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ASSESSMENT OF GENETIC VARIATION AMONG DIVERSE TARAMIRA (*ERUCA SATIVA* MILL.) GERMPLASM BASED ON MOLECULAR MARKERS

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Abstract

Genetic diversity is a key to identify elite genotypes for any crop improvement programme. In present study, diverse Taramira (*Eruca sativa* Mill.) genotypes were characterized using molecular markers in order to assess the inherited patterns of these genotypes for identification of elite and genetically diverse genotypes. Sixty (60) genotypes were provided by Bio-resources Conservation Institute (BCI), National Agricultural Research Center (NARC), Islamabad, Pakistan. Simple Sequence Repeats (SSR) analysis revealed that 54 ploymorphic bands were generated using 40 primer pairs. Polymorphism Information Content (PIC) value calculated for 10 SSR selected primers determined allele variations at each locus. These values varied from 0.234 for primer PBCGSSRBo36 to 0.720 for primer Na10-G10 with an average PIC value of 0.495 per locus. Each primer produced an average of 1.4 polymorphic bands. All the selected primers produced polymorphism among studied genotypes. The allele's size varied from 90bp-700bp is the evolution of proposed molecular approach. These results inferred that the accessions studied in present research have low variation revealing narrow genetic background of these accessions. Present investigation concerning molecular analyses, illustrated the highest level of inter-species and middle level of intra-species diversity amongst evaluated genotypes.

Key words: Genetic variability, Polymorphic bands, SSR marker, Elite genotypes, Taramira.

Introduction

Taramira (*Eruca sativa* Mill.) is a minor crop of global world. It was ignored by plant researchers for long time. Its importance of genetic diversity was realized by the agricultural scientists in the last few years. These results enhance the interest in improvement of some minor crop such as *Eruca sativa,* familiar for its outstanding resistance to diseases and drought. Other minor crops may also get benefit because it is believed to be a genetic resource for all crops in *Brassiceae* family (Gomez-Campo, 1980; Sun, 2000). The success of breeding programme relies on genetic variability for economically important traits in the population and its management for exploitation. Available diversity is important for crop improvement and development of cultivars with wide genetic bases and adaptability. Species with genetic variation are able to adapt themselves in changeable environment and insured the plants to meet the harsh environment, therefore allow stability in world agricultural system.

Molecular marker or DNA markers can be applied for study of genetic and molecular variation (Rehman *et al*., 2015; Shah *et al*., 2015; Shinwari *et al*., 2018; Khan *et al*., 2019). They are not affected by phenotypic traits as they are presented near or associated to genes controlling the characters. These markers can be detected through specific primer or probe (Barcaccia *et al*., 2000). SSR markers are simply automated, highly polymorphic and reproducible. These markers are mostly used for genotyping of individual, genome mapping, genetic variability studies, genotypes evaluation, evolutionary and phylogenetic studies (Rabbani *et al*., 2010).

In plant breeding as well as for germplasm conservation, eco-geographical and genetic diversity are the most important and basic method of variability (Alemayehu & Becker, 2002). A number of approaches *i.e.* physiological, biochemical and morphological characterization have been developed to evaluate genetic diversity (Greene *et al*., 2004; Geleta *et al*., 2005). Therefore, the current study was conducted to check molecular diversity among local and exotic Taramira germplasm.

Materials and Methods

DNA isolation and PCR analysis: Total genomic DNA was isolated from all tested genotypes (Table 1) using CTAB method of Doyle & Doyle (1990). PCR analysis was carried out with a set of available 40 SSR markers in 60 diverse genotypes for the presence of monomorphic and polymorphic band variation.

DNA quantification: Nano drop technique was used to find out the concentration of DNA and was further diluted to working concentration for PCR analysis. The samples were then run through agarose gel electrophoresis.

PCR amplification of target fragments: For DNA amplification, protocols of Hasan *et al*., (2006) along with some modification was used. The final volume of reaction mixture was 20µl including 1x PCR buffer, 0.2mM each dNTPs, 0.5µM of forward and reverse primer, 2mM MgCl2, 1 unit of Taq Polymerase (Fermentas Life Sciences) and 25ng of sample genomic DNA. Amplification reactions were performed in Veriti thermocycler (Model # 9902 Applied Biosystems Inc, USA) and reaction profile was programmed as follows: initial strand separation had 1 cycle at 94ºC for 4 minutes, followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC – 60ºC for 0.35 minute and primer extension at 72ºC for 2 minutes and one cycle of final extension at 72ºC for 7 minutes and followed by soaking at 4ºC temperature to hold samples until the whole amplification process completed and removed from PCR machine.

