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CHARACTERIZATION OF THE GENETIC DIVERSITY OF *EPIMEDIUM BREVICORNUM* (BERBERIDACEAE) VIA ISSR AND CDDP MARKERS

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Abstract

Epimedium brevicornum is a traditional and edible medicinal herb, used to tonify kidneys and strengthen tendons and bones. In this study, two types of molecular markers, namely, inter-simple sequence repeat (ISSR) and conserved DNA-derived polymorphism (CDDP) markers, were used to evaluate the genetic diversity of *E. brevicornum*. The results showed that both ISSR and CDDP markers were useful for estimating the genetic diversity. Forty-six polymorphic (86.79%) bands were generated by 7 random ISSR primers, while 82 polymorphic (95.35%) bands were generated by 11 CDDP primers. The genetic similarity coefficients of the ISSR markers ranged from 0.019 to 0.60, while those of the CDDP markers varied from 0.048 to 0.871. Moreover, the CDDP molecular marker technique was a preferable method for marker-assisted selection of stress-resistant varieties. Two superior resistant germplasms (BQ2 and MPC2) were screened, which would enable the selection and breeding of stress-resistant varieties of *E. brevicornum.*

Key words: *Epimedium brevicornum*; ISSR; CDDP; Resistance germplasm classification.

Introduction

Epimedium brevicornum, a perennial herbaceous species belonging to the Berberidaceae family, has a long history of use as an edible medical plant species in China (Yang *et al*., 2016). The aerial parts of *E. brevicornum*, known in China as "Yin-Yang-Huo", are used to tonify kidneys, strengthen tendons and bones and treat impotence (Xue *et al*., 2016, Ren *et al*., 2018). This plant species has been traditionally harvested from the wild. In China, this species is distributed mainly in Hubei, Hunan, Guizhou, Sichuan and Shaanxi, and the wild resources from these different locations are genetically distinct (Jiang & Zang, 2017). However, in recent years, the number of wild plant resources has decreased because of excessive collection. Additionally, the seed germination rate of this species is generally low, limiting artificial cultivation. As a result, *E. brevicornum* resources cannot meet the needs of the market. It is therefore necessary to obtain an in-depth understanding of the genetic variation among the different endemic resources to better protect them.

The assessment of genetic diversity, on which selection acts, leading to the evolution of superior genotypes, is fundamental for the crop breeding industry (Hajibarat *et al*., 2015). Various molecular markers have been widely used to evaluate the genetic variation of different species, including inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), and start codon targeted (SCoT) markers (Hamidi *et al*., 2014, Cui *et al*., 2017, Alotaibi, 2020). Among these molecular markers, ISSR markers are superior because their reproducibility and simplicity overcome many of the technical limitations associated with random markers (Seth & Panigrahi, 2018). In recent years, ISSR molecular markers have proven useful in analyses of genetic diversity, genetic relationships and germplasm of *E. brevicornum* (Liu *et al*., 2017, Guan *et al*., 2018, Cao *et al*., 2020). With the movement away from random DNA markers toward gene-targeted markers, conserved DNA-derived polymorphism (CDDP) markers were developed. CDDP markers are based on conserved regions, which are typically functional domains that correspond to conserved DNA sequences within genes (Collard & Mackill, 2009; Hamidi *et al*., 2014). In principle, CDDP markers are similar to ISSR markers because the same primer is used as the forward and reverse primer (Hamidi *et al*., 2014). Furthermore, CDDP markers focus on gene regions, which may give them advantages over random markers for applications in quantitative trait locus (QTL) mapping (Anders & Lübberstedt, 2003). CDDP markers are designed on the basis of conserved DNA sequences within plant genes, such as genes involved in the response to biotic and abiotic stresses (Guo *et al*., 2016). WRKY transcription factors (TFs) are involved processes affected by various biotic and abiotic stresses, such as biosynthesis, senescence, and hormone signal transduction (Wang *et al*., 2017). MYB TFs play regulatory roles in physiological and biochemical processes such as the response to stress, hormones, signal transduction, and pathogen defense (Wang *et al*., 2015). ERFs are often involved in regulating gene expression in response to biotic and abiotic stresses in plants (Zhai *et al*., 2013). Therefore, amplified bands corresponding to ERFs obtained by three types of CDDP primers might indicate tolerance to the corresponding stress applied; the plants associated with those bands could potentially present superior resistance (Wu *et al*., 2017).

