ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIOTECHNOLOGY

ISSR MARKERS ASSISTED STUDIES ON GENETIC DIVERSITY IN BRASSICA JUNCEA

MANJU YADAV AND J. S. RANA*

Department of Bio&Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana (India)

J. S. RANA
Department of Bio&Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana (India)

ABSTRACT

Genetic polymorphism was studied among thirty Indian mustard genotypes using 20 ISSR primers. This study revealed 156 amplified bands with an average of 7.8 bands per primer out of which 115 were polymorphic. Size of amplified fragments ranged from 100 bp to 1500 bp and number varied from 4 to 14. ISSR data were used to compute genetic similarities based on Jaccrad's similarity coefficient, it ranged from 0.50 to 1.0. Similarity matrix was utilized to construct the UPGMA (unweighted pair- group method with arithmetic average) dendrogram. In cluster analysis, based on similarity coefficient, Indian mustard genotypes were categorized into three major clusters Two dimensional and three dimensional analyses further strengthened the cluster analysis observations. Genotypes, Kranti and Rohini were found to be least diverse. BEC 144 was most diverse genotype with a separate cluster. Phenotypic variation was also analyzed and revealed some genetic relationship among genotypes which were evaluated for seed yield and other phenotypic traits.

KEYWORDS

Genetic polymorphism, Indian mustard, Similarity matrix, dendrogram.

INTRODUCTION

Brassica is an agriculturally important genus containing species with highly diverse morphology and wide ranging utility. Indian mustard (*Brassica juncea* (L.) Czern. et Coss., 2n = 36, AABB) is cultivated in Asia, USA and Canada especially for oil production. It is the second largest oilseed crop in India after soybean and accounts for about 62-65% of the total Rabi oil-seed crop. It is cultivated mainly in Northwest India in an area of 70 lac ha with an average yield of 1149 kg/ha and contributes nearly 27 per cent of edible oil pool of the country. Major mustard growing states are Rajasthan, M.P., U.P., Haryana, Gujarat, Bihar, Punjab, West Bengal and Assam. Of the total production (5.08 m tones) of the country, Rajasthan, Uttar Pradesh and Haryana accounts for over 71%. Information on genetic diversity would provide a much improved basis to understand broad-scale patterns of genetic variation. A variety of molecular markers have been used to study the extent of genetic variation among the diverse group of important crops in the genus *Brassica*. Zietjiewicz *et al.,* (1994) developed a new molecular marker, inter simple sequence repeat (ISSR). This approach employs oligonucleotides with 2–4 purine or pyrimidine residues, as primers to amplify mainly the inter-SSR regions. SSRs or micro satellites are short tandem repeats (STRs) or variable number of tandem repeats (VNTRs) of 1–4 bases of DNA ubiquitously presented in eukaryote genomes (Tautz and Renz 1984). They dispersed throughout the genome and varied in the number of repeat units, because of the characters of SSRs, ISSR marker could give information mainly about unrepeated sequences and have the advantage of genome coverage. The utility of PCR-ISSR as phylogenetic markers for investigating evolutionary relationships among plants has been clearly established (Charpter *et al.,* 1996; Panda *et al.,* 2002; Ghariani *et al.,* 2003). ISSR markers have been used to determine the genetic diversity (e.g. Qian *et al.,* 2001). It was also applied to the study of genetic relationships and phylogenetic analysis of various crop plants (e.g. Martin and Sanchez-Yelemo 2000).

Inter simple sequence repeat (ISSR) markers with low cost and low labor requirement but with high reliability have been developed since 1994 (Zietkiewicz *et al.,* 1994). Like any other PCR-based marker, are rapid and require only small amount of the template DNA. ISSR amplification does not require genome sequence information but produce highly polymorphic patterns, and it seem to have the reproducibility of SSR's and the usefulness of RAPD's (Bornet and Branchard 2001), and thus combine the advantages of SSR and AFLP and the utility of RAPD. Thus, in the present study, ISSR fingerprinting data has been used to examine the genetic relationship among thirty *B. juncea* genotypes.

