriculture and Forestry
GKTAE: Transitional Zone Agricultural Research Institute
ETAE: Aegean Agricultural Research Institute
TAGEM: Directorate General of Agricultural Research and Policies
TTAE: Thrace Agricultural Research Institute

Molecular screening

For the molecular characterization of wheat genotypes, six SSR and eight ISSR primers that were previously used in drought studies conducted by different researchers were used (Table 2).

A total of 45 SSR and ISSR primers were pre-screened for control genotypes and selected 15 most polymorphic primers were used for molecular characterization. Based on Doyle & Doyle (1990), DNA was extracted from the leaf of bread wheat genotypes by some modifications. PCR reactions were carried out in a 40 µL mixture containing 50–60 ng of genomic DNA, 0.25 µM of each primer, 0.2 µM dNTP mix, 2.5 µM $MgCl_2$, 10x PCR buffer, and 0.1 units of *Taq* DNA polymerase. PCR cycles were performed an initial denaturation step of 5 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 45 sec at 50-62°C (depending upon the annealing temperature of the primers), extension for 1 min at 72°C, and with a final extension step for 5 min at 72°C. The PCR products were resolved on 2% agarose gels (Figure 1a, 1b). Electrophoresis was performed at constant power of 90 V for 3-4 h. In addition, 100 bp ladder was used for SSR markers, and 1000 bp ladder was used for ISSR markers.

The markers were scored for the presence (1) or absence (0) of amplified bands. Comparison of genotypes and examination of genetic relationships between genotypes were done by the help of Numerical Taxonomy and Multivariate Analysis System software (NTSYSpc, version 2.1) (Rohlf, 1998). To be able to obtain a dendrogram of wheat genotypes, the DendroUPGMA (D-UPGMA) program (http://genomes.urv.cat/UPGMA) was used. The genetic similarity index of wheat genotypes was calculated according to Jaccard (1908). SSR and ISSR marker polymorphism rates were determined using Polymorphism Information Content (PIC) values, which were calculated based on the following formula:

PIC = 1 − ∑ Pij^2'where Pi is the frequency of the i^{th} allele (Anderson et al., 1993). The heterozygosity (He) was calculated according to Liu and Wu (1998).

Results and Discussion

In the study, eight ISSR (UBC 811, UBC 815, UBC 826 UBC 834, UBC 835, UBC 852, UBC 857 and ISSR 827) and six SSR (Xgwm 11, Xbarc 101, Xgwm 165, Xgwm 325, Xgwm 603 and Xgwm 99) markers, which showed polymorphism among control genotypes, were used to determine genetic diversity in 27 bread wheat genotypes in terms of drought tolerance (Figure 1a, 1b).

According to molecular screening (Table 3), a total of 40 alleles were determined by six SSR primers of bread wheat genotypes. The number of alleles in PCR amplification products obtained using SSR primers differed according to the primers and was determined to be between six-eight alleles. The average number of alleles was 6.7. When the allele numbers of the primers were analyzed, it was determined that the Xgwm 11 primer gave the highest allele number with eight alleles, followed by Xbarc 101 with seven alleles. PIC values for SSR primers ranged from 0.74 to 0.81. While the highest PIC value was observed in Xgwm 11 primer with 0.81, the lowest PIC was determined in the Xgwm 603 primer with a value of 0.74 (Table 3). The mean PIC value for all SSR primers was calculated as 0.77. In terms of heterozygosity values, whereas the highest He value was determined in Xgwm 11 primer with 0.84, the lowest He value was determined in Xgwm 603 primer with 0.75. In this study, the mean He value was determined as 0.79 (Table 3).

