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Original scientific article

INVESTIGATION OF MOLECULAR VARIABILITY IN SOME *Aegilops* SPECIES USING START CODON TARGETED POLYMORPHISM (SCoT) AND CAAT-BOX DERIVED POLYMORPHISM (CBDP) MARKERS

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Among wild relatives of wheat, *Aegilops* species are ideal genetic resources for the discovery of new characteristics such as resistance to environmental stresses and even grain quality for wheat improvement. Hence, knowledge of the population structure and genetic diversity of this germplasm is very important for their conservation and further utilization. In the present study, 80 accessions of the *Aegilops* including *Ae. tauschii*, *Ae. cylindrica* and *Ae. triuncialis* were investigated for genetic diversity using SCoT and CBDP markers. Eight SCOT and twelve CBDP primers amplified a total of 84 and 94 fragments with a mean of 10.50 and 7.83 fragments per primer, respectively. Resolving power (Rp) for SCoT and CBDP primers varied between 6.04 and 11.65, and 13.08 and 28.02, with the polymorphic information content (PIC) from 0.40 to 0.49 and 0.35 to 0.48, respectively. The results of analysis of molecular variance (AMOVA) indicated that the highest proportion of genetic variance referred to between species. SCoT primers

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indicated high values for all informativeness parameter (except resolving power) than CBDP primers across all tested accessions. However, CBDP primers indicated higher values of the genetic parameters than using SCoT primers. As a result, the maximum values for genetic parameters such as number of effective alleles (*Ne*), Nei's gene diversity (*H*) and Shannon's information index (*I*) were detected in *Ae. cylindrica* and *Ae. triuncialis* using SCoT and CBDP markers, respectively. Cluster analysis based on those molecular system grouped all accessions into three main clusters. The grouping pattern observed by CBDP primers indicated more clear phylogenetic relationship among some *Aegilops* species, so that PCoA's results confirmed the grouping pattern. In conclusion, it was observed that SCoT and CBDP displayed good efficiency in depicting polymorphism among the tested accessions, however, CBDP markers provided a clear grouping pattern of evaluated accessions. Hence, the use of CBDP markers in determining population structure and estimating genetic diversity in other plant species is recommended.

Keywords: Aegilops species, gene-targeted markers, PCoA, genetic diversity

INTRODUCTION

The wild relatives of common wheat, especially the *Aegilops* species are a source of genetic variability for wheat improvement (ZAHARIEVA *et al.,* 2003). *Aegilops* species have a long history in wheat breeding so that numerous studies indicated a good capability of these species predominantly cope with both biotic and abiotic stresses (YESAYAN *et al.* (2009); ARABBEIGI *et al.* 2014; 2009; KIANI *et al.,* 2015; POUR-ABOUGHADAREH *et al.,* 2017a, 2019; AHMADI *et al.,* 2018a). Moreover, this germplasm has been identified as a source of desirable genes conferring higher quality protein content (AHMADI *et al.,* 2018d). Furthermore, some of *Aegilops* species could have an impact on wheat quality improvement due to high amount of zinc and iron contents in their grain (CHHUNEJA *et al.,* 2006). Evaluation of genetic diversity and phylogenetic relationship among the *Aegilops* species—which are known as the main source of variability for wheat—becomes present day need due to its role as an ideal source of novel genetic variations which can be a useful for the breeders to develop new wheat cultivars for farmer and end-user point of interests (BALOCH *et al.,* 2017). One of the tools for dissecting population structure and genetic variability is DNA-based molecular markers. In this regard, different molecular marker systems are currently available. Investigation of molecular variability using different molecular markers has been exhibited to be an inexpensive and efficient task to investigate genetic diversity. In previous studies, numerous efforts have been put into the evaluation of the genetic diversity of different wild wheat species using different molecular markers (GULBITTI-ONARICI *et al.,* 2007; WANG *et al.,* 2013; MORADKHANI *et al.,* 2012 and 2015; MOUSAVIFARD *et al.,* 2015; POUR-ABOUGHADAREH *et al.,* 2017b, c; POUR-ABOUGHADAREH *et al.,* 2018; ETMINAN *et al.,* 2019). In recent years, few novel gene-based markers have been developed to aid studies of genetic analyses. Of these novel techniques, CAAT box-derived polymorphism (CBDP) and start codon-targeted polymorphism (SCoT) are suitable marker techniques to detection of genetic diversity and phylogenetic relationships among many plants. These markers have been developed based on the specific and conserved region of the genome in plant genes (COLLARD and MACKILL, 2009; SINGH *et al.,* 2014, respectively). SCoT is the simple and reliable marker system based on the short-conserved sequence and has used single primers