In this study, ISSR and CDDP markers were used to evaluate the genetic diversity and genetic relationships of *E. brevicornum*. Additionally, potential resistant germplasms were screened. The findings of this study may provide valuable insight for the genetic characterization of this species particularly for breeding resistant varieties.

Materials and Methods

Plant materials and DNA extraction: Seventeen *E. brevicornum* samples were collected from different geographical locations in the Wuling mountainous area in China (Table 1). The samples were used to study the genetic diversity of the species on the basis of ISSR and CDDP molecular markers. The leaves were frozen in liquid nitrogen and then ground into a fine powder. Genomic DNA was extracted from the 17 samples using a plant genomic DNA extraction kit (Tiangen, China) according to the manufacturer's instructions. DNA quality was checked via 1% agarose gel electrophoresis and spectrophotometry. The DNA was stored at -20°C until use.

ISSR and CDDP analysis: Seven random ISSR primers screened from microsatellite primers designed by the University of British Columbia (Canada) were synthesized by Sangon (Shanghai, China) (Table 2). The 11 CDDP primer sequences used in this study were designed by Collard and Mackill *et al*., (2009) and were based on the protein sequences of well-characterized genes (Table 2).

ISSR amplification was performed in a 20 µl total volume consisting of 30 ng of template DNA, 9.8 µl of 2× Taq Master Mix (Tiandz, China), 1.5 µl of 2.5 µmol·L^{-1} primers, and 4.6 µl of double-distilled H_2O (ddH_2O). PCR was carried out in a Bio-Rad thermal cycler (USA) in accordance with the following procedure: 94°C for 5 min; 40 cycles of denaturation at 94°C for 5 min, annealing at 46.8~65°C for 0.5 min, and extension at 72°C for 0.75 min; and a final extension step of 72°C for 10 min. The PCR products were subsequently stored at 4°C, after which they were analyzed via 1.0% agarose gel electrophoresis and visualized by DNA green (UA, Tiandz, China) staining under UV light.

CDDP amplification was performed in a 20 µl volume containing 30 ng of template DNA, 10.5 µl of 2× Taq Master Mix, 5.4 µl of 2.5 µmol·L^{-1} primer, and 2.6 µl of ddH_2O. PCR was carried out in a Bio-Rad thermal cycler (USA) according to the following procedure: 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 0.5 min, annealing at 45~54.6°C for 1 min, and extension at 72°C for 1.5 min. A final extension step of 72°C for 10 min followed. PCR products were stored at 4°C. The PCR products were analyzed via 1.0% agarose gel electrophoresis and visualized by DNA green staining under UV light.

DNA bands obtained with ISSR and CDDP markers were generated by scoring the presence (1) and absence (0) of individual alleles. The obtained data matrices were used for phylogenetic reconstruction according to the unweighted pair-group method with arithmetic mean (UPGMA). The Dice coefficient was used to compute a distance matrix between accessions in NTSYS-pc 2.10 software.

The amplified bands of WRKY, MYB and ERF primers were counted, and the average of the number of bands obtained by each type was calculated. If the number of amplified bands obtained by a certain type of primer in an individual was greater than the average, it was considered to have potentially superior resistance. A Venn diagram showing potential superior resistant germplasms on the basis of the three types of primers was constructed by the use of online tools (http://www.omicshare.com/tools/Home/Soft/venn).

Results

ISSR fingerprinting analysis: total of 100 ISSR primers were used for initial testing. Only 7 primers that exhibited legible and reproducible bands were selected in this study (Table 2). In total, 53 amplified bands were obtained on the basis of the ISSR analysis, 46 of which were polymorphic (86.79% polymorphism). The number of fragments produced per primer ranged from 4 to 12, and the degree of polymorphism ranged from 71% to 100% (Table 2). A representative amplification profile obtained with ISSR primers was constructed (Fig. 1a). Overall, *E. brevicornum* exhibited high levels of polymorphism, and there was abundant genetic diversity among the 17 samples.