Table 2. SSR and ISSR primers were used in molecular identification

Primers	Primer Sequence (5' →3')	References
SSR Primers		
Xgwm 99	F-5'AAGATGGACGTATGCATCACA3' R-5' GCCATATTTGATGACGCATA 3'	(Röder et al., 1998)
Xgwm 11	F-5 GGATAGTCAGACAATTCTTGTG 3' R-5' GTGAATTGTGTCTTGTATGCTTCC 3'	(Yang et al., 2002)
Xbarc 101	F-5' GCTCCTCTCACGATCACGCAAAG 3' R-5' GCGAGTCGATCACACTATGAGCCAATG 3'	(Kumar et al., 2018)
Xgwm 165	F-5' TGCAGTGGTCAGATGTTTCC 3' R-5' CTTTTCTTTCAGATTGCGCC 3'	(Iqbal, 2019)
Xgwm 325	F-5' TTTCTTCTGTCGTTCTCTTCCC 3' R-5' TTTTTACGCGTCAACGACG 3'	(Mason et al., 2010)
Xgwm 603	F-5'ACAAACGGTGACAATGCAAGGA3' R-5' CGCCTCTCTCGTAAGCCTCAAC 3'	(Somers et al., 2004)
ISSR Primers		
UBC 811	5'GAGAGAGAGAGAGAGAC 3'	(Khaled et al., 2015)
UBC 815	5'CTCTCTCTCTCTCTCTG 3'	(Khaled et al., 2015)
UBC 826	5' ACACACACACACACACC3'	(Sen et al., 2017)
UBC 834	5' AGAGAGAGAGAGAGAGYT 3'	(Khaled et al., 2015)
UBC 835	5' AGAGAGAGAGAGAGAGY*C 3'	(Sen et al., 2017)
UBC 852	5' TCTCTCTCTCTCTCRA C3'	(Sen et al., 2017)
UBC 857	5' ACACACAC CACACACYG 3'	(Sen et al., 2017)
ISSR 827	5'ACACACACACACACACG 3'	(Barakat et al., 2010)

