Turkish Journal of FIELD CROPS

Turk J
Field Crops
2020, 25(2), 245-253
DOI: 10.17557/tjfc.834403

POPULATION STRUCTURE AND LINKAGE DISEQUILIBRIUM ANALYSES OF MODEL PLANT *Brachypodium distachion* THROUGH iPBS MARKERS

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Received: 13.10.2020

ABSTRACT

In the current study, a total of 235 genotypes of *Brachypodium distachyon (BD)* were studied and targeted to evaluate the genetic diversity, population structure and relative kinship of these genotypes using inter-primer binding site (iPBS) markers. Twenty-eight iPBS markers were used, and 184 clear and sharp polymorphic bands were produced. The polymorphism information content and diversity parameters indicated the existence of an appropriate amount of genetic diversity in the analysed genotypes. The results of the dendrogram with heat map and principal component analysis (PCoA) revealed that 235 *BD genotypes* were grouped into two separate clusters. The population structure was calculated using the STRUCTURE software, and two major sub-groups (K = 2) were established. A total of 95.1% of the relative kinship estimates being less than 0.4 for all genotypes. The findings of this study concluded that iPBS markers are highly polymorphic and are very efficient in the evaluation of the genetic diversity of *B. distachyon*.

Keywords: *Brachypodium distachyon*, genetic diversity, inter-primer binding site (iPBS), linkage disequilibrium, population structure.

INTRODUCTION

BD has a very small genome size among cereals and consists of approximately 272 Mbp with a C value of 0.36 pg / haploid genome and haploid chromosomes (x = 5) with 6 unmapped scaffolds (Ozdemir et al., 2008). It is a wild plant with a combination of many features required by a desirable and traceable experimental model, such as having the smallest genome, a self-fertilizing plant, easy growth conditions, a short generation time (eight weeks), a short physical length (approximately 20 cm), a life cycle of less than four months, and easy genetic transformation (Bevan et al., 2010). Although, *Arabidopsis thaliana*, a model for all flowering plants, has a small genome (135 Mbp), it is quite distant from the Poaceae family. On the other hand, *Oryza sativa* is used as a model for the genomes of all temperate crop species, including major grains, such as barley and wheat. Among these, *BD* is a better model for the grass genome than *O. sativa*, possessing the smallest genome in the Poaceae family (Draper et al., 2001).

The natural habitat of *BD* covers the Middle East and the Mediterranean (Syria, Lebanon and Israel), including Turkey (Wilson et al., 2019). It is mainly distributed in Turkey's Southeast Anatolia. To date, several genetic diversity studies have been carried out on Turkish *BD* (Filiz et al., 2009; Vogel et al., 2009; Gordon et al., 2014; Tyler et al., 2016; Stritt et al., 2018). In these studies, the genotypes were mainly collected from inland Turkey, and the results indicated a great genetic variation among the Turkish genotypes. to the best of our knowledge, the genotypes collected mainly from coastal regions of Turkey have not been studied in detail.

Apart from a few rare species, most of the genome of the eukaryotic organism is composed of dispersed repeat sequences and large-scale DNA sequencing. These are mainly transposable elements (TEs) and they can cause various types of mutation (Kalendar et al., 2011). They can also trigger other genetic rearrangements which they recombine in the plant genome. These unique properties make them a significant source of spontaneous mutation (Pereira and Ryan, 2019). These mutations sometimes can be advantageous to the plants, and certain TEs have been related to plant adaptation to various abiotic and biotic stress conditions (Pereira and Ryan, 2019). In addition, these mutations are generally related with the origin of new phenotypic and genetic diversity of plants (Serrato-Capuchina and Matute, 2018).

Molecular markers originating from retrotransposons are powerful resources for plant genomic and phylogenetic analyses, because a significant proportion of genomic variation is sourced from TEs (Roy et al., 2014). In

particular, the iPBS marker system has some advantages in comparison with other retrotransposon-based marker systems, such as the sequence information not being necessary when designing the primer, requiring a low amount of DNA, and ease of utilisation. In previous studies, several genetic diversity studies have been conducted in various crops using this marker system (Gedik et al., 2017; Ali et al., 2019). However, to the best of our knowledge, the present study is the first report on the identification and molecular characterization of *BD genotypes* using iPBS markers.

Due to their genomic properties, TEs are used in various ways as a molecular marker (Kalendar et al., 2010). In the transposon studies performed on *B. distachyon*, retrotransposons constitute 21.4% of the genome (Vogel et al., 2010). The iPBS approach has recently been used for isolation and visualisation of the polymorphism of retrotransposons since it does not require predetermined sequence information and has high reproducibility due to its binding temperature and primary length (Guo et al., 2014). Recognising the degree, quantity and pattern of genetic diversity studies to be carried out with *BD* population is important for their future utilisation in breeding studies which is expected to lead to rapid developments in genomic information production in order to improve all temperate crops, especially cereals (Bevan et al., 2010). However, iPBS markers have not been previously identified in *B. distachyon*; therefore, the current research was undertaken using these markers to determine the genetic diversity and population structure of *BD genotypes* collected from Turkey.

