

Original Research Article

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## Genetic Diversity Analysis of Indian Mustard (*Brassica* spp.) Germplasm Lines using SSR Molecular Markers

Ravi Baghel<sup>1</sup>, A. K. Sharma<sup>1</sup>, Sushma Tiwari<sup>1</sup>, M. K. Tripathi<sup>1\*</sup> and Niraj Tripathi<sup>2</sup>

<sup>1</sup>Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior-474002, India

<sup>2</sup>Directorate of Research Services, Jawaharlal Nehru Agricultural University, Jabalpur-482004, India

\*Corresponding author

### ABSTRACT

#### Keywords

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Molecular categorization of 48 mustard (*Brassica* spp.) genotypes by employing 20 SSR markers divulged that eight primers viz., BRMS-240, BRMS-324, SR-7223, SR-9222, SR-94102, OI10-C05, SSRNa10-DO9 and SSRNa 10-D11 illustrated good technical resolution and out of these eight seven furnished adequate variations among diverse genotypes. In total 50% polymorphism was detected. Major group restrained 48 genotypes that divided into three major groups, first main group 'I' contained 17 genotypes, second chief group 'II' hold 24 genotypes and third core group 'III' included 7 genotypes. Polymorphic information content (PIC) was estimated for each of the 7 SSR markers. The highest PIC value (0.6851) was documented for the molecular marker OI10-CO 5. It had 8 alleles among the 48 genotypes; whereas, the lowest PIC (0.4038) was examined for the molecular marker SR-7223.

### Introduction

Oilseed *Brassica* occupies an imperative spot in the agriculture due to useful oil content. In most of the provinces of the world, its cultivation has risen tremendously during the last periods and, by now; it is the third largest contributor of the world supply of vegetable oil. Rapeseed is one of the most significant oil and protein rich annual crops in the world. Seed provides oil both for industrial and cooking purposes. The oils extracted from mustard contain high protein (37%) and feed

concentration which is highly palatable to livestock. So, it is urgent to analyse the genetic diversity and its response for the selection of short duration mustard genotypes for increasing our cropping intensity.

Presence of diversity at marker loci is presently the most feasible strategy for characterizing diversity among mustard germplasm lines. Molecular markers offer the best estimate of genetic diversity, since these are independent from the difficult effects of environmental factors. In mustard, various

marker systems have been applied for detecting the genetic diversity. There are increasing number of reports where molecular markers like Restriction fragment length polymorphism (RFLP) (Diers and Osborn, 1994; Hallden *et al.*, 1994), random amplified polymorphic DNAs, (RAPDs) (Ghosh *et al.*, 2009; Khan *et al.*, 2011, Tripathi *et al.*, 2012), amplified fragment length polymorphism (AFLP) (Sun *et al.*, 2001; Zhao *et al.*, 2005, Tripathi *et al.*, 2011) and microsatellites or simple sequence repeats (SSRs) (Abbas *et al.*, 2009; Wang *et al.*, 2009; Tiwari *et al.*, 2019) have been used to study genome organization, varietal differences and diversity analysis in *Brassicac*s. Among all molecular markers, Simple Sequence Repeats (SSRs) or microsatellites have been distinguished as constructive molecular markers in marker assisted selection (MAS), for the analysis of genetic diversity (Kachare *et al.*, 2019), population analysis (Tiwari *et al.*, 2019) and other purposes in various species (Tripathi and Khare, 2016; Mishra *et al.*, 2020; Pramanik *et al.*, 2019; Shyam *et al.*, 2020; Upadhayay *et al.*, 2020). Several hundred SSR markers have been developed for *Brassica* genotypes (Kumar *et al.*, 2015). But, no systematic work on molecular diversity analysis among the germplasm lines selected for the current study has been performed. So, the present investigation was undertaken to study the genetic diversity among germplasm lines of mustard at molecular level to see inherent relationship between and among the germplasm lines for incorporation in mustard breeding programme.