Table 1. List of Taramira (*Eruca sativa* Mill.) genotypes used for SSR study.

Sr. No.	Accession	Origin	Sr.No.	Accession	Origin	Sr.No.	Accession	Origin
1.	34749	US	21	34780	Pakistan	41	348083	Pakistan
2.	34750	US	22	34781	Pakistan	42	34809	Pakistan
3.	34751	Turkey	23	34784	Pakistan	43	34812	Pakistan
4.	34752	India	24	34786	Pakistan	44	34813	Pakistan
5.	34753	Turkey	25	34791	Pakistan	45	34815	Pakistan
6.	34754	Turkey	26	34792	Pakistan	46	34845	Pakistan
7.	34755	India	27	34793	Pakistan	47	34847	Pakistan
8.	34757	Turkey	28	34794	Pakistan	48	34848	Pakistan
9.	34760	India	29	34795	Pakistan	49	34854	Pakistan
10.	34761	India	30	34796	Pakistan	50	34862	Pakistan
11.	34763	Egypt	31	34797	Pakistan	51	34866	Pakistan
12.	34764	India	32	34799	Pakistan	52	34867	Pakistan
13.	34766	Pakistan	33	34800	Pakistan	53	34878	Pakistan
14.	34767	Egypt	34	34801	Pakistan	54	34884	Pakistan
15.	34769	Iran	35	34802	Pakistan	55	34907	India
16.	34772	Iran	36	34803	Pakistan	56	34917	Italy
17.	34774	Iran	37	34804	Pakistan	57	34927	Italy
18.	34775	Iran	38	34805	Pakistan	58	34930	Italy
19.	34776	Afghanistan	39	34806	Pakistan	59	**26187**	Netherlands
20.	34778	Turkey	40	34807	Pakistan	60	**27460**	Pakistan

Table 2. List of 40 microsatellites, primer sequences and their length size used in present studies.

S. No.	Primers	Forward (F)	Reverse (R)	F(bp)	R(bp)
1.	PBCESSRJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	20	21
2.	PBCESSRJU4	CACCTTATCATCTCTCTATCCC	CCTCTGTTTCTCTCCTTGTG	22	20
3.	PBCESSRJU7	TACCACTCCCTAACCGCA	ATCACCTTGAGAGCGAAG	18	18
4.	PBCESSRJU9	CCCTACCGCTGGCTAGACTT	GCATCATGACCAACTATCAACC	20	22
5.	PBCESSRJU16	TCCTCACTTTCTTGGCATC	ACTGAAAAGACCACTACCACCA	19	22
6.	Na12-F03	GGCGACATAGATTTGAACCG	TCCACTTTCTCTCTCTTCCCC	20	21
7.	Ni4-D09	AAAGGACAAAGAGGAAGGGC	TTGAAATCAAATGAGAGTGACG	20	22
8.	Ra2-D04.	TGGATTCTCTTTACACACGCC.	CAAACCAAAATGTGTGAAGCC.	21	21
9.	Ra2-E03.	AGGTAGGCCCATCTCTCTCC.	CCAAAACTTGCTCAAAACCC.	20	20
10.	Ra2-E07	ATTGCTGAGATTGGCTCAG	CCTACACTTGCGATCTTCACC	20	21
11.	Ra2-E11.	GGAGCCAGGAGAGAAGAAGG.	CCCAAAACTTCCAAGAAAAGC.	20	21
12.	Ra2-E12.	TGTCAGTGTGTCCACTTCGC.	AAGAGAAACCCAATAAAGTAGAACC.	20	25
13.	Ra2-F11	TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC	20	20
14.	Ra2-H06	GAATTCAGAGGTATCTACACGGC	TAACAAAGACCCTGCGTTCC	23	20
15.	Ra3-H10	TAATCGCGATCTGGATTCAC	ATCAGAACAGCGACGAGGTC	20	20
16.	Na10-B08	AGAGAAAAACACTTCCCGCC	GTGAGCTTTGCGAAACACG	20	19
17.	Ni2-B03.	ACTTCTTGCCCTCCTCACC.	AAATACTCACTGCAATACCCAGG.	19	23
18.	Ni2-C12.	ACATTCTTGGATCTTGATTCG.	AAAGGTCAAGTCCTTCCTTCG.	21	21
19.	Ni3-G05.	AGGAAGCATTTGCGCTAGTC.	TCTACAACCACAACGTCCAAG.	20	21
20.	Ni4-A03.	ACACAGAAACATCAAACATACC.	GGACCGGTTTTATTTGTTCG.	22	20
21.	Ol10-A05	TGTAATAACCCGACCCATCC	CTCTCTCGCTCTCTCGATCC	20	20
22.	Ol10-F12	TCCATGTTTCATGTTGGAGG	CTCTCCGGCTTCACTTTCC	20	19
23.	Ol11-B03	ATGAAAACCAATCCAGTGCC	GATAGCAGATGGAAGAGCCG	20	20
24.	Ol11-H02	TCTTCAGGGTTTCCAACGAC	AGGCTCCTTCATTTGATCCC	20	20
25.	Ol12-E03	CTTGAAGAGCTTCCGACACC	GACGGCTAACAGTGGTGGAC	20	20
26.	Na10-G10	TGGAAACATTGGTGTTAAGGC	CATAGATTCCATCTCAAATCCG	21	22
27.	Na10-D09	AAGAACGTCAAGATCCTCTGC	ACCACCACGGTAGTAGAGCG	21	20
28.	Ni2-B01	AAGGAGATTGTTTTTGGGGC	AAGACTAATAAACACACGGCG	20	21
29.	PBCGSSRBo6	TGAAGAGGAAGCACCAGACC	ATCCGAAACCAACACTCGTC	20	20
30.	PBCGSSRBo7	TAGGCCCATGTTCTAGACGG	GAAGGTTCACGATCAAGGGA	20	20
31.	PBCGSSRBo36	AAACGAGGCTTCCACAGAGA	GGGTACCCGTTCGGTTCT	20	18
32.	PBCGSSRBo40	AGACATCCCACATCGGCTAC	GACCCAAGACCCAAGACTCA	20	20
33.	PBCESSRNA18	TTAAAATGAAACCCACCCGA	TGTTGGGCAACATCCATTTA	20	20
34.	PBCESSRNA26	AAGAACGTGATCTCCATCGC	CGAGCTCCAAAGCAGATACC	20	20
35.	BRMS-001	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTCACCAACCCC	20	20
36.	BRMS-007	AAATTGTTTCTCTTCCCCAT	GTGTTAGGGAGCTGGAGAAT	20	20
37.	BRMS-020	AACAAGAGAAGGAGAGCCACCG	CGCTTATAAAATGGCAGTCGCA	22	22
38.	BRMS-036	GGTCCATTCCTTTTTGCATCTG	CATGGCAAGGGGTAACAAACAT	22	22
39.	BRMS-037	CTGCTCGCATTTTTTATCATAC	TACGCTTGGGAGAGAAAACTAT	22	22
40.	BN83B1	GCCTTTCTTCACAACTGATAGCTAA	TCAGGTGCCTCGTTGAGTTC	25	20