designed to the flanking regions of ATG codon on both DNA strands (COLLARD and MACKILL, 2009). Besides, CBDP is another promoter-targeted marker, which uses the nucleotide sequence of CAAT box, a consensus sequence GGCCAATCT, of plant promoters (BENOIST *et al.*, 1980). Moreover, several advantages such as inexpensive, reproducibility, and producing high polymorphism have caused this technique to be useful for plant genetic studies (POUR-ABOUGHADAREH *et al.*, 2022a). As the usefulness molecular marker systems, SCoT and CBDP with the high efficiency have been successfully used in peanut, orchids, *Dendrobium*, durum wheat, maize, *Salvia* spp., *Aegilops* species, wild wheat species, castor, and poppy (XIONG *et al.*, 2011; BHATTACHARYYA *et al.*, 2013; FENG *et al.*, 2015; ETMINAN *et al.*, 2016; 2018b; VIVODIC *et al.*, 2016; ETMINAN *et al.*, 2018a; POUR-ABOUGHADAREH *et al.*, 2017, 2018b; ETMINAN *et al.*, 2019; VIVODIK *et al.*, 2019; QADERI *et al.*, 2019). With this in mind, the objectives of the present study were to estimate genetic diversity, analyze molecular variance, and principal coordinate analysis using SCoT and CNDP marker systems in a core collection of *Ae. tauschii* (DD genome), *Ae. cylindrica* (CCDD genome), and *Ae. triuncialis* (UUCC genome) species.

MATERIALS AND METHODS

Genetic material and DNA isolation

A total of 80 accessions from three species, *Ae. tauschii* (35 accessions), *Ae. cylindrica* (19 accessions), and *Ae. triuncialis* (26 accessions) provided by the Ilam University Genebank (IUGB) were investigated in the present study (Table 1). All materials originated from different regions of Iran and possess different genomic constitutions. Five seeds from each accession were planted in the glasshouse and after early growth and seedling development, genomic DNA was isolated from freshly leaves based on CTAB protocol (DOYLE and DOYLE, 1987).

Table 1. The studied accessions belong to three Aegilops species with their genebank codes.

No.	Species	Genebank code	No.	Species	Genebank code	No.	Species	Genebank code
1	*Ae. triuncialis*	IUGB-03000	29	*Ae. cylindrica*	IUGB-00035	57	*Ae. tauschii*	IUGB-00141
2	*Ae. triuncialis*	IUGB-03001	30	*Ae. cvlindrica*	IUGB-00137	58	*Ae. tauschii*	IUGB-00143
3	*Ae. triuncialis*	IUGB-03002	31	*Ae. cylindrica*	IUGB-00173	59	*Ae. tauschii*	IUGB-00144
4	*Ae. triuncialis*	IUGB-03003	32	*Ae. cylindrica*	IUGB-00194	60	*Ae. tauschii*	IUGB-00151
5	*Ae. triuncialis*	IUGB-03004	33	*Ae. cylindrica*	IUGB-00217	61	*Ae. tauschii*	IUGB-00157
6	*Ae. triuncialis*	IUGB-03005	34	*Ae. cylindrica*	IUGB-00240	62	*Ae. tauschii*	IUGB-00164
7	*Ae. triuncialis*	IUGB-03006	35	*Ae. cylindrica*	IUGB-00246	63	*Ae. tauschii*	IUGB-00193
9	*Ae. triuncialis*	IUGB-03008	37	*Ae. cylindrica*	IUGB-00421	65	*Ae. tauschii*	IUGB-00198
10	*Ae. triuncialis*	IUGB-03009	38	*Ae. cylindrica*	IUGB-00035	66	*Ae. tauschii*	IUGB-00223
11	*Ae. triuncialis*	IUGB-03010	39	*Ae. cylindrica*	IUGB-00137	67	*Ae. tauschii*	IUGB-00224
12	*Ae. triuncialis*	IUGB-03011	40	*Ae. cylindrica*	IUGB-00173	68	*Ae. tauschii*	IUGB-00238
13	*Ae. triuncialis*	IUGB-03012	41	*Ae. cylindrica*	IUGB-00194	69	*Ae. tauschii*	IUGB-00245
14	*Ae. triuncialis*	IUGB-03013	42	*Ae. cylindrica*	IUGB-00217	70	*Ae. tauschii*	IUGB-00247
15	*Ae. triuncialis*	IUGB-03014	43	*Ae. cylindrica*	IUGB-00240	71	*Ae. tauschii*	IUGB-00249
16	*Ae. triuncialis*	IUGB-03015	44	*Ae. cylindrica*	IUGB-00246	72	*Ae. tauschii*	IUGB-00260
17	*Ae. triuncialis*	IUGB-03016	45	*Ae. cylindrica*	IUGB-00387	73	*Ae. tauschii*	IUGB-00261