MATERIALS AND METHOD

(i) Plant material

Thirty Indian mustard genotypes were grown in HLRDC field, Hisar. Phenotypic data [Plant height (cm), main shoot length (cm), point to 1st branch (cm), point to 1st siliqua (cm), no. of primary branches, no. of secondary branches, no of siliqua on main shoot, seed yield/plant(g), test weight (g)] was recorded from five randomly selected plants (Table 3). All genotypes were also grown in greenhouse of department of Bio & Nano Tecnology, GJUS&T, Hisar. Leaf tissues of 3 - 4 weeks old plant of each genotype were collected to isolate genomic DNA. Details of 30

genotypes of *Brassica juncea,* used in the present work, are given in Table 1.

Table 1
Detail of thirty genotype of Indian mustard

Sr.No	Code	Name	Source	Specific features/traits
1	G1	RC- 199	CCSHAU,Hisar	Frost resistant
2	G2	PM	Collected from CCSHAU Germplasm	High lectin
3	G3	RH- 8812	CCSHAU,Hisar	Bold seeded, Suitable for irrigated condition
4	G4	T- 6342	IARI, New Delhi	Tolerant to mustard aphid
5	G5	PJK	IARI, New Delhi	High productivity, Suitable for irrigated conditions
6	G6	PCR- 7	DRMR, Bharatpur	Drought resistant
7	G7	B -85	PORS, Behrampur	Glossy plant
8	G8	RH- 7846	CCSHAU,Hisar	Hairy leaf surface
9	G9	Kranti	CSA, Kanpur	Tolerant to high temperature at seedling stage
10	G10	Rohini	CSA, Kanpur	Drought tolerant, Suitable for irrigated conditions
11	G11	Varuna	CSA, Kanpur	Bold seeded, Smooth leaf surface,Thermotolerant
12	G12	RW white glossy	Collected from IARI Germplasm	White petals
13	G13	BEC- 144	Exotic collection (Polish)	White rust resistant
14	G14	RW 2-2	Collected from IARI Germplasm	Tolerant to aphid
15	G15	RLM -514	PAU,Ludhiyana	High glucosinolates, High erucic acid
16	G16	RLM -198	PAU,Ludhiyana	High erucic acid
17	G17	RGN -73	ARS,RAU,Shriganganagar	Suitable for growing under irrigated and draught condition
18	G18	RH- 30	CCSHAU,Hisar	Bold seeded, Non shattering
19	G19	RH- 819	CCSHAU,Hisar	Drought tolerant ,Thermo tolerant, White rust resistant
20	G20	RN-393(Arawali)	ARS,RAU,Navgaon	Suitable for rainfed conditions
21	G21	P. Bold	IARI, New Delhi	Bold seeded, Non shattering
22	G22	RH -8701	CCSHAUHisar	Frost resistant , Suitable for late sown conditions
23	G23	NPJ -93	IARI, New Delhi	High temperature tolerant at seedling stage
24	G24	Krishna	GBPAUT,Pantnagar	Higher oil content
25	G25	CS -54	CSSRI, Krnal	Suitable for salt affect areas
26	G26	RL- 18	PAU,Ludhiyana	Salt tolerant
27	G27	P. Jagganath	IARI, New Delhi	Suitable for irrigated condition
28	G28	Vardan	CSA, Kanpur	Suitable for late sown conditions
29	G29	RB- 50	CCSHAU, Bawal,(Haryana)	Suitable for salinity and rain fed conditions
30	G30	RK- 9501	CSA, Kanpur	Shattering resistant, suitable for late sown conditions