Primer for analysis of genetic diversity: Forty SSR primer combinations were selected and tested for their suitability (Table 2). On the basis of ease of score, level of polymorphism, compatible allele size range and similar optimal reaction conditions of 40 primer pair's ten markers were selected after pre-screening.

Electrophoresis of amplified products: After completion of amplification, 3µl gel loading dye (6x) were added to amplified target fragment and analyzed directly on 3% (weight/volume) Gene Choice High Resolution agarose (CLP, USA) gels in 1 x TBE buffer (1mM EDTA, 10mM Tris Borate) having 5µl ethidium bromide. Fermentas Life Sciences DNA marker of 100bp was used as a size indicator to compare the molecular weights of all PCR amplified SSR products. Only 50bp marker was run with PBCSSRJUI, Na12–Fo3, Ra2-D04, Ra2-F11, Ni2-B03 and Ra2-E07. A 8-10µl of amplified SSR samples containing dye along with ladder were loaded into the wells of gel. The number of wells and samples were noted to evade any mistake or repetition while loading of wells. Red (+) and black (–) electrodes of power supply were connected with the apparatus. The voltage of apparatus was kept constant at 96V and 80 mA for 40 minutes, until a blue DNA lines came at the end of the gel plates.

Allele scoring and data analysis: Electrophoresis of SSR amplified products on agarose gel created many bands. The size of DNA bands were compared with the DNA marker. In case of absence of bands data were score as 0 and those present were scored as one and entered into an excel binary matrix as a distinct variables. The high polymorphic primers were selected as they are highly informative primers. The polymorphic information content (PIC) value of a DNA ladder was calculated according to formula of Anderson *et al*., (1993). The bands with identical mobility were considered to be identical fragments and therefore were not scored. Only major bands were considered and faint bands were not scored. Mean allele numbers, and genetic similarities were calculated using software (NTSys PC 2.1).