18	*Ae. triuncialis*	IUGB-03017	46	*Ae. tauschii*	IUGB-00205	74	*Ae. tauschii*	IUGB-00263
19	*Ae. triuncialis*	IUGB-03018	47	*Ae. tauschii*	IUGB-00222	75	*Ae. tauschii*	IUGB-00269
20	*Ae. triuncialis*	IUGB-03019	48	*Ae. tauschii*	IUGB-00275	76	*Ae. tauschii*	IUGB-00273
21	*Ae. triuncialis*	IUGB-03020	49	*Ae. tauschii*	IUGB-00381	77	*Ae. tauschii*	IUGB-00274
22	*Ae. triuncialis*	IUGB-03021	50	*Ae. tauschii*	IUGB-00382	78	*Ae. tauschii*	IUGB-00276
23	*Ae. triuncialis*	IUGB-03022	51	*Ae. tauschii*	IUGB-02076	79	*Ae. tauschii*	IUGB-00279
24	*Ae. triuncialis*	IUGB-03023	52	*Ae. tauschii*	IUGB-00020	80	*Ae. tauschii*	IUGB-00289
25	*Ae. triuncialis*	IUGB-03024	53	*Ae. tauschii*	IUGB-00039			
26	*Ae. triuncialis*	IUGB-03025	54	*Ae. tauschii*	IUGB-00051			
27	*Ae. cylindrica*	IUGB-00030	55	*Ae. tauschii*	IUGB-00080			
28	*Ae. cylindrica*	IUGB-00032	56	*Ae. tauschii*	IUGB-00108			

Genotyping with SCoT markers

Eight primers were designed based on COLLARD and MACKILL (2009) (Table 2).

Table 2. CAAT box-derived polymorphism (CBDP) and start codon targeted polymorphism (SCoT) primers and their amplification results generated in the 80 Aegilops accession.

Primers	Sequences	*Ta (˚C)**	*TAB*	*NPB*	*PPB*	*PIC*	*Rp*	*MI*
SCoT1	CAACAATGGCTACCACCG	56	10	10	100	0.40	6.04	4.21
SCoT2	CAACAATGGCTACCACCT	56	11	11	100	0.41	7.20	11
SCoT3	CAACAATGGCTACCACGA	56	7	7	100	0.43	4.57	7
SCoT4	CAACAATGGCTACCAGCA	56	11	11	100	0.45	8.42	5.19
SCoT5	CCATGGCTACCACCGGCC	56	11	11	100	0.46	9.35	5.37
SCoT6	CAATGGCTACCATTAGCC	56	11	11	100	0.49	10.02	5.45
SCoT7	CCATGGCTACCACCGCCA	56	12	12	100	0.49	11.65	5.99
SCoT8	CACCATGGCTACCACCAT	56	11	11	100	0.48	10.95	5.49
			10.50	10.50	100	0.45	8.52	6.21
CBDP1	TGAGCACGATCCAATAGC	50	8	8	100	0.39	7.55	3.98
CBDP2	TGAGCACGATCCAATAAT	50	9	9	100	0.40	8.72	4.49
CBDP3	TGAGCACGATCCAATACC	50	7	7	100	0.35	7	3.46
CBDP4	TGAGCACGATCCAATAAG	50	6	6	100	0.41	5.92	2.99
CBDP5	TGAGCACGATCCAATCTA	50	5	5	100	0.40	5.07	2.49
CBDP6	TGAGCACGATCCAATCGA	50	5	5	100	0.45	5.64	2.45
CBDP7	TGAGCACGATCCAATGAT	50	7	7	100	0.46	8.62	3.31
CBDP8	TGAGCACGATCCAATGTT	50	7	7	100	0.47	6.77	3.49
CBDP9	TGAGCACGATCCAATATA	50	9	9	100	0.48	10.2	4.42
CBDP10	TGAGCACGATCCAATGAG	50	9	9	100	0.46	11.39	4.18
CBDP11	TGAGCACGATCCAATGCG	50	14	14	100	0.48	16.47	6.78
CBDP12	TGAGCACGATCCAATTGA	50	8	8	100	0.47	9.87	3.78
	Mean		7.83	7.83	100	0.44	8.60	3.82