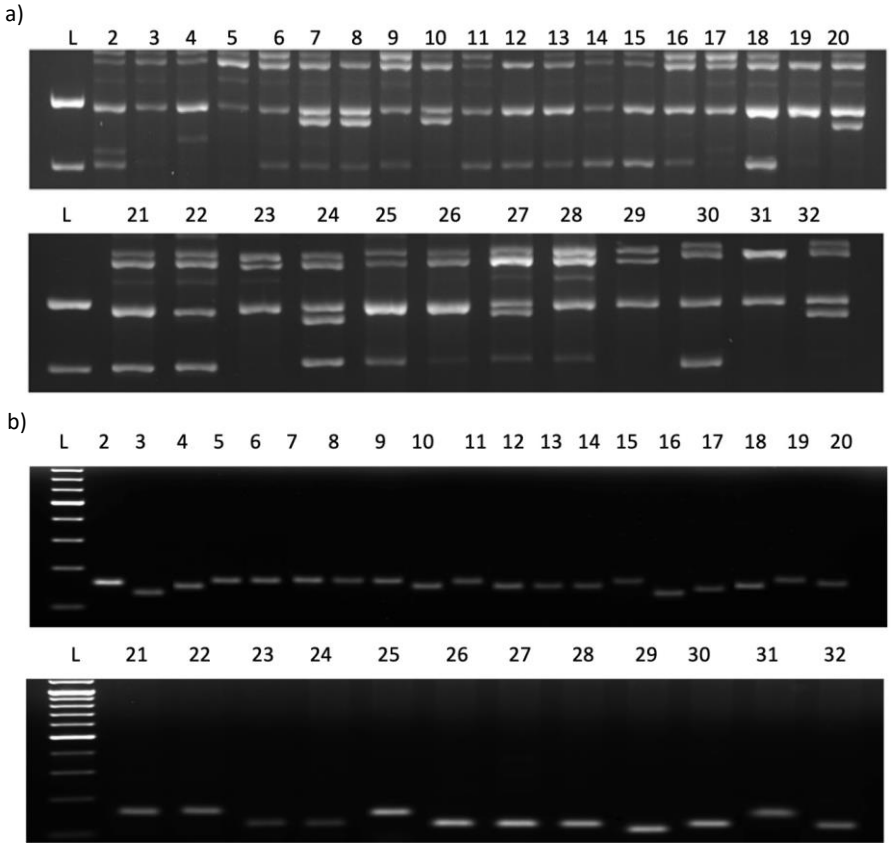

Figure 1. Gel image for molecular characterization of genotypes with a) UBC 811 ISSR primer (L: 1000 bp), b) Xgwm 165 SSR primer (L: 100 bp).
Control Genotypes; Drought-sensitive: Aldane (17, 29) and Bezostaja (18, 30) Drought-tolerant: Mufitbey (19, 31) and Gun 91 (20, 32)

This indicates the presence of significant genetic variation (Mkhabela et al., 2020). For six SSRs, all with more than six alleles, indicated PIC higher than 0.75. Many researchers have suggested that an objective evaluation of genetic diversity in wheat genotypes should be reflected by both PIC values and the number of alleles per locus in combination as in this study (Hao et al., 2006; Hai et al., 2007; Dodig et al., 2010). Another study was conducted to determine the genetic diversity among seven bread wheat genotypes, and phylogenetic relationships of wheat genotypes by using SSR and RAPD markers (Al-Tamimi & Al-Janabi, 2019). Among the DNA markers used, the highest PIC value was produced by SSR marker, and in the genetic diversity study on wheat genotypes, the SSRs were found to be quite informative. It was also stated that such studies would be the basis for the breeders in terms of selecting the appropriate parental genotypes to be able to achieve the highest desired heterosis in wheat populations.

A total of 49 bands were observed in 27 bread wheat genotypes by the alleles obtained from eight ISSR markers, and 25 of these bands were polymorphic (Table 4).

The average number of polymorphic bands was 2.9. While the highest polymorphism was given by UBC 811 and UBC 835 primers with 100%, the lowest polymorphism percentage was obtained by UBC 826 primer with 50%. PIC values for ISSR primers ranged from 0.13 to 0.82 (Table 4). Whereas the highest PIC value (0.82) was determined in UBC 857 primer, the lowest PIC value (0.13) was determined in UBC 826 ISSR primer (Table 4). In another study, the characterization of drought tolerance in three wheat varieties, including sensitive and tolerant, was carried out by using 14 ISSR markers (Eid, 2018). In the study, the number of alleles per locus ranged from 2 to 3, while the PIC value ranged from an average of 0.34 to 0.59. Similar to our study results, they obtained two main clusters (drought-

Table 3. SSR primers, major allele number, allele number, band sizes, heterozygosity ratio (He) and PIC values

Primer Name	Number of Major Alleles	Number of Alleles	Heterozygosity Ratio (He)	PIC	Band Sizes (bp)
Xgwm 11	0.24	8	0.84	0.81	165-195
Xgwm 99	0.36	6	0.78	0.75	115-170
Xbarc101	0.30	7	0.80	0.79	165-200
Xgwm 165	0.31	7	0.80	0.77	115-170
Xgwm 325	0.29	6	0.77	0.76	135-150
Xgwm 603	0.32	6	0.75	0.74	100-130
Total	1.82	40	4.74	4.62	-
Average	0.30	6.7	0.79	0.77	-

Table 4. ISSR primers, total number of bands, percentage of polymorphic bands (P%), PIC values and band sizes

Primer Name	Number of Bands	Number of Polymorphic Bands	P%	PIC	Band Sizes (bp)
UBC 811	5	5	100	0.55	955-2000
UBC 815	3	2	66.7	0.28	650-951
UBC 826	4	2	50.0	0.13	435-750
UBC 834	5	4	80.0	0.75	700-1250
UBC 835	3	3	100	0.50	630-1500
UBC 852	5	3	60.0	0.62	500-1500
UBC 857	4	3	75.0	0.82	600-1000
ISSR 827	4	3	75.0	0.80	500-750
Total	333	25	606.7	4.42	435-2000
Average	4.1	2.9	75.8	0.55	-

tolerant and drought-sensitive genotypes) as a result of the cluster analysis. They emphasized that ISSR markers were a valuable tool for studying genetic diversity in wheat varieties.