Results

SSR markers were selected with a minimum acceptable length to evaluate genetic diversity at molecular level for 60 Taramira genotypes of diverse origin. A set of 40 SSR markers were applied to assess variation of Taramira germplasm. In some cases, banding pattern of accessions was different showing DNA polymorphism. A total of 54 different DNA bands were produced by applying 40 primers, these all were polymorphic. On average, each primer produced 1.4 polymorphic bands base on polymorphic average. Among the evaluated SSR markers, three markers were documented the foremost for assessing genetic diversity on account of producing excellent polymorphic results. Among all the studied primers: N10-G10 produced 4 polymorphic bands, Ol10-A05 and PBCGSSRBo6 producing 3 polymorphic bands and **Na**12-F03, Ra2-F11, Ra3-H10, Ni2-B03, Ol11-B03, Ol12-E03, PBCESSRNA18 produced 2 polymorphic bands. Whereas the minimum number of polymorphic bands (i.e. 1) was produced by markers PBCESSRJU1, PBCESSRJU4, PBCESSRJU7, PBCESSRJU9, PBCESSRJU16, Ni4-D09, Ra2-D04, Ra2-E03, Ra2-E12, Ra2-E07, Ra2-H06, Na10-B08, Ni2-C12, Ni3-G05, Ni4-A03, Ol10-F12, Ol11-H02, Na10-D09, Ni2-B01, PBCGSSRBo7, PBCGSSRBo36, PBCGSSRBo40, PBCESSRNA26, BRMS-001, BRMS-036BRMS-020, BRMS-007, BRMS-037 and BN83B1. Amplified band size ranged from 95 bp (Ra2-E07) to 700 bp (Ni4-A03) (Table 3). The polymorphic banding pattern of some primers is shown in Fig. 1a & b.

Table 3. List of the 40 SSR, amplified alleles, polymorphic allele, size of alleles and size range used in present studies.

Primers	Total amplified alleles	Polymorphic allele	Size of alleles (bp)
PBCESSRJU1	1	1	400
PBCESSRJU4	1	1	260
PBCESSRJU7	1	1	210
PBCESSRJU9	1	1	110
PBCESSRJU16	1	1	250
Na12-F03	2	2	190,450
Ni4-D09	1	1	160
Ra2-D04.	1	1	190
Ra2-E03.	1	1	260
Ra2-E07	1	1	95
Ra2-E11	1	1	195
Ra2-E12	1	1	125
Ra2-F11	2	2	70,250
Ra2-H06	1	1	220
Ra3-H10	2	2	205
Na10-B08	1	1	260
Ni2-B03	2	2	400,700
Ni2-C12	1	1	210
Ni3-G05	1	1	600
Ni4-A03	1	1	190
Ol10-A05	3	3	100-380
Ol10-F12	1	1	110
Ol11-B03	2	2	150,550
Ol11-H02	1	1	180
Ol12-E03	2	2	250-510
Na10-G10	4	4	230-500
Na10-D09	1	1	160
Ni2-B01	1	1	600
PBCGSSRBo6	3	3	160-500
PBCGSSRBo7	1	1	150
PBCGSSRBo36	1	1	400,600
PBCGSSRBo40	1	1	380
PBCESSRNA18	2	2	100-450
PBCESSRNA26	1	1	215
BRMS-001	1	1	190
BRMS-007	1	1	180
BRMS-020	1	1	210
BRMS-036	1	1	210
BRMS-037	1	1	270
BN83B1	1	1	210
	54	**54**	

