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Assessment of molecular diversity in Indian mustard [*Brassica juncea* (L.) Czern and Coss.] using SSR markers

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Abstract
Molecular diversity analysis of 38 Indian mustard genotypes was carried out employing 18 Simple Sequence Repeats (SSR) related to Resistant Gene Homologue (RGH) loci in the Department of Genetics and Plant Breeding, Dharwad, during 2018-19. These SSRs grouped the genotypes into five major clusters at similarity coefficient of 0.001, with cluster I consisted of 13 genotypes, cluster II and cluster II had six genotypes each. While cluster IV consisted of nine genotypes and cluster V with four genotypes. From 18 markers used for amplification, 130 alleles were amplified across 38 genotypes. Among them 128 alleles were scored polymorphic with 77 per cent average polymorphism. The dissimilarity value was lowest for the genotypes RH 1650 and RH 1656 which shared considerably high bootstrap value of 90.

Keywords: Indian mustard, molecular diversity, simple sequence repeats, cluster

Introduction
Indian mustard [*Brassica juncea* (L.) Czern and Coss.] is an important *rabi* oil seed crop belongs to the family Brassicaceae. Mustard having chromosome number 2n=36, is a natural amphidiploid derived from interspecific cross between *B. nigra* and *B. campestris* (Nagaharu, 1935) [6]. Oil content of mustard ranges from 37 to 48 per cent, similarly protein content ranges from 24 to 30 per cent and 35 to 40 per cent based on whole seed and meal respectively. Mustard oil finds its usage in preparation of dishes, medicines, hair oils, detergents and biodegradable plastics.

Existence of variation at the genetic level is the basic need to practise selection. The variability observed at the phenotypic level is not always reliable due to the influence of various environmental factors. Thus, variability present in the genomic sequences is highly reliable which is known as molecular diversity (Singh and Singh, 2015) [15]. The utilization of a species in any crop improvement programme depends not only on the degree of genetic diversity it holds, but also on the precise information regarding genetic divergence and relatedness among breeding materials (Ladole *et al*., 2015) [15]. Assessment of genetic variation and genomic diversity among the genotypes will be utilized to classify them into different clusters, to determine the evolutionary relationship with the wild relatives, to check pedigrees and to remove the gaps in lineage (Singh and Singh, 2015) [15].

Simple Sequence Repeats (SSRs) are type of DNA markers which are extensively used in crop improvement program as they offer many advantages like locus specificity, co-dominance, high polymorphism, high reproducibility, automation amenability and genome wide distribution (Roder *et al.,* 1998) [11]. SSR markers are often transferable across different species of the same genus and even across closely related genera (Choumane *et al*., 2000; Narasimhan *et al*., 2016; Satya *et al*., 2016) [1, 8, 13]. Transferability of SSR markers means that the primers for SSR markers developed for one plant species can be successfully used in related plant species. In this era of molecular markers, variability can be studied at molecular level employing various markers and diverse genotypes so identified can be employed in the crossing programs to yield transgressive segregations. Similarly they can also be utilized for widening genetic base of cultivated varieties.

Materials and methods

Thirty eight *Brassica juncea* genotypes obtained from Bhaba Atomic Research Centre, Trombay, Mumbai; Directorate of Rapeseed Mustard Research, Bharatpur, Rajasthan and Indian Agricultural Research Institute, New Delhi were used for molecular diversity assessment. The genotypes were sown in coco pit filled pro-trays to obtain seedlings for DNA isolation. The DNA was isolated from seven to eight actively growing fresh young seedlings following CTAB (Cetyl Trimethyl-Ammonium Bromide) method suggested by Doyle and Doyle in 1990 with slight modifications.

A set of 18 Resistant Gene Homologue (RGH) related simple sequence repeats (SSR) markers (Table 1) developed in *Vigna* sp. were employed for examination of molecular diversity. The PCR reactions were carried out using Axygen PCR strips with caps in Eppendorf Mastercycler nexus Thermal Cycler. The PCR reactions were set for 10 µl volume with components. The amplification products from PCR were then subjected to electrophoretic separation using horizontal agarose gel electrophoresis. Agarose gel of 3.5 percent concentration was prepared with ethidium bromide for partitioning SSR amplicons. Gel electrophoresis was taken up with 1X TAE buffer for 2.5 hours at 65 volts with 100 bp ladder (Bangalore Genei) as reference for partitioned amplicons. The gel was visually examined under UV light and documented using UVITECH Cambridge documentation system.

PCR banding pattern of all primers against all genotypes was recorded by following binary system. Variable bands were scored manually as '1' for presence of amplicons and '0' for absence. The size of allele was approximately determined with reference to 100 kb ladder (Bangalore Genie) used in the gel electrophoresis. Bands with clear visibility in the image were used for the analysis of phylogeny. DAR win version 6.0.17 was used for construction of dendrogram. Method given by Sokal-Michener (Singh and Singh, 2015) [15] and neighbour-joining algorithm (Perrier *et al*., 2003) [10] was used for construction and representation of phylogenic relationship between the genotypes.

