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Applicability of CAAT box-derived polymorphism markers in the study of genetic diversity in *Aegilops tauschii*

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ABSTRACT

In this study, the molecular diversity among 95 accessions of *Aegilops tauschii* was investigated using a CAAT-box derived polymorphism (CBDP) marker system. Fifteen CBDP primers produced a total of 73 bands (4.87 bands per primer), of which 66 bands (91.9%) were polymorphic. The mean of polymorphic information content (PIC=0.26) and resolving power (Rp=5.62) indicated the capability of this marker system in evaluating genetic diversity in *Ae. tauschii*. In addition, the mean percentage of polymorphic loci, the number of observed alleles, effective alleles, the Shannon information index, and genetic diversity criterion were estimated to be 91.90%, 1.83, 1.47, 0.44, and 0.27, respectively. Analysis of molecular variance revealed that intra-population variation accounted for 96% of the total variance, while the remaining 4% was related to inter-population variation. Cluster analysis grouped the accessions into 7 main clusters. Also, according to principal coordinate analysis (PCoA), accessions were classified into six main groups, where the first two principal coordinates explained 24.19% of the total variation. The results of cluster analysis were relatively consistent with the results of PCoA. In this study, although the CBDP marker system did not completely separate Iranian accessions from other accessions however, accessions from Turkmenistan, Russia, Georgia, Turkey, Japan, and Kosovo were grouped in separate clades. Overall, the results showed that the CBDP marker system effectively identifies polymorphisms in the studied accessions. The results of this research suggest that the studied CBDP markers can be used in genetic fingerprinting and analysis of relationships between wheat and its related germplasm to evaluate various agronomic traits as well as tolerance to environmental stresses.

***Key words*: *Aegilops tauschii*, CBDP markers, Genetic resources, Molecular markers.**

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INTRODUCTION

Due to their adaptation to different environmental conditions wild relatives are considered a rich genetic source for finding genes and alleles to deal with various biotic and abiotic stresses and even grain quality (Schneider *et al*., 2008; Pour-Aboughadareh *et al*., 2017). *Aegilops tauschii* (2n=2x=14) is a diploid and self-pollinated species that has played an essential role in wheat domestication. This species is an important source of genetic diversity and has been frequently used in wheat breeding programs (Luo *et al*., 2013). *Ae. tauschii* (donor of the D genome to common wheat) has shown resistance to various pests and pathogens, which are significant in agriculture, and there are reports of its valuable physiological characteristics such as resistance to low temperature, salt tolerance, and remarkable performance in low-yielding as well as dry environments (Hojjatollah *et al*., 2006). Moreover, *Ae. tauschii* is one of the primary ancestors of several *Aegilops* species, including *Ae. cylindrica* (DDCC-genome), *Ae. ventricosa* (DDNN-genome), *Ae. crassa* (DDMM- and DDDDMM-genomes), *Ae. juvenalis* (DDMMNN-genome) and *Ae. syriacum* (DDMMSS-genome) (Kimber and Sears, 1983).

Estimating the diversity of wheat wild relatives is one of the main conditions for breeding objectives (Moradkhani *et al*., 2012). Genetic diversity in wheat germplasm opens up new scenarios to discover valuable genes or alleles and improves varieties with traits of interest to both the farmers and the breeders such as yield potential, large seed, high seed weight, and tolerance to both biotic and abiotic stresses (Moradkhani *et al*., 2015). According to reports, different regions of Iran have the most diverse wheat germplasm in the world. The western regions of the country are the main distribution centers of Einkorn and *Aegilops* wheat species, which are a good source of valuable genes for wheat breeding purposes (Tabatabaei and Maassoumi, 2001).

CAAT box-derived polymorphism (CBDP) is a dominant gene-targeted marker that targets the sequences of the CAAT box region of the promoter. This class of primers consists of 18 nucleotides with a central CCAAT core flanked by a filler sequence at the 5' end and two or three nucleotides at the 3' end (Singh *et al*., 2014). The advantages of this technique include extensive genetic information, low cost, and high polymorphism (Heikrujam *et al*., 2015; Rajesh *et al*., 2015; Etminan *et al*., 2018; Collard and Mackill, 2019).

