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Research article

Agro-morphological traits and SSR markers reveal genetic variations in germplasm accessions of Indian mustard – An industrially important oilseed crop

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ABSTRACT

Indian mustard is an economic and highly important industrial oilseed crop. In this study, genetic diversity among 135 Indian mustard germplasm accessions was evaluated using 11 agro-morphological descriptors and 227 SSRs. Morphological characterization of Indian mustard germplasm accessions exhibited a broad range of variation for characters including biological yield (CV = 25.63%), seed yield (CV = 23.23% and 1000-seed weight (CV = 23.14%); whereas traits such as days to maturity (CV = 2.91%) showed lowest degree of variation. Out of 227 SSR markers evaluated, a total of 159 (70.04%) SSRs produced polymorphic products and 68 (29.96%) SSRs resulted into monomorphic amplicons. The polymorphic markers amplified 575 alleles and the number of alleles ranged from 2-7 with 3.61 average number of alleles per locus. SSR markers BRMS-030, Ra2-E11, Ra2-G05, Ni4-G10 and Ol10B11 generated the highest number of alleles (7). SSR marker Ra2-G05 was having the highest allele frequency (0.84), while BRMS-002 was having the lowest major allele frequency (0.33). Polymorphism information content (PIC) values ranged from 0.24-0.61 with an average value of 0.39 per primer pair. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on morphological traits grouped Indian mustard genotypes into three clusters, while two clusters were obtained based on SSR based clustering. Population structure analysis provided a better estimate of genetic diversity and divided all the genotypes into five sub-populations. Genetically diverse accessions identified may be used for hybridization in Indian mustard crop improvement programs in future.

1. Introduction

Indian mustard (*Brassica juncea* L. Czern. & Coss) is an economically important oilseed crop belonging to rapeseed-mustard (RM, oilseed *Brassica*) group and is being cultivated mainly in Indian subcontinent and in some parts of Canada, China, Australia and Russia. It is a predominantly self-pollinating crop with an extent of 8–12% cross-pollination. It has been developed in nature by crossing between *B. nigra* (BB, 2n = 16) and *B. rapa* (AA, 2n = 20) followed by subsequent chromosome duplication thousands of years ago (Redden et al., 2009). Indian mustard is the most dominating crop of RM group which is presently being cultivated over >85% of RM acreage in India. It accounts for 23.3% of the total acreage and 26.2% of the total oilseed production in India (Govt. of India, 2019). Besides its use as cooking oil, Indian mustard oil has found numerous applications in food, chemical, biofertilizer and paint industries. Its oil contains higher amount of erucic acid, which makes it quite useful in paint and varnishes industry. In addition, the presence of high erucic acid in its oil is the main reason for its use in biodiesel production in automobile industry (Premi et al., 2013). Mustard oil is an enriched source of various antioxidants and α-tocopherol, which provides health benefits to the consumers. Its oil has both culinary and therapeutic uses. It contains rich amount of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) and optimum ratio of ω-3 and ω-6 fatty acids, which is very much essential for curing cardiac disorders.

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Due to antimicrobial properties of mustard oil, it is also used in pickle industry for preservation purpose. Besides mustard oil, its seedmeal is an excellent source of feed and nutrition for chicken industry and India has now become world's largest exporter of mustard seedmeal (Thakur et al., 2020). However, due to the exponential increase in population and consumers' changing taste preferences towards mustard oil, it will become a challenging assignment to fulfil the growing edible oil demand in future. On the other hand, several biotic and abiotic stress constraints leading to considerable yield losses seriously hampers overall production of this crop. This necessitates the development of high yielding mustard varieties having resistance to both abiotic and biotic stresses so that they can grow in a diverse array of agroclimatic conditions.

Information about the genetic diversity patterns of various germplasm accessions and their genetic relationships is of paramount relevance in formulating breeding strategies and germplasm management programs. Utilization of genetically diverse germplasm lines in breeding programs can be used to harness allelic richness to create new gene combinations. Traditionally, various agro-morphological traits had been used to estimate genetic diversity among Indian mustard germplasms (Singh et al., 2018, 2020). Morphological characterization of germplasm has certain advantages including less cost involvement, less technically demanding and simplicity of characterization protocols. Estimates of genetic variability can be indirectly done using morphological traits, but these estimates do not provide the exact picture of genetic diversity as they work under the influence of environmental factors (Pandey et al., 2018) and developmental stages of the plant. Further, the lesser number of available morphological markers render them unfit for diversity characterization in crops. Whereas, molecular markers are not influenced by the environmental factors or any developmental stage of the plant. Nowadays, it has been suggested to evaluate genetic diversity using both agro-mor phological traits and molecular markers to get more precise estimates of the genetic variability (Vinu et al., 2013; Thakur et al., 2017).

To date, several type of DNA-based marker systems were used for evaluation of genetic diversity in Indian mustard germplasm accessions including random amplified polymorphic DNA (RAPD) (Khan et al., 2008; Sharma et al., 2015), inter simple sequence repeat (ISSR) markers (Huangfu et al., 2009; Yadav and Rana, 2012), restriction fragment length polymorphism (RFLP) (Mir et al., 2015), amplified fragment length polymorphism (AFLP) (Srivastava et al., 2001; Qi et al., 2008) and simple sequence repeat (SSR) markers (Vinu et al., 2013; Pratap et al., 2015; Thakur et al., 2015; Sudan et al., 2016). Among these marker systems, SSR markers have proved highly useful in genetic diversity analysis in Indian mustard owing to their abundance in eukaryotic systems, ease of doing and scorability, high reproducibility, co-dominant and multi-allelic inheritance patterns and less cost involvement (Viera et al., 2016). With this background knowledge, the present research investigation was planned to evaluate genetic diversity in Indian mustard germplasm accessions using various agro-morphological characters and SSR markers.

2. Materials and methods

2.1. Plant material

A total of 135 Indian mustard germplasm accessions were used as plant material (Table 1). The pure, selfed seeds of germplasm accessions were obtained from the Germplasm Division of ICAR-DRMR, Bharatpur. All 135 germplasm accessions were grown in augmented block design in a 3 m length row with a row-to-row spacing of 45 cm along with plant-to-plant spacing of 10 cm for two consecutive years in the rabi season of 2017 and 2018, following standard package of practices. Two Indian mustard varieties viz. Giriraj and NRCHB 101 were kept as check genotypes. The composition of the soil was kept same for growing different genotypes of Indian mustard.

2.2. Morphological characterization

Observations of 11 agro-morphological traits were taken as per the DUS guidelines (Singh et al., 2006). Data was recorded on five randomly selected plants per genotype for various traits including days to flower initiation (DFI, 50%), days to maturity (DM), plant height (PH, cm), main shoot length (MSL, cm), siliqua length (SL, cm), siliquae on main shoot (SMS), seeds per siliqua (SS), 1000-seed weight (SW, g), seed yield (SY, Kg/ha), biological yield i.e. dry matter production excluding roots (BY, Kg/ha) and harvest index (HI, %).

2.3. Genomic-DNA isolation and purification

Genomic-DNA was isolated from the pooled leaf tissues collected from five randomly selected healthy plants per genotype and purified using the protocol of Thakur et al. (2013).

2.4. Genotyping

A set of 227 genomic-SSR markers covering all the linkage groups of Indian mustard as reported by Singh et al. (2021) were used in present study for genotyping. The mastermix was prepared in a 2.0 ml centrifuge

Table 1. List of *Brassica juncea* germplasm accessions used in the present study.