Table 3
Pooled phenotypic data of thirty Indian mustard genotypes

S.No.	Genotype	PH	SL	Point br	Point sil	NPB	NSB	No sili	SY	TW
1	RC-199	212.0	60.8	51.2	21.0	7.6	15.4	36.2	9.49	1.92
2	PM	223.2	35.2	93.4	3.2	5.2	12.6	33.4	7.31	1.12
3	RH-8812	215.8	55.4	87.8	14.6	7.2	15.6	37.2	19.43	2.79
4	T-6342	215.0	70.0	83.0	6.6	5.8	16.0	36.8	14.00	2.20
5	Pusa Jai Kisan	209.0	83.4	61.2	6.8	4.8	13.0	55.8	19.04	3.02
6	PCR-7	213.6	85.8	38.4	16.6	5.0	12.8	45.0	13.40	1.72
7	B-85	210.6	78.2	66.8	15.0	4.8	15.4	42.2	9.47	2.38
8	RH-7846	225.2	72.0	62.2	13.3	4.0	14.6	40.0	14.00	2.10
9	Kranti	215.4	73.8	68.2	14.2	4.0	13.4	41.8	12.48	1.84
10	Rohini	220.4	69.4	83.6	7.2	4.8	12.6	41.8	16.32	1.87
11	Varuna	218.2	69.4	93.2	5.0	7.0	13.8	57.0	13.31	1.81
12	RW white glossy	190.0	73.0	74.0	9.0	6.2	15.2	42.2	13.90	1.90
13	BEC-144	281.0	50.0	133.0	3.0	6.0	16.0	17.0	8.26	1.40
14	RW-2-2	227.6	84.8	89.0	6.4	5.6	8.2	44.0	10.28	3.25
15	RLM-514	201.4	75.0	45.4	12.4	4.4	13.0	46.0	12.90	2.52
16	RLM-198	244.6	73.2	73.6	4.6	5.4	8.6	48.4	11.85	2.72
17	RGN-73	233.8	70.0	86.0	7.0	6.0	16.6	43.8	15.61	2.57
18	RH-30	231.6	71.2	105.6	16.2	6.4	16.0	38.8	13.47	2.52
19	RH-819	220.0	69.2	93.0	14.4	6.0	11.8	45.8	15.17	2.12
20	Arawali (RN-393)	222.0	53.6	109.8	5.0	4.6	11.4	35.2	12.25	2.58
21	Pusa Bold	187.0	68.6	23.6	6.6	6.6	18.4	40.0	17.28	3.12
22	RH-8701	198.8	80.0	54.6	16.2	4.0	5.4	45.8	11.41	3.16
23	NPJ-93	191.2	74.2	61.8	7.6	4.2	13.8	42.2	18.35	3.49
24	Karishna	245.8	63.2	99.4	17.4	6.2	19.8	42.0	16.70	2.51
25	CS-54	239.8	75.6	74.8	18.6	4.8	16.0	44.4	14.53	1.77
26	RL-18	231.2	65.0	79.4	19.8	5.2	13.6	42.4	9.69	1.72
27	Pusa Jagganath	214.6	71.2	51.4	4.4	8.0	18.4	55.2	19.83	2.65
28	Vardan	218.6	64.4	75.0	5.2	6.6	19.6	49.0	10.69	1.43
29	RB-50	198.6	75.8	55.4	8.2	3.6	7.2	39.6	14.10	2.53
30	RK-9501	219.4	76.0	68.0	6.2	5.8	13.4	46.0	13.40	3.38

PH- Plant height (cm), SL- main shoot length, Point br-Point to 1st branch(cm), Point sil- Point to 1st siliqua(cm), NPB- No. of primary branches, NSB- No. of Secondary branches, No sili- no of siliqua on main shoot, SY -Seed yield/plant(g), TW -Test weight (g)

(ii) DNA extraction

Total genomic DNA was extracted from the fresh leaf tissues of genotypes by the CTAB method of Doyle and Doyle (1990). The concentration of DNA was visually monitored by the band intensity in comparison with the lambda DNA of known concentration using 0.8% agarose gel. Further, the concentration of DNA was

determined by Nano drop spectrophotometer (ND-1000). Dilution was made accordingly so that the final DNA concentrations become 20ng/µl. This concentration was used as working concentration.

(iii) ISSR amplification:

Twenty primers were used in the present study (Table 2). Amplification of DNA was carried out in a reaction mixture containing 40 ng of DNA; 1.5 mM of $MgCl_2$ in the buffer (10x, with 25 mM $MgCl_2$); 0.25 mM of dNTPs; 0.2 µM primers and 1.0 U Taq DNA polymerase per 20 µl reaction mixture. The PCR amplification conditions were introduced by Martin and Sanchez-Yelemo (2000), with a little modification of the annealing temperature. PCR amplification was performed in thermal cycler Gene Pro (China), programmed for 35 cycles as: 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 45s, 50°C for 45 s, and 72°C for 2 min followed by 1 cycle of 72°C for 7 min. The amplification product was visualized on 1.5% agarose gel by staining with ethidium bromide and photographed under UV light using gel documentation system (Syngene, USA). The molecular size of fragments was estimated by reference to a 100-bp DNA ladder fig.1. At least two PCR amplifications were done for each sample, and only reproducible bands in several runs were considered for analysis.

