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Characterization of Indian Mustard Germplasm on the Basis of Morphological Traits and SSR Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NSR, MKT, RST and ST designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors VSK and ST managed the analyses of the study. Authors NSR, ST and MKT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The genus Brassica is one of the most important oil seed crops in India with high degree of genetic diversity. In present study, genetic diversity was studied in forty germplasm lines and eight cultivars of Indian mustard using morphological traits and SSR markers. Morphological characters were taken for days to 50% flowering, days to maturity, plant height (cm), length of main raceme (cm), number of primary branches/plant, number of secondary branches/plant, number of silique per plant, number of seeds per silique, 1000 seed weight (g) and seed yield per plant (g). Total 50 SSR markers were used for characterization of these lines, out of which 7 SSR markers were highly polymorphic between all the germplasms of mustard. An UPGMA phonogram was constructed for all 48 Germplasms and the similarity coefficient ranged from 0.00 to 0.91. Number of alleles ranged from 3 to 4, genetic diversity ranged from 71% to 65% with average value of 67%, heterozygosity raged from 20 to 10% with average of 12% and PIC value for markers ranged from 0.65 to 0.59 with mean PIC value 0.61. All seven SSR primers showed PIC value above 0.5 (50%) indicating high genetic diversity in the studied plant material.

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Keywords: Genetic diversity; Brassica juncea L.; morphological traits; polymorphic information content; similarity coefficient.

1. INTRODUCTION

Mustard is mainly self-pollinated crop, however, up to 30% out-crossing does occur under natural field conditions depending upon wind and bee activities [1]. The important mustard growing countries of the world are India, Canada, China, Pakistan, Poland, Bangladesh and Sweden. In India, its cultivation is mainly confined in the states *viz*., Uttar Pradesh, Rajasthan, Madhya Pradesh, Haryana, Punjab, Assam, Bihar, Gujarat and West Bengal. In Madhya Pradesh, this crop is mainly cultivated in Morena, Bhind, Gwalior and Sheopur districts. Due to low cost of cultivation and high economic profit, the area and productivity of mustard is continuously increasing, however productivity is low. In most of the agricultural crops, the first movement in crop improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Therefore, the most proficient approach to further improve the performance of crop varieties is to access to large diverse pool of genetic diversity. Moreover, information on the genetic diversity of *B. juncea* germplasm collection scan provide breeders and geneticists important information on the allelic diversity present in *B. juncea* materials. It may help to identify genetically diverse pools for use in cross combinations to improve important agronomic traits or to better exploit heterosis [2]. Traditionally, morphological, phenological and agronomical traits have been employed as criteria for the introgression of new variation into oilseed rape breeding lines.

In *Brassica*, genome research with the application of marker assisted programme began to emerge in the late 1980^{s} when the first RFLP linkage map for *B. oleracea* [3], B. *napus* [4] and *B. rapa* [5], was developed. For phylogenetic studies and genetic mapping in *Brassica*, RFLPs and RAPDs have been extensively used [6]. The number of available *Brassica* SSRs (microsatellite) primers is increasing day by day. *Brassica* genome integration greatly assisted the release of highly polymorphic map based, robust SSR markers of the entire *B.* nigra, *B. rapa*, B. *napus* and B. *oleracea* genome into public domain. However, the discovery of the PCR [7], leads the potential to increase the variety and density of marker in the already existing genetic maps with ISSR, AFLP and with the microsatellites [8,9], also called as SSR. SSRs are highly important resource of map-based alignment among distinct crosses, because of their robust, simple analysis and highly polymorphic nature [10]. Molecular markers closely linked to genes of interest are very useful for marker-assisted selection and pyramiding of two or more genes into a single genotype in *Brassica* breeding programmes [11]. Among different types of molecular markers, microsatellite also known as simple sequence repeats (SSRs) have been utilized for different purposes in plant breeding and genetics including gene mapping, cultivar discrimination and detection of genetic diversity [10]. It has been proven that SSR markers are useful for genetic diversity and structure studies of *Brassica*. Soengas et al. [12] established the genetic relationship among eight populations and studied the genetic structure by analyzing the polymorphic alleles of 18 SSR markers. The objectives of present study were to use a set of SSR markers to detect DNA polymorphism among cultivated *B. napus* accessions. This will provide useful information for *Brassica* breeding programme in the future.