Code	Name/identity of the genotype	IC/EC No.	Source	Country of origin
1	B 123	IC 494164	Unknown	India
2	JBT 41/15	IC 520747	Jharkhand	India
3	03–530	IC 609262	Unknown	Unknown
4	KLM 4	IC 609278	PAU, Ludhiana	India
5	BT 15	IC 609452	Unknown	Unknown
6	SNTM 53	IC 411750	Jammu and Kashmir	India
7	HUJM 05-01	IC 511426	BHU, Varanasi	India
8	JBT 41/60	IC 520759	Jharkhand	India
9	SN 99	IC 609983	Andhra Pradesh	India
10	EC 511481	EC 511481	Saskatchewan, Canada	Canada
11	BT 31	IC 607046	Unknown	Unknown
12	NE 19	IC 609882	Assam	India
13	HP 35	IC 609380	Himachal Pradesh	India
14	UP I-23	IC 346131	Uttar Pradesh	India
15	UP I-75	IC 346183	Uttar Pradesh	India
16	DU 10	IC 609529	Unknown	Unknown

(*continued on next page*)

Table 1 (*continued*)

Code	Name/identity of the genotype	IC/EC No.	Source	Country of origin
17	SN 24	IC 609927	Andhra Pradesh	India
18	RH 08-2	IC 511367	CCSHAU, Hisar	India
19	SEL 4	IC 511598	Unknown	India
20	SEL 9	IC 511602	Unknown	India
21	VKG 29/80	IC 447832	Bihar	India
22	JBT 41/4	IC 520766	Jharkhand	India
23	JCR 914	IC 427136	Himachal Pradesh	India
24	PSR 11226	IC 436220	Telangana	India
25	SN 29	IC 609935	Andhra Pradesh	India
26	P 83	IC 346769	Punjab	India
27	SV 41708	EC 182920	Sweden	Sweden
28	B 267	IC 494266	Unknown	India
29	RESJ 564	EC 399288	Rothamsted	England
30	Pusa Bold x BJ-1058	IC 609897	Unknown	India
31	NBPG 29	IC 609235	Unknown	Unknown
32	HPLM 06-46	IC 609255	Unknown	Unknown
33	Parasmani 8	IC 609293	Shakti Vardhak Hybrid Seeds Pvt.Ltd., Hisar	India
34	BBM 06-02	IC 511432	RRS, IGKVV, Jagdalpur	India
35	RRN 615	IC 511431	ARS, SKRAU, Navgaon	India
36	RRN 605	IC 511434	ARS, SKRAU, Navgaon	India
37	HUJM 05-02	IC 609321	BHU, Varanasi	India
38	Kranti	IC 491638	CSAUA & T, Kanpur	India
39	DMRS 186	IC 266917	Uttarakhand	India
40	UP II-76	IC 345930	Uttar Pradesh	India
41	UP II-6	IC 345860	Uttar Pradesh	India
42	P 4	IC 346690	Punjab	India
43	PR 2001-84	IC 609324	GBPUA&T, Pantnagar	India
44	HP 57	IC 609363	Himachal Pradesh	India
45	SKM 125	IC 609378	SDAU, SK Nagar	India
46	P 63	IC 346749	Punjab	India
47	HP 14	IC 347670	Himachal Pradesh	India
48	HP 30	IC 609402	Himachal Pradesh	India
49	UP II-62	IC 345916	Uttar Pradesh	India
50	UP II-18	IC 345872	Uttar Pradesh	India
51	P 37	IC 346723	Punjab	India
52	JGM 01-15	IC 609490	RVSKVV, Gwalior	India
53	RN 593	IC 609494	ARS, SKRAU, Navgaon	India
54	DU 32	IC 609533	Unknown	Unknown
55	VKG 13/75	IC-267715	Bihar	India
56	RGN 42	IC 609543	ARS, SKRAU, Sriganganagar	India
57	B/K/S 67	IC 310767	Haryana	India
58	B/K/S 135	IC 310807	Madhya Pradesh	India
59	B/K/S 42	IC 310748	Rajasthan	India
60	CS 1100-1-2-2-3	IC 511389	CSSRI, Karnal	India
61	CS 3000-1-1-1-2-4	IC 511390	CSSRI, Karnal	India
62	NDRS 2010-5-1	IC 511393	NDUA&T, Faizabad	India
63	CS 610-5-2-5P1	IC 511455	CSSRI, Karnal	India
64	NDR 05-02	IC 511503	NDUA&T, Faizabad	India
65	YS,SKJ 5	IC 312514	Haryana	India
66	SKJ/DPS 9	IC 312524	Haryana	India
67	JBT 41/32	IC 520768	Jharkhand	India
68	VKG 31/8	IC 521376	Jharkhand	India
69	EC 511436	EC 511436	Saskatoon, Canada	Canada
70	EC 511589	EC 511589	Saskatoon, Canada	Canada
71	PAB 9511	IC 607054	GBPUA&T, Pantnagar	India
72	EC 511682	EC 511682	Saskatoon, Canada	Canada
73	EC 511447	EC 511447	Saskatoon, Canada	Canada
74	EC 511684	EC 511684	Saskatoon, Canada	Canada
75	SKM 158	IC 609336	SDAU, SK Nagar	India

(*continued on next page*)

Table 1 (*continued*)

Code	Name/identity of the genotype	IC/EC No.	Source	Country of origin
76	P 18	IC 346704	Punjab	India
77	Krishna Shorty	IC 609188	Krishna Agril. Res. & Dev., Agra	India
78	RGN 13	IC 296337	ARS, SKRAU, Sriganganagar	India
79	PRB 2006-5	IC 573439	GBPUA&T, Pantnagar	India
80	Parasmani 3	IC 609946	DRMR, Bharatpur	India
81	MRN J 2001-12	IC 511467	ZARS, RVSKVV, Morena	India
82	PBR 2004-06	IC 511487	PAU, Ludhiana	India
83	CS 219-1	IC 511537	CSSRI, Karnal	India
84	RH 118	IC 609894	CCSHAU, Hisar	India
85	NRC 323-1	IC 511525	DRMR, Bharatpur	India
86	UP II-9	IC 345863	Uttar Pradesh	India
87	HP 10	IC 609511	Himachal Pradesh	India
88	UP II-75	IC 345929	Uttar Pradesh	India
89	SN 92	IC 609923	Andhra Pradesh	India
90	SN 57	IC 609931	Andhra Pradesh	India
91	SN 55	IC 609933	Andhra Pradesh	India
92	SN 34	IC 609938	Andhra Pradesh	India
93	SN 28	IC 609941	Andhra Pradesh	India
94	SKACV 09-62	IC 571685	Karnataka	India
95	UP I-21	IC 346129	Uttar Pradesh	India
96	NE 68	IC 609916	Nagaland	India
97	UP I-24	IC 346132	Uttar Pradesh	India
98	UP II-111	IC 345965	Uttar Pradesh	India
99	P 11	IC 346697	Punjab	India
100	UP II-25	IC 345879	Uttar Pradesh	India
101	UP II-49	IC 345903	Uttarakhand	India
102	UP II-4	IC 345858	Uttar Pradesh	India
103	UP I-62	IC 346170	Uttar Pradesh	India
104	PR 2006	IC 511618	GBPUA&T, Pantnagar	India
105	UP II-21	IC 345875	Uttar Pradesh	India
106	B/K/S 130	IC 310802	NBPGR, New Delhi	India
107	NRCHB 06-5912	IC 609944	DRMR, Bharatpur	India
108	JBT 42/RP-3/43	IC 526318	Bihar	India
109	IB 1521	IC 493679	Unknown	India
110	LET 3	IC 511546	IARI, New Delhi	India
111	Domo 1	IC 609210	Unknown	Unknown
112	OMK 15	IC 609257	SAREC, CSKHPKVV, Kangra	India
113	BT 13	IC 609451	Unknown	Unknown
114	DU 25	IC 609532	Unknown	Unknown
115	B/K/S 1	IC 609565	Unknown	Unknown
116	SEL 15	IC 511608	Unknown	India
117	VKG 21/208	IC 342778	Bihar	India
118	RCC 4 x Zem-2	IC 609905	Unknown	Unknown
119	HPLM 06-35	IC 609250	Unknown	Unknown
120	HPLM 06-36	IC 609251	Unknown	Unknown
121	Pusa Basant	IC 491533	IARI, New Delhi	India
122	RK 07-2	IC 609288	CSAUA&T, Kanpur	India
123	RH 0305	IC 575576	CCSHAU, Hisar	India
124	DMRS 97	IC 266828	Uttarakhand	India
125	UP II-119	IC 345973	Uttar Pradesh	India
126	DU 15	IC 609531	Unknown	Unknown
127	B/K/S 19	IC 310730	Rajasthan	India
128	SEL 23	IC 511483	UAS, Bangalore	India
129	SEL 68	IC 511485	UAS, Bangalore	India
130	SEL 6	IC 511600	Unknown	India
131	SEL 14	IC 511607	Unknown	India
132	SKJ/DPS 10	IC 312525	NBPGR, New Delhi	India
133	YSR/SKJ 45	IC 355345	Uttar Pradesh	India
134	JCR 996	IC 427206	Himachal Pradesh	India
135	SN 66	IC 609940	Andhra Pradesh	India

Table 2. Range, mean and coefficient of variation for agro-morphological traits among 135 germplasm accessions of Indian mustard.