MATERIALS AND METHODS

Plant materials and DNA extraction

In the present study, 235 *BD genotypes* collected from 63 different locations (mainly coastal regions) of Turkey were analysed (Table 1 and Figure 1). Plants were grown in greenhouses in Ege University (Izmir, Turkey) and fresh young leaves from each single plant representing genotypes (four-six weeks old) were harvested from each seedling for DNA isolation.

In accordance with the procedure of DNA isolation defined by Doyle and Doyle (1987), genomic DNA from fresh young leaves of each sample were isolated with minor modifications. The DNA purity was controlled by 1% agarose gel electrophoresis, and the DNA concentration was measured by a Nanodrop spectrophotometer (ND-1000, Thermo Fisher Scientific Inc., USA). All DNA samples were diluted to 5 ng μL^{-1} concentration and stored at -20°C until the start of iPBS analyses.

iPBS PCR amplification

A total of 35 iPBS primers (Table 2) designed by Kalendar et al. (2010) were tested for display on six randomly selected genotypes of *BD* to observe which primers generated polymorphic and clear bands. DNA amplification was performed following the protocol of Kalendar et al. (2010) with minor modifications.

Data analyses

Sharp and clear amplified bands were scored manually for the absence and presence of the bands as 0 and 1, respectively. Effective alleles number (Ne), polymorphism information content (PIC), Nei's gene diversity (He), and Shannon's information index (I) were calculated for each marker by the use of R Studio statistical program. The PIC of the markers was identified based on the formula defined by Anderson et al. (1993):

$$PIC = 1 - \sum_{i} Pi^2$$

where *P* is the frequency of the presence of each band. Additionally, resolving power (Rp) was utilised to test the ability of iPBS primers to discriminate all individuals (Prevost and Wilkinson, 1999). The Rp analysis was performed based on the following formula:

$$Rp = \sum Ib \quad \text{and} \quad Ib = 1 - [2 * (0.5 - P)]$$

A cluster test was conducted to construct a dendrogram on the basis of Jaccard index using the unweighted pair-group method with arithmetic averages (UPGMA). The neighbour-joining tree was build and principal coordinate analysis (PCoA) was performed using the R studio software (Pal et al., 1996).

Analysis of population structure

The software STRUCTURE (v.2.2) (Pritchard et al., 2000) was used to ascertain the structure of the population consisting of 235 *BD genotypes*. The values of hypothetical subpopulations (K) was calculated from 1 to 10, estimating the associated allele frequencies [100,000 Monte Carlo Markov chain (MCMC) replicates and a burn-in period of 100,000]. STRUCTURE HARVESTER (Earl and VonHoldt, 2012) was used to measure the subpopulation number-Delta K (Evanno et al., 2005), the expected heterozygosity, and the fixation index (*Fst*) (Earl and VonHoldt, 2012).

Table 1. List of 235 *B. distachyon* accessions *genotypes* used for molecular characterisation analyses