2. MATERIALS AND METHODS

2.1 Plant Material

Materials for the present study included 40 germplasm accessions and 8 varieties of mustard collected from Bastar Jagadalpur, Jaipur, Rajasthan and NBPGR, New Delhi. The field trial on 48 entries was conducted in randomized block design (RBD) with two replications Plot size of entries was represented by 2m x 22.5m with a spacing of 30 cm x l0 cm. A fertilizer dose of 80:40:40 kg NPK/ha was applied. Hoeing and hand weeding were done at the time of top dressing and need based plant protection measures were followed.

2.1.1 List of germplasm lines

MRNJ-1, MRNJ-2, MRNJ-3, MRNJ-4, MRNJ-5, MRNJ-6, MRNJ-7 and MRNJ-8 Collected from Bastar Jagadalpur; MRNJ-9, MRNJ-10, MRNJ-11, MRNJ-12, MRNJ-13, MRNJ-14 MRNJ-15, MRNJ-16, MRNJ-17, MRNJ-18, MRNJ-19, MRNJ-20, MRNJ-21, MRNJ-22 were collected from Rajasthan Jaipur; MRNJ-23, MRNJ-24, MRNJ-25, MRNJ-26, MRNJ-27, MRNJ-28, MRNJ-29 from Rape seed Buster; MRNJ-30, MRNJ-31, MRNJ-33, MRNJ-34, MRNJ-35, MRNJ-36, MRNJ-37, MRNJ-38 from NBPGR,

New Delhi; and MRNJ-39, MRNJ-40 and MRNJ-41 were collected from NRC Bustar.

2.1.2 List of cultivars

Kranti, Maya, Rvm-1, Rvm-2 Rvm-3, Jm-1, Jm-2, IDM-69

2.2 Methods

2.2.1 Morphological characterization of mustard germplasm lines and cultivars

The field trial with 48 genotypes was conducted in randomized block design (RBD) with two replications. The trial was shown on November, 2016 in Rabi season. Plot size was 2m x 22.5m with spacing of 30 cm x l0 cm. A fertilizer dose of 80:40:40 kg NPKha^{-1} was applied. Hoeing and hand weeding were done at the time of top dressing and need based plant protection measures were followed. The crop was harvested on March 2017 during Rabi season. Observations for 10 traits were taken for morphological characterization of germplasm lines and cultivars *i.e.*, days to 50% flowering, days to maturity, plant height (cm), length of main raceme (cm), no. of primary branches/plant, no. of secondary branches/plant, no. of silique per plant, no. of seeds per silique, 1000 seed weight (g), seed yield per plant (g). The analysis of variance (ANOVA) of morphological traits for standard error (S.E.), critical difference (CD) and coefficient of variation (CV) was performed using OP Stat software [13]. The coefficient of correlation among all morphological traits at maturity was calculated using SPSS ver.19 software. The similarity matrices was used to construct a dendrogram for all the genotypes using NTSYS-pc (Rohlf's et al., 2000) based on Unweighted Pair Group Method with Arithmetic Mean UPGMA .The phenotypic and genotypic coefficients of variation in per cent were computed by the formulae given by Burton (1952).

2.3 Molecular Characterization using SSR Markers

2.3.1 Genomic DNA isolation

Two to three healthy leaves were collected from each plant of mustered genotypes at seedling stage and leaf samples were grinded to a fine powder with DNA extraction buffer (1M Tris-CL pH 8.0, 0.5 M EDTA pH 8.0, 4.0 M NaCl, 10% CTAB, β-mercaptoethanol and Milli-Q water) in a mortar and pestle. DNA extraction was done using method as described by Murray and Thompson [14], with little modification [15]. The DNA was diluted to a final concentration of 10-25 ngµl^{-1} for SSR analysis.