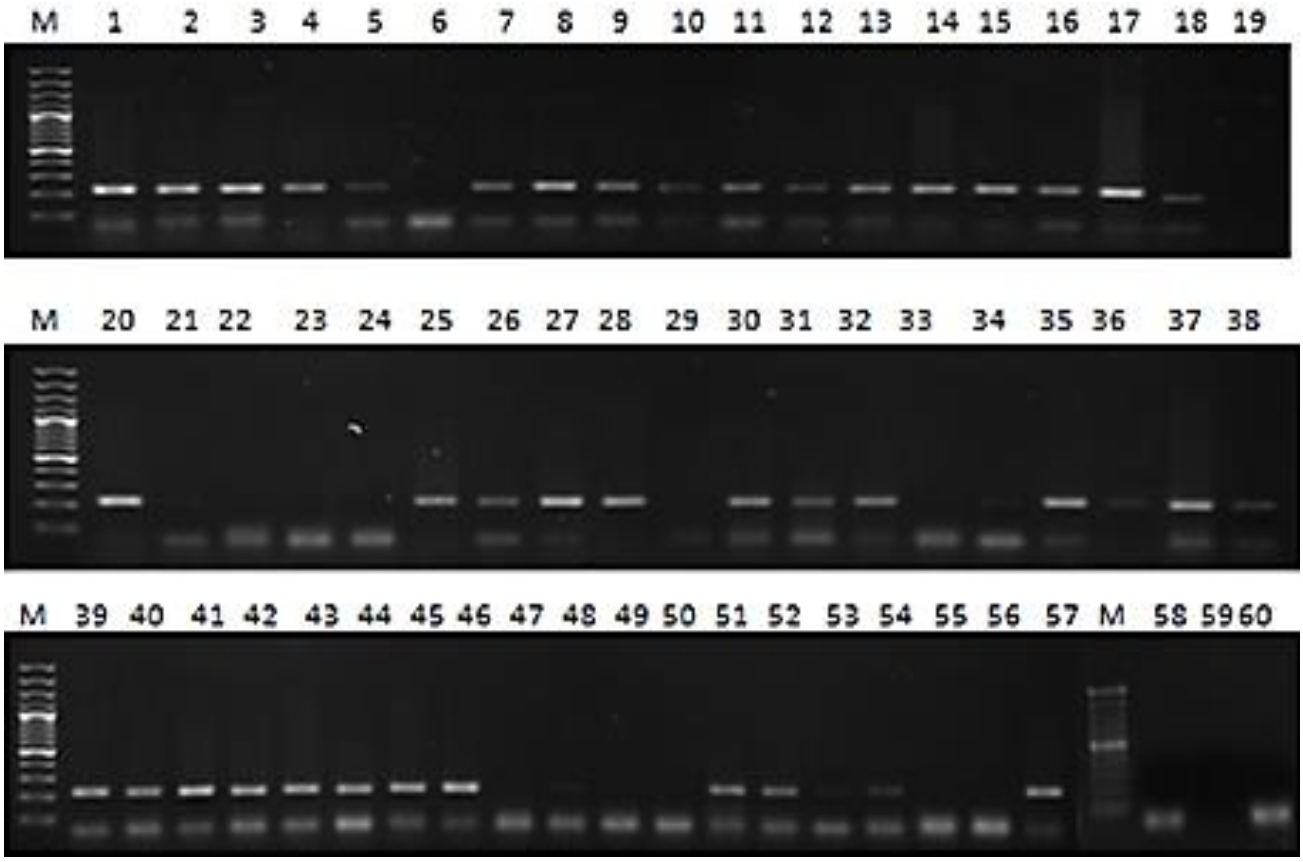

Fig. 1a. Agarose gel showing banding pattern of 60 genotypes of *Eruca sativa* analyzed with primer PBCGSSRNA26. M-present 100 bp molecular marker.