Different molecular marker systems have been used to investigate genetic diversity in wheat germplasm sources and relatives. Saeidi *et al*. (2006) reported a significant genetic diversity in an *Ae. tauschii* population collected from different regions of Iran using Simple Sequence Repeats (SSR) markers. Also, the genetic diversity of *Ae. tauschii* species have been studied through morphological indices, isozymes, Restriction Fragment Length Polymorphisms (RFLPs), and microsatellite markers (Gholamian *et al*., 2019). Bokaei *et al.* (2023) investigated the level of genetic diversity among 157 *Aegilops* accessions consisting of *Ae. tauschii* Coss. (DD genome), *Ae. crassa* Boiss. (DDMM genome), and *Ae. cylindrica* Host. (CCDD genome) using two sets of CBDP and SCoT markers. The SCoT and CBDP primers yielded 171 and 174 fragments, out of which 145 (90.23%) and 167 (97.66%) fragments were polymorphic, respectively. Pandey *et al*. (2023) used the CBDP marker to evaluate genetic assortment and interrelatedness among and within wild inhabitants of *Cannabis sativa*. The twenty-one CBDP primers generated a total of 80 alleles with an average of 6.65 alleles per primer. The average polymorphic information content (PIC) and resolving power (Rp) per primer indicated that CBDP is an ideal marker system for studying genetic diversity in *C. sativa*. The population diversity analysis based on the CBDP marker dataset revealed a high genetic differentiation (Gst>0.15), and a low estimated gene flow (Nm<1.0) between the studied populations.

This study aimed to evaluate genetic diversity within and among *Ae. tauschii* accessions collected from Iran and other countries using the CBDP marker system, and demonstrating the capability of this marker system in differentiating and grouping the studied accessions is another goal of this research.

MATERIALS AND METHODS

Plant materials and DNA extraction

The plant materials of this study included 95 *Aegilops tauschii* accessions collected from 15 different countries, whose characteristics are presented in Table 1. The seeds were provided by Ilam University Gene Bank (IUGB). After sowing in pots at greenhouse conditions, genomic DNA was extracted from young leaves according to the CTAB method (Doyle and Doyle, 1987). The quality and quantity of DNA samples were then determined using 0.8% agarose gel electrophoresis and spectrophotometrically (Nanodrop, Thermo; 2000) methods. The amount of DNA extracts was measured by light absorption at 260, and 280 nm, and all samples were diluted with sterile distilled water and delivered to a concentration of 50 ng L^{-1}.

Table 1. Codes and origin of the 95 accessions of *Aegilops tauschii*.

Genotypes	Accession code	Source	Genotypes	Accession code	Source
4	BW_01027	Armenia	291	BW_01138	Iran
16	BW_01046	Armenia	292	BW_01139	Iran
32	BW_01047	Armenia	293	BW_01143	Iran
36	BW_01048	Armenia	296	BW_01147	Iran
37	BW_01183	Armenia	298	BW_01149	Iran
38	BW_01184	Armenia	303	BW_01156	Iran
44	BW_01192	Armenia	307	BW_01157	Iran
63	BW_01007	Azerbaijan	314	BW_01160	Iran
65	BW_01008	Azerbaijan	316	BW_01163	Iran
66	BW_01009	Azerbaijan	325	BW_01164	Iran
67	BW_01010	Azerbaijan	328	BW_01166	Iran
70	BW_01011	Azerbaijan	330	BW_01168	Iran
71	BW_01012	Azerbaijan	331	BW_01173	Iran
72	BW_01040	Azerbaijan	333	BW_01189	Iran
73	BW_01043	Azerbaijan	337	BW_01126	Japan
75	BW_01044	Azerbaijan	338	BW_01128	Japan
76	BW_01045	Azerbaijan	339	BW_01129	Japan
115	BW_01067	Azerbaijan	341	BW_01014	Kazakhstan
123	BW_01069	Azerbaijan	342	BW_01013	Pakistan
125	BW_01072	Azerbaijan	344	BW_01036	Pakistan
126	BW_01091	Azerbaijan	345	BW_01037	Pakistan
127	BW_01102	Azerbaijan	346	BW_01055	Kosovo
128	BW_01104	Azerbaijan	347	BW_01085	Kosovo
137	BW_01108	Azerbaijan	348	BW_01093	Kosovo
140	BW_01119	Azerbaijan	225	BW_01087	Russia
166	BW_01120	Azerbaijan	352	BW_01030	Russia
167	BW_01121	Azerbaijan	353	BW_01088	Russia
214	BW_01122	Azerbaijan	356	BW_01134	Russia
217	BW_01123	Azerbaijan	360	BW_01050	Syria
219	BW_01034	China	361	BW_01029	Tajikistan
221	BW_01038	China	372	BW_01035	Tajikistan
229	BW_01028	Georgia	373	BW_01101	Tajikistan
233	BW_01056	Georgia	374	BW_01032	Turkey
238	BW_01057	Georgia	376	BW_01033	Turkey
244	BW_01058	Georgia	379	BW_01080	Turkey
246	BW_01059	Georgia	380	BW_01081	Turkey
249	BW_01185	Georgia	381	BW_01082	Turkey
271	BW_01186	Georgia	382	BW_01131	Turkey
272	BW_01002	Iran	385	BW_01026	Turkmenistan
273	BW_01003	Iran	387	BW_01051	Turkmenistan
274	BW_01006	Iran	405	BW_01053	Turkmenistan
277	BW_01086	Iran	409	BW_01054	Turkmenistan
279	BW_01094	Iran	447	BW_01089	Turkmenistan
280	BW_01095	Iran	448	BW_01115	Turkmenistan
282	BW_01099	Iran	449	BW_01127	Turkmenistan
283	BW_01132	Iran	450	BW_01031	Uzbekistan
287	BW_01133	Iran	452	BW_01049	Uzbekistan
289	BW_01136	Iran			