**Ta - temperature annealing; TAB - total amplified bands; NPB - number of polymorphic; PPB - percentage of polymorphism; PIC - polymorphism information content; Rp - resolving power; MI - marker index*

Each 20 µl amplification reaction consisted of 2 µl template DNA from each sample, 2 µl of each primer, 6 µl double distilled water and 10 µl master mix 2XPCR. The PCR reaction was carried out as follows: an initial denaturation step at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 45 s, primer annealing at 56°C (for all used primers) for 45 s and primer elongation at 72°C for 90 s; the final extension at 72°C was held for 10 min. PCR products were visualized on a 1.5% agarose gel, stained with SafeView II (Parstous, Mashhad, Iran) and finally photographed under UV light.

Genotyping with CBDP markers

Twelve primers were designed based on SINGH *et al.* (2014) (Table 2). Each 20 µl amplification reaction consisted of 2 µl template DNA from each sample, 2 µl of each primer, 6 µl double distilled water and 10 µl master mix 2XPCR. The PCR reaction was carried out as follows: an initial denaturation step at 94 °C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C (for all used primers) for 1 min and primer elongation at 72°C for 2 min; the final extension at 72 °C was held for 7 min. PCR products were visualized on a 1.5% agarose gel, stained with SafeView II and finally photographed under UV light.

Data analysis

Based on the absence and presence of the bands, all PCR products were scored as 0 and 1, respectively. To investigate efficiency of used primers, several informativeness parameters such as marker index (MI), resolving power (Rp) and polymorphism information content (PIC) were estimated as proposed by PREVOST and WILKINSON (1999). To partitioning of genetic diversity between and within *Aegilops* species, analysis of molecular variance (AMOVA) was computed using GenAlEx package (PEAKALL and SMOUSE, 2006). Moreover, several genetic parameters such as the number of observed (*Na*) and effective alleles (*Ne*), Shannon's information index (*I*), Nei's gene diversity (*H*) and percentage of polymorphic loci (*PPL*) were also estimated using GenAlEx software. Cluster analysis was computed based on the Jaccard's dissimilarity matrix to investigate relationships among *Aegilops* accessions using DARwin software (PERRIER *et al.*, 2003). To demonstrate distribution of accessions a principal coordinate Analysis (PCoA) was carried out GenAlEx software.

RESULTS

Polymorphism of SCoT and CBDP markers

Our results showed that eight SCoT primers produced a total of 84 fragments across the 80 accessions, which out of all fragments were polymorphic (Table 2). The number of polymorphic bands (NPB) varied between 7 (for SCoT3) and 12 (for SCoT7) with a mean of 10.50 per primer. The average of polymorphism information content (*PIC*) indicated a high value of polymorphism (0.45) across the tested samples, ranging from 0.40 (for SCoT1) to 0.49 (for SCoT6 and SCoT7). The resolving power (*Rp*) mean value of the eight SCoT primers was 8.52 with a range of 6.04 (for SCoT1) to 11.65 (for SCoT7) as indicated in Table 2. The marker index (MI) parameter showed a mean of 6.21 and the maximum and minimum values estimated for SCoT2 (5.19) and SCoT4 (6.21), respectively. In the CBDP analysis, 12 primers amplified 94

loci, all of which were polymorphic fragments. The number of polymorphic fragments ranged from a minimum of 5 in primers CBDP5 and CBDP6 to a maximum of 14 in CBDP11. The *PIC* parameter varied between 0.35 and 0.48 with an average of 0.44. The primers CBDP11 and CBDP3 indicated the maximum and minimum value for this parameter, respectively. The mean of *Rp* was 8.60, and the highest value observed in primer CBDP11 (16.47), while the lowest was for CBDP5 (5.07). The MI parameter with a mean of 3.82 ranged from 2.45 (CBDP6) to 6.78 (CBDP11).