Table 1. Details of the plant materials used in this study.

No	Accession No.	Locality	Altitude (m)	Latitude (N)	Longitude (E)
1.	MPC1	Mupingcun, Guizhou	950	28°25'	108°14'
2.	MPC2	Mupingcun, Guizhou	950	28°25'	108°14'
3.	MPC3	Mupingcun, Guizhou	950	28°25'	108°14'
4.	MPC4	Mupingcun, Guizhou	950	28°25'	108°14'
5.	QSC1	Qingshancun, Guizhou	1210	26°24'	107°59'
6.	QSC2	Qingshancun, Guizhou	1210	26°24'	107°59'
7.	QSC3	Qingshancun, Guizhou	1210	26°24'	107°59'
8.	TPS1	Tianpingshan, Hunan	1180	29°41'	110°3'
9.	TPS2	Tianpingshan, Hunan	1180	29°41'	110°3'
10.	MSC1	Mashicun, Hunan	750	29°22'	109°36'
11.	MSC2	Mashicun, Hunan	750	29°22'	109°36'
12.	LHC1	Lianhuacun, Guizhou	1160	26°26'	106°57'
13.	LHC2	Lianhuacun, Guizhou	1160	26°26'	106°57'
14.	TZG1	Taozizhai, Guizhou	790	26°26'	108°3'
15.	TZG2	Taozizhai, Guizhou	790	26°26'	108°3'
16.	BQ1	Banqiao, Hubei	1700	30°57′	109°22'
17.	BQ2	Banqiao, Hubei	1700	30°57′	109°22'

Table 2. ISSR and CDDP markers and amplification results.

Marker	Primer	Sequence (5'-3')	Annealing temperature (°C)	Total number of bands	Number of polymorphic bands	Polymorphism (%)
ISSR	UBC807	AGAGAGAGAGAGAGAGT	52.2	4	3	75
	UBC817	CACACACACACACACAA	52.2	7	6	86
	UBC826	ACACACACACACACACC	60.3	7	6	86
	UBC840	GAGAGAGAGAGAGAGAYT	52.4	12	12	100
	UBC846	CACACACACACACACART	54.3	11	10	91
	UBC847	CACACACACACACACARC	56.2	7	5	71
	UBC856	ACACACACACACACACYA	54.3	5	4	80
CDDP	ABP1-1	ACSCCSATCCACCGC	45	8	7	87.5
	ERF2	GCSGAGATCCGSGACCC	51.5	10	10	100
	ERF3	TGGCTSGGCACSTTCGA	51.5	5	4	80
	KNOX-1	AAGGGSAAGCTSCCSAAG	47.5	6	6	100
	KNOX-2	CACTGGTGGGAGCTSCAC	49.7	11	10	91
	KNOX-3	AAGCGSCACTGGAAGCC	46.7	8	8	100
	MADS-2	ATGGGCCGSGGCAAGGTGG	54.6	10	9	90
	MYB1	GGCAAGGGCTGCCGC	47.7	7	6	86
	WRKY-R2	GCCCTCGTASGTSGT	42.2	9	8	89
	WRKY-R2B	TGSTGSATGCTCCCG	42.2	5	2	40
	WRKY-3B	CCGCTCGTGTGSACG	45	7	7	100