Sr. No.	Trait	Range		Mean	Standard deviation (SD)	Coefficient of variation (%)
		Minimum	Maximum			
1	Time of flowering initiation (IF, 50%)	33.00	77.00	53.13	8.23	15.49
2	Days to maturity (DM)	123.00	143.00	130.31	3.78	2.91
3	Plant height (PH, cm)	148.00	268.00	205.4	22.16	10.79
4	Main shoot length (MSL, cm)	45.50	115.00	83.73	11.01	13.16
5	Siliqua length (SL, cm)	2.64	5.63	4.16	0.62	14.95
6	Siliquae on main shoot (SMS)	77	130	108.00	11.57	10.73
7	Number of seeds per siliqua (SS)	8	18	14.29	1.67	11.74
8	1000-seed weight (SW, g)	2.20	7.5	4.60	1.06	23.14
9	Seed yield (SY, Kg/Ha)	800.00	3088.8	2198.9	509.85	23.23
10	Biological Yield (BY, Kg/Ha)	3955.55	30074	10901	2816.31	25.63
11	Harvest index (HI, %age)	9.93	27.57	20.4	2.94	14.43

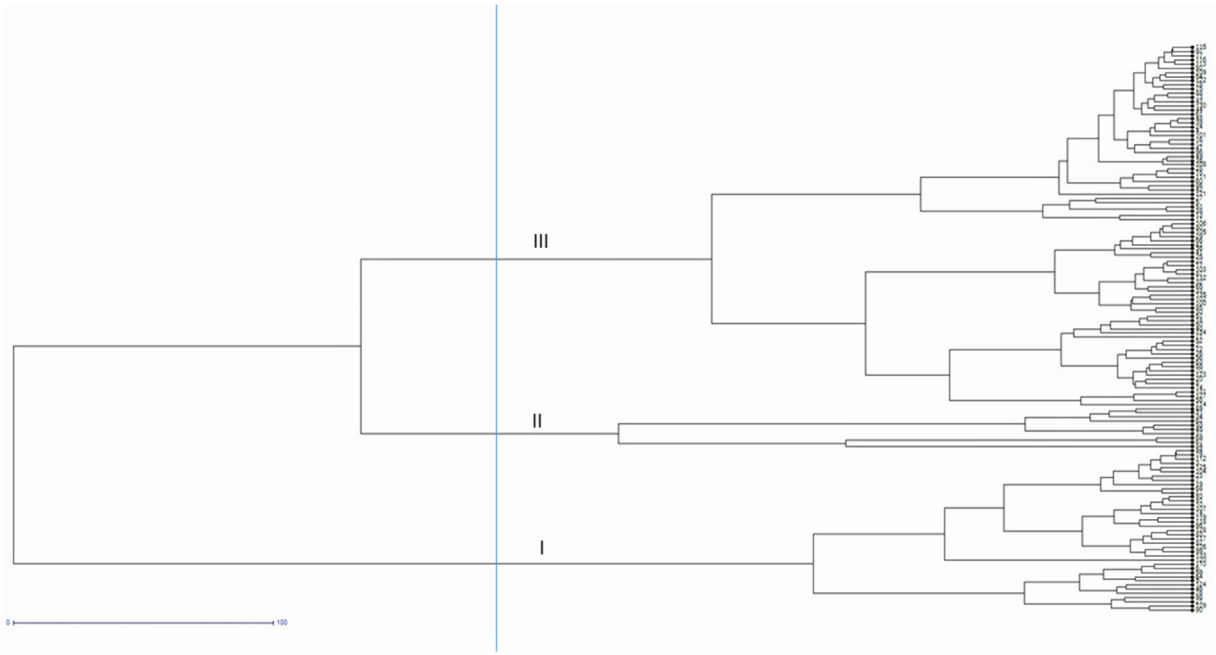

Figure 1. UPGMA dendrogram showing genetic relationship among 135 Indian mustard germplasm accessions based on 11 agro-morphological traits.

tube in 25 μl volume by taking 50 ng genomic-DNA, 1XPCR buffer, 2.0 mM $Mgcl_2$, 0.2 mM of each dNTP, 1.0 U *Taq DNA polymerase* (GCC Biotech, India) and 400 nM primers using Verity 96-w PCR machine. In thermal cycler, initial denaturation cycle comprised of 94 °C temperature for 5 min which was followed by 45 cycles at 94 °C for 30 s, 50–60 °C (depending on the annealing temperature of the SSR primers) for 30 s, 45 s of extension at 72 °C and in the last, primer extension step at 72 °C for 7 min. PCR amplicons containing 5 μl loading dye were resolved in a 3.5% Super Fine Resolution (SFR) agarose (Amresco, USA). Further, gel pictures were taken in a gel documentation unit (Syngene Gel Doc, UK).

2.5. Data analysis

Statistical analysis of mean values of each of agro-morphological trait was carried out using the Fit Model of SAS JMP9.2. DARwin 5 software (Perrier and Jacquemoud-Collet, 2006) was used to construct UPGMA-based dendrogram on the basis of similarity coefficients based on Euclidean distances.

SSR bands were scored based on allele size and allelic data was subjected to using Power Marker software v3.25 (Liu and Muse, 2005) for computation of major allele frequency, PIC value and gene diversity for individual SSR marker. UPGMA-dendrogram was developed to demonstrate the relationship among different accessions under investigation using MEGA version 5.03 (Tamanna and Khan, 2005).

2.6. Population structure analysis

Population structure was analyzed to determine number of sub-populations in the germplasm panel using STRUCTURE v. 2.3.4 software (Pritchard and Wen, 2003). Ten independent runs were carried out for each number of population (K) set from 1-10. Burn-in time and Markov Chain Monte Carlo (MCMC) replication number were both set to 100,000 for each run. The optimal value of K was determined by examining delK statistic and L(K) (Evanno et al., 2005) using Structure Harvester software (Earl and VonHoldt 2012). Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) was computed using software GenALex6.5 (Peakall and Smouse, 2012).

3. Results

3.1. Germplasm performance based on agro-morphological characters

The genotype panel used in present study consisted of 135 *B juncea* germplasm lines, both exotic and indigenous as-well-as some advanced

Table 3. Genetic diversity parameters of 159 polymorphic SSR markers used in the present study.