Geno no	Location	Geno no	Location	Geno no	Location	Geno no	Location	Geno no	Location	Geno no	Location
1	Kozluk (Batman)	40	Dursunbey-Harmancik	79	Biyikali, Tekirdag (2)	118	İnecik	157	Yenice	196	Polatli- Haymana
2	18 Mart Univ. Kampus	41	Mugla	80	Pada Koyu	119	İnecik	158	İnecik	197	Polatli- Haymana
3	TUBITAK Gen Merkezi	42	Kozluk (Batman)	81	Balya Yenice II	120	18 Mart Univ.	159	Baragi, Kesan	198	Polatli Haymana
4	Burdur-Bucak-2	43	Eskisehir	82	Avanos III Nevsehir	121	İnecik	160	Yenice	199	Kaymaz cikisi Eskisehir
5	Canakkale Serceler Koyu	44	Dursunbey- Balikesir	83	Haskoy, Enez	122	Agva Sile yolu II	161	Yenice	200	Eskisehir
6	Kayi Koyu, Tekirdag	45	Polatli Haymana	84	Sarkoy	123	Sehitlik II Canakkale	162	Cenekoy, Hayrabolu	201	Polatli Haymana
7	Harmancik- Kutahya	46	Mugla	85	Harmancik- Kutahya	124	İnecik	163	TUBITAK Gen Merk.	202	Kaymaz cikisi Eskisehir
8	Harmancik- Kutahya	47	Eskisehir	86	Agva Sile yolu II	125	Agva Sile yolu II	164	Balikesir Merkez	203	Burdur-Bucak-1
9	Cenekoy, Hayrabolu	48	Balli, Sarkoy	87	Biyikali, Tekirdag (3)	126	18 Mart Univ.	165	Golcuk, Sarkoy	204	Dursunbey- Balikesir
10	Balikesir merkez II	49	Dursunbey-Harmancik	88	Avanos III Nevsehir	127	Avanos-Nevsehir	166	Canakkale sehitlik	205	İskender, Edirne
11	Danisment	50	Kutahya-Eskisehir	89	Sarkoy	128	18 Mart Univ.	167	Avanos III Nevsehir	206	İskender, Edirne
12	Olacak, Meric	51	Dursunbey- Balikesir	90	Buzagici, Hayrabolu	129	Avanos 4 Nevsehir	168	Nevsehir	207	İskender, Edirne
13	Burdur-Bucak-1	52	Kahta (Adiyaman)	91	Avanos III Nevsehir	130	İnecik	169	Canakkale Bursa yolu	208	Balli, Sarkoy
14	Kozluk (Batman)	53	Biyikali, Tekirdag (2)	92	Avanos-Nevsehir	131	Sehitlik II Canakkale	170	Biyikali, Tekirdag (2)	209	Ilgardere-Gelibolu
15	Olacak, Meric	54	Kutahya Tavsanli	93	Buzagici, Hayrabolu	132	Avanos 4 Nevsehir	171	Polatli Haymana	210	Kayi Koyu, Tekirdag
16	Olacak, Meric	55	Gaziantep-1	94	Cakmak, Edirne	133	Balikesir merkez II	172	Eskisehir	211	Cenekoy, Hayrabolu
17	Olacak, Meric	56	Balikesir Merkez	95	Danisment	134	18 Mart Univ.	173	Kaymaz Eskisehir	212	_
18	Begendik	57	Dursunbey- Balikesir	96	Biyikali, Tekirdag (2)	135	Balikesir merkez II	174	Kutahya	213	İnecik
19	18 Mart Univ.	58	Kozluk (Batman)	97	Kilickoy, Kesan	136	Inecik	175	Pada Koyu	214	Yenice
20	Ahmetli, Sile	59	Balya Yenice II	98	Golcuk, Sarkoy	137	Pada Koyu	176	Kaymaz/Eskisehir	215	İskender, Edirne
21	Burdur-Bucak-2	60	Can - Yenice	99	İzzetiye	138	Danisment	177	Polatli Haymana	216	İnecik
22	Uveyizli, Sile	61	Dursunbey-Harmancik	100	Dursunbey- Balikesir	139	İzzetiye	178	Kutahya Tavsanli	217	Cenekoy, Hayrabolu
23	Uveyizli, Sile	62	Vize	101	Nevsehir	140	Nevsehir	179	Kutahya-Eskisehir	218	İskender, Edirne
24	Balya Yenice II	63	Kilickoy, Kesan	102	Avanos 4 Nevsehir	141	Burdur-Bucak-1	180	Kutahya	219	Yenice Balya
25	Biyikali, Tekirdag (3)	64	Balya Yenice II	103	Burdur-Bucak-1	142	Buzagici, Hayrabolu	181	Polatli- Haymana	220	Haskoy, Enez
26	Canakkale Bursa Yolu	65	Sehitlik II Canakkale	104	Dursunbey-Harmancik	143	Biyikali, Tekirdag (3)	182	Pada Koyu	221	Disbudak
27	Dursunbey-Harmancik	66	Vize	105	Canakkale-Serceler	144	Yenice	183	Polatli- Haymana	222	Baragi, Kesan
28	Kozluk (Batman)	67	Sehitlik II Canakkale	106	İnecik	145	Uveyizli, Sile	184	Canakkale Bursa Yolu	223	Biyikali, Tekirdag
29	Adiyaman	68	Vize	107	Balikesir merkez II	146	Disbudak	185	Kutahya-Eskisehir	224	Balli, Sarkoy
30	Kozluk (Batman)	69	Buzagici, Hayrabolu	108	İnecik	147	Danisment	186	Polatli- Haymana	225	Yesilsirt Koyu, Muratli
31	Sile	70	İnecik	109	Avanos-Nevsehir	148	Yesilsirt Koyu, Muratli	187	Balya	226	Dursunbey- Balikesir
32	Haskoy, Enez	71	Ciftlik, Uzunkopru	110	Agva Sile yolu II	149	Agva Sile yolu II	188	Pada Koyu	227	Can - Yenice
33	Kilickoy, Kesan	72	Ciftlik, Uzunkopru	111	Kutahya Tavsanli	150	TUBITAK gen merk.	189	Polatli- Haymana	228	Baragi, Kesan
34	Gaziantep-1	73	Cenekoy, Hayrabolu	112	Mugla	151	Burdur-Bucak-2	190	Nevsehir	229	Yenice Balya
35	Gaziantep-1	74	Ilgardere-Gelibolu	113	Kutahya Tavsanli	152	Kayi-Tekirdag	191	Kutahya-Eskisehir	230	Avanos 4 Nevsehir
36	Dursunbey- Balikesir	75	Haskoy, Enez	114	İnecik	153	Balli, Sarkoy	192	Eskisehir	231	Burdur-Bucak-1
37	Sehitlik II Canakkale	76	Vize	115	Kutahya Tavsanli	154	İzzetiye	193	Polatli- Haymana	232	Yenice Balya
38	Sile	77	Ilgardere-Gelibolu	116	İnecik	155	Agva Sile yolu II	194	Polatli- Haymana	233	İzzetiye
39	Ahmetli, Sile	78	Cakmak, Edirne	117	Balya	156	Burdur-Bucak-2	195	Polatli- Haymana	234	Seymen, Corlu
										235	Buzagici, Hayrabolu