Table 1: List of SSR primers used in the study

Sl. No.	Primer	Forward sequence	Reverse sequence	T_m value (ºC)
1	Primer 1	GCAGCAACTTGTGC	TCTTCAGCTATCTGC	48.55
2	Primer 2	GCGGGTCTGGGAAATCTACG	CTGCAGTAAGCAAAGCAACG	65.10
3	Primer 3	CGCACGAGTTGGATATGATG	GCTCGAGTCATGGAGGGTAA	64.05
4	Primer 4	TCAAACTCAAGCCACCACAA	CGTCGCACGAGTTTACTGAT	63.55
5	Primer 5	CTTGGCAAAATGTCAACTCT	GGAAAGGGGTTAGGTGAGT	59.35
6	Primer 6	GGAATGGGNGGNGTNGGNA	YCTAGTTGTRAYDATDAYYYRC	56.75
7	Primer 7	GCATTGGAACAAGGTGAA	AGGGGGACCACCACGTAG	62.25
8	Primer 8	GAAAGATGAGTAAATTACTT	TGAGGGTCAGGCATGCAG	58.15
9	Primer 9	TGAGCAATCTTTCCCCAATC	CCACGCTCTCTCACCTCTCT	63.80
10	Primer 10	CTGTTCTGCTTGTTGGTTTTCA	AGGTTTGTTGATCGTCAGGAAG	64.05
11	Primer 11	GCCAATTCAGCACAAGGTTT	TAGGTGGAGGATGTGCATTG	63.70
12	Primer 12	CCAGCGTAGTGATGTTCTTGAG	GCAACCCTTGATAGCTTATGGA	63.75
13	Primer 13	GTGGCTTGATGAGGATATGAAA	GGAGAAGTGTATTTGTTGTTGAGAG	62.25
14	Primer 14	CCAATGCCTTGAGGATTAAAA	CGGTCTAAGTCGGTCATGAAG	63.00
15	Primer 15	CAGAATACACGAAACGAAAGTG	CCGATTGGACAGTTTAAGAAGA	61.50
16	Primer 16	TACGTGTGAAAATTGCTTGACC	TAGAATTGGGAGATTTGGAACG	63.65
17	Primer 17	GAAAGATGAGTAAATTACTT	TGAGGGTCAGGCATGCAG	50.70
18	Primer 18	GAACCCACAACCCTGAAATG	TGAGAGGACTTGGGTTCGAG	64.20

Results and Discussion

The SSR markers related to RGH's were used to screen the genotypes at molecular level. From 18 markers used for amplification 130 alleles were amplified across 38 genotypes. Among them 128 alleles were scored polymorphic with 77 per cent average polymorphism. The present investigation grouped the genotypes into five clusters at similarity coefficient of 0.001 as shown in Fig. 1, which indicated considerable dissimilarity among the clusters (Patel *et al.*, 2018) [9]. It was observed that, at similarity coefficient of 0.1, genotypes were grouped into 35 clusters. Cluster I consisted of 13 genotypes, cluster II and cluster II had six genotypes each, cluster IV with nine genotypes and cluster V with four genotypes.

The genotypes *viz*., TM-138-1, TM 277, RH 1590, DEMRIJ 16-51, NPJ-210, JD 6 (ZC), Filler (JD 6), Pusa Jagannath, Pusa Mahak, Pusa Tarak, Pusa Mustard 26, Pusa Mustard 28 and Dodda Sasive fell into cluster I. The bootstrap values recorded for cluster I ranged from 10 to 80. Cluster II included TM-210, TM-267-1, DRMRCI 106, SVJ-104, Pusa Agrani and NRCHB-101. Cluster II registered bootstrap values ranged from 10 to 60. While, cluster III exhibited bootstrap values of 30 and consisted of six genotypes *viz.*, NPJ-209, TM-136, KMR (E) 16-1 Pusa Mustard 25, PRE-2013-3 and PRE-2015-1. Clusters IV consisted of TM 2776, PRE-2013-19, CAU-RM1, NDRE 8-14-1, DRMR 2017-14, DRMR 4005, DRMRCI 98, KMR (E) 17-1 and KMR (E) 17-2. The bootstrap values for cluster IV ranged from 10 to 70. Cluster V revealed bootstrap values from 20 to 90 and consisted of genotypes *viz*., Pusa Mustard 27, RRN 921, RH 1650 and RH 1656.

The phylogenic relationship was not based on the geographic location from which the genotypes were collected since there was no clear cut partition between the clusters based on place of collection which indicated sharing of common ancestry between the genotypes from DRMR, Bharatpur, BARC, Trombay and IARI, New Delhi at least to some extent at RGH loci. Cluster I was largest which includes 13 genotypes. The results are in accordance with Kumar *et al.*, (2013) [4].

The bootstrap values indicate the repeatability of the phylogenic representation. The bootstrap value more than 70 is considered better for more accurate grouping which gives probability of more than 95 per cent and corresponding clade is most probable under the cluster (Hillis and Bull, 1993) [3]. The phylogenic representation of genotypes had a bootstrap values which ranged from 10 to 90, which were in agreement with Narasimhan (2011) [7]. The bootstrap value of more than 70 was recorded by only six pair of genotypes which

indicated low repeatability of the phylogenic representation. According to Rokas and Carroll (2005) [12], movement from one taxa to another decreases phylogenic accuracy, which is evident in the present study where primers from *Vigna* spp. were amplified in family Brassicaceae. Additionally, genes are linked to RGH's which are conserved across the species retaining the same amino acids component. Since they are prone to frequent modifications, every allele amplified may not be related to phylogeny of the genotypes (Scott, 2015) [14]. Thus low bootstrap values and less representation of phylogeny of the genotypes is justifiable.

The Jaccard's dissimilarity matrix placed the genotypes TM 277 and Pusa Jagannath in the same cluster, which shared maximum dissimilarity value with genotypes RH 1590, TM 138-1 and Dodda Saasive. Thus, any of these combinations can be used for hybridization in development of mapping populations for mapping Resistant Gene Homologues. The dissimilarity value was lowest for RH 1650 and RH 1656 which shared considerably high bootstrap value of 90 an indicative of common ancestry between the genotypes.