2.3.2 PCR amplification SSR markers

A total of 50 microsatellite markers (SSR) were used to study the polymorphism in selected accessions or germplasm lines to find out polymorphic markers for diversity analysis among 48 genotypes. Among them, only 8 SSR primers were selected for further analysis (Table 1). The SSR primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was performed in 10µl reaction mixture comprising of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 20 ng/µl of genomic DNA in a thermocycler (Bio-Rad, USA). The Polymerase Chain Reaction protocol comprised of initial denaturation step of 94°C for 5 min followed by 35 cycles of 94°C for 1 min, annealing at 55°C for 30 sec, elongation at 72°C for 1 min with final extension at 72°C for 7 min. The PCR products were resolved on 3% agarose gel at 100V for 2-3 hrs and documented using Zenith, Gel Documentation System. The SSR banding patterns were scored based on the size of fragments amplified across all mustered genotypes with known ladder of 100 bp as size standard. The major allele frequency, number of alleles per locus, polymorphism information content (PIC) and gene diversity was analyzed using Power Marker v3.25 software [16], The dendrogram based on unweighted pair group method for arithmetic average (UPGMA) and bootstrap value of 1000 permutations was constructed using MEGA 6.0 software [17].

3. RESULTS

3.1 Phenotypic and Genotypic Correlation Coefficient

A correlation study was conducted between the different traits studied and it predicts the germplasm lines that should be given priority while selecting the accessions for further improvement. In general, genotypic correlations were higher than phenotypic ones in magnitude for all the characters. The character which showed negative association at genotypic level also showed negative association at phenotypic level. Phenotypic correlation coefficient revealed that seed yield per plant was significantly correlated with the length of main raceme at 1% significant level. Among the yield attributing characters days to 50% flowering showed

significant phenotypic correlation coefficient with number (s) of primary branches per plant, number of secondary branches per plant and number (s) of siliqua per plant at p value 0.01 (Table 2). Days of maturity showed significant correlation coefficient with plant height, number of primary branches per plant and number of secondary branches per plant and number of siliqua per plant, length of main raceme also showed significant correlation coefficient with harvest index and days of maturity. Plant height, number of siliqua per plant were found highly significant with number of secondary branches. Seed yield per plant was also highly significant with harvest index. Mean value of morphological traits was also analysed for correlation coefficient using SPSS V19 software. Days to 50% flowering was positively significantly correlated with number of primary branches per plant, number of secondary branches per plant and number of silique per plant at 1% significant level (Table 3). Number of secondary branches are showing positively highly significance with Days to 50% flowering (r=0.473) and number of primary branches (r=0.589) at 1% significance level. Number of seeds per siliqua are positively highly significant with number of siliquae per plant (r=0.495**) at 1% significant level.

3.2 Morphological Trait Analysis and Cluster Formation

Cluster analysis revealed that accessions fell into two major groups that further subdivided into 8 sub-groups. Group-'I' carried 2 genotypes MRNJ-39 and MRNJ-11; while, Group-'II' carried 46 genotypes. Group -'II' further classified into two sub-groups 'II-A' and 'II-B'. Sub-group 'II-A' contained 6 genotypes; whereas, sub-group 'II-B' carried 40 genotypes (Fig 1). According to Jaccard's Similarity Coefficient, highest similarity was observed between JM-1 and JM-2; MRNJ-12 and MRNJ-19; MRNJ-21 and MRNJ-3; MRNJ-41 and RVM-3; Kranti and RVM-1; MRNJ-11 and MRNJ-39 and MRNJ-8 and MRNJ-22. Therefore, both genotypes were grouped together. Based on genetic diversity of morphological traits, highest genetic diversity was observed between MRNJ-39 and MRNJ-1 (Table 4).