DNA fingerprinting

The primer sequences used in this research were made based on the study of Singh *et al*. (2014). After optimizing the PCR reactions, fifteen primers having polymorphism and amplified bands, that could be scored, were selected. The reaction mixture for all 15 primers included 7.5 µl of ready-to-use PCR master mix 1X (Ampliqon), 5.5 µl of sterile distilled water, 1 µl of template DNA from each sample, and 1 µl of each primer with a final volume of 15 µl. DNA amplifications

Table 2. Characteristics and the estimated marker indices of the CBDP primers studied (Singh *et al.*, 2014).

Primer	Sequence (5´→3´)	TAB	NPB	PPB	PIC	Rp	MI
CBDP-1	TGAGCACGATCCAATAGC	4	4	100	0.24	5.08	0.96
CBDP-2	TGAGCACGATCCAATAAT	7	7	100	0.38	5.98	2.66
CBDP-3	TGAGCACGATCCAATAAG	7	7	100	0.30	8.78	2.10
CBDP-4	TGAGCACGATCCAATCTA	8	8	100	0.35	8.62	2.80
CBDP-5	TGAGCACGATCCAATCGA	4	4	100	0.32	6.02	1.28
CBDP-6	TGAGCACGATCCAATGTT	5	4	80	0.15	4.9	.75
CBDP-7	TGAGCACGATCCAATATA	4	4	100	0.37	5.78	1.48
CBDP-8	TGAGCACGATCCAATTGA	4	3	75	0.25	4.72	1.00
CBDP-9	TGAGCACGATCCAATTTG	3	3	100	0.36	3.3	1.08
CBDP-10	CTGAGCACGATCCAATAG	5	5	100	0.27	6.22	1.35
CBDP-11	CTGAGCACGATCCAATAT	3	3	100	0.22	4.9	0.66
CBDP-12	CTGAGCACGATCCAATCA	7	4	75	0.07	6.74	0.03
CBDP-13	CTGAGCACGATCCAATCG	4	4	100	0.29	2.62	1.16
CBDP-14	CTGAGCACGATCCAATGA	2	2	100	0.40	2.76	0.80
CBDP-15	CTGAGCACGATCCAATGT	6	4	66.6	0.25	7.9	0.20
	Mean	4.87	4.4	91.9	0.26	5.62	1.22

TAB: Total amplified bands, NPB: Number of polymorphic bands, PPB: Percentage of polymorphic bands, PIC: Polymorphism information content, MI: Marker index, Rp: Resolving power.

were carried out in a thermocycler (Techne-5000). CBDP-PCR amplifications were performed with a first cycle of initial DNA denaturation at 94 °C for 5 min, followed by five cycles of 1 min denaturation at 94 °C, 1 min annealing at 35 °C, and 1 min of extension at 72 °C. In the following 35 cycles, the annealing temperature went up to 50 °C with a final extension of 72 °C for 10 min. The PCR product was separated in 3% agarose gel and stained with a safe stain (Sinaclon) to observe the amplified fragments (Figure 1).