In the grouping of wheat genotypes, evaluation of dendrogram and genetic similarity coefficients together is a more accurate approach. The dendrograms are given in Figure 2-4, and genetic similarity values are given in Table S1-S3 Bread wheat genotypes were divided into two main groups based on the SSR markers (Figure 2). While the wheat lines Aldane and Bezostaja (drought-sensitive control varieties), and Dagdas94 were placed in subgroup I, all other genotypes were included in subgroup II with branching. While subgroup II showed branching within itself, the drought-tolerant control varieties (Gun 91 and Mufitbey) were included in the same subgroup.

Based on the ISSR markers (Figure 3), wheat genotypes were divided into two main groups. While the wheat line TGB 003232 was placed in subgroup I, all other genotypes were included in subgroup II with branching. Subgroup II showed branching within itself, while the drought-sensitive control varieties (Aldane and Bezostaja) were included in the subgroup IIa and the drought-tolerant control varieties (Gun 91 and Mufitbey) were included in the subgroup IIb. Ahmad et al. (2019) also reported high level of genetic diversity in the wheat genotypes and grouped the wheat genotypes in four clusters based on the dendrogram results obtained using similar ISSR markers.

The dendrogram generated based on SSR and ISSR markers identified control genotypes as genetically distinct. The use of them in breeding studies is recommended to increase the probability of additive genes that increase yield (Mkhabela et al., 2020).

Based on the dendrogram of combined ISSR and SSR markers (Figure 4), wheat genotypes were divided into two main groups. While the drought-sensitive control varieties Aldane and Bezostaja were placed was placed in same subgroup, the drought-tolerant wheat varieties Mufitbey and Gun 91 were in the other same subgroup.

When the dendrogram obtained by UPGMA analysis and genetic similarity coefficients were evaluated together, reliable results were obtained. According to the data obtained by combined ISSR and SSR markers, drought-sensitive wheat varieties Aldane and Bezostaja gave the high (0.87) genetic similarity