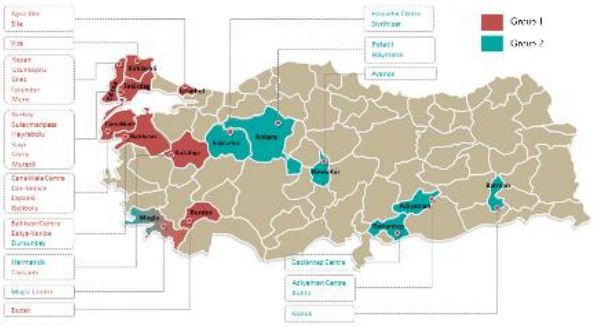

Figure 1. Geographical locations where 235 *Brachypodium distachion genotypes* were collected in Turkey

RESULTS

Analyses of iPBS markers

Thirty-five iPBS primers were evaluated with 28 indicating a clear, sharp and polymorphic bands profile (Table 2). Therefore, analyses were continued with these 28 primers, which produced 184 polymorphic bands with the proportion of 86% (Table 3). The average band number and polymorphic band number per primer were 8 and 7, respectively. While the highest polymorphic band was detected as 13 (iPBS 2401), the lowest polymorphic band was found as 2 (iPBS 2273 and 2402). The primers having a polymorphic band ratio of 100% were iPBS 2085, 2255, 2257, 2273, 2298, 2389, 2393, 2399 and 2401 (Table 3).

PIC, He, I, Ne and Rp were obtained to measure the ability of 28 iPBS markers in the present study. The maximum PIC value was 0.87 for iPBS 2245, and the lowest was 0.18 for iPBS 2273 (Table 3). The average number of PIC for all primers was calculated as 0.66, and this result being close to 1 indicated that the primers used in this study were considerably informative. The mean value of Shannon's information index (I) was found to be 1.29 and ranged from 0.18 (iPBS 2273) to 2.91 (iPBS 2401) (Table 3). On the other hand, the average number of Ne was 0.69 (Table 3). The quantity of gene diversity (He) was observed in iPBS 2224 with a maximum value of 0.40 and in iPBS 2085 and 2392 with a minimum value of 0.08. The average amount of gene diversity (He) was 0.25 for 235 individuals in the entire study, showing a high level of variation (Table 3).

Cluster and principal component analyses

Using the iPBS data and the similarity coefficient method of Jaccard, a genetic distance matrix of *BD genotypes* was determined in the R studio software. The genetic distance matrix results can be found at the website of https://www.dropbox.com/s/omtrp72qllevd9y/genetic%20distance%20matrix.xlsx?dl=0. The genetic distance level analyses indicated that all values varied from 0.04 to 0.67, indicating a high level of variation. In addition, the closest genotypes in the population were Geno65 (Sehitlik, Canakkale) and Geno68 (Sehitlik, Canakkale), while the most distant genotypes were Geno58 (Kozluk, Batman) and Geno115 (Tavsanli, Kutahya).

The UPGMA dendrogram with a heat map showed that the population mainly consisted of two groups, and as shown in Figure 2, while group 1 mainly comprised 161 genotypes collected from coastal regions of Turkey, group 2 mainly included 74 genotypes collected from inland Turkey (Figure 2; cluster 1 in red and cluster 2 in green). In addition, these groups were split into various subgroups. Supporting the results of UPGMA and heat map, PCoA diagram also demonstrated that the population split into two groups (Figure 3; cluster 1 in red and cluster 2 in green). These results present a clear and sharp variation between the 235 *BD genotypes*.