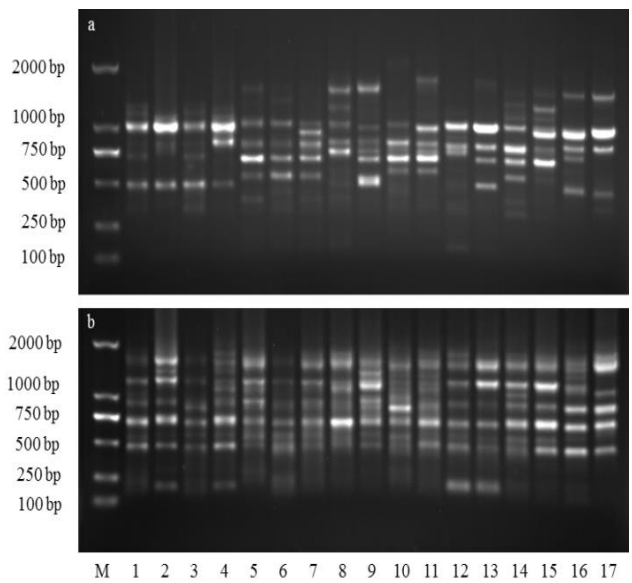

Fig. 1. Representative amplification profile obtained with the ISSR primer UBC840 (a) and the CDDP primer ERF2 (b). M, DL2000 DNA marker. Lanes 1-17 correspond to the samples listed in Table 1.

As shown in the genetic similarity and distance matrix (Table 3), the genetic similarity ranged from 0.019 to 0.603, and the genetic distance ranged from 0.547 to 0.981. The genetic similarity between *E. brevicornum* (BQ1) and *E. brevicornum* (MPC2) was the lowest (0.547); accordingly, their genetic distance was the greatest (0.603). The genetic similarity between *E. brevicornum* (MPC3) and *E. brevicornum* (MPC1) was the greatest (0.981); accordingly, their genetic distance was the lowest (0.019). Moreover, the observed number of alleles (*Na*) was 1.8679±0.3418, the effective number of alleles (*Ne*) was 1.4063±0.3388, Shannon's information index (*I*) was 0.3881±0.2277, and Nei's gene diversity (H) was 0.2494±0.1698. The UPGMA dendrogram (Fig. 2) divided the samples into four major groups, with a genetic coefficient of 0.75. Samples from the same geographical location were generally clustered, except for the *E. brevicornum* (TPS1) and *E. brevicornum* (TPS2) samples and the *E. brevicornum* (TZG1) and *E. brevicornum* (TZG2) samples.

CDDP fingerprinting analysis: A total of 21 CDDP markers were used for initial testing. Only 11 primers that presented legible and reproducible bands were selected in this study (Table 2). In total, 86 amplified bands were obtained on the basis of CDDP analysis, 82 of which were polymorphic (95.35% polymorphism). The number of fragments produced per primer ranged from 5 to 11, and the degree of polymorphism ranged from 40% to 100% (Table 2). The primers ERF2, KNOX-1 and KNOX-3 were 100% polymorphic. A representative amplification profile obtained via CDDP markers was constructed (Fig. 1b). *E. brevicornum* presented high levels of polymorphism, and there was an abundance of genetic diversity among the 17 samples.

As shown in the genetic similarity and distance matrix (Table 4), the genetic similarity ranged from 0.048 to 0.871, and the genetic distance ranged from 0.419 to 0.954. The genetic similarity between *E. brevicornum* (MSC1) and *E. brevicornum* (MPC2) was the lowest (0.419); accordingly, their genetic distance was the greatest (0.871). In addition, the genetic similarity between *E. brevicornum* (MPC3) and *E. brevicornum* (MPC1) was the greatest (0.954); accordingly, their genetic distance was the lowest (0.048). The observed *Na* was 1.9535±0.2118, the *Ne* was 1.5629±0.3070, *I* was 0.4995±0.1845, and *H* was 0.3327±0.1440. The UPGMA dendrogram (Fig. 3) divided the samples into three major groups, with a genetic coefficient of 0.73. Samples from the same geographical location generally clustered, except for *E. brevicornum* (BQ1) and *E. brevicornum* (BQ2).

Screening of resistant germplasm: In total, 153, 73 and 89 bands were amplified by the WRKY, Myb, and ERF primers, respectively. A Venn diagram based on the amplification results was constructed (Fig. 4, Table 5). Two superior resistant germplasms, BQ2 and MPC2, were screened by the three kinds of CDDP primers. Three other germplasms, BQ1, TZG1 and TZG2, were screened by the ERF and WRKY primers.