S. No.	Marker ID	Annealing temperature (°C)	Total amplified bands (TB)	Polymorphic bands (PB)	Percentage of polymorphic bands (PPB)	Major allele frequency	PIC value	Gene diversity
1	BRMS-002	51	3	1	33.33	0.33	0.59	0.67
2	BRMS-003	55	3	1	33.33	0.34	0.59	0.66
3	BRMS-005	50	3	1	33.33	0.63	0.37	0.47
4	BRMS-006	49	3	1	33.33	0.34	0.59	0.66
5	BRMS-007	53	4	4	100.00	0.56	0.49	0.56
6	BRMS-011	50	6	6	100.00	0.70	0.40	0.45
7	BRMS-015	56	3	1	33.33	0.52	0.46	0.54
8	BRMS-017	56	4	4	100.00	0.66	0.38	0.45
9	BRMS-027	50	5	5	100.00	0.65	0.44	0.49
10	BRMS-029	55	2	1	50.00	0.50	0.38	0.50
11	BRMS-030	57	7	7	100.00	0.74	0.37	0.41
12	BRMS-033	53	4	4	100.00	0.66	0.40	0.46
13	Ra1-F03	53	3	2	66.66	0.34	0.58	0.66
14	Ra2-A01	53	4	4	100.00	0.69	0.36	0.43
15	Ra2-A11	55	5	5	100.00	0.73	0.35	0.41
16	Ra2-C09	55	4	1	25.00	0.51	0.54	0.61
17	Ra2-D04	54	3	1	33.33	0.53	0.45	0.54
18	Ra2-E04	54	4	4	100.00	0.69	0.37	0.44
19	Ra2-E11	54	7	7	100.00	0.67	0.45	0.49
20	Ra2-E12	56	3	1	33.33	0.35	0.58	0.65
21	Ra2-F11	54	6	6	100.00	0.77	0.32	0.36
22	Ra2-G05	56	7	7	100.00	0.84	0.24	0.27
23	Ra2-G08	54	5	5	100.00	0.76	0.31	0.36
24	Ra2-G09	55	2	2	100.00	0.53	0.35	0.47
25	Ra2-C11	56	4	4	100.00	0.71	0.34	0.41
26	Ra3-H09	56	3	3	100.00	0.64	0.36	0.46
27	Ra3-H10	54	6	6	100.00	0.75	0.34	0.38
28	BrgMS60	54	3	1	33.33	0.66	0.35	0.45
29	BrgMS63	54	3	1	33.33	0.67	0.34	0.44
30	BrgMS68	55	5	1	20.00	0.60	0.37	0.48
31	BrgMS70	54	4	4	100.00	0.72	0.33	0.40
32	BrgMS75	54	3	2	66.66	0.63	0.37	0.47
33	BrgMS309	54	2	1	50.00	0.50	0.38	0.50
34	BrgMS316	53	3	1	33.33	0.66	0.35	0.45
35	BrgMS329	54	2	1	50.00	0.66	0.26	0.34
36	BrgMS334	57	3	2	66.66	0.63	0.37	0.47
37	BrgMS337	53	5	1	20.00	0.70	0.38	0.44
38	BrgMS338	56	3	3	100.00	0.70	0.30	0.37
39	BrgMS344	53	4	3	75.00	0.73	0.32	0.39
40	BrgMS372	55	4	3	75.00	0.70	0.35	0.42
41	BrgMS388	56	4	4	100.00	0.71	0.34	0.41
42	BrgMS397	54	4	1	25.00	0.69	0.36	0.43
43	BrgMS399	55	4	4	100.00	0.68	0.37	0.44
44	BrgMS409	55	3	3	100.00	0.67	0.34	0.44
45	BrgMS412	53	5	5	100.00	0.74	0.34	0.40
46	BrgMS418	56	2	1	50.00	0.50	0.38	0.50
47	BrgMS421	54	3	2	66.66	0.60	0.39	0.48
48	BrgMS422	55	3	2	66.66	0.59	0.41	0.50
49	BrgMS429	54	3	2	66.66	0.41	0.54	0.61
50	BrgMS430	55	3	2	66.66	0.65	0.36	0.45
51	BrgMS432	54	3	1	33.33	0.66	0.35	0.44
52	BrgMS521	55	3	3	100.00	0.67	0.34	0.44
53	BrgMS590	54	4	1	25.00	0.72	0.33	0.40
54	BrgMS638	53	3	3	100.00	0.34	0.59	0.67
55	BrgMS710	54	2	2	100.00	0.52	0.36	0.48
56	BrgMS713	55	3	1	33.33	0.34	0.59	0.67
57	BrgMS751	54	2	1	50.00	0.50	0.38	0.50
58	BrgMS776	55	4	4	100.00	0.74	0.31	0.38

(*continued on next page*)

Table 3 (*continued*)

S. No.	Marker ID	Annealing temperature (°C)	Total amplified bands (TB)	Polymorphic bands (PB)	Percentage of polymorphic bands (PPB)	Major allele frequency	PIC value	Gene diversity
59	BrgMS778	54	3	1	33.33	0.67	0.35	0.44
60	BrgMS780	55	3	3	100.00	0.65	0.36	0.46
61	BrgMS782	54	4	4	100.00	0.69	0.36	0.43
62	BrgMS783	54	2	1	50.00	0.51	0.37	0.49
63	BrgMS787	54	6	5	83.33	0.66	0.46	0.51
64	BrgMS794	53	2	2	100.00	0.51	0.37	0.49
65	BrgMS799	54	2	1	50.00	0.50	0.38	0.50
66	BrgMS801	56	4	4	100.00	0.69	0.36	0.43
67	BrgMS802	54	2	1	50.00	0.50	0.38	0.50
68	BrgMS848	54	2	1	50.00	0.50	0.38	0.50
69	BrgMS961	55	3	2	66.66	0.57	0.42	0.51
70	BrgMS1237	55	4	3	75.00	0.74	0.32	0.39
71	BrGMS2766	51	4	1	25.00	0.74	0.32	0.39
72	BrGMS2767	54	3	1	33.33	0.61	0.38	0.48
73	BrgMS4508	55	4	4	100.00	0.71	0.34	0.41
74	BrgMS4533	55	3	1	33.33	0.65	0.35	0.45
75	Ni2A01	55	2	2	100.00	0.54	0.34	0.46
76	Ni2A02	55	2	1	50.00	0.50	0.38	0.50
77	Ni2A07	55	3	1	33.33	0.67	0.34	0.43
78	Ni2A08	50	6	1	16.66	0.71	0.40	0.44
79	Ni2A12	54	3	1	33.33	0.62	0.38	0.47
80	Ni2B01	52	3	3	100.00	0.66	0.35	0.45
81	Ni2B02	54	4	4	100.00	0.51	0.54	0.61
82	Ni2B03	55	5	5	100.00	0.61	0.48	0.54
83	Ni2B07	55	4	4	100.00	0.63	0.43	0.49
84	Ni2B08	53	3	3	100.00	0.66	0.35	0.45
85	Ni2C09	52	5	5	100.00	0.78	0.29	0.34
86	Ni2D03	48	4	4	100.00	0.76	0.29	0.36
87	Ni2D10	52	4	1	25.00	0.73	0.32	0.39
88	Ni2E05	58	6	6	100.00	0.75	0.34	0.38
89	Ni2F11	52	3	1	33.33	0.64	0.36	0.46
90	Ni4A06	54	2	1	50.00	0.51	0.37	0.49
91	Ni4A09	53	4	4	100.00	0.75	0.31	0.38
92	Ni4B04	51	3	2	66.66	0.68	0.34	0.43
93	Ni4C02	56	3	3	100.00	0.60	0.40	0.49
94	Ni4C06	55	3	3	100.00	0.55	0.43	0.52
95	Ni4C09	53	5	1	20.00	0.53	0.56	0.62
96	Ni4C11	54	4	4	100.00	0.61	0.44	0.51
97	Ni4F09	54	5	1	20.00	0.74	0.33	0.38
98	Ni4F11	55	5	1	20.00	0.78	0.29	0.34
99	Ni4G06	50	4	1	25.00	0.71	0.34	0.41
100	Ni4G10	55	7	7	100.00	0.77	0.33	0.37
101	nia-m091a	52	3	1	33.33	0.65	0.36	0.46
102	nia-m066a	53	3	1	33.33	0.64	0.36	0.46
103	SJ3838	51	5	5	100.00	0.79	0.28	0.33
104	SJ4933	51	5	5	100.00	0.80	0.27	0.32
105	SJ6846	52	5	5	100.00	0.76	0.31	0.36
106	SJ3302RI	52	2	1	50.00	0.50	0.38	0.50
107	SJ03104	51	2	1	50.00	0.50	0.37	0.50
108	SJ3627R	52	4	4	100.00	0.71	0.34	0.41
109	SB1672	48	5	5	100.00	0.69	0.39	0.44
110	SB1752	53	4	4	100.00	0.61	0.44	0.51
111	SB2131	50	2	1	50.00	0.50	0.38	0.50
112	SA0306	51	5	5	100.00	0.52	0.56	0.61
113	SB0372	53	3	3	100.00	0.62	0.38	0.48
114	SB1935A	51	5	3	60.00	0.72	0.35	0.40
115	SJ8033	51	4	4	100.00	0.68	0.38	0.45
116	SB0202I	51	2	2	100.00	0.50	0.38	0.50