Genetic diversity analysis

Based on each marker system, analysis of molecular variance (AMOVA) was computed (Table 3). In SCoT analysis the percentage molecular variance was higher between species (59%) than within species (41%). Furthermore, based on CBDP data the high portion of genetic variance was observed between species (52%) compared to within species (42%). Based on SCoT data, average value of *Na* across three species was 1.55, and *Ae. tauschii* (1.70) showed the maximum value. The *Ne* ranged from 1.29 (*Ae. triuncialis*) to 1.44 (*Ae. cylindrica*) with an average of 1.36. The *I* parameter varied between 0.28 and 0.39 with a mean of 0.33, and *Ae. cylindrica* and *Ae. triuncialis* displayed the maximum and minimum amounts, respectively. The *H* parameter with an average of 0.22 varied between 0.18 (*Ae. triuncialis*) and 0.26 (*Ae. cylindrica*). The average of *PPL* was 73.41% and it varied between 64.29 and 78.57%. Also, the highest and lowest values of this parameter was estimated in *Ae. cylindrica* and *Ae. triuncialis*, respectively. The number of private alleles (*PL*) varied between 0 and 6, and *Ae. tauschii* had the maximum *PL* compared to other species. In CBDP analysis, *Na* ranged from 1.51 (*Ae. cylindrica*) to 1.63 (*Ae. tauschii*) with an average of 1.61. Four genetic parameters *Ne*, *I*, *H* and *PPL* ranged between 1.32–1.56, 0.30–0.46, 0.20–0.31 and 65.96%–85.11%, respectively. The *Ae. triuncialis* and *Ae. tauschii* species had the highest and lowest values for these parameters, respectively (Table 4). In contrast, the highest private number of alleles (4) was recorded in *Ae. tauschii* accessions.

Table 3. Analysis of molecular variance (AMOVA) in Aegilops species.

Source of variation	SCoT		CDBP	
	Between species	Within species	Between species	Within species
df	2	77	2	77
SS	545.85	741.58	622.29	840.12
MS	272.92	9.63	311.15	10.91
Est.Var	10.18	9.63	11.61	10.91
Var	51%	49%	52%	48%

SS, sum of squares; MS, mean squares; Est. Var, estimated variance components; Var, total variance

Table 4. Estimated genetic variation parameters for different Aegilops species using SCoT and CBDP primers

Marker	Species	*Na*	*Ne*	*I*	*H*	*PL*	*PPL*
SCOT	*Ae. triuncialis*	1.35	1.29	0.27	0.18	0	64.29%
	Ae. cylindrica	1.59	1.43	0.38	0.25	1	78.57%
	Ae. tauschii	1.70	1.35	0.32	0.21	6	77.38%
	Mean	1.55	1.36	0.33	0.21	2.33	73.41
CBDP	*Ae. triuncialis*	1.70	1.56	0.46	0.31	0	85.11%
	Ae. cylindrica	1.51	1.38	0.33	0.22	0	67.02%
	Ae. tauschii	1.63	1.32	0.30	0.20	4	65.96%
	Mean	1.61	1.41	0.36	.24	1.33	72.70%

Na, Ne, I, H, PL and PPL indicate Number of observed alleles, observed number of alleles, Shannon's information index, Nei's gene diversity, number of private alleles and percentage of polymorphic loci

Genetic distance coefficients and grouping of the accessions

In SCoT analysis, the pairwise genetic distance coefficients ranged from 0.05 to 0.93 with an average of 0.63 among the 80 samples of *Aegilops*. The highest genetic distance (0.93) was recorded between two samples of *Ae. triuncialis* and *Ae. cylindrica* species, while the minimum distance (0.05) was estimated between two samples from *Ae. cylindrica* species. Besides, the genetic distance coefficients estimated by CBDP data ranged from 0.03 to 0.96 with an average of 0.52. The maximum distance (0.96) was showed between two samples from *Ae. triuncialis* and *Ae. tauschii* species, whereas the minimum distance (0.03) was estimated between two samples from *Ae. triuncialis* species (distance coefficient not shown).

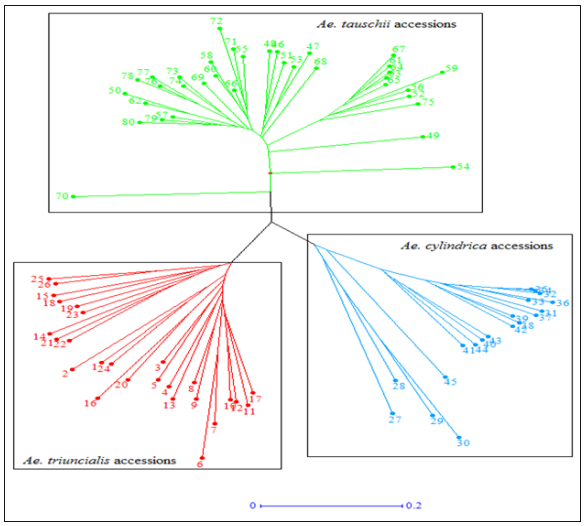

Figure 1. Radial dendrogram created with a neighbor-joining clustering algorithm from the distance coefficient matrix among 80 *Aegilops* accessions using SCoT markers.