3.3 Molecular Characterization

Molecular characterization of 48 genotypes was based on electrophoretic banding pattern of all the polymorphic SSR primers (Table 5). Cluster analysis revealed that accessions of *Brassica juncea* L. under study fell into major group and sub groups. The dendrogram which is formed by molecular characterization of SSR marker formed 8 main groups and 13 subgroups which gives a recognizable result of the characteristics of all the germplasm lines based on its origin, its virtue towards the check varieties and data interpretation through base pair analysis (Fig 2). According to Jaccard's similarity coefficient, highest similarity was observed between MRNJ-4 and MRNJ-8 in group-'I'. RVM-1 and MRNJ-19 in sub group- 8(B), KRANTI and MRNJ-22. In sub group- 8 (A). MRNJ-39 and Maya in Sub group-7 (A), MRNJ35 and IDM-69 in sub group- 6 (B), MRNJ-24 and JM-2 in sub group -6 (B), JM-1 and MRNJ-36 in sub group -3 (A), MRNJ-3 and RVM-2 in sub group- 2(A).

Table 1. Details of SSR primers selected for mustard germplasm screening

S.	Name of SSR	Forward primer sequence (5'-3')	Ref.
1	BRMS- 240	Forward-CAAGAGTATTTGTGTGGGTTGACTC Reverse-AAATAACGAACGGAGAGAGAGAGAG	He et al. (2003)
2	BRMS- 324	Forward-AACTTAACCGAAACCGAGSTAGGTG Reverse-AATCTCGAAATTCATCGACTTCCTC	He et al. (2003)
3	SR- 7223	Forward-AGGACCCGACTTTCCTTGTT Reverse-ACCAAACTCGGCGTACAAAT	Wang et al. (2007)
4	SR- 9222	Forward-CACCGAACAAAACTGAGGGT Reverse-CGTTTCACTGCGTTCTACCA	Wang et al. (2007)
5	SR – 94102	Forward-ATCCCCAAACTACCCTCACC Reverse-AGGATGAGCAAAGGAAAGCA	Wang et al. (2007)
6	OI 10-CO 5	Forward-GGCTACAAAATGTTTGATAAGCTCT Reverse-ACCTGAAAGAGAGGCTACACAT	Wang et al. (2007)
7	SSR Na10-D09	Forward-AAGAACGTCAAGATCCTCTGC Reverse-ACCACCACGGTAGTAGAGCG	Wang et al. (2007)
8	SSR Na10-D11	Forward-GAGACATAGATGAGTGAATCTGGC Reverse-CATTAGTTGTGGACGGTCGG	Wang et al. (2007)

Table 2. Genotypic and phenotypic correlation coefficient of grain yield and its attributes in mustard germplasm

	Day of 50% flowering	Day of maturity	Plant height (cm)	Length of main raceme	No. of pri. branches per plant	No. of sec. branches per plant	No. of Siliqua per plant	No. of seed per siliqua	1000 seed weight	Seed yield per plant	Harve-st Index
Day of 50% flowering	1	0.246	0.171	-0.154	0.566**	0.455**	0.464**	0.243	0.116	-0.004	0.068
Day of maturity	0.126	1	0.427**	-0.180	0.151	-0.053	-0.160	0.153	-0.029	0.156	-0.125
Plant height (cm)	0.160	0.251	1	0.219	0.319*	0.342*	0.343*	-0.004	-0.109	0.214	0.157
Length of main raceme	-0.137	-0.027	0.168	1	0.150	-0.025	0.148	-0.240	0.093	0.327*	0.461**
No. of pri. branches per plant	0.465**	-0.003	0.256	0.059	1	0.652**	0.634**	0.030	0.140	0.013	0.085
No. of sec. branches per plant	0.415**	-0.016	0.311	-0.018	0.517**	1	0.813**	0.228	0.173	0.071	0.207
No. of Siliqua per plant	0.413**	-0.091	0.337*	0.136	0.506**	0.742**	1	0.216	0.155	0.075	0.228
No. of seed per siliqua	-0.076	0.022	-0.003	-0.206	0.034	0.226	0.186	1	0.288	-0.084	-0.013
1000 seed weight	0.049	0.070	-0.104	0.043	0.168	0.141	0.138	0.238	1	0.008	-0.130
Seed yield per plant	-0.002	0.100	0.214	0.501**	0.015	0.070	0.073	-0.078	0.011	1	0.130
Harvest Index	0.065	-0.075	0.155	0.374**	0.073	0.200	0.221	-0.007	-0.128	-1.231**	1