(*continued on next page*)

Table 3 (*continued*)

S. No.	Marker ID	Annealing temperature (°C)	Total amplified bands (TB)	Polymorphic bands (PB)	Percentage of polymorphic bands (PPB)	Major allele frequency	PIC value	Gene diversity
117	SB3140	52	6	6	100.00	0.63	0.48	0.52
118	SJ6842	53	4	1	25.00	0.57	0.48	0.55
119	SB2556	52	3	1	33.33	0.63	0.37	0.46
120	SB3872	51	2	2	100.00	0.50	0.38	0.50
121	SJ0338	52	3	3	100.00	0.63	0.37	0.47
122	SJ1505	49	2	1	50.00	0.51	0.37	0.49
123	SJ3640I	50	3	3	100.00	0.59	0.40	0.49
124	SJ39119I	51	4	4	100.00	0.68	0.37	0.44
125	SJ13133	51	4	3	75.00	0.62	0.42	0.49
126	SJ1536	51	5	5	100.00	0.68	0.40	0.46
127	SB1937	50	3	2	66.66	0.64	0.36	0.46
128	SJ4633	47	2	2	100.00	0.50	0.38	0.50
129	SB1728	52	3	3	100.00	0.62	0.38	0.47
130	SJ34121	51	4	3	75.00	0.66	0.39	0.46
131	SJ1668I	50	2	1	50.00	0.50	0.38	0.50
132	SB3751	51	2	1	50.00	0.50	0.37	0.50
133	cnu_m587a	52	3	3	100.00	0.65	0.35	0.45
134	cnu_m593a	52	2	1	50.00	0.50	0.38	0.50
135	cnu_m596a	53	4	1	25.00	0.72	0.33	0.40
136	cnu_m597a	54	4	4	100.00	0.72	0.33	0.40
137	cnu_m600a	51	4	1	25.00	0.75	0.30	0.37
138	cnu_m602a	56	3	1	33.33	0.65	0.35	0.45
139	cnu_m604a	53	6	1	16.66	0.51	0.61	0.66
140	cnu_m611a	55	2	2	100.00	0.51	0.37	0.49
141	cnu_m626a	54	5	5	100.00	0.73	0.34	0.40
142	sORA43	55	4	1	25.00	0.70	0.35	0.42
143	Ol09A01	54	5	5	100.00	0.79	0.28	0.33
144	Ol10A11	55	3	1	33.33	0.34	0.59	0.66
145	Ol10B01	54	4	1	25.00	0.45	0.58	0.64
146	Ol10B11	52	7	7	100.00	0.79	0.31	0.34
147	KBRH138G23	52	3	3	100.00	0.67	0.34	0.44
148	KBRH139B23	52	3	3	100.00	0.66	0.35	0.45
149	E018	55	3	3	100.00	0.66	0.35	0.45
150	GOL2	56	3	2	66.66	0.38	0.56	0.63
151	GOL3	55	3	3	100.00	0.34	0.59	0.66
152	EJU1	53	2	1	50.00	0.50	0.38	0.50
153	EJU3	52	4	1	25.00	0.73	0.32	0.39
154	EJU5	55	3	1	33.33	0.57	0.41	0.51
155	ENA2	55	5	5	100.00	0.60	0.50	0.56
156	ENA20	54	4	4	100.00	0.72	0.33	0.40
157	ENA28	54	2	1	50.00	0.51	0.37	0.49
158	ENA9	54	3	1	33.33	0.45	0.50	0.59
159	MB4	46	2	2	100.00	0.51	0.37	0.49
Mean			**3.61**			**0.62**	**0.39**	**0.47**

breeding lines also. For each of the traits evaluated under field conditions, their descriptive statistics including mean, standard deviation and coefficient of variation (CV) are described in Table 2. Morphological characterization of Indian mustard germplasm accessions exhibited a wide range of variation for traits including biological yield (CV = 25.63%), seed yield (CV = 23.23%) and 1000-seed weight (CV = 23.14%); whereas traits such as days to maturity (CV = 2.91%) showed lowest degree of variation (Table 2). In the present study, genotype KLM 4 was the earliest flowering line (33 days), while genotype P 63 was the late flowering genotype (77 days). The plant height varied from 148 cm to 268 cm. NDRS 2010-5-1 was the tallest with plant height of 268 cm and HP 35 had the shortest plant height (148 cm). Main shoot length and the number of siliqua it bears are the critical factors determining seed yield. In this study, main shoot length ranged from 45.5 cm (BT 15) to 115 cm (UP II-76) and the number of siliqua on main shoot varied from 77 (HP 35) to 130 (SEL 68). In this study, Indian mustard genotypes had seeds per siliqua in the range of 8–18. While 1000-seed weight ranged from 2.2 to 7.5 g. Further, for overall yield enhancement, such genotypes are required which bear more seeds per siliqua and have higher 1000-test seed weight. Four such genotypes identified in this study are RRN 605 (17 seeds/siliqua, 6.01 g seed weight), SKJ/DPS 9 (14.72 seeds/siliqua, 6.07 g seed weight), P 4 (14 seeds/siliqua, 6.61 g seed weight) and HP 10 (14.20 seeds/siliqua, 6.87 g seed weight). Overall, seed yield is the most important and critical trait of any crop. Seed yield showed extensive range between genotypes and varied from 800 kg/ha to 3088.9 kg/ha. Genotype YSR/SKJ 45 was the highest yielding genotype. The results of ANOVA inferred that there is enough genetic diversity among the Indian mustard germplasm lines for formulating breeding programs with the aim of improving seed size and grain yield. The UPGMA-dendrogram grouped all the 135 germplasm accessions into three main clusters