Population structure analyses

A total of 184 polymorphic bands produced by 28 iPBS primers (Table 2) were used for genotyping. The population structure of 235 *BD genotypes* was identified using STRUCTURE HARVESTER (v.2.2) (Earl and VonHoldt, 2012) based on the most appropriate group number estimation (Delta K). The highest value of Delta K was indicated K = 2 on the graph (Figure 5). At this level (K = 2) of discrimination, the genotypes were split into two main groups (Figure 4; cluster 1 in red and cluster 2 in green).

The expected heterozygosity, which helps to examine the probability of separation between two randomly chosen genotypes, was measured as 0.14 for the first group and 0.15 for the second group at K = 2. Additionally, the average *Fst* values were computed as 0.43 for the first group and 0.33 for the second group with an average of 0.38, at K = 2. This high expected heterozygosity and *Fst* values display the presence of a great heterozygosity among the *BD genotypes*.

Table 2. List of iPBS primers used in the current study

Primer ID	Optimal Annealing Temperature (°C)	Nucleotide Number	Sequence
iPBS 2085	55	12	ATGCCGATACCA
iPBS 2087	48	12	GCAATGGAACCA
iPBS 2389	48	12	ACATCCTTCCCA
iPBS 2391	45	12	ATCTGTCAGCCA
iPBS 2392	48	12	TAGATGGTGCCA
iPBS 2393	45	12	TACGGTACGCCA
iPBS 2273	45	13	GCTCATCATGCCA
iPBS 2217	48	18	ACTTGGATGTCGATACCA
iPBS 2221	51	18	ACCTAGCTCACGATGCCA
iPBS 2222	46	18	ACTTGGATGCCGATACCA
iPBS 2224	51	18	ATCCTGGCAATGGAACCA
iPBS 2228	51	18	CATTGGCTCTTGATACCA
iPBS 2229	48	18	CGACCTGTTCTGATACCA
iPBS 2230	45	18	TCTAGGCGTCTGATACCA
iPBS 2241	51	18	ACCTAGCTCATCATGCCA
iPBS 2243	51	18	AGTCAGGCTCTGTTACCA
iPBS 2245	51	18	GAGGTGGCTCTTATACCA
iPBS 2251	54	18	GAACAGGCGATGATACCA
iPBS 2252	45	18	TCATGGCTCATGATACCA
iPBS 2255	45	18	GCGTGTGCTCTCATACCA
iPBS 2257	48	18	CTCTCAATGAAAGCACCA
iPBS 2295	51	18	AGAACGGCTCTGATACCA
iPBS 2298	45	18	AGAAGAGCTCTGATACCA
iPBS 2373	51	18	GAACTTGCTCCGATGCCA
iPBS 2399	45	18	AAACTGGCAACGGCGCCA
iPBS 2400	51	18	CCCCTCCTTCTAGCGCCA
iPBS 2401	53	18	AGTTAAGCTTTGATACCA
iPBS 2402	51	18	TCTAAGCTCTTGATACCA

Table 3. Information of the iPBS primers used in the current study

Primer ID	TB	PB	PR (%)	PIC	Ne	I	He	Rp
iPBS 2085	8	8	100	0.75	0.10	0.77	0.08	0.75
iPBS 2087	9	7	78	0.78	0.65	1.49	0.24	2.41
iPBS 2217	5	4	80	0.76	1.05	0.61	0.12	0.52
iPBS 2221	10	9	90	0.59	0.27	1.49	0.20	2.46
iPBS 2222	7	5	71	0.72	0.71	0.68	0.15	0.97
iPBS 2224	7	5	71	0.76	0.85	1.56	0.40	3.13
iPBS 2228	13	12	92	0.68	0.26	2.54	0.26	4.63
iPBS 2229	10	9	90	0.60	0.28	2.07	0.31	4.47
iPBS 2230	5	3	60	0.85	2.26	0.75	0.49	1.50
iPBS 2241	9	8	89	0.68	0.34	2.04	0.35	4.42
iPBS 2243	11	8	73	0.71	0.43	1.33	0.19	2.50
iPBS 2245	8	6	75	0.87	1.33	1.59	0.30	2.67
iPBS 2251	5	4	80	0.47	0.47	0.79	0.28	1.56
iPBS 2252	5	4	80	0.74	0.95	0.97	0.30	1.67
iPBS 2255	11	11	100	0.73	0.34	2.46	0.28	4.40
iPBS 2257	4	4	100	0.21	0.32	0.42	0.19	0.95
iPBS 2273	2	2	100	0.18	0.61	0.18	0.17	0.37
iPBS 2295	10	9	90	0.82	0.61	0.75	0.20	2.45
iPBS 2298	12	12	100	0.86	0.58	2.65	0.23	3.70
iPBS 2373	10	8	80	0.61	0.32	1.99	0.35	4.52
iPBS 2389	7	7	100	0.60	0.36	1.14	0.21	1.95
iPBS 2391	7	5	71	0.77	0.88	0.69	0.17	1.21
iPBS 2392	6	5	83	0.46	0.37	0.32	0.08	0.42
iPBS 2393	4	4	100	0.54	0.55	0.98	0.37	2.14
iPBS 2399	6	6	100	0.50	0.35	0.87	0.20	1.71
iPBS 2400	5	4	80	0.76	1.02	0.55	0.12	0.14
iPBS 2401	13	13	100	0.74	0.29	2.91	0.25	4.64
iPBS 2402	3	2	67	0.82	2.72	0.61	0.38	1.15
Average	7.57	6.57	86	0.66	0.69	1.29	0.25	2.27
Total	212	184	**TB**: Total bands number, **PB**: Polymorphic bands number, **PR**: Polymorphism rate, **PIC**: Polymorphism information contents, **Ne**: Effective allele number, **I**: Shannon's information index, **He**: Nei's gene diversity, **Rp**: Resolving power.					