The cluster analysis was computed based on the neighbour-joining (NJ) method. The radial dendrogram generated by SCoT data indicated a clear grouping pattern among the studied samples (Figure 1). All samples were grouped in three main. All accessions from *Ae. triuncialis* (samples 1–26) and *Ae. cylindrica* (samples 27–47) were grouped in two separate clusters. Moreover, all *Ae. tauschii* accessions (samples 48–80) far from other samples placed into a distinct cluster. The dendrogram rendered by CBDP data ambiguously grouped all samples in different clusters (Figure 2). Out of 26 accessions from *Ae. triuncialis*, five samples were clustered far from other accessions and grouped with *Ae. triuncialis* accessions in the same cluster. Other samples from *Ae. triuncialis* separately make a separated cluster. All *Ae. tauschii* accessions separated from other samples into a distinct cluster. To confirm of these results, the PCoA was done based on the data of SCoT and CBDP. In each analysis the first two components jointly accounted for more than 50% of the total variation (SCoT = 55.88% and CBDP = 57.73%) and all the samples from each species were clustered into a distinct group, so that the grouping patterns confirmed the results of cluster analysis (Figure 3).

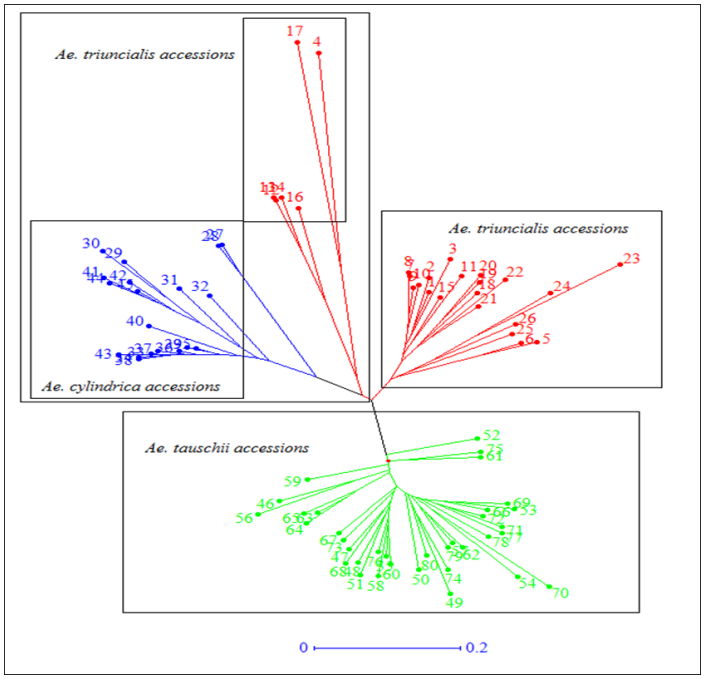

Figure 2. Radial dendrogram created with a neighbor-joining clustering algorithm from the distance coefficient matrix among 80 *Aegilops* accessions using CBDP markers