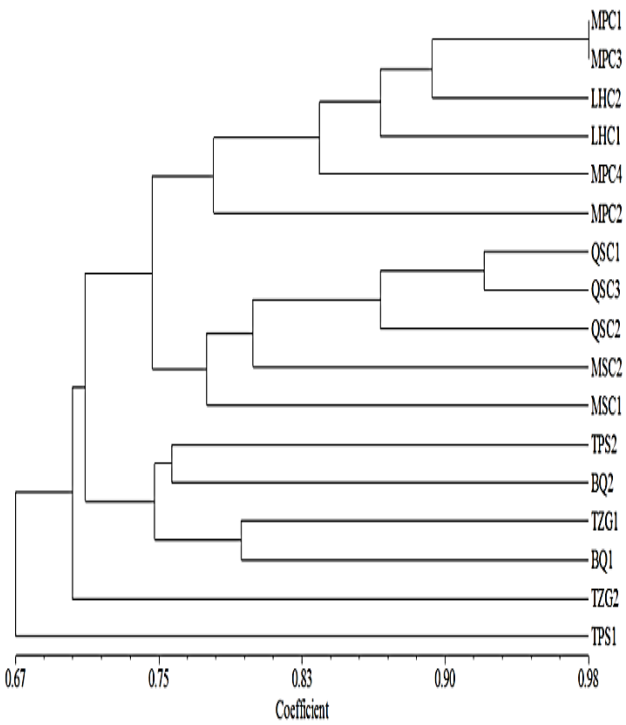

Fig. 2. UPGMA dendrogram of *E. brevicornum* samples. The dendrogram is based on 53 PCR bands amplified by 7 ISSR primers.

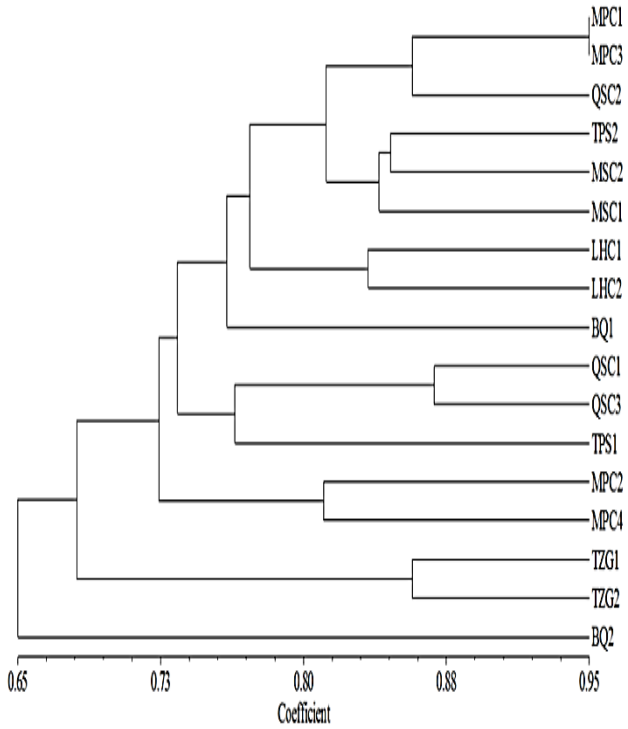

Fig. 3. UPGMA dendrogram of *E. brevicornum* samples. The dendrogram is based on 86 PCR bands amplified by 11 CDDP primers.

Table 5. Filtered collections.