**, ** significant at 5 & 1 % level of significance, Upper diagonal represent genotypic correlation coefficient and lower diagonal represent phenotypic correlation*

Table 3. Analysis of correlation coefficient of mean value of yield attributing morphological traits in mustard germplasm using SPSS V19 software

Correlations										
	DF	**DM**	**PH**	**LMR**	**NPB**	**NSB**	**NS**	**NSS**	**TKW**	**GYD**
DF	1	0.336*	-0.053	0.014	0.411**	0.473**	0.455**	0.284	0.024	-0.019
DM		1	-0.073	-0.050	0.057	-0.067	-0.047	-0.050	0.070	-0.004
PH			1	0.202	-0.119	-0.076	-0.063	-0.184	-0.204	0.092
LMR				1	.156	0.111	0.203	-0.001	0.161	0.349*
NPB					1	0.589**	0.539**	0.206	0.099	0.012
NSB						1	0.819**	0.495**	0.112	0.041
NS							1	0.396**	0.120	0.077
NSS								1	-0.072	-0.001
TKW									1	0.046
GYD										1

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

DF=Day of 50% flowering, DM=Day of maturity, PH=Plant height (cm), LMR=Length of main raceme, NPB=No. of primary branches per plant, NSB=No. of sec. branches per plant, NS=No. of Siliqua per plant, NSS=No. of seed per silique, TKW=1000 seed weight, GYD=Seed yield per plant

Table 4. Important features of Clusters formed in analysis of morpho-physiological traits of mustard germplasms using NTSYS software

Cluster/Sub cluster	No. of Accessions	Important features	Remark
Cluster-I	2	MRNJ-11, MRNJ-39	
Sub-Cluster-I(A)	1	MRNJ-39	
Sub-Cluster-I(B)	1	MRNJ-11	
Cluster-II	46		
Sub Cluster-II(A)	6		
Sub Cluster-II(A)-I	2	Kranti, RVM-1	Highest number of secondry branches per plant observed in Kranti (29)
Sub-Cluster-II(A)-II		MRNJ-14, MRNJ-28, MRNJ-41, RVM-3	Highest day of maturity observed in MRNJ-28(124)
Sub-Cluster-II(B)	40		

Sub-Cluster-II(B)-I	26	MRNJ-37, MRNJ-24, MRNJ-15,MRNJ-17, MRNJ-36, MRNJ-34, MRNJ-30, MRNJ-13, MRNJ-26, MRNJ-33, MRNJ-12, MRNJ-29, MRNJ-22, MRNJ-8, MRNJ-6, MRNJ-27, MRNJ-23, MRNJ-21, MRNJ-3, MRNJ-40, MRNJ-35, MRNJ-25, MRNJ-19, MRNJ-7, MRNJ-9, MRNJ-2,	Highest 1000 seed weight observed in MRNJ-24(6.63gm) and highest number of primary branches per plant observed in MRNJ-26(9)
Sub-Cluster-II(B)-II	14	MRNJ-1, RVM-2, JM-1, JM-2, MRNJ-4, MRNJ-31, MRNJ-5, MRNJ-38, MRNJ-20, MAYA, MRNJ-10, MRNJ-16, MRNJ-18, IDM-69	Highest plant height observed in MRNJ-1 (251.4cm),highest number of siliqua per plant observed in RVM-2 (796) ,highest seed yield per plant observed in MRNJ-10

Table 5. Details of highly polymorphic SSR primers used for diversity analysis of mustard germplasms