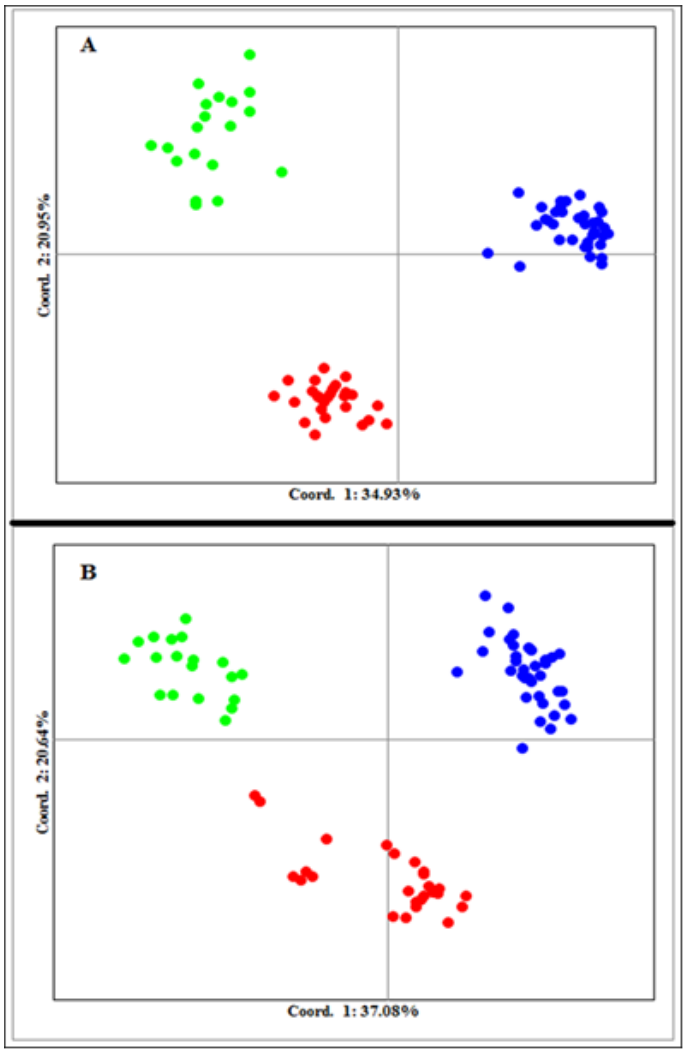

Figure 3. Biplot derived from the PCoA of the 80 Aegilops accessions using (A) SCoT and (B) CBDP markers. Green, blue and red circles indicate *Ae. cylindrica*, *Ae. tauschii* and *Ae. triuncialis*, respectively

DISCUSSION

In this study, SCoT and CBDP techniques were applied to investigate the genetic diversity in the 80 accessions of three *Aegilops* species. Our results indicated that there is a high level genetic diversity between and within the studied species. Likewise, several studies reported a good level of polymorphism between species of *Aegilops* (THOMAS and BEBELI, 2010;

MORADKHANI *et al.*, 2015; POUR-ABOUGHADAREH *et al.*, 2018; ETMINAN *et al.*, 2019). In addition, in the present study the efficiencies of SCoT and CBDP for estimating the genetic diversity were compared. Both marker techniques represented 100% polymorphism. In general, SCoT markers revealed higher values of *NPB*, *PIC* and *MI*. However, the mean of *Rp* value for CBDP was found to be 8.60 *vs*. 8.52 for SCoT markers (Table 2). It has been reported that, *PIC*, *Rp* and *MI* provide a useful criterion in determination of efficiency of markers in genetic analyses (POUR-ABOUGHADAREH *et al.*, 2018). Our results indicated that average of *PIC* value for SCoT ranged from 0.40 to 0.49 with an average of 0.45 per primer and the range of values in these primers was greater than CBDP. This result suggesting that the good efficiency of SCoT technique to display level of polymorphism in *Aegilops* germplasm. In contrast, the mean of *MI* for SCoT primers was more than CBDP, which indicated the high capability of the SCoT in the estimation of genetic diversity in *Aegilops* spp. These results were supported by ETMINAN *et al.* (2016 and 2018a), POUR-ABOUGHADAREH *et al.* (2018) and QADERI *et al.* (2019), who reported that SCoT primers were more useful for the represent of the high level of diversity and structure analysis compared to other molecular marker techniques.

In this study, significant variation was observed among and within *Aegilops* species based on SCoT and CBDP markers. The portion of estimated variation by the SCoT marker was approximately equal and in the CBDP marker, so that both marker systems indicated that the portion of within diversity was less than among variation (Table 3). Likewise, a previous study on *Aegilops* germplasm showed a greater variation between populations than within them (POUR-ABOUGHADAREH *et al.*, 2022a, b). From viewpoint of the genetic parameters, CBDP marker indicated more values for all genetic parameters (*Na*, *Ne*, *I* and *H*) compared to SCoT (Table 4), suggesting that this marker has a capability to represent of genetic variation between different species. However, each marker system revealed a different result in identification of species with high level of diversity. Based on SCoT marker, *Ae. cylindrica* showed the maximum values for *Ne*, *I*, *H* and *PPL*, while *Ae. triuncialis* was recognized as the species with highest values for these parameters using CBDP markers. Several studies previously demonstrated that *Ae. cylindrica* and *Ae. triuncialis* have huge allelic variation and these species due to their useful genes and even alleles can response well to abiotic stresses such as drought and salinity. AHMADI *et al.* (2018a, b, c) reported that among *Aegilops* species, *Ae. cylindrica* has a good ability in cope with drought and salinity stresses. In another studies, POUR-ABOUGHADAREH *et al.* (2017) and AHMADI *et al.* (2018a) indicated that a considerable root system and photosynthetic capacity of *Ae. cylindrica* under drought stress conditions. Hence, observation the high level of genetic diversity in this species can refer to these useful abilities.