Table 2

ISSR primers used for the molecular characterization of Brassica juncea.

Primer	Primer sequence*	Annealing temperature (°C)	Fragment size range	Fraction polymorphic fragments†
MM1	$(CT)_8T$	50	430–1225	4/4
MM2	$(CT)_8A$	50	300–1180	6/12
MM3	$(CT)_8G$	52	395–1090	5/5
MM4	$(CA)_8A$	52	200–1355	6/7
MM5	$(GT)_8T$	52	575–1255	5/7
MM6	$(TC)_8G$	50	100–1030	7/9
MM7	$(AC)_8C$	52	200–1135	5/5
MM8	$(AG)_8YT$	52	370–1490	4/6
MM9	$(AG)_8YA$	52	325–1290	7/8
MM10	$(GA)_8YT$	54	255–1640	4/5
MM11	$(CT)_8RA$	54	350–1160	6/8
MM12	$(CT)_8RG$	54	100-1500	5/9
MM13	$(CA)_8RG$	55	300–875	4/10
MM14	$(CT)_8RA$	52	100–1190	7/7
MM15	$(AC)_8YT$	55	150–825	7/8
MM16	$(AC)_8YA$	55	480–1100	8/10
MM17	$(AC)_8YG$	54	250–1300	5/12
MM18	$(TG)_8RT$	52	330–1400	8/10
MM19	$(TG)_8RC$	55	300–1340	5/6
MM20	$(TG)_8RA$	52	330–1400	7/8
Total				115/156

**Single letter abbreviations for mixed-base positions: (Y = T or C, R = G or A)*

†Number of polymorphic fragments/number of fragments amplified.

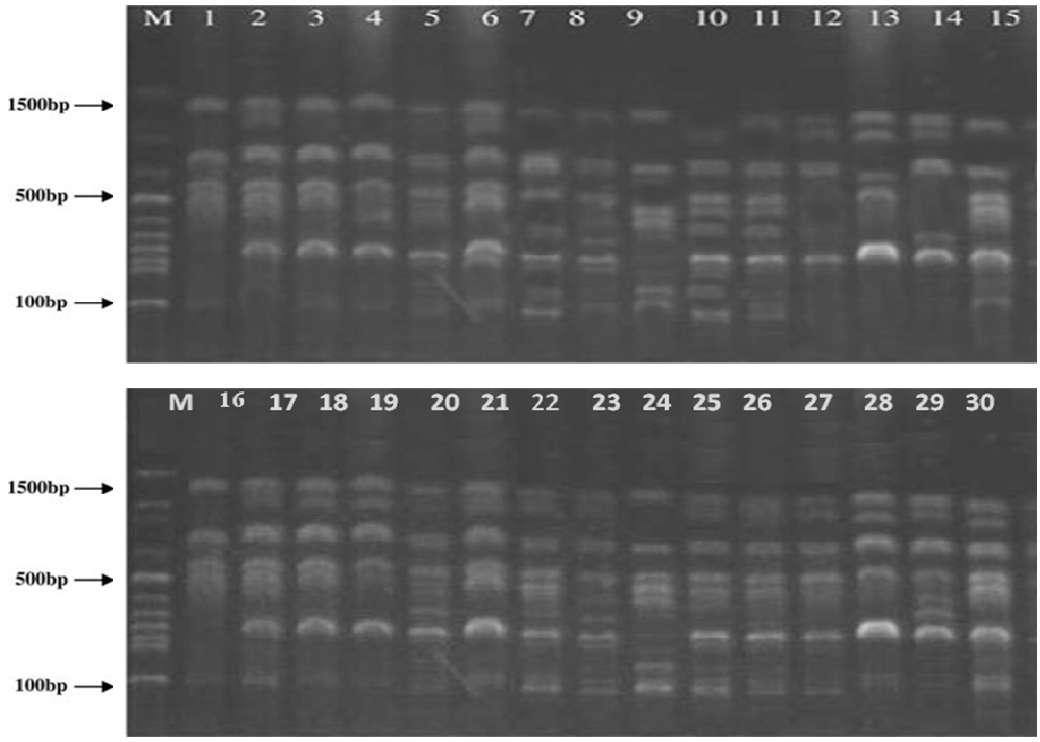

Figure 1
PCR profile of 30 genotypes of Brassica juncea using ISSR marker MM12.