S.	Name of SSR	Forward primer sequence (5'-3')	Ref.
1	BRMS- 240	Forward-CAAGAGTATTTGTGTGGGTTGACTC Reverse-AAATAACGAACGGAGAGAGAGAGAG	He et al. (2003)
2	BRMS- 324	Forward-AACTTAACCGAAACCGAGSTAGGTG Reverse-AATCTCGAAATTCATCGACTTCCTC	He et al. (2003)
3	SR- 7223	Forward-AGGACCCGACTTTCCTTGTT Reverse-ACCAAACTCGGCGTACAAAT	Wang et al. (2007)
4	SR- 9222	Forward-CACCGAACAAAACTGAGGGT Reverse-CGTTTCACTGCGTTCTACCA	Wang et al. (2007)
5	SR – 94102	Forward-ATCCCCAAACTACCCTCACC Reverse-AGGATGAGCAAAGGAAAGCA	Wang et al. (2007)
6	SSR Na10-D09	Forward-AAGAACGTCAAGATCCTCTGC Reverse-ACCACCACGGTAGTAGAGCG	Wang et al. (2007)
7	SSR Na10-D11	Forward-GAGACATAGATGAGTGAATCTGGC Reverse-CATTAGTTGTGGACGGTCGG	Wang et al. (2007)

The summary statistics extracted from Power Marker was observed for Allele number, Major Allele Frequency, Gene Diversity, Heterozygosity and Polymorphism Information Content (PIC). Highest PIC value (0.6888) was observed for SSRNa10D09 which has 8 alleles among the 48 genotypes. Markers SR-9222, and BRMS-324 also had high PIC scores and high number of alleles (Table 6). Lowest PIC value was obtained for SR - 7223 (0.6231). Mean PIC value was 0.6154, representing high diverse nature of marker, which can be utilized for further characterization. All SSR primers showed PIC value above 0.5 (50%) indicating high genetic diversity in the studied plant material. Major allele frequency was between 0.33 to 0.44 with average 0.389. Number of alleles ranged from 3 to 4, genetic diversity ranged from 71% to 65% with average 67%. Heterozygosity ranged from 20 to 10% with average of 12% (Table 6).

Table 6. Statistical analysis data of polymorphic SSR markers for Polymorphic Information Contents, and other parameters of mustard germplasms

Marker	Major Allele Frequency	Genotype No.	Allele	Gene Diversity	Hetero-zygosity	PIC
BRMS324	0.3750	5.0000	4.0000	0.6866	0.2500	0.6231
SR7223	0.4375	3.0000	3.0000	0.6398	0.0000	0.5642
SSRNa10D11	0.4167	5.0000	4.0000	0.6443	0.0625	0.5731
BRMS224	0.4479	7.0000	4.0000	0.6586	0.1042	0.5966
SSRNa10D09	0.3438	7.0000	4.0000	0.7368	0.2292	0.6888
SR94102	0.3750	4.0000	4.0000	0.6745	0.0000	0.6060
SR9222	0.3333	7.0000	4.0000	0.7118	0.2083	0.6556
Mean	0.3899	5.4286	3.8571	0.6789	0.1220	0.6154