In our study, averages of genetic distance of studied samples estimated by SCoT and CBDP were 0.63 and 0.52, respectively, which results in a different grouping pattern of studied accessions by multivariate analyses. Dendrogram resulted from the neighbor-joining method demonstrated that the grouping pattern obtained by SCoT was clear than CBDP (Figures 1 and 2). However, in both grouping patterns, there is a phylogenetic relationship among species so that grouping of *Ae. cylindrica* with *Ae. triuncialis* accessions together in a same group shows that the genome of these species more similar to each other due to their common parent (*Ae. caudata* as the donor of C genome) (KIMBER and ZHAO, 1983; BADAEVA *et al.,* 2004). Moreover,

PCoA confirmed these results and revealed that accessions were grouped into different clusters according to their species classification pattern (Figure3).

CONCLUSIONS

Knowledge of the genetic diversity in wheat germplasm is one of the first strategies their conservation as well as utilization of them in wheat breeding programs. Our results suggested that there was a high level of genetic diversity in *Ae. tauschii*, *Ae. triuncialis* and *Ae. cylindrica*, species as revealed by SCoT and CBDP analyses. It can, therefore, be assumed that these markers are not only useful in differentiating wild species with different genomic constitutions (D, UC and DC genome, respectively), but also in evaluating the genetic diversity of accessions within species. Hence, we recommend that these novel DNA-based marker could be used in other genetic analyses.

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ISTRAŽIVANJE MOLEKULARNE VARIJABILNOSTI U NEKIM VRSTAMA Aegilopsa KORIŠĆENJEM POLIMORFIZMA CILJANOG POČETNOG KODONA (SCoT) I MARKERA POLIMORFIZMA IZVEDENIH CAAT-BOKS-om (CBDP)

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Izvod

Među divljim srodnicima pšenice, vrste *Aegilops* su idealni genetski resursi za otkrivanje novih karakteristika kao što su otpornost na stresove okoline, pa čak i kvalitet zrna za poboljšanje pšenice. Zbog toga je poznavanje populacione strukture i genetičkog diverziteta ove germplazme veoma važno za njihovo očuvanje i dalje korišćenje. U ovoj studiji, kod 80 uzoraka *Aegilops* uključujući *Ae. tauschii, Ae. cilindrica i Ae. triuncialis* istraživani su genetski diverzitet korišćenjem SCoT i CBDP markera. Osam SCOT i dvanaest CBDP prajmera amplificiralo je ukupno 84 i 94 fragmenta sa srednjim vrednostima od 10,50 i 7,83 fragmenata po prajmeru, respektivno. Rezoluciona moć (Rp) za SCoT i CBDP prajmere varirala je između 6,04 i 11,65 i 13,08 i 28,02, sa sadržajem polimorfne informacije (PIC) od 0,40 do 0,49 i 0,35 do 0,48, respektivno. Rezultati analize molekularne varijanse (AMOVA) pokazali su da je najveći udeo genetičke varijanse između vrsta. SCoT prajmeri su pokazali više vrednosti za sve parametre informativnosti (osim moći rezolucije) nego CBDP prajmeri u svim testiranim uzorcima. Međutim, CBDP prajmeri su ukazivali na veće vrednosti genetskih parametara nego korišćenje SCoT prajmera. Kao rezultat toga, maksimalne vrednosti za genetske parametre kao što su broj efektivnih alela (Ne), Nei gensk diverzitet (H) i Šenonov indeks (I) su otkrivene u *Ae. cilindrica* i *Ae. triuncialis* koristeći SCoT i CBDP markere, respektivno. Klaster analiza zasnovana na tim molekularnim sistemima grupisala je sve uzorke u tri glavna klastera. Obrazac grupisanja koji su dali CBDP prajmeri ukazao je na jasniji filogenetski odnos među nekim vrstama *Aegilops*, tako da su rezultati PCoA potvrdili obrazac grupisanja. U zaključku, primećeno je da su SCoT i CBDP pokazali dobru efikasnost u prikazivanju polimorfizma među testiranim uzorcima, ali su CBDP markeri dali veoma jasan obrazac grupisanja evaluiranih uzoraka. Stoga se preporučuje upotreba CBDP markera u određivanju strukture populacije i proceni genetičkog diverziteta drugih biljnih vrsta.

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