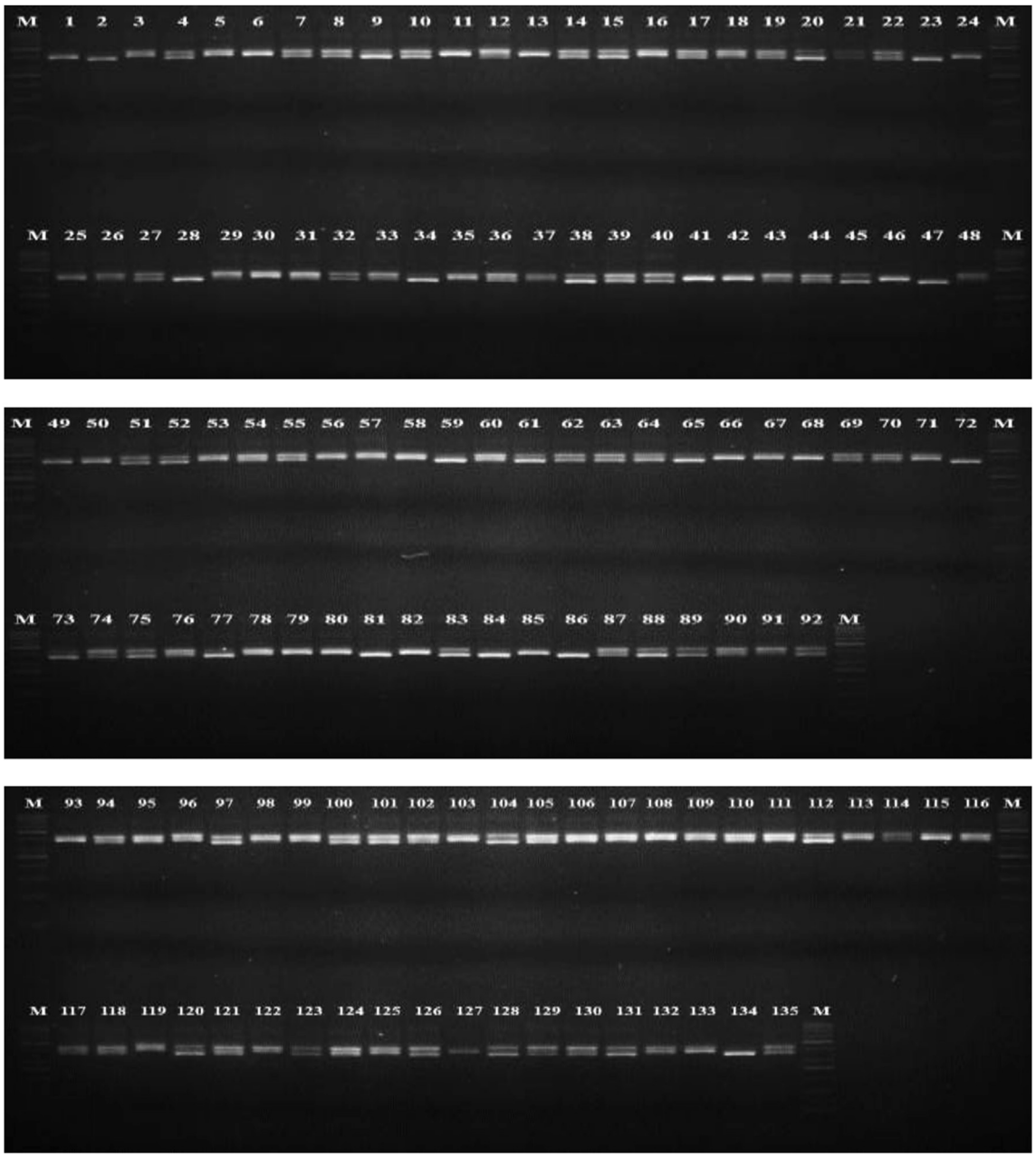

Figure 2. A representative gel picture depicting the amplification profile of SSR marker SA0306 in 135 Indian mustard germplasm accessions (M: 50 bp DNA ladder; Samples 1–135: Indian mustard germplasm accessions).

(Figure 1) on the basis of agro-morphological traits. Cluster I consisted of 39 genotypes, cluster II with 10 genotypes and cluster III was comprised of 86 genotypes.

3.2. Allelic diversity

Of 227 SSR markers evaluated, a total of 159 (70.04%) SSRs generated polymorphic products, while 68 (29.96%) SSRs resulted into monomorphic amplicons. The results for allele number per SSR locus, major allele frequency, PIC value and gene diversity for polymorphic markers have been presented in Table 3. PCR amplification profile of SSR marker SA0306 is presented as Figure 2. The polymorphic markers amplified 575 alleles with allele number varying from 2-7 and 3.61 average number of alleles per locus. SSR markers BRMS-030, Ra2-E11, Ra2-G05, Ni4-G10 and Ol10B11 generated the highest number of alleles (7). Major allele frequency denotes the frequency of main key allele of a molecular marker and it varied from 33% to 84%. SSR marker Ra2-G05 was having the highest major allele frequency (84%), while BRMS-002 was having the lowest major allele frequency (33%) along with 62% as the average major allele frequency per SSR locus (Table 3). PIC values varied from 0.24-0.61 with an average value of 0.39 per SSR locus. Thus, cnu_m604a marker was the most efficient primer pair having the maximum PIC value (0.61). Three SSR markers viz. BRMS-002, BrgMS638 and BrgMS713 depicted the highest gene diversity value (0.67); while SSR marker Ra2-G05 resulted into the lowest gene diversity value (0.27) along with 0.47 as average gene diversity value per SSR marker. UPGMA-dendrogram divided all 135 accessions into two separate clusters (Figure 3). Cluster I comprised of only one germplasm line i.e. HPLM 06–36 (120), while cluster II consisted of two sub-clusters – IIa and IIb. Sub-cluster IIa again comprised of one genotype only i.e. EC 511684 (74), which is a Canadian genotype. These two germplasm accessions viz. HPLM 06–36 and EC 511684 were found to be the most

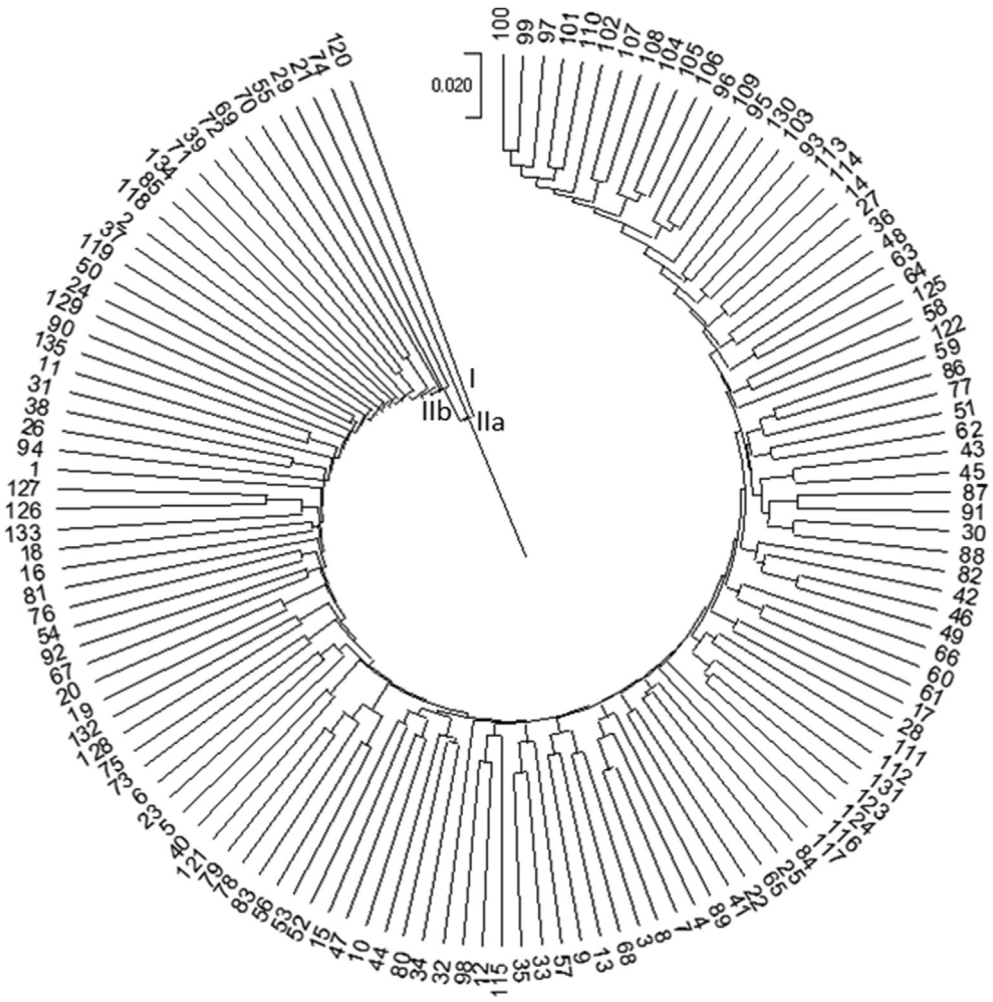

Figure 3. UPGMA dendrogram showing genetic relationship among 135 Indian mustard germplasm accessions based on 159 SSR loci.

divergent in the present study, hence may be deployed in breeding programs for creation of genetic variability. Rest of 133 germplasm accessions were grouped into sub-cluster IIb. PCoA was also performed to depict the genetic relationship among genotypes, which could better distinguish the genetically diverse accessions (Figure 4).

3.3. Population structure analysis and AMOVA

The log likelihood displayed by structure revealed the optimum K value as 5 (K = 5), which inferred that whole germplasm panel can be divided into five subpopulations (Figure 5). The summary statistics obtained from STRUCTURE software as depicted in color chart inferred varying degree of genetic intermixing between genotypes under study (Figure 6). AMOVA statistics of Indian mustard genotypes exhibited 3% variation among populations and 97% variation within individuals (Table 4, Figure 7).