Collection name	Number of germplasms	Name of germplasms
WRKY ERF Myb	2	BQ2, MPC2
ERF WRKY	3	BQ1, TZG1, TZG2
WRKY	1	QSC3
ERF	2	TPS2, LHC2
Myb	1	QSC1, TPS1

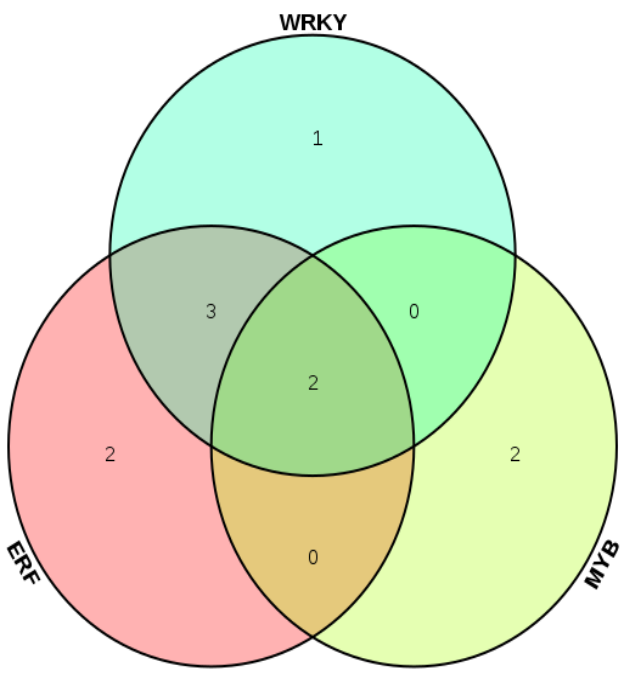

Fig. 4. Venn diagram of potential resistant materials.

Discussion

Comparison of the genetic diversity of *E. brevicornum* according to ISSR and CDDP markers: DNA-based molecular identification of medicinal plants has become a promising tool for quality control in the production of herbal medicines (Peng *et al*., 2016). A recent study showed that both ISSR and CDDP marker techniques could be widely used to analyze the genetic diversity and phylogenetic relationships of medicinal plants (Li, 2013, Natarajan *et al*., 2018). In this study, ISSR and CDDP markers were used to evaluate the genetic diversity among 17 genotypes from different endemic resources. A comparison of genetic similarity coefficients obtained via ISSR and CDDP markers showed that the coefficients of the former ranged from 0.019 to 0.603, while those of the latter varied from 0.048 to 0.871. *I* was 0.3881±0.2277 for the ISSR markers, while it was 1.5629±0.3070 for the CDDP markers, and *H* was 0.2494±0.1698 for the ISSR markers, while it was 0.3327±0.1440 for the CDDP markers. Thus, both the ISSR and CDDP markers were effective in assessing the 17 genotypes from different endemic resources. In addition, 46 and 82 polymorphic loci were obtained with ISSR and CDDP markers, respectively. Overall, *E. brevicornum* exhibited obvious polymorphism and a high level of diversity. Previous studies have shown that CDDP markers are more reliable than ISSR markers for studying phylogenetic relationships among plant species (Guo *et al*., 2016). The results of our study revealed that each method was effective for the assessment of genetic diversity. However, few studies have shown that ISSR markers are structurally important (Hamidi *et al*., 2014). More polymorphic loci were revealed by CDDP markers than by ISSR markers in our study. Thus, CDDP molecular markers have more advantages than ISSR markers with respect to analyses of the genetic diversity of *E. brevicornum*.