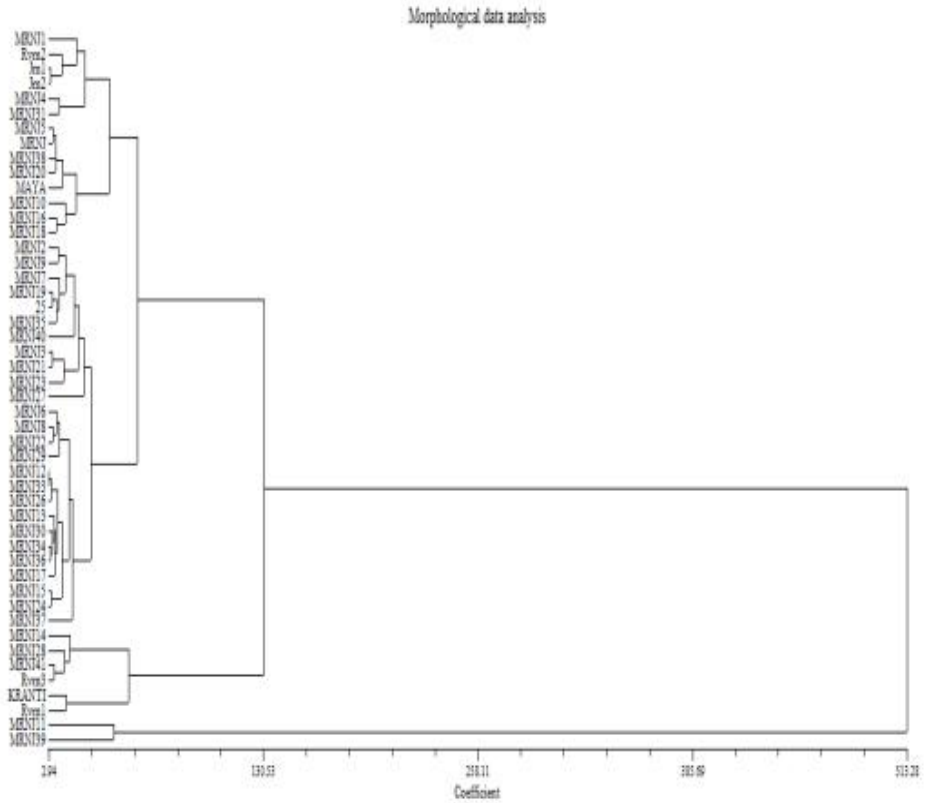

Fig. 1. Dendrogram of *Brassica* germplasm for mean value of yield attributing morphological traits