Data analysis

Scoring of band patterns was done based on criteria of 0 (absence of band) and 1 (presence of band), and also, the values of indices determining the marker system efficiency were calculated for each primer. The calculated performance indices included total amplified bands (TAB), percentage of polymorphism bands (PPB), polymorphism information content (PIC), resolving power (Rp), and the marker index (MI). PIC was calculated based on the formula $PIC=2\times fi\times(1-fi)$ (Roldan-Ruiz *et al.*, 2000), where fi is the frequency of the amplified allele (band present) and (1-fi) is the frequency of the null allele (band absent). MI is the product of the PIC and the effective multiplex ratio (Varshney *et al.*, 2007). The Rp of each primer was calculated using the formula $Rp=\sum Ib$, where Ib is band informativeness (the Ib can be represented on a scale of 0-1 by the following formula: $Ib=1-[2\times(0.5-p)]$, where p is the proportion of individual containing the band) (Prevost and Wilkinson, 1999).

Analysis of molecular variance (AMOVA) was performed using the GenAlEx package ver. 6.5 to evaluate the distribution of genetic diversity between and within Iranian and foreign accessions (Peakal and Smouse, 2006). Using POPGENE software ver. 1.31, genetic parameters such as the number of alleles (Na), the effective number of alleles (Ne), Shannon's information index (I), Nei genetic diversity (H), and the percentage of polymorphic loci (PPL) were calculated (Yeh *et al.*, 1997). The values of genetic distances between pairs of accessions were calculated based on the Jaccard distance coefficient with NTSYS software (Rholf, 2000). To group the accessions, cluster analysis was performed using the UPGMA method as well as principal coordinate analysis (PCoA) using XLSTAT software.

RESULTS

CBDP Polymorphism

Fifteen CBDP primers produced a total of 73 bands with an average of 4.87 bands per primer, of which 66 bands were shown polymorphism (91.9%). Total amplified bands (TAB) ranged from 2 (for CBDP-14) to 8 (for CBDP-4), with an average of 4.87. The mean PIC was 0.26, ranging from 0.07 (CBDP-12) to 0.40 (CBDP-14). The Rp ranged from 2.62 (CBDP-13) to 8.78 (CBDP-3), with an average of 5.62 per primer. The average marker index (MI) was estimated to be 1.22, ranging from 0.03 for CBDP-12 to 2.80 for CBDP-4, which indicated the existence of a wide range of diversity among the examined primers (Table 2).

Table 3. Analysis of molecular variance based on CBDP markers in Iranian and foreign accessions of *Ae. tauschii*.

Source	df	SS	MS	Est. Var	Var (%)
Among pops	1	24.24	24.24	0.396	4
Within pops	93	932.68	10.03	10.03	96
Total	94	956.93	–	10.43	100

Table 4. Estimation of genetic parameters in Iranian and foreign accessions of *Ae. tauschii* using CBDP markers.

Parameters	Iranian genotypes	Foreign genotypes
Observed number of alleles (*Na*)	1.86	1.80
Effective number of alleles (*Ne*)	1.48	1.46
Nei's genetic diversity (*H*)	0.28	0.27
Shannon's information index (*I*)	0.43	0.41
Percentage of polymorphic loci (*PPL* %)	86.30	82.19

Genetic diversity analysis

To partition the genetic diversity, an analysis of molecular variance (AMOVA) was performed (Peakall and Smouse, 2006). The result of the AMOVA showed that more than 96% of the genetic diversity belonged to within populations (Iranian and foreign accessions), while the variation between the populations was only 4% (PhiPT=0.038; p=0.01) (Table 3). The estimated genetic parameters for Iranian and foreign accessions are shown separately in Table 4. On average, the number of observed alleles (Na) was 1.83, and Iranian accessions, with an average of 1.86, had more Na than foreign accessions (1.8). Also, the average number of effective alleles was calculated to be 1.47 and foreign accessions had fewer effective alleles than Iranian accessions. In addition, Shannon's information index (I) was found to be 0.44 on average, ranging from a minimum of 0.41 to a maximum of 0.48. Moreover, the average Nei's genetic diversity (H) was recorded as 0.27 which was 0.27 and 0.28 for foreign and Iranian accessions, respectively. Furthermore, the percentage of polymorphic loci (PPL) had a mean of 84.25 and showed a value of 82.19 for foreign and 86.30 for Iranian accessions.