4. Discussion

A high volume of genetic information pertaining to genetic diversity is required to formulate breeding strategies (Carvalho et al., 2019). Since Indian mustard germplasm has a narrow genetic base (Chauhan et al., 2011), so we used both agro-morphological traits and SSR marker systems to evaluate genetic diversity in a panel of Indian mustard germplasm collection. In the present investigation, we characterized 135 Indian mustard germplasm accessions using 11 agro-morphological traits. Various traits including biological yield, seed yield and 1000-seed weight expressed wide range of variation. Whereas, almost all the Indian mustard accessions exhibited a narrow range of variation for days to maturity. In a similar study, Singh et al. (2018) also reported least variability for days to maturity in Indian mustard varieties. In another study conducted to evaluate genetic variability in Indian mustard accessions, maximum variation for seed yield per plant followed by secondary branches per plant, primary branches per plant, 1000-seed weight and the number of siliqua on main shoot was recorded (Singh et al., 2020). In another attempt to decipher the genetic variation in 50 Indian mustard germplasm and breeding lines, maximum variability for biological yield followed by seed yield and minimum variability for days to maturity followed by days to flower initiation was reported (Singh et al., 2013). All the germplasm lines initiated flowering between 33-77 days interval. Lesser plant height is a desirable agronomic trait in Indian mustard as it eases various agronomic operations. Hence, genotypes HP 35 (148 cm), BT 31 (154 cm) and JCR 996 (158 cm) are having low plant height and may be used as donor for breeding for low plant height trait. Number of seeds per siliqua and 1000-seed weight are important yield component traits. A total of 14 germplasm lines were having 1000-seed weight above 6.0 g, out of which genotype JBT 41/32, UP II-21 and BT 13 were having 1000-seed weight above 7.0 g, inferring the utility of these genotypes for breeding for bold seededness trait. Overall, a high degree of genetic diversity was observed in Indian mustard germplasm accessions for agro-morphological traits, which is consistent with the previous reports (Vinu et al., 2013; Singh et al., 2018). UPGMA-based dendrogram grouped all the Indian mustard germplasm accessions into three major groups.

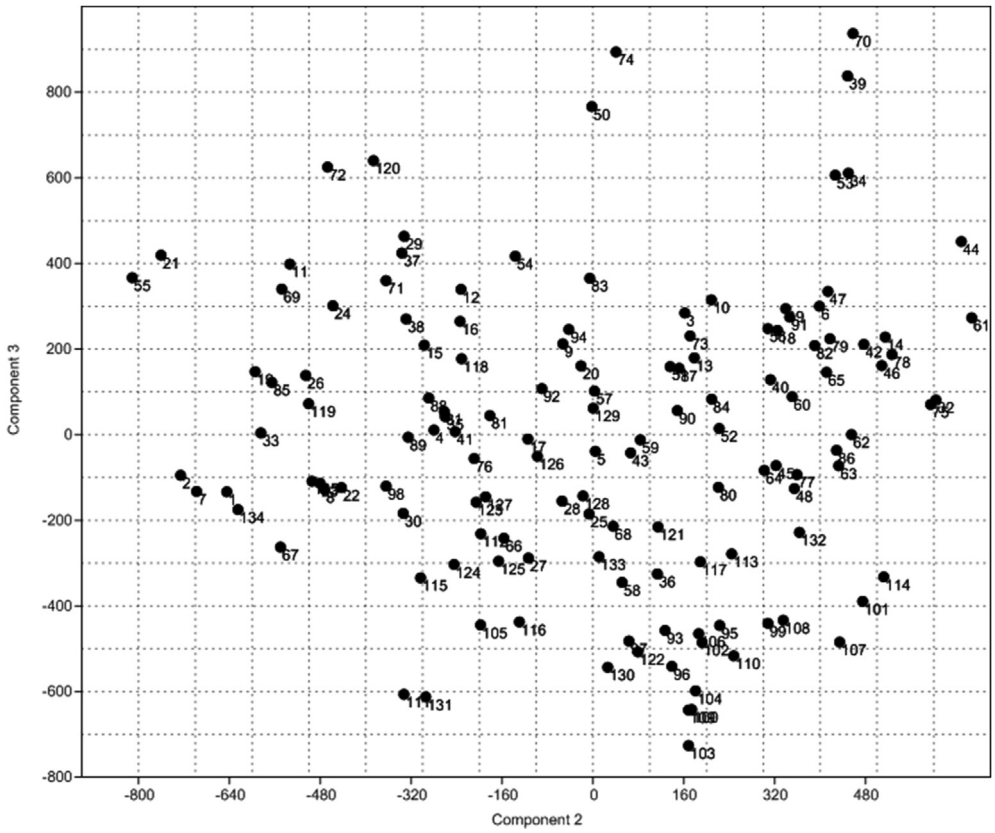

Figure 4. Principal coordinate analysis (PCoA) of Indian mustard germplasm accessions on the basis of SSR markers.

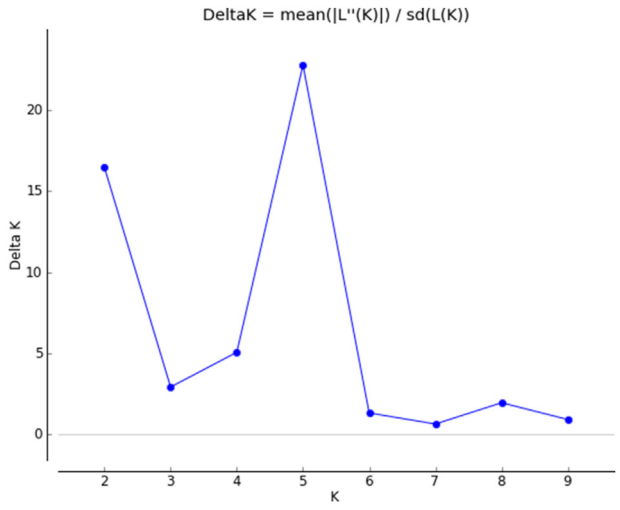

Figure 5. Population structure analysis using LnP(D) derived delta K for determining optimum number of subpopulations. The maximum of adhoc measure delta K determined by Structure Harvester was found to be K = 5, which inferred that the whole population can be divided into 5 subpopulations.

Genetic diversity estimation aids crop improvement programs by establishing genetic relationship in germplasm accessions, which assists in effective utilization of genetic resources. In the present investigation, 159 SSRs resulted into polymorphic amplicons, which were further considered for computation of various allelic diversity parameters. The number of alleles ranged from 2-7 with a mean value of 3.61 per SSR locus. A lesser average allele number (2.37) per SSR marker than the present study was reported in a similar study by Sudan et al. (2016), where 23 genotypes of Indian mustard were characterized using 16 SSRs. Thakur et al. (2015) also reported 2.17 average number of alleles per SSR marker when they characterized 12 popular varieties of *B. juncea*. Higher allele number reported in the present study can be credited to the large number of genotypes evaluated and higher number of SSRs used than earlier studies. PIC value describes the discriminatory potential of a molecular marker and it ranged from 0.24-0.61 with a mean value of 0.39 per SSR locus. A lesser average PIC value was reported in a similar study involving evaluation of genetic diversity in *B. juncea* genotypes for *Alternaria* blight tolerance using 25 SSRs (Pratap et al., 2015). Similarly, Singh et al. (2016) observed a lesser average PIC value (0.30) than the present study when they assessed the polymorphic potential of 134 genic-SSRs in six *B. juncea* genotypes. Lower PIC value in these studies might be due to the lesser diversity in the germplasm used. Botstein et al. (1980) developed a PIC value scale to estimate the level of genetic variability. According to them, the locus having PIC value > 0.5 was considered of high genetic diversity; with PIC value < 0.25 means of low genetic diversity and with PIC value in the range of 0.25–0.5, with intermediate diversity. In this study, an average PIC value of 0.39 per SSR locus was obtained, which inferred that the present data set consists of intermediate genetic diversity level. Further, it was also reported by another group of scientists (Akkaya and Buyukunal Bal, 2004) that markers having PIC values of 0.5 or above 0.5 are considered highly useful in differentiating the genotypes. In this study, out of 159 polymorphic SSRs, 16 markers recorded PIC values > 0.5, which can be considered as highly polymorphic and thus informative and useful for genetic diversity studies, linkage map construction and QTL mapping in Indian mustard. Gene diversity is the probability that two randomly chosen alleles are differing in the genotype (Choukan and Warburton, 2005). In the present study, gene diversity values were in the range of 0.27–0.67 with a mean value of 0.47 per SSR marker.