Table 3. Genetic similarity (above the diagonal) and genetic distance (below the diagonal) matrix for *E. brevicornum*, obtained with ISSR marker data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	***	0.755	0.981	0.830	0.717	0.774	0.679	0.736	0.755	0.736	0.774	0.849	0.887	0.774	0.755	0.679	0.698
2.	0.281	***	0.774	0.774	0.736	0.717	0.698	0.604	0.660	0.717	0.679	0.755	0.830	0.642	0.585	0.547	0.604
3.	0.019	0.257	***	0.849	0.736	0.793	0.698	0.755	0.774	0.755	0.793	0.868	0.906	0.793	0.736	0.698	0.717
4.	0.186	0.257	0.164	***	0.698	0.717	0.660	0.717	0.698	0.679	0.717	0.793	0.868	0.679	0.623	0.660	0.679
5.	0.333	0.307	0.307	0.360	***	0.868	0.925	0.604	0.736	0.793	0.830	0.830	0.755	0.793	0.736	0.698	0.717
6.	0.257	0.333	0.233	0.333	0.142	***	0.868	0.660	0.755	0.774	0.811	0.811	0.774	0.811	0.793	0.717	0.698
7.	0.387	0.359	0.359	0.415	0.079	0.142	***	0.642	0.698	0.755	0.755	0.793	0.717	0.717	0.736	0.660	0.679
8.	0.307	0.505	0.281	0.333	0.505	0.415	0.444	***	0.717	0.623	0.660	0.698	0.660	0.698	0.642	0.679	0.623
9.	0.281	0.415	0.257	0.359	0.307	0.281	0.359	0.333	***	0.717	0.755	0.755	0.717	0.755	0.660	0.736	0.755
10.	0.307	0.333	0.281	0.387	0.233	0.257	0.281	0.474	0.333	***	0.774	0.811	0.774	0.660	0.679	0.679	0.623
11.	0.257	0.387	0.233	0.333	0.186	0.209	0.281	0.415	0.281	0.257	***	0.849	0.736	0.774	0.717	0.717	0.736
12.	0.164	0.281	0.142	0.233	0.186	0.209	0.233	0.359	0.281	0.209	0.164	***	0.887	0.774	0.717	0.717	0.736
13.	0.120	0.186	0.099	0.142	0.281	0.257	0.333	0.415	0.333	0.257	0.307	0.120	***	0.698	0.679	0.679	0.660
14.	0.257	0.444	0.233	0.387	0.233	0.210	0.333	0.359	0.281	0.415	0.257	0.257	0.359	***	0.755	0.793	0.736
15.	0.281	0.536	0.307	0.474	0.307	0.233	0.307	0.444	0.415	0.387	0.333	0.333	0.387	0.281	***	0.698	0.642
16.	0.387	0.603	0.359	0.415	0.359	0.333	0.415	0.387	0.307	0.387	0.333	0.333	0.387	0.233	0.359	***	0.755
17.	0.359	0.505	0.333	0.387	0.333	0.359	0.387	0.474	0.281	0.474	0.307	0.307	0.415	0.307	0.444	0.281	***

Table 4. Genetic similarity (above the diagonal) and genetic distance (below the diagonal) matrix for *E. brevicornum*, obtained with CDDP markers data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	***	0.826	0.954	0.826	0.640	0.570	0.547	0.488	0.500	0.523	0.593	0.581	0.523	0.465	0.523	0.605	0.535
2.	0.192	***	0.779	0.814	0.628	0.465	0.535	0.430	0.465	0.419	0.488	0.500	0.488	0.477	0.535	0.523	0.593
3.	0.048	0.250	***	0.802	0.616	0.593	0.570	0.512	0.523	0.523	0.616	0.605	0.547	0.512	0.570	0.605	0.535
4.	0.192	0.206	0.220	***	0.674	0.558	0.558	0.454	0.512	0.512	0.558	0.547	0.512	0.477	0.535	0.547	0.523
5.	0.447	0.465	0.484	0.394	***	0.581	0.674	0.640	0.628	0.605	0.581	0.593	0.558	0.570	0.605	0.570	0.523
6.	0.563	0.766	0.523	0.583	0.542	***	0.744	0.733	0.791	0.814	0.814	0.802	0.767	0.709	0.744	0.802	0.639
7.	0.604	0.626	0.563	0.583	0.394	0.296	***	0.756	0.791	0.767	0.814	0.756	0.744	0.733	0.767	0.686	0.616
8.	0.717	0.843	0.670	0.791	0.447	0.311	0.280	***	0.802	0.802	0.756	0.721	0.640	0.605	0.640	0.651	0.651
9.	0.693	0.766	0.648	0.670	0.465	0.235	0.235	0.220	***	0.837	0.837	0.756	0.721	0.709	0.698	0.709	0.640
10.	0.648	0.871	0.648	0.670	0.503	0.206	0.265	0.220	0.178	***	0.861	0.779	0.721	0.663	0.674	0.733	0.640
11.	0.523	0.717	0.484	0.583	0.542	0.206	0.206	0.280	0.178	0.150	***	0.826	0.744	0.686	0.744	0.756	0.686
12.	0.542	0.693	0.503	0.604	0.523	0.220	0.280	0.327	0.280	0.250	0.192	***	0.826	0.651	0.733	0.767	0.581
13.	0.648	0.717	0.604	0.670	0.583	0.265	0.296	0.447	0.327	0.327	0.296	0.192	***	0.663	0.721	0.756	0.593
14.	0.766	0.741	0.670	0.741	0.563	0.344	0.311	0.503	0.344	0.411	0.377	0.430	0.411	***	0.849	0.651	0.581
15.	0.648	0.626	0.563	0.626	0.503	0.296	0.265	0.447	0.360	0.394	0.296	0.311	0.327	0.164	***	0.709	0.663
16.	0.503	0.648	0.503	0.604	0.563	0.220	0.377	0.429	0.344	0.311	0.280	0.265	0.280	0.429	0.344	***	0.651
17.	0.626	0.523	0.626	0.648	0.648	0.447	0.484	0.429	0.447	0.447	0.377	0.542	0.523	0.542	0.411	0.430	***