Genetic distances and grouping the accessions

Estimation of the Jaccard distance coefficient showed that genetic distances among accessions ranged from 0.48 to 0.96 (data not shown). The lowest distance was observed between two accessions belonging to Azerbaijan and Iran. In contrast, the highest genetic distances were found between two accessions from Japan, two accessions from Turkmenistan, and two accessions belonging to Turkmenistan and Uzbekistan. In addition, the population analysis results showed that the diversity among populations (Gst) and gene flow (Nm) were 0.034 and 14.53, respectively (data not shown).

To investigate the genetic relationships among *Ae. tauschii* accessions, cluster analysis was performed using the Jaccard similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA) algorithm. The UPGMA method was used because its cophenetic correlation coefficient (r_{cop}=0.87) was higher compared to the other clustering methods. According to the obtained cluster dendrogram, 95 *Ae. tauschii* accessions were put into seven main groups (Figure 2). Group one included two accessions from Armenia and Azerbaijan. Similarly, in group two, most of the accessions were from Armenia and Azerbaijan. The three accessions in the third group belonged to Iran. Group four, containing accessions from most countries, was further divided into four subgroups. All accessions of group five were from Azerbaijan. The sixth group included two accessions from Iran, and one accession from Iran was placed separately in the seventh group. The PCoA results indicated that the first two principal coordinates explained 24.19% of the total variation (15.04 and 9.15% by pc1 and pc2, respectively). As shown in Figure 3, the PCoA separated the accessions roughly into six main groups. The results obtained from the cluster analysis were almost identical to the results of the PCoA. Also, the results of both cluster and PCoA analyses revealed that although the use of CBDP primers could not thoroughly separate Iranian accessions from other foreign accessions, accessions from some countries such as Turkmenistan, Russia, Georgia, Turkey, Japan, and the Republic of Kosovo were grouped into separate clads.

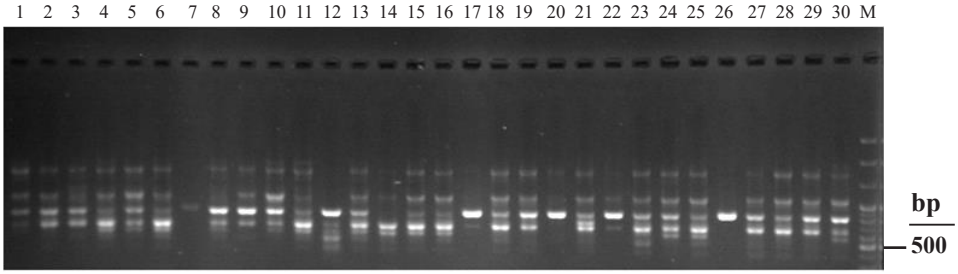

Figure 1. Amplification of genomic DNA from different *Ae. tauschii* genotypes with CBDP7. M: 100bp marker, accession 1 to 8 from Armenia and 9 to 30 from Azerbaijan.