(iv) Data analysis:
The molecular data was analyzed using statistical software package NTSYS-pc (version 2.1, Rohlf F.J. 2000). For statistical interpretation of DNA fingerprinting results; binary matrix was constructed by scoring the ISSR gels as presence (1) and absence (0) of bands across genotypes. On the basis of binary matrix, similarity matrix was calculated using Jaccard's coefficient (Jaccard, 1908) and a dendrogram based on UPGMA (Unweighted Pair Group Method of Arithmetic Averages) analysis was constructed with ISSR data. A principal coordinate analysis (PCA) was performed in order to highlight the resolving power of the ordination (fig, 3). Genetic diversity was measured by the percentage of polymorphic bands (P), which was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. The phenotypic data was analyzed by statistical software package SPSS (version 13) using Sequential Agglomerative Hierarical Nested Clustering (SAHN) to group individuals into discrete clusters.

RESULTS AND DISCUSSION

ISSR primers produced a total of 156 bands with an average frequency of 7.8 bands per primer. The number of the bands produced by each primer ranged from 4 to 14. Of the total 156 bands produced, 115 (73.71%) were polymorphic. Figure 1 showed the amplification profiles of primer MM12. The similarity matrix values were found from 50 to 100% among different genotypes. The dendrogram constructed on the basis of Jaccard's similarity matrix, followed by UPGMA based clustering analysis (Figure 2) showed that the genotypes were grouped into three major clusters viz., cluster I, cluster II and Cluster-III.

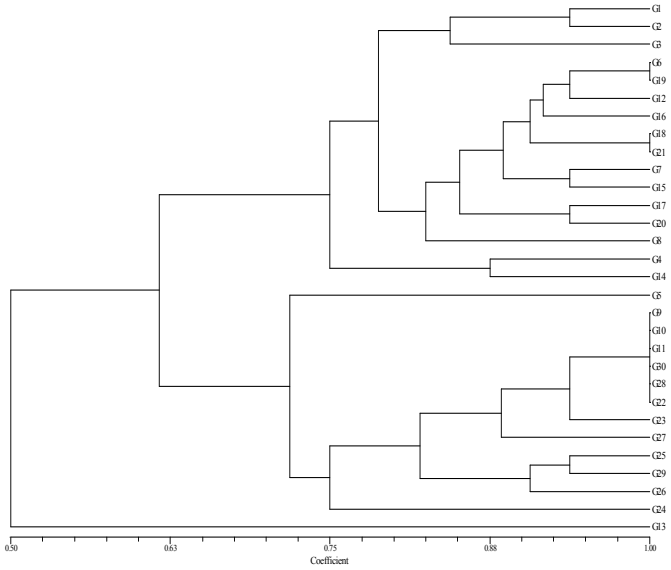

Figure 2
Dendrogram of thirty B.juncea genotypes based on UPGMA analysis of ISSR polymorphisms.

Cluster-I comprised 16 genotypes with a genetic similarity of 72% to 100%. Cluster-II comprised 13 genotypes with a genetic similarity of 70% to100%. Cluster-III comprised of 1 genotype which is an exotic collection having 50% similarity with other genotypes. Cluster I was further subdivided in three sub clusters Cluster-II was also subdivided in three sub clusters. Thirty genotypes were clustered into four clusters on the basis of phenotypic traits by using Sequential Agglomerative Hierarical Nested Clustering (SAHN). A comparison between two types of clustering revealed some genetic relationship based on phenotypic traits. Cluster membership table (Table 4) showed that some genotypes viz. Kranti, Rohini, Varuna, Vardan, Krishna and RK-9501 were grouped together in both types of cluster analysis. BEC 144 was in separate group in both type of analysis. On the basis of salt tolerant trait CS-54, RL-18, RB-50 were clustered together.