## Materials and Methods

Experimental material consists of forty-eight Indian mustard genotypes (Table 1) obtained from the Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalay, Gwalior, Madhya Pradesh (AICRP on Rapeseed and Mustard).The

present investigation was conducted at the Department of Plant Molecular Biology and Biotechnology to characterize mustard germplasm lines at molecular level with the use of SSR markers. DNA was isolated from actively growing fresh leaves of 20 to 25 days old seedlings using Cetyl Trimethyl Ammonium Bromide (CTAB) method. DNA quality was confirmed by electrophoresis on 0.8% agarose gel and quantified using a spectrophotometer. Initially a total of 20 SSR primers were tested and only polymorphic markers were used for further analysis. For polymerase chain reaction initial denaturation at 94°C for 3 min; denaturation at 94°C for 1 min; annealing at 35°C for 45 sec; elongation or extension at 72°C for 45 sec.; Cycle to step 2 for 40 more time; final extension at 72°C for 5 min were performed. The amplified products were separated electrophoretically on 2.5% agarose gel containing ethidium bromide. Molecular weight marker of 100 bp DNA ladder was electrophoresed alongside with the PCR products. DNA bands were observed on UV-transilluminator in the dark chamber of the Image Documentation System. Bands were scored according to their sizes. The scores obtained using all primers in the SSR analysis were pooled to create a single data matrix. This was used to construct a UPGMA dendrogram.

## Results and Discussion

Genetic diversity investigation is the first and leading footstep in any crop advancement agenda. Usually, to have a reliable estimate of genetic relationship and genetic diversity, a large number of polymorphic markers are applied. DNA analysis with markers is a very effective tool for analyzing genetic diversity. Out of all types of molecular markers, SSR markers have been extensively used in many crops as genetic markers for the assessment of genetic diversity and found to be successful in the characterization of individual with their

pedigree. These markers are dominant and can detect variation in non-coding regions of the genome.

The randomly selected 20 SSR primers were shortlisted based on banding patterns by using DNA samples of two mustard germplasm lines. Out of them, the best eight amplified SSR markers were picked for an advance work. Among these 8 markers, only one marker showed monomorphic bands while 7 markers confirmed polymorphic bands. In total 50% polymorphism was observed. Mishra *et al.*, (2011) and Prajapat *et al.*, (2014) also found analogous results. They documented 72% polymorphism. Comparative genomics in *Brassica* have demonstrated that microsatellite

characteristics in related species are highly similar (Shi *et al.*, 2014). Intra-generic transferability of SSRs had been reported earlier in many studies, *i.e.* SSRs from *Pennisetum glaucum* to *P. Purpureum*, *Brassica species* to *B. tournefortii*, *B. Fruticulosa* and *B. Spinescens* (Singh *et al.*, 2012). Transferability of SSR markers between A- and C- genomes of *Brassica* species had also been assessed by Saal *et al.*, (2001), which corresponded to the already established evolutionary relationship. In a genetic diversity analysis with Indian mustard genotypes and SSR markers, Parida *et al.*, (2010) reported only 2 alleles per locus, where the fragment size varied from 100 to 2000 bp.