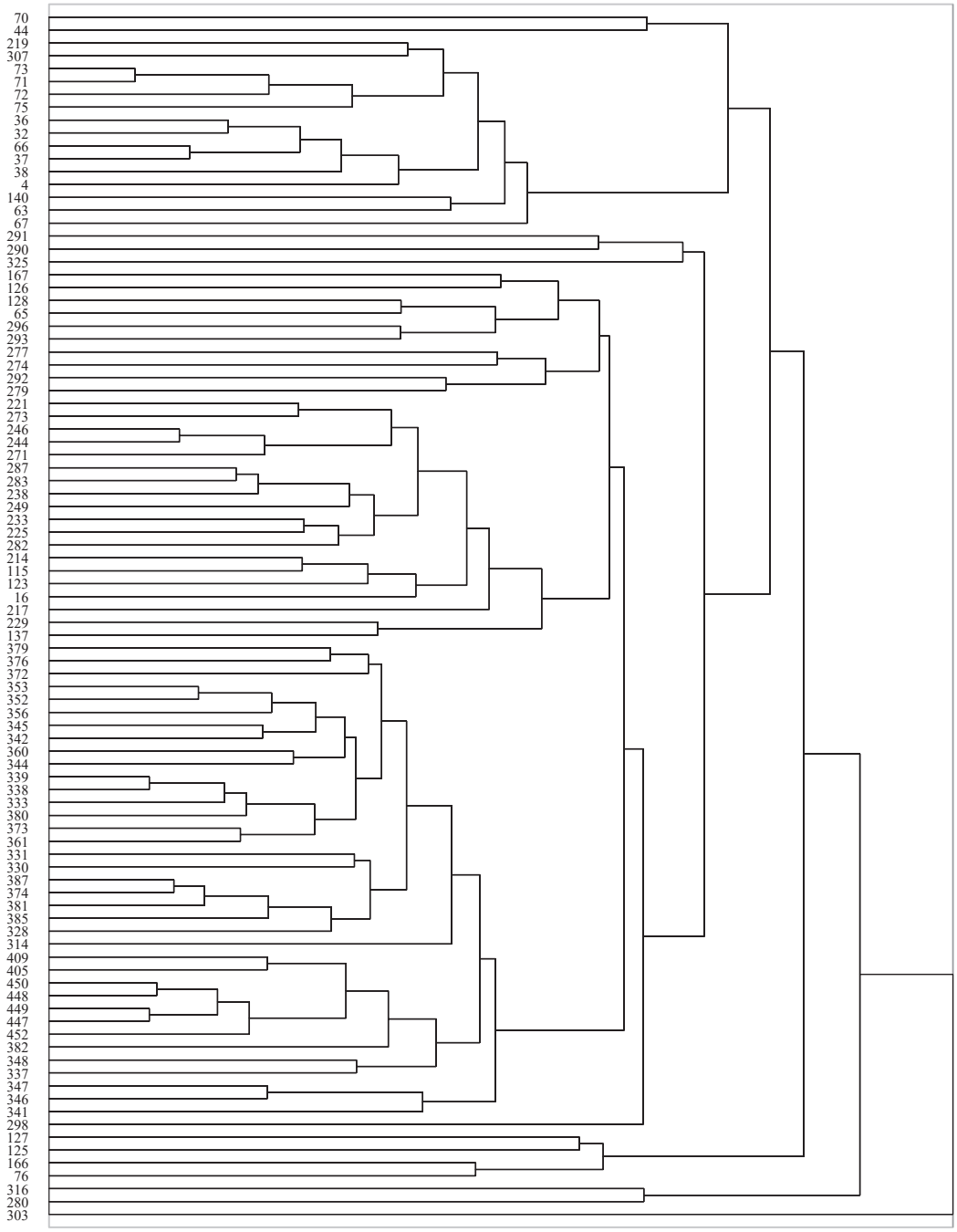

Figure 2. Dendrogram rendered for 95 accessions of *Ae. tauschii* using Jaccard's coefficients and the UPGMA method based on CBDP primers.