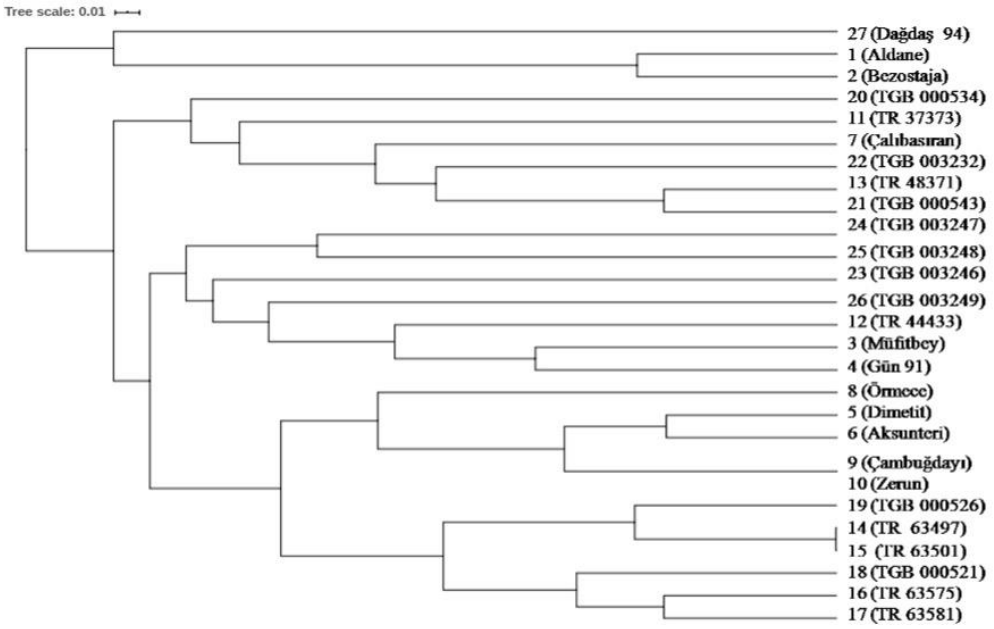

Figure 2. Dendrogram showing the genetic relationship among bread wheat genotypes based on SSR data using UPGMA.
Control Genotypes: Drought-sensitive: Aldane (1) and Bezostaja (2); Drought-tolerant: Mufitbey (3) and Gun 91 (4)

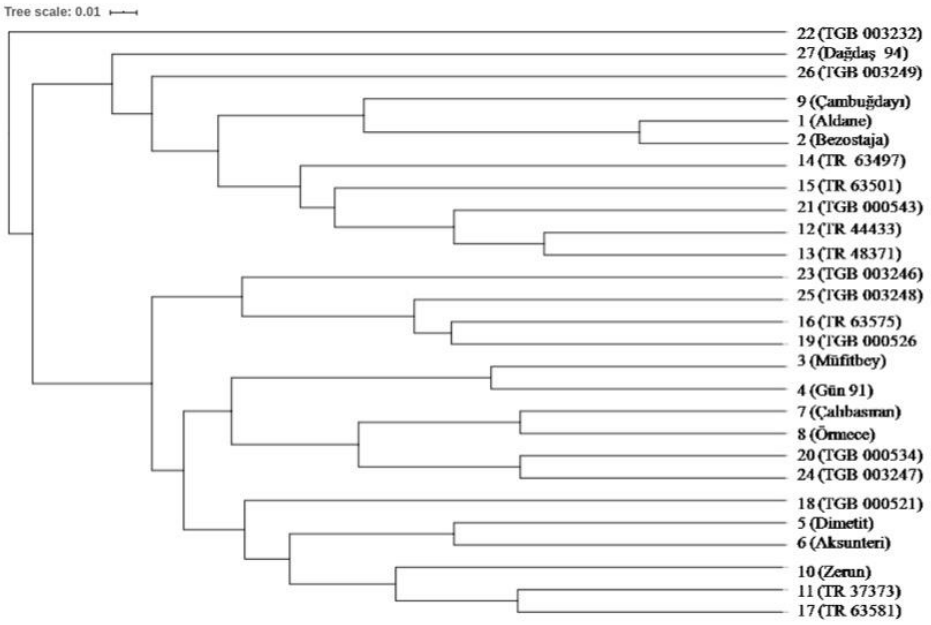

Figure 3. Dendrogram showing the genetic relationship among bread wheat genotypes based on ISSR data using UPGMA.
Control Genotypes: Drought-sensitive: Aldane (1) and Bezostaja (2); Drought-tolerant: Mufitbey (3) and Gun 91 (4)

coefficient (Table S3). Based on the data obtained by SSR markers, drought-tolerant wheat varieties Mufitbey and Gun 91 gave the high (0.75) genetic similarity coefficient (Table S1). The closest genotypes of drought-tolerant wheat variety Mufitbey were Gun 91 and TGB 0003526, while the most distant genotype was Calibasiran. The most distant genotype of drought-tolerant wheat variety Gun91 was line TGB 003232. The closest genotype to the drought-sensitive control Aldane was Bezostaja. On the other hand, the most distant genotypes to the Aldane were TR 37373 and Gun 91. Here, it can be said that cluster analysis is a useful tool in the determination of genotypes based on drought tolerance.