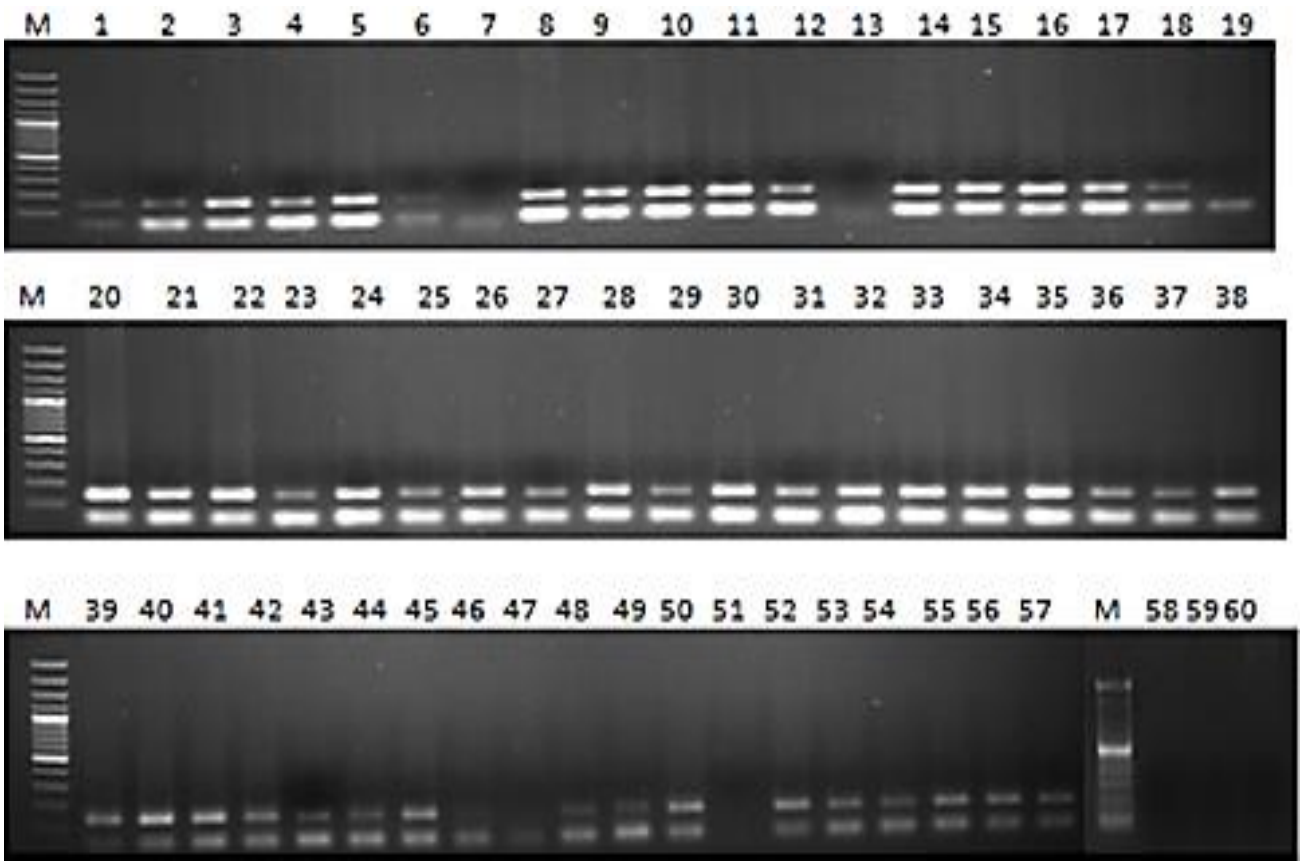

Fig. 1b. Agarose gel showing banding pattern of 60 genotypes of *Eruca sativa* analyzed with primer NI4-D09. M-present 100 bp molecular marker.