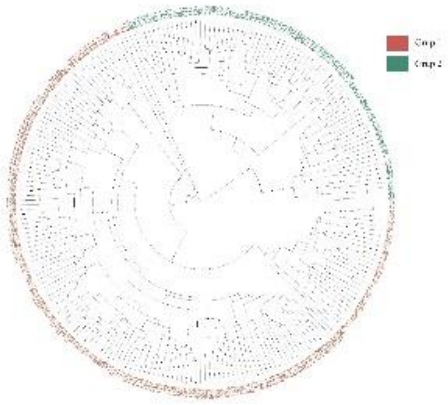

Figure 2. UPGMA dendrogram of 235 *B. distachyon genotypes*

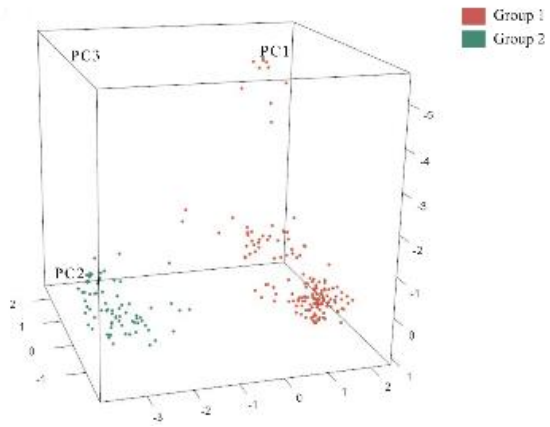

Figure 3. Principal coordinate analysis (PCoA) plot for 235 *B. distachyon* genotypes based on iPBS data

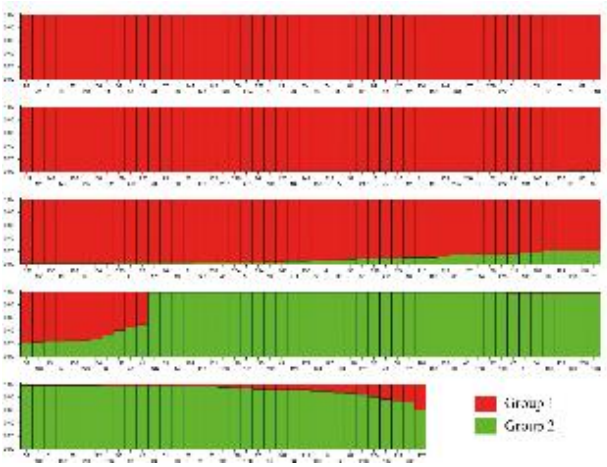

Figure 4. Population structure of 235 *B. distachyon* accessions genotypes used in the study (K = 2)

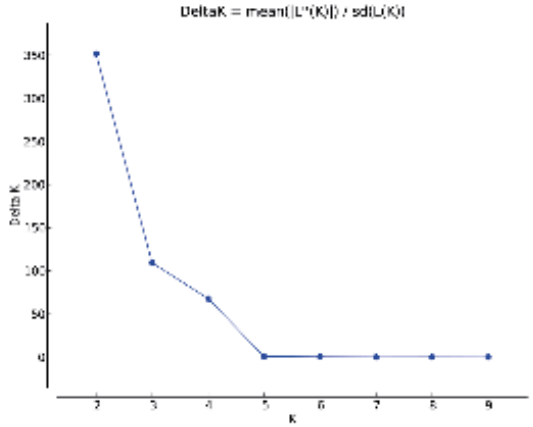

Figure 5. The Delta K of 10 repeats based on the STRUCTURE analyses

Relative kinship

In the current study, 184 polymorphic loci were used to indicate the relative kinship in the *BD genotypes*, and the pairwise kinship values were between 0 and 1. The calculations indicated that more than 82.3% of the pairwise kinship estimates ranged from 0 to 0.2 kinship values (Figure 6). In addition, 97.2% of the relative kinship estimates were less than 0.4 (Figure 6), meaning that while a great majority of the genotypes were a distant relative, some presented high similarity.