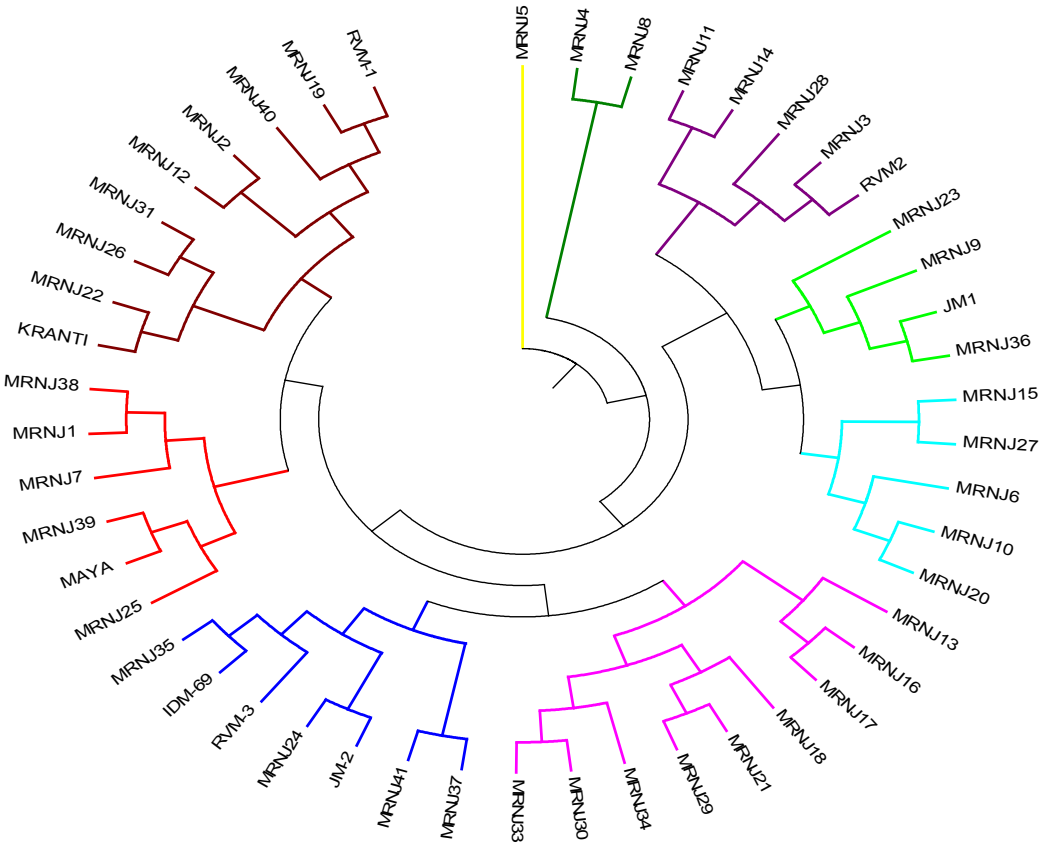

Fig. 2. UPGMA tree based on dissimilarity index for mustard germplasms using polymorphic SSR markers

4. DISCUSSION AND CONCLUSION

Current study consisted morphological variability among mustard germplasms for eleven yield attributing traits

Among the yield attributing characters days to 50% flowering showed significant phenotypic correlation coefficient with number of primary branches per plant, number of secondary branches per plant and number of siliqua per plant. Seed yield per plant was also found highly significantly correlated with harvest index. All these characters showed high significance at both the probability level (5% and 1%). These traits are highly influence by each other and if seed yield per plant is high, harvest index will also increase. Similarly, if days to 50% flowering is more, it will affect primary branches per plant, number of secondary branches per plant and number of siliqua per plant positively. Earlier Ray et al. [18], showed positive correlations for association of primary branches with plant height. Dawar et al. [19], reported that the phenotypic coefficients of variation were higher than genotypic coefficient of variation for all the characters which support the finding of present study.

The assessment of genetic diversity is not only important in crop improvement programmes but also for the competent management and conservation of existing genetic variability. Molecular profiling has been the preferred choice of breeding being more reliable and authentic and less influenced by environmental fluctuations [20]. As such it is important to evaluate newly developed genotypes for their stability in production and screen for variability present which could provide a source material for further improvement in mustard crop. Genetic diversity analysis is not only important for crop improvement efforts but also for the efficient management and protection of available genetic variability. Molecular profiling has been the preferred choice of breeding for almost all major crops as these are more reliable and authentic and less influenced by environmental fluctuations

[20-24]. In current study all SSR primers showed PIC value above 0.5 (50%) indicating high genetic diversity in the studied plant material. Major allele frequency was between 0.33 to 0.44 with average 0.389. The results of present study showed somewhat resemblance with the study conducted by Qu et al [25], using 217 genotypes and 37 markers, as the markers were selected from the same research work. Recently, Verma et al, [26] and Baghel et al. [27], have also done similar kind of study in mustard and used 11 and 7 markers respectively for diversity analysis. Shyam et al. [28], used 48 brassica genotype (s) with the aim to identify genotypes with low and high erucic acid content on the basis of 23 SSR markers. They have identified a total of 109 alleles with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity varied from minimum 0.55 of SSR marker Na10-D07 to maximum 0.77 of BRMS-098 with mean value of 0.68. Polymorphism information content (PIC) value of the markers varied from minimum 0.51 for SSR Na10-D07 to maximum 0.73 with primer BRMS-098 with a mean value of 0.62. In our study, the average PIC values were found to be higher than that of reported by Patel et al. [29], where the PIC value ranged from 0.427-0.730 with an average of 0.555. PIC values reported by Bharti et al. (2018) ranged from 0.34 to 0.49 with an average of 0.41. Gupta et al. [30], also reported low PIC value 0.281. In the present experimentation; it came out to be 3 to 4 with an average of 3.85 allele which shows that the markers came out with even better results with the present tested genotypes. Current study accomplished morphological characterization of Indian mustard germplasm using yield attributing traits and SSR markers. Phenotypic and genotypic correlation between traits was observed to see environmental effect as well as highly polymorphic SSR markers were used to study of genetic diversity at molecular level. Diverse germplasms formed distinct group based on morphological and SSR markers data. Diversified parents identified from this study may be utilized as resource for further improvement programme in mustard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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