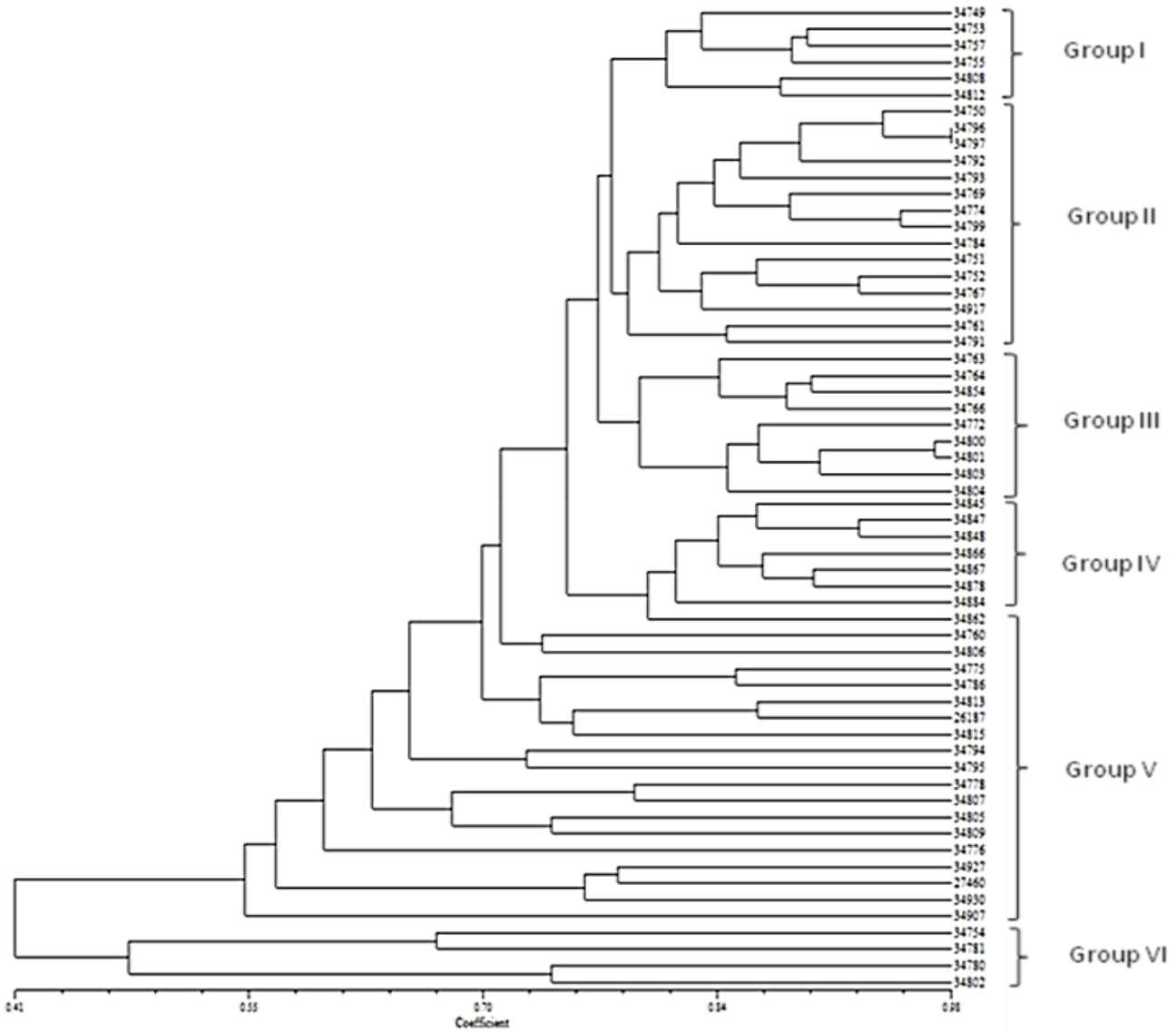

Fig. 2. The dendrogram constructed to study genetic diversity using 60 *Eruca sativa* genotypes and 40 SSR markers.

Cluster analysis: To determine the genetic relationships among 60 Taramira genotypes based on SSR markers, cluster analysis was carried out. Dendrogram was created by using UPGMA, clustering illustrated different grouping of these genotypes. The dendogram created based on similarity index divided 60 Taramira genotypes into six groups (Fig. 2). Group I consisted of 6 genotypes, group II to VI consisted of 15, 9, 7, 19 and 4 genotypes, respectively (Table 4) The similarity coefficient values ranged from 13 to 93% in present study. The highest coefficient of similarity (0.93) was observed between 34767 and 34752, while minimum (0.13) was observed between check accession 27460 and 34802.

Polymorphism information content (PIC): Polymorphism information content value was calculated for each of the 10 SSR primers to measure allele diversity at each locus. The PIC values varied from 0.234 (PBCGSSRBo36) to 0.720 (Na10-G10) with an average value of 0.495 per locus (Table 5). Five primers revealed PIC values greater than 0.50 (50%) representing high genetic diversity in plant genotypes under studies. Primers with PIC values greater than 0.50, such as Na10-G10 (0.720), O110- A05 (0.668) will be highly informative for genetic studies as they were very helpful in differentiating the polymorphism rate of the marker at specific locus.

Discussion

Assessment of genetic diversity of plant species is the foundation of exploitation and utilization of genesof interest for genetic development. Molecular classification is essential for identifying genetic association than morphological and biochemical characterization (Azam *et al*., 2013). These markers can identify polymorphism at high level and are insensitive to surroundings and developmental differences (Ahmad *et al*., 2014). Microsatellites markers are used for several purposes e.g., genome mapping, varieties identification and genetic diversity at molecular level. First report of SSRs on plant was given by Condit & Hubbel (1991) by suggesting that SSRs are rich in plant system. It is the most preferable marker, can easily be automated, highly reproducible, easily exchangeable between laboratories, transferable among populations, highly polymorphic marker, can differentiate closely related lines and a lesser amount DNA are required by SSRs markers.

Table 4. Sixty *Eruca sativa* genotypes, groups and accessions numbers of present research studies.

Groups	No. of genotypes	Accessions numbers
I	6	34749, 34753, 34757, 34755, 34808, 34812.
II	15	34750, 34796, 34797, 34792, 34793, 34769, 34774, 34799, 34784, 34751, 34752, 34767, 34917, 34761, 34791.
III	9	34763, 34764, 34754, 34766, 34722, 34800, 34801, 34803, 34804
IV	7	34845, 34847, 34848, 34866, 34867, 34878, 34884
V	19	34862, 34760, 34806, 34775, 34786, 34813, 26187, 34815, 34794, 34795, 34778, 34807, 34805, 34809, 34776, 34927, 27460, 34930, 34907
VI	4	34754, 34781, 34780, 34802

Table 5. List of the SSR markers analyzed, alleles amplified, polymorphic alleles, PIC values and rate of polymorphic loci.