**Table.1** List of germplasm lines used in present study

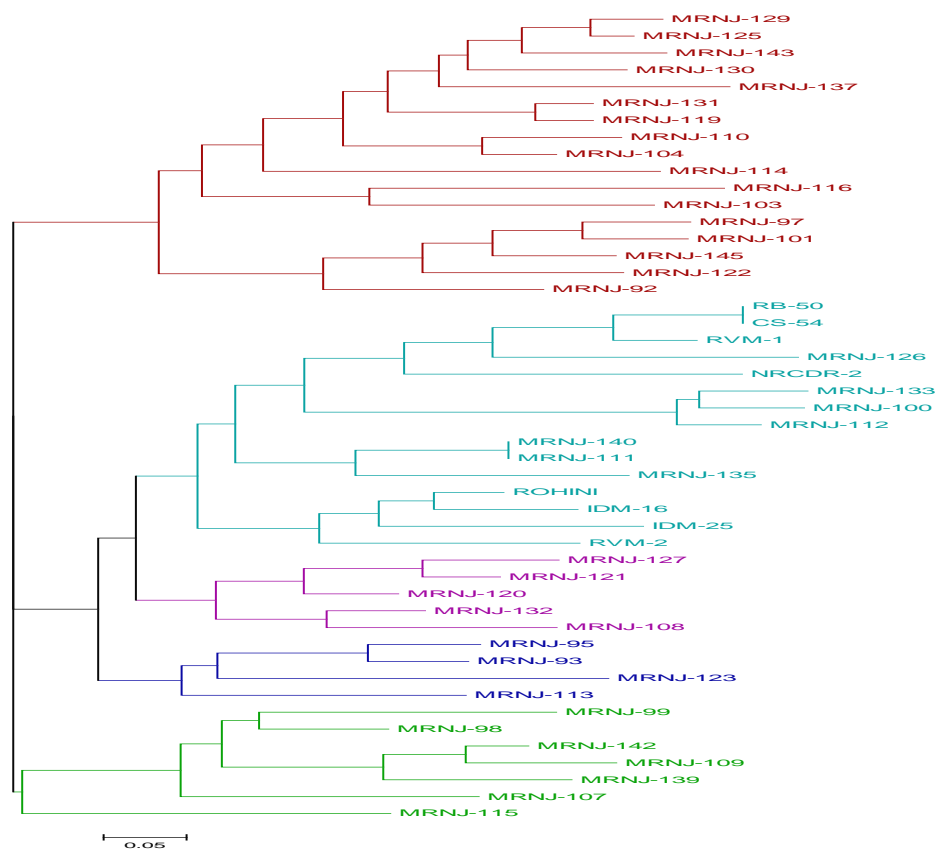
S. No.	Germplasm lines	Collection site	S. No.	Germplasm lines	Collection site
1.	MRNJ-92	BTE	25.	MRNJ-123	BTE
2.	MRNJ-93	BTE	26.	MRNJ-125	BTE
3.	MRNJ-95	BTE	27.	MRNJ-126	BTE
4.	MRNJ-97	BTE	28.	MRNJ-127	BTE
5.	MRNJ-98	BTE	29.	MRNJ-129	BTE
6.	MRNJ-99	BTE	30.	MRNJ-130	BTE
7.	MRNJ-100	BTE	31.	MRNJ-131	BTE
8.	MRNJ-101	BTE	32.	MRNJ-132	BTE
9.	MRNJ-103	BTE	33.	MRNJ-133	BTE
10.	MRNJ-104	BTE	34.	MRNJ-135	BTE
11.	MRNJ-107	BTE	35.	MRNJ-137	BTE
12.	MRNJ-108	BTE	36.	MRNJ-139	MRA
13.	MRNJ-109	BTE	37.	MRNJ-140	MRA
14.	MRNJ-110	BTE	38.	MRNJ-142	MRA
15.	MRNJ-111	BTE	39.	MRNJ-143	GCT
16.	MRNJ-112	BTE	40.	MRNJ-145	GCT
17.	MRNJ-113	BTE	41.	RB-50	BWL
18.	MRNJ-114	BTE	42.	IDM-25	MRA
19.	MRNJ-115	BTE	43.	CS-54	KUN
20.	MRNJ-116	BTE	44.	RVM-1	GWL
21.	MRNJ-119	BTE	45.	NRCDR-2	BTE
22.	MRNJ-120	BTE	46.	RVM-2	GWL
23.	MRNJ-121	BTE	47.	IDM-16	MRA
24.	MRNJ-122	BTE	48.	ROHINI	CNB

BTE – Bharatpur, MRA – Morena, GCT – Ghazipur, BWL – Bawal, KUN – Karnal, CNB – Kanpur, GWL – Gwalior.