As a result of molecular screenings performed with ISSR markers, drought-sensitive wheat varieties Mufitbey and Gun 91 showed the highest (0.88) genetic similarity (Table S2). Also, the closest genotypes of drought-tolerant wheat variety Gun 91 were Mufitbey and TR 63581, while the most distant genotypes of the Gun 91 variety were TGB 003248 and TGB 003249 wheat genotypes. In addition, the closest genotype to the Aldane, which was a drought-sensitive control type, was TGB 003249. Yadav et al. (2018) used 15 ISSR markers for six drought-tolerant and six drought-sensitive wheat varieties, and 14 of markers gave reproducible band results. As a result of the study, it was stated that the

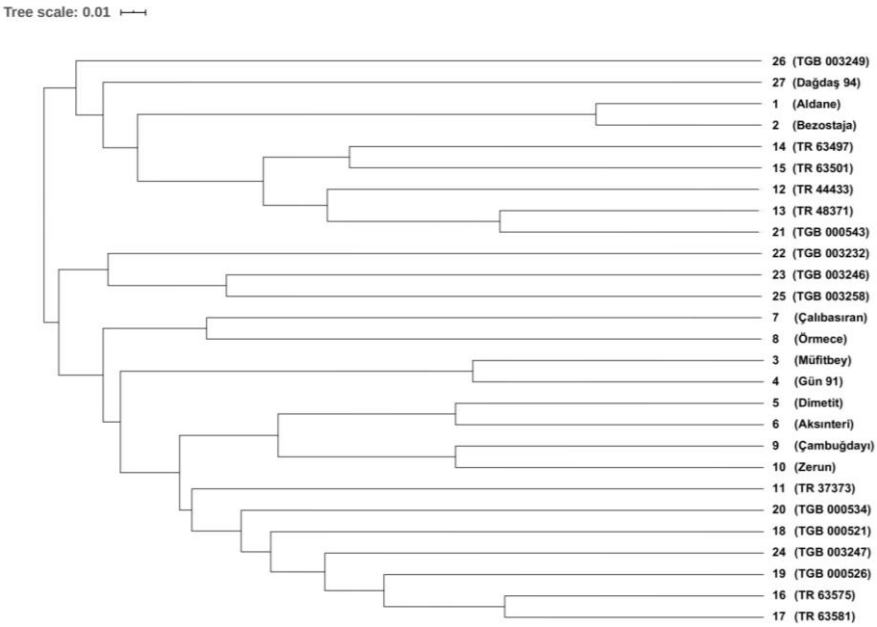

Figure 4. UPGMA dendrogram of combined ISSR and SSR markers for bread wheat genotypes.
Control Genotypes: Drought-sensitive: Aldane (1) and Bezostaja (2); Drought-tolerant: Mufitbey (3) and Gun 91 (4)

genetic diversity of drought-tolerant and drought-sensitive wheat genotypes was determined reliably and successfully by using molecular markers. Tungalag et al. (2018) used 17 ISSR markers to define variants in six Mongolian local wheat varieties. They reported that ISSRs could be used to determine genetic relationships and the fact that these markers did not require target sequence information was an advantage.

Conclusion

In the current study, genetic variations of 27 bread wheat (*Triticum aestivum* L.) genotypes were determined by using 14 SSR and ISSR markers associated with drought-related gene regions. As a result, the mean PIC values were 0.55 for ISSRs and 0.77 for SSRs. Also, all SSR primers gave PIC values higher than 74%. In conclusion, it can be said that the ISSR and SSR markers can be used successfully in genetic diversity, marker-assisted selection, and breeding studies related to drought tolerance in wheat.

When dendrogram results and genetic similarity coefficients were evaluated together, it can be stated that the SSRs and ISSRs used in the study are quite informative for genetic characterization studies related to drought resistance. In this study, based on the results of genetic similarity coefficient and dendrogram distribution, a preliminary data was provided for the use of the examined genotypes in later breeding studies. High genetic diversity was observed among the wheat genotypes, which allowed the identification and selection of drought-related genotypes. The identified genotypes are useful genetic resources for drought-related breeding studies to be conducted on wheat.

Author Contributions

OAS: conceptualization, supervision and designed; EC: experiments, investigation and analyzed the data BTA: writing, review and editing. All contributing authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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