Marker	Amplified alleles	Polymorphic alleles	Rate of polymorphic loci (%)	PIC
Na12-F03	2	2	100	0.500
Ra2-F11	2	2	100	0.484
Ni2-B03	2	2	100	0.355
O110- A05	3	3	100	0.668
OI 11-B03	2	2	100	0.499
0112 –E03	2	2	100	0.500
Na10-G10	4	4	100	0.720
PBCGSSRBo6	2	2	100	0.500
PBCGSSRBo36	3	3	100	0.234
PBCESSRNA18	2	2	100	0.499
Total	**24**	**24**	**------**	**4.959**
Average	**4.36**	**4.36**	**100**	**0.495**

In present study, 40 SSR markers were used as molecular markers in *Eruca sativa* for their possible exploitation. This work may be essential for future plants breeders who want to use various SSRs for study genetic diversity. Microsatellites markers evaluate better genetic variability than all other molecular markers (Eujayl *et al*., 2001). These markers offered high rate of polymorphisms in crops species such as maize (Senior *et al*., 1998), soybean (Maughan *et al*., 1995), barley, wheat and rice (Pillen *et al*., 2000; Prasad *et al*., 2000). Microsatellites proved to be helpful in evaluation of association among brassica species and various sequences of microsatellite primer were recognized (Snowdon & Friedt, 2004). Distribution of SSR loci among diverse species of mustard family exposed variation at high level, particularly in species of U-triangle. The results of present work are contrary to the findings of high level of variation in *Brassica*. Our results were not agreed with the findings of Plieseke & Struss (2001), they explained genetic variation at high level in U-triangle species. Present research studies showed low level of variation at molecular level in the genotypes of *Eruca sativa.*

Average number of 2.66 polymorphic alleles per locus was stated by Farhatullah *et al*., (2014) by using a set of 12 SSR primers in *B. napus* and *B. campestris*. Other researchers had also accounted the polymorphic average as 4.44 (Tonguc & Griffiths, 2004), 3.9 (Rudolph *et al*., 2000), and 2 (Uzunova & Ecke, 1999). Our findings were not coinciding with the findings those scientists. A total of 54 different DNA bands were produced by applying 40 primers, all were polymorphic alleles. Each primer produced 1.4 polymorphic bands based on polymorphic average. The results of DNA base analysis by microsatellite explained low level of genetic diversity in the genotyps used. Our results agreed with the earlier findings of Chen *et al*., (2000), Osborn & Lukensd (2003), and Stephanie *et al*., (2009) who described low level of polymorphism of microsatellite markers in *Brassica.* Among the studied sets of SSR markers, two primers sets were recognized as the best markers for evaluating of genetic diversity in Taramira crop as these generated the best polymorphic bands. In present study, Na10-G10 (0.720), and O110-A05 (0.668), will be very helpful in differentiating the polymorphic rate of marker at specific locus as they are more informative for genetic studies. Findings gained from present study are very helpful and essential for future studies and researchers are working for genetic variation of genotypes by utilizing SSR markers.

In present investigation PIC value was calculated for each of the 10 SSR primers to measure allele diversity at each locus. The PIC values varied from 0.234 (PBCGSSRBo36) to 0.720 (Na10-G10) with an average value of 0.495/locus. Similar findings were reported by Plieske & Struss (2001), who observed an average of 0.50 PIC values ranged from 0.04-0.80 within *B. napus.* Similar investigations were reported by Fayyaz *et al.*, (2014), they reported that primers BRMS-040 and BRMS-019 had large number of alleles i.e. 4 and had 0.72 PIC value. In present investigation Na10-G10 has large number of alleles (4) with 0.720 PIC value.

The similarity matrix of bands developed for these SSR used for degree of similarity among different Taramira genotypes. The similarity coefficient values ranged between 13% and 93%. The highest coefficient of similarity (0.93) was observed between 34767 and 34752, while minimum (0.13) was observed between check line 27460 and 34802. This result disagreed with Fayyaz *et al*., (2014), who reported similarity matrix ranging from 0.3 to 0.95 among 90 accessions of Barasica species using 12 SSR markers. Cluster analysis revealed that most of similar species fell into close subgroups. Study of genetic diversity has good breeding values. Germplasm having low level of polymorphism showed similarity within study genotypes. Results of study can be used for assessment of genetic diversity, mapping and sequencing of Taramira genomes for development of crop improvement. Information of the genetic associations created from present research work might be important in utilization of available genotypes.

Conclusion

High level of polymorphism was noted among local and exotic Taramira germplasm. However, the level of SSR based variability varies among genotypes. SSR markers are the best sources for genetic studies of Taramira germplasm, and offered an easy and efficient means for comparative genomics studies in *Eruca sativa.* The unique and novel genotypes will be used further for further genome wide association studies.

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