UPGMA-dendrogram grouped all 135 accessions into two separate clusters (Figure 3). It has been observed that even exotic accessions from Canada except for EC 511684, Sweden and England used in the present

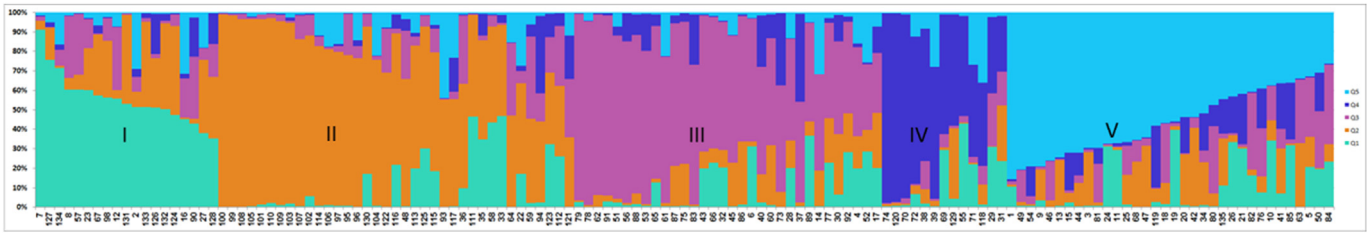

Figure 6. Population structure of 135 Indian mustard germplasm accessions with K = 5 estimated using 159 SSR loci.

Table 4. Summary of Analysis of variance (AMOVA).

Source of variation	df	SS	MSS	Est. Var.	%age
Among populations	4	683.039	170.760	1.572	3
Among individuals	130	11494.709	88.421	44.210	97
Total	134	12177.748		45.782	100

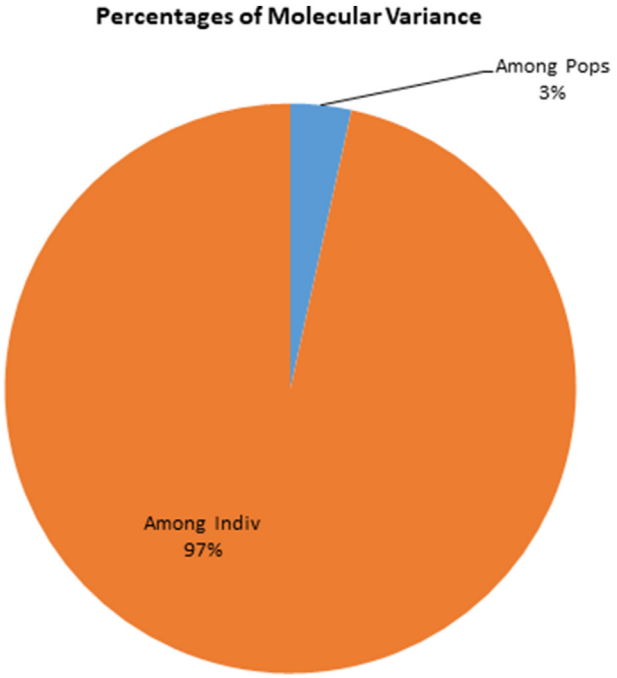

Figure 7. Analysis of molecular variance of 135 Indian mustard accessions based on SSR markers.

study showed grouping with the Indian genotypes. There was no grouping of genotypes observed on the basis of geographical origin. The most plausible explanation for that these exotic germplasm lines might have been imported from India earlier in various international collaborative programs or these genotypes might have involved Indian genotypes as parents somewhere. Further, the SSR-based grouping of Indian mustard genotypes did not show any coherence with the quantitative trait-based grouping. The reason for such a mismatch might be due to that molecular marker differentiation is affected by a number of factors including genetic drift, mutation and gene flow; whereas, the differentiation in agro-morphological traits is more dependent on environmental factors' influence (Govindaraj et al., 2015). In a similar study, Singh et al. (2013) also obtained different clustering patterns of Indian mustard germplasm lines based on agro-morphological trait and RAPD marker data showing no coherence between genetic and phenotypic diversity. In another study, different clustering patterns were obtained in rice genotypes using both morphological and SSR markers showing no correspondence with each other (Sruthi et al., 2020). In the present study, PCoA could provide a better estimate of genetic diversity inherent in Indian mustard germplasm accessions than the morphological trait and SSR allele-based UPGMA. Indian mustard genotypes EC 511589 (70) from Canada and DMRS 186 (39) from Uttarakhand, India were the most genetically diverse accessions, followed by EC 511684 (74) from Canada and UP II-18 (50) from U.P., India. Two germplasm lines from India viz. RN 593 (53) from Navgaon, Rajasthan and BBM 06-02 (34) from Jagdalpur, Chhatisgarh formed a separate cluster. Similarly, two germplasm accessions from Bihar, India viz. VKG 29/80 (21) and VKG 13/75 (55) clustered together in a separate group (Figure 4).

It has been observed that the genetically diverse accessions identified in the present study using SSR allelic data viz. HPLM 06–36 (120) is having 127 siliquae on main shoot and thus may be a potential donor for this trait. Similarly, EC 511684 (74), an exotic accession from Canada is high yielding genotype (seed yield 2986 kg/ha). EC 51189 (70) from Canada and HPLM 06–36 (120) are also having a higher siliqua length (4.59 cm). Germplasm accessions viz. DMRS 186 (39) from India and EC 51189 (70) from Canada attained maturity in 126–127 days, hence can be used for breeding for early maturity. DMRS 186 (39) also exhibited the trait of bearing higher number of seeds per siliqua (16 seeds/siliqua), thus can be deployed for breeding for both early maturity and higher yield. Population structure analysis is useful for distinguishing the number of subpopulations based on the distribution of allele frequency among genotypes. It can further be utilized to identify pure genotypes from the admixtures in the gene banks and while formulating hybridization strategies (Chen et al., 2017). In the present study, five subpopulations were obtained based on STRUCTURE analysis and all the subpopulations exhibited significant number of admixture genotypes. Actually, *B. juncea* has a narrow genetic base as reported by Chauhan et al. (2011). It has been further reported that Indian mustard variety Varuna or its derivatives had been used as parent in the development of a number of Indian mustard varieties and breeding lines. Further, there might have occurred the exchange of genetic material over the time among various genotypes used in the present study resulting into admixture genotypes in structure analysis. AMOVA partitioned the genetic variation as 3% variation among populations and 97% variation within individuals.

5. Conclusion

In the present study, agro-morphological traits along with SSR markers were used for characterization of Indian mustard germplasm panel. The study inferred that Indian mustard germplasm expressed considerable diversity based on agro-morphological traits. However, the genetic diversity at molecular level was found relatively low. Finally, dissimilarities between agro-morphological and molecular markers-based clustering of genotypes inferred that geographic and genetic distances are not related in Indian mustard accessions. The results of cluster analysis may be utilized to formulate strategies to enhance genetic diversity by crossing genetically diverse parents in future. The second approach may involve crossing of high yielding genotypes that possess many random genetic differences, which may increase the number of transgressive segregants. This study inferred that both morphological

and molecular marker systems are supplementing each other as they have identified separate list of genotypes as diverse ones. The genetically diverse material identified in the present study may be utilized for Indian mustard crop improvement programs in future.

Declarations

Author contribution statement

Lal Singh, M.Sc; Deepika Sharma, M.Sc; Nehanjali Parmar, Ph.D: Performed the experiments.

J Nanjundan, Ph.D.: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

K H Singh, Ph.D: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Rohit Jain, Ph.D: Analyzed and interpreted the data.

Ajay Kumar Thakur: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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