The dendrogram clusters revealed the genetic relationships between varieties. On the basis of the ISSR and CDDP marker results, the UPGMA dendrogram divided the samples into four and three major groups, respectively. The different results obtained by the ISSR and CDDP markers may be due to the two methods being associated with different portions of the genome. In general, samples from the same geographic area tend to cluster together, but this is not always the case (Jiang & Zang, 2017). In our study, the results obtained with both ISSR and CDDP markers showed that the genetic diversity was not solely due to geographical distance; there was also high genetic diversity within *E. brevicornum* at the same geographical location.

Screening of resistant germplasm: ERF, MYB, and WRKY proteins are characterized by the presence of highly conserved DNA-binding sequence at the N-terminus (Liu *et al*., 2014, Liu *et al*., 2017). These proteins are involved in the response to biotic and abiotic stresses. Qiu and Yu (2009) revealed that overexpression of WRKY45 increased disease resistance and drought tolerance in Arabidopsis. Wang *et al*., (2015) showed that the WRKY27 and MYB174 genes improved the stress tolerance of soybean and other crop species. CDDP markers are designed on the basis of these TFs (Collard & Mackill, 2009; Wu *et al*., 2017), and the amplified bands obtained by those primers might indicate tolerance of those plants to the corresponding stress. As a result, the germplasms screened via the amplified bands, which are obtained by CDDP markers, are considered to have potentially superior resistance. Two superior resistant germplasms (BQ2 and MPC2) were screened via three kinds of primers. The number of amplified bands obtained by three kinds of CDDP markers was greater than the average, which suggested that these two germplasms might display resistance corresponding to the three different types of TFs. BQ1, TZG1, and TZG2 might be involved in tolerance to the same stress as ERF and WRKY TFs. QSC3 might be associated with resistance corresponding to that associated with WRKY TFs, and TPS2 and LHC2 might be associated with resistance corresponding to that associated with ERF TFs. In addition, QSC1 and TPS1 might be associated with tolerance to the corresponding stress associated with MYB TFs. Taken together, these results ultimately highlight two potentially resistant germplasm resources: BQ2 and MPC2. Thus, molecular marker-assisted breeding may improve the breeding efficiency of this species.

Conclusion

In our study, both ISSR and CDDP markers were effective for assessing the genetic diversity of *E. brevicornum*. The genetic diversity obtained with CDDP molecular markers was greater than that obtained with ISSR markers. On the basis of these findings, CDDP molecular markers could be used as a rapid and effective method for marker-assisted selection of stress-resistant varieties. Thus, CDDP molecular markers have more advantages than do ISSR markers in terms of analyzing the genetic diversity of *E. brevicornum*. Two superior resistant germplasms (BQ2 and MPC2) were subsequently screened by CDDP markers, facilitating the selection and breeding of stress-resistant varieties of *E. brevicornum*.

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