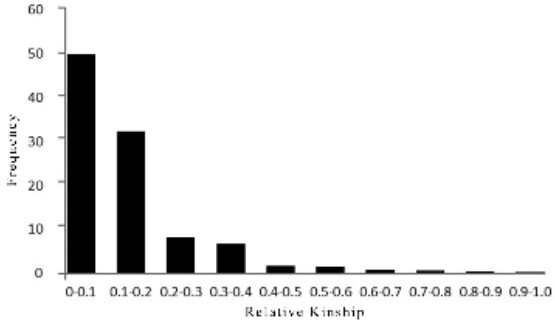

Figure 6. The distribution of pairwise relative kinship values

DISCUSSION

Analyses of genetic diversity and relationships between genotypes are important for their effective utilisation in crop improvement studies. Genetic diversity analyses provide an understanding of the genetic variation level and population structure (Ellegren and Galtier, 2016). *BD* has the smallest genome in the Poaceae and is phylogenetically close to wheat, rice and sorghum; therefore, it acts as a powerful functional genomics resource for all grasses (Draper et al., 2001; Vogel et al., 2010).

Analyses of iPBS markers and genetic diversity in B. distachyon

The results of band polymorphisms per primer were greater than reported as 3.8 in the common bean (Nemli et al., 2015), and 2.7 in fig (Belttar et al., 2017). These results indicate that iPBS primers were more conserved for *BD* compared to these species used in previous studies (Nemli et al., 2015; Belttar et al., 2017). On the other hand, a few iPBS studies have reported polymorphic alleles amplified per primer more than in the current study; for instance, apricot (Baránek et al., 2012), pea (Baloch et al., 2015), grape (Guo et al., 2014), rice (Comertpay et al., 2016), chickpea (Andeden et al., 2013) and saffron (Gedik et al., 2017) detected the number of polymorphic bands as 6.6, 6.7, 7.1, 8.5, 13 and 25.1, respectively. This is because the *BD* genome is very small and the amount of retrotransposons is variable among different species (Draper et al., 2001; Kalendar et al., 2019). Additionally, the average polymorphism found in this study was greater than previous studies in *B. distachyon*, such as AFLP (%70) (Filiz et al., 2009), SSR (%66) (Vogel et al., 2009), SSR, and inter simple sequence repeat (ISSR) (26% and 16%, respectively) (Hammami et al., 2014).

The PIC value indicates a stronger prediction of genetic diversity than the number of bands, since it calculates the comparative frequencies of each band (Comertpay et al., 2016). In the present study, the calculated mean PIC value was 0.66 per primer (Table 3). In previous iPBS studies, the mean PIC values were detected as 0.61 in pea (Baloch et al., 2015), 0.35 in rice (Comertpay et al., 2016), and 0.17 in cotton (Tyagi et al., 2014). Our average PIC value (0.66) was greater than these studies. On the other hand, the RP value is closely associated with a primer's ability to distinguish the genotypes, and the RP values ranged from 0.14 (iPBS 2400) to 4.64 (iPBS 2401) with an average of 2.27 in this study (Table 3). In previous iPBS studies, the RP values ranged from 1.43 (iPBS 2231) to 5.60 (iPBS 2230) in grape (Guo et al., 2014) and from 4.32 (iPBS 2377) to 11.20 (iPBS 2230) in *Tetradium ruticarpum* (Xu et al., 2018). The result of the RP analysis indicates that the type of germplasm being examined affects the resolving power of the primers (Sarla et al., 2003). These PIC and RP results not only demonstrated that all primers studied in this research were sufficiently powerful to establish the polymorphism but also the iPBS markers were notably useful in identifying genetic diversity in *B. distachyon*.

The mean values of Shannon's information index (I) and Nei's genetic diversity (H) among *BD genotypes* were calculated as 1.29 and 0.25, respectively (Table 3). For these calculations, higher values indicate high genetic diversity, and for this reason, there was a great variation between the *BD genotypes*. The mean Shannon's information index (I) (1.29) and Nei's genetic diversity (H) (0.25) in this study was greater than previous studies, such as in rice as 0.30 and 0.23 (Comertpay et al., 2016) and in saffron as 0.29 and 0.16 (Gedik et al., 2017). In addition, in this study, the number of effective alleles belonging to the locus of lines (Ne) ranged from 0.10 (iPBS 2085) to 2.72 (iPBS 2402) with an average of 0.69 (Table 3).