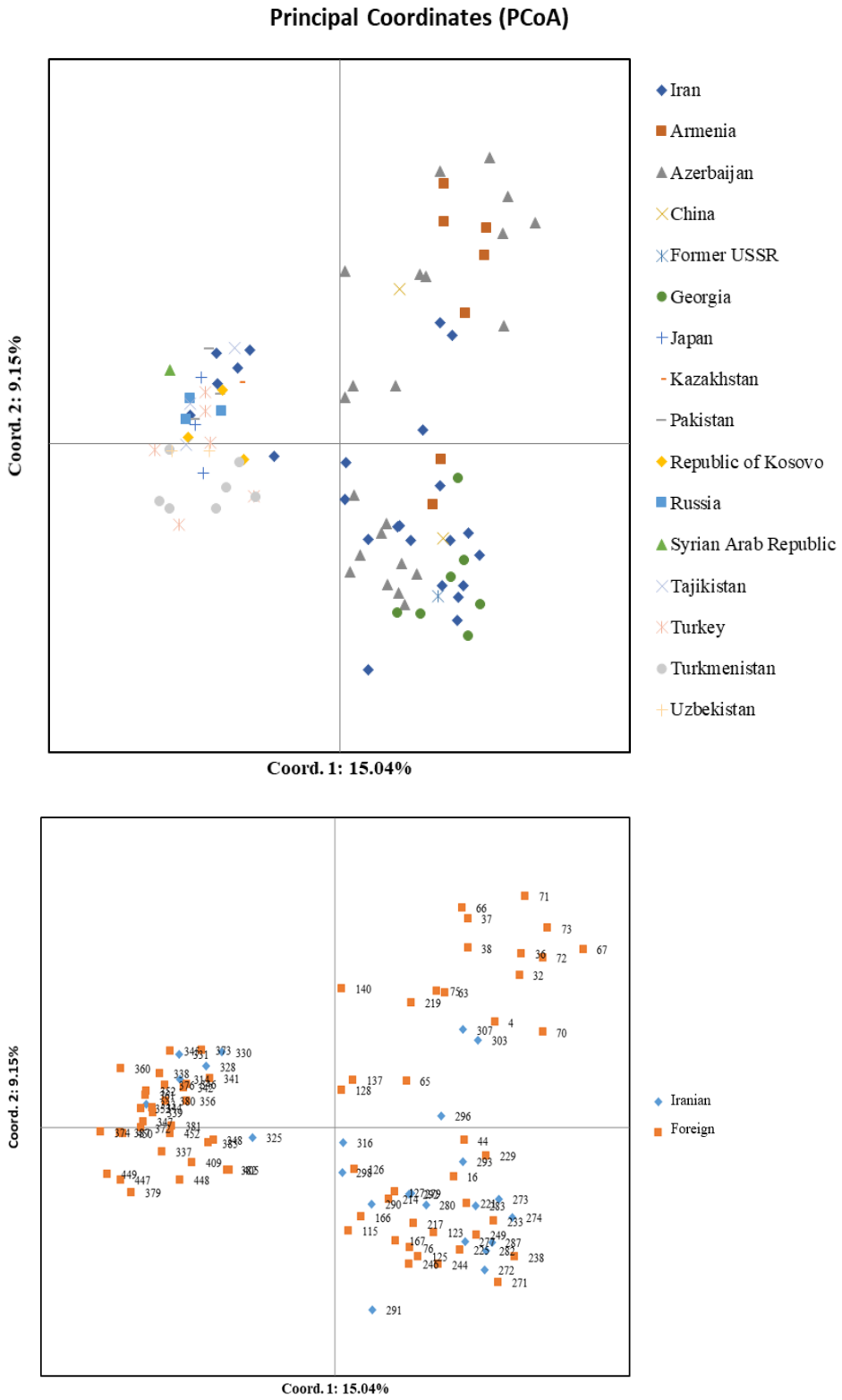

Figure 3. Biplots (Coord 1 and Coord 2) for 95 accessions of *Ae. tauschii* using CBDP primers.

DISCUSSION

Advances in molecular tools have led to a paradigm shift in the use of random, and DNA-based markers to gene-based markers, as well as the introduction of new marker systems (Poczia *et al*., 2013). In this regard, CBDP markers can be considered among new marker systems. Although various studies have been conducted concerning the evaluation of genetic diversity and the investigation of relationships between relatives in different plants using this technique, however, little information is available regarding the ability of this technique in the evaluation of genetic diversity and grouping of wheat wild relatives. Therefore; the results of this study would be helpful in determining the efficiency of this marker system. The primers used in this research showed a high polymorphism of about 91.9%, which was higher compared to that reported for other plants. such as Jojoba with 51.90% (Heikrujam *et al*., 2015), Fenel with 66.14% (Ydav and Malik, 2017), Kalmeg with 85.57% (Tiwari *et al*., 2017) and Centaurea with 73.18% (Mohamed Atia *et al*., 2021). Resolving power and PIC are among the most widely used indices to compare the efficiency of markers in genetic analysis (Pour-Aboughadareh *et al*., 2018). The PIC index shows the ability of a primer to detect polymorphism between samples and therefore depends on the number of detectable alleles. On the other hand, the Rp index differentiates between the primers used in terms of generating informative fragments (Powell *et al*., 1996). The estimated PIC and Rp values confirm the usefulness of the primers used in this research and thus their suitability in the analysis of genetic diversity and grouping of populations belonging to *Ae. tauschii* (Table 2). Likewise, Gholamian *et al*. (2019) investigated accessions of the *T. urartu* by using CBDP markers, the values of PIC and Rp indices were obtained as 0.45 and 10.66, respectively. Also, Pour-Aboughadareh *et al*. (2022) examined accessions of *Triticum* and *Aegilops* species using CBDP markers and obtained values of 0.45 and 8.42, respectively, for PIC and Rp indices. Khodaee *et al*. (2021) also obtained values of 0.34 and 3.57 for PIC and Rp, respectively, in examining the genetic diversity of Iranian *Aegilops triuncialis* accessions using CBDP primers. In a study (Bokaei *et al*., 2023) the level of genetic diversity among 157 *Aegilops* accessions using two sets of CBDP and SCoT markers, the average of PIC, MI, and Rp for SCoT and CBDP markers were 0.32, 3.59, 16.03 and 0.29, 3.01, 16.26, respectively. Also, in the investigation of the genetic diversity of Triticum and *Aegilops* genotypes using SCoT, CBDP, and SSR marker systems, it was found that the CBDP marker was more effective due to its higher PIC, Rp, and MI (Pour-Aboughadareh *et al*., 2022). Therefore, according to the obtained results, it can be stated that there is a high level of genetic diversity among the *Ae. tauschii* genotypes studied in this research.