Table 4
Cluster membership table

Cluster	Name of Genotypes	
	On the basis of Phenotypic traits	**On the basis of ISSR analysis**
I	NPJ-93, P.Jagganath, RLM-514, P.Bold, PCR-7, P.Jai Kisan, RH-8701,T-6342, RB-50, RW white glossy,	RC-199, PM, RH-8812, PCR-7, RH-819, RW white glossy, RLM-198, RH-30, P.Bold, B-85, RLM-514, RGN-73, RN-393, RH-7846, T-6342, RW 2-2
II	Kranti, Rohini, RH -8812, RH- 30, RH- 819, RW 2-2, RLM -198, Varuna, RK- 9501, Vardan, Krishna, RGN- 73, CS- 54, RL -18, B-85,RH-7846, RC-199	Kranti, Rohini, Varuna, RK- 9501,Vardan, RH-8701, NPJ-93, P.Jagganath, CS-54, RB-50, RL-18, Krishna
III	BEC-144	BEC-144
IV	RN-393, PM	

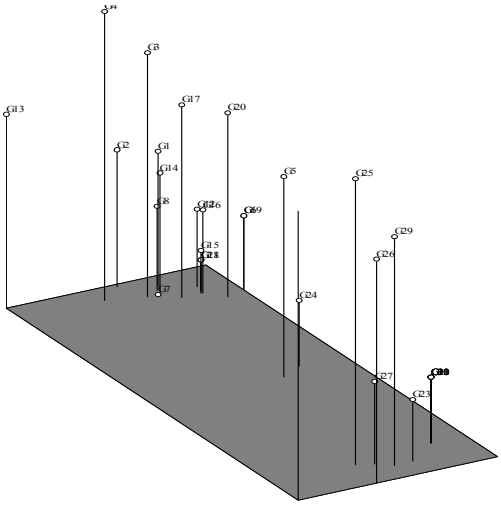

Figure 3
Principal-Coordinate Analysis (PCA) of thirty genotypes of Brassica juncea.

PCA analysis also showed very clearly that BEC 144 genotype was distinguished from other genotypes.

Huangfu *et al.,* (2009) also used Inter-simple sequence repeat (ISSR) to assess level and patterns of genetic diversity in wild *Brassica juncea* (L.) Czern. et Coss. Populations. A total of 93 plants from 24 wild populations in China were analyzed by eight primers resulting in 86 highly reproducible ISSR bands. The analysis of molecular variance (AMOVA) with distances among individuals corrected for the dominant nature of ISSRs showed that most of the variation (54.09%) occurred among populations, and the remaining 45.91% variance was attributed to differences among individuals within populations.

Liu *et al.,* (2006) used *Brassica* triangle, including three allopolyploids and three diploids, to study genomic evolution after the formation of polyploids. Based on the inter-simple sequence repeat (ISSR) analysis, the different degree of A, B or C genomic modifications were observed. ISSR data supported that a higher degree of ancestral genomic divergence gave rise to a greater frequency of genomic change of polyploids.

The genetic diversity in nine genotypes of *Brassica* and their two wild relative genera *Sinapis alba* and *Eruca sativa* were studied by Kumari *et al.,* (2009) using Inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) markers. The perusal of dendrogram showed the highest similarity Index of 0.595 between *B. campestris* var. *yellow sarson* and *B. campestris* var. *toria.* The lowest similarity coefficient (0.125) between *B. napus* and *Eruca sativa* shows that they have highest diversity between genome. *B. juncea* (yellow seeded) and *B. juncea* (brown seeded) had high similarity coefficient (0.425) that Indicates the gene controlling the seed coat color also contribute towards the genetic diversity. Similarity coefficient (0.425) between *B. juncea* yellow seeded and brown seeded varieties clustered separately indicates that the genes controlling the seed coat color also contribute towards the genetic diversity between these two cultivars of *B. juncea.*

Gupta *et al.,* (2004) used ISSR UBC primers to characterize a total of 21 F_1's involving *B. juncea* as female and *B. campestris* and *B. carinata* as male donors. The size of amplified bands ranged from 400 to 1000 bp resulting into a polymorphism of 92%. Bands specific to female and male parents were present in all hybrids ascertaining the hybrid nature of F_1 progeny. The dendrogram constructed using

Jaccard's similarity coefficient grouped parent genotypes and hybrids into four major distinct clusters. All hybrids were first grouped into two clusters based on the presence of male donors, *B. campestris* and *B. carinata*, and then further sub-grouped depending upon the presence of female parent in specific crosses. Hybrids generated from cross *B. juncea* x *B. campestris* resembled the female and male parents, at a genetic similarity value of 0.71 and 0.45, respectively, while those of cross *B. juncea* x *B. carinata* resembled the female and male parent at a genetic similarity value of 0.67 and 0.37, respectively. The genetic similarity values indicated more influence of female parent in the hybrids in both types of crosses.