**Table.2** Polymorphism revealed by SSR markers assays of mustard germplasm lines

SSR Marker	Major Allele Frequency	Genotype Number	Allele Number	Gene Diversity	PIC value
BRMS- 240	0.5000	4.0000	4.0000	0.5877	0.5047
BRMS- 324	0.4583	6.0000	5.0000	0.6727	0.6164
SR- 7223	0.5208	3.0000	3.0000	0.5182	0.4038
SR- 9222	0.3958	5.0000	5.0000	0.7196	0.6723
SR 94102	0.3958	5.0000	4.0000	0.6968	0.6395
OI 10-CO 5	0.3750	5.0000	5.0000	0.7300	0.6851
SSR Na10-D09	0.3750	5.0000	5.0000	0.7222	0.6740
<b>Mean</b>	<b>0.4315</b>	<b>4.7143</b>	<b>4.4286</b>	<b>0.6639</b>	<b>0.5994</b>

**Fig.1** The diagrammatic representation of clusters based on SSR data prepared using power marker 3.25 software



The summary statistics extracted from Power Marker was scrutinized for allele number, major allele frequency, genotype number, gene diversity, heterozygosity and Polymorphism Information Content (PIC). Major Allele Frequency ranged from 0.3750

to 0.52 with a mean value of 0.43. Heterozygosity ranged between 0 and 20. The mean heterozygosity was 0.03. Polymorphism information content (PIC) was estimated for each of the 7 SSR markers. Higher value of PIC indicated higher polymorphism of the

SSR markers and assists to select the best SSR markers in phylogenetic analysis. Highest PIC value (0.6851) was documented for SSR marker OI10-CO 5 which has 8 alleles among the 48 genotypes. Molecular markers SSR Na10-D09, SR- 9222 and BRMS-324 also had higher PIC scores and high number of alleles (Table 2). The lowest PIC value was attained with SR-7223 (0.4038) marker. Mean PIC value was 0.59, representing high diverse nature of marker which can be employed for further characterization. Pandey *et al.*, (2012) found that only 15.7 percent of markers gave PIC values greater than 0.5. They found that allelic values ranging from 5 to 11. Thus, the number of alleles directly influenced the PIC value (Tantasawat *et al.*, 2011; Tiwari *et al.*, 2019). The PIC ranged from 0.75 to 0.993 and genetic similarity varied between 0.478 and 1.000.

Based on electrophoretic banding pattern of SSR primers, pair-wise genetic similarity among 48 genotypes was estimated and a dendrogram was generated (Fig. 1). Cluster analysis revealed that accessions of *Brassica juncea* L. under study, fell into major group and sub groups. The cluster analysis disclosed two main clusters with highest 58% similarity percentage (Gohel *et al.*, 2014). Kumar *et al.*, (2011) also estimated Jaccard's similarity coefficients and constructed dendrogram by using UPGMA revealed the presence and extent of genetic similarities among mutants ranged from 0.54 to 0.91. In present investigation, major group contained 48 genotypes that divided into three main groups, first main group 'I' included 17 genotypes, second main group 'II' hold 24 genotypes and third main group 'III' possessed 7 genotypes. According to Jaccard's similarity coefficient, highest similarity was found between MRNJ-119 and MRNJ-131 in sub group 'A'. Both genotypes were grouped together. The uppermost genetic diversity was observed in main group 'A' between MRNJ-92 and

MRNJ-129. Both of the germplasm lines were grouped distantly. In subgroup 'B' maximum similarity was recorded between RB- 50 and CS – 54, and highest genetic diversity was observed between RVM -1 and MRNJ-113. At last in sub group 'C' utmost similarity and highest genetic diversity was observed between germplasm lines MRNJ-142 and MRNJ-109 and MRNJ-99 and MRNJ-115 correspondingly. Some genotypes are falling in same group existing possibility of many traits to be similar whereas the genotypes falling extremely apart from whole of the cluster shows the possibility of discovering new traits in them which could be further assisted in conventional and /or molecular breeding programmes.

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