The results of molecular variance analysis indicated the existence of high genetic diversity within the studied populations, which can be helpful in wheat breeding projects. In the report of Bokaei *et al*. (2023), the AMOVA results revealed that the genetic variability within the three *Aegilops* species was greater than the observed variation among them (Bokaei *et al.*, 2023). The presence of high genetic diversity within wheat germplasm species using different molecular markers such as RAPD, SCoT, SSR, and ISSR has also been reported by other researchers (Thomas *et al*., 2010; Etminan *et al*., 2016; Etminan *et al*., 2017; Gholamian *et al*., 2019; Ghobadi *et al*., 2021; Pour-Aboughadareh *et al*., 2022). The genetic diversity index (H) reflects diversity and differentiation among the germplasm collections, while Shannon's index (I) reflects genetic diversity within and between the populations (Heikrujam *et al*., 2015). The higher the indices, the greater the genetic diversity. The extent of variability among Na, Ne, H, and I indices in CBDP markers indicated a high level of genetic diversity among the Iranian and foreign accessions .In studying the genetic diversity of the wild relatives of wheat using CBDP markers, Etminan *et al*. (2019) have observed a high level of genetic diversity in *Ae. tauschii*. Wild relatives of wheat, including *Ae. cylindrica* and *Ae. tauschii* carry new genes responsible for drought tolerance and therefore have a valuable application in bread wheat breeding programs. (Pour-Aboughadareh *et al*., 2017; Pour-Aboughadareh *et al*., 2019; Pour-Aboughadareh *et al*., 2020; Pour-Aboughadareh *et al*., 2021; Pour-Aboughadareh *et al*., 2022). In addition, Ahmadi *et al*. (2020) found the appropriate response of *Ae. tauschii* to different levels of salt stress compared to other wild relatives of wheat.

Both multivariate methods, UPGMA and PCoA, used in the analysis of genetic relationships among the test species, generated comparable results. Based on the results of cluster analysis and PCoA, Iranian and foreign accessions were not completely placed in separate clusters. Hence, the CBDP primers used in this study showed a relative ability to discriminate the accessions studied based on their geographic region. Eslamzadeh-Hesari *et al*. (2023) reported that the CBDP marker system provided a clear grouping pattern of the evaluated *Aegilops* accessions compared to the SCoT marker system. Therefore, they recommended

the use of CBDP markers in determining population structure and estimating genetic diversity in other plant species (Eslamzadeh-Hesari *et al*., 2023). Our results showed that there was no full agreement between the clustering patterns and the geographical distribution of the examined accessions, which indicates high gene flow among accessions provided from different areas. As mentioned above, the results of population analysis showed that gene flow (*Nm*) was 14.53, which indicates a very high gene flow. Also, it was found that *Ae. tauschii* accessions provided from different regions investigated in this research are genetically diverse. The accessions with higher diversity should be used in breeding programs and germplasm collection/ management. According to the calculated indices, it was found that the accessions collected from different regions of Iran had higher genetic diversity compared to the accessions collected from the other countries therefore they can be used in the wheat breeding programs and should be given more attention.

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