Analyses of population structure

The genetic diversity analysis between 235 *BD genotypes* using 28 iPBS primers was clearly illustrated in the UPGMA dendrogram (Figure 2) based on the pairwise genetic distance coefficient scores. The genetic distance values ranged between 0.04 and 0.67, and the closest genotypes were Geno66 (Sehitlik, Canakkale) and Geno67 (Sehitlik, Canakkale), while the maximum genetic variation was identified between Geno58 (Kozluk, Batman) and Geno115 (Tavsanli, Kutahya). In line with this result, genetically distant genotypes (Geno58 and Geno115) could be chosen as candidate parents to observe great segregation in F_2. On the other hand, in the results of the dendrogram (Figure 2; cluster 1 in red and cluster 2 in green) and PCoA (Figure 3; cluster 1 in red and cluster 2 in green), the population was made up of two groups with great diversity. In an earlier study of Turkish *BD* using AFLP, a UPGMA dendrogram divided the population into five groups according to their geographic origins (Filiz et al., 2009). The reason for this can be considered as the use of different genotypes and DNA being divided into very small meaningless parts with DNA restriction enzymes in the AFLP method.

The analysis of the population structure indicated that the genotypes were clustered into two groups (Figure 4; cluster 1 in red and cluster 2 in green). Group 1 consisted of genotypes collected mainly from coastal cities, while group 2 comprised genotypes collected from inland Turkey (Figure 1; cluster 1 in red and cluster 2 in green). Thus, this result demonstrated that the distribution of Turkish *BD genotypes* used in the current study depended on the locations (coastal regions or inland Turkey) with a few exceptions. This clustering result also supports a previous study on Turkish *BD* using SSR markers (Vogel et al., 2009). In that SSR study, inland lines generated one of the two groups, while the line forming the other group was collected from a coastal city (Vogel et al., 2009). These findings agree with the results of population structure analyses of *BD* using the DNA barcoding method (López-Alvarez et al., 2012), SNP (Tyler et al., 2016) and SSR (Vogel et al., 2009). In another previous population structure analysis of *BD* using SSR, similar to our results, the genotypes were split into two main clusters based on the geographic regions and one of the clusters was made of Californian genotypes, while the other cluster comprised of mainly Eurasian genotypes (Bakker et al., 2009). Furthermore, in the current study some genotypes belonging to close locations were found to have high genetic distance, showing that there is a low gene flow among genotypes, consistent with high self-fertilization rates in *BD* (Tyler et al., 2016).

The *Fst* inbreeding coefficient presents a measure of the genetic heterozygosity between subpopulations (Ochoa and Storey, 2019). The calculation of *Fst* using the structure analysis of the current study was 0.43 for the first group and 0.33 for the second group, and these results indicated a

great differentiation within subgroups. Moreover, group 1 had ahigher *Fst* value than group 2 as expected since the genotypes collected from the coastal regions of Turkey had a wide range of geographic location. In the previous AFLP study of Turkish *B. distachyon*, for all pairwise comparisons between different locations, the *Fst* values were considerably different from zero; thus, there was a large variation between populations (Filiz et al., 2009). A considerable amount of natural diversity was also observed in the wild grass *BD* according to a phenotypic characterisation study (Tyler et al., 2014). In previous studies, the *Fst* values were detected as 0.36 in barley (Forsberg et al., 2019), 0.18 in durum wheat (Maccaferri et al., 2005) and 0.85 in rice (Garris et al., 2003). The differences in the genetic variation levels between these close grain species may be due to differences in their breeding history and cultivation rates in the habitats of the genotypes (Casler, 2012). Furthermore, the expected heterozygosity values (0.15 for group 1 and 0.14 for group 2) demonstrated that *BD genotypes* in Turkey had a great level of genetic variation, supporting the *Fst* value.

CONCLUSION

It was demonstrated that 235 B. distachyon genotypes collected from coastal and inland regions of Turkey had large genetic variation. The iPBS marker system differentiated successfully the population collected from Turkey. This marker system presented a high level of genome diversity among the *BD genotypes* despite its small genome size. A clear distinction was found between the *BD genotypes* collected from the coastal and inland areas of Turkey. The major reason for broad genetic differences between coastal and inner land regions might be resulted from changes in the TE regions due to adaptation for different climatic conditions.

The data obtained from *B. distachyon* population will be helpful for the researchers of the Poaceae family.

ACKNOWLEDGEMENTS

We are grateful to Ege University, Faculty of Agriculture for permission to use their greenhouses.

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