Chao-zhi *et al.,* (2003) compared the genetic diversity of 24 Chinese weak-winter, Swedish winter and spring *Brassica napus* accessions by inter-simple sequence repeats (ISSRs). Using cluster analysis (UPGMA) based on 125 polymorphism bands amplified with 20 primers, the 24 accessions were divided into three groups. Results from cluster analysis and PCO analysis showed very clearly that Chinese weak-winter, Swedish spring and winter accessions were distinguished from each other The Chinese weak-winter accessions had larger diversity than the Swedish spring or winter accessions.

CONCLUSION

Experimental results evidenced the role of ISSR markers, which allowed estimating the genetic diversity for *Brassica juncea* genotypes. In this work ISSR analysis has provided the information on the genetic relatedness within selected genotypes of *Brassica juncea* on the basis of phenotypic traits. Thus the present study indicates that ISSR is a suitable and effective tool to evaluate genetic diversity among *Brassica juncea* genotypes.

REFERENCES

1. Bornet B., Branchard M., Non anchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Rep, 19:209–215, (2001)
2. Chao–zhi M.A., Ting-dong F. U., Tuevesson S., and Bo Gertsson, Genetic diversity of Chinese and Swedish rapeseed (*Brassica napus* L.) analysed by inter-simple sequence repeats (ISSRs). Plant Genetic Resources, 1: 169-174, (2003)
3. Charpter Y.M., Robertson A., Wilkinson M.J. and Ramsay G., PCR analysis of oilseed rape cultivars (*Brassica napus* L. sp. *oleifera*) using 5'-anchored simple sequence repeat (SSR) primers. Theor. Appl. Genet, 92: 442–447, (1996)
4. Doyle, J.J., Doyle, J.L., Isolation of plant DNA from fresh tissue. Focus, 12: 13 -15, (1990)
5. Ghariani S., Trifi-Farah N., Chakroun M., Marghali S. and Marrakchi M., Genetic diversity in Tunisian perennial ryegrass revealed by ISSR markers. Genet. Resour. Crop Evol, 50: 809–815, (2003)
6. Gupta K., Prem D., Negi M.S., and Agnihotri A., ISSRs: An efficient tool to characterize interspecific F_1 hybrids of *Brassica* species. 4th International Crop Science Congress. Brisbane, Australia, (2004)
7. Huangfu C., Song X. and Qiang S. G., ISSR variation within and among wild *Brassica juncea* populations: implication for herbicide resistance evolution Genetic Resources and Crop Evolution., 56: 913–924, (2009)
8. Jaccard P., Nouvelles recherches sur la distribution florale. Bulletin de la Societe Vaudoise des Sciences Naturelles 44: 223–270, (1908)

9. Kumari P., Rathore R.K.S., Yadav R., Singh K. P., and Kumar R., Utility of SSR and ISSR markers for assessment of genetic diversity in *Brassicas* and their related genera. Prog. Agrlc, 9 (1): 71-78 (2009)
10. Liu Ai-hua and Wang Jian-bo, Genomic evolution of *Brassica* allopolyploids revealed by ISSR marker. Genetic Resources and Crop Evolution, 53: 603–611, (2006)
11. Martin JP., Sanchez-Yelemo MD, Genetic relationships among species of the genus Diplotaxis (*Brassica*ceae) using inter simple sequence repeat markers. Theor Appl Genet, 101:1234–1241, (2000)
12. Panda S., Martin J.P. and Aguinagalde I., Chloroplast and nuclear DNA studies in a few members of the *Brassica* oleracea L. group using PCR–RFLP and ISSR-PCR markers: a population genetic analysis. Theor. Appl. Genet, 106: 1122–1128, (2002)
13. Qian W., Ge S., Hong D-Y., Genetic variation within and among populations of a wild rice Oryza granulata from China detected by RAPD and ISSR markers. Theor Appl Genet, 102:440–449, (2001)
14. Rohlf FJ, NTSYSpc. Numerical taxonomy and multivariate analysis system. Version 2.1. Department of Ecology and Evolution. State University of New York, Stony Brook, NY (2000)
15. Tautz D. and Renz M., Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res, 12: 4127–4138, (1984)
16. Zietkiewicz E., Rafalski A., Labuda D., Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20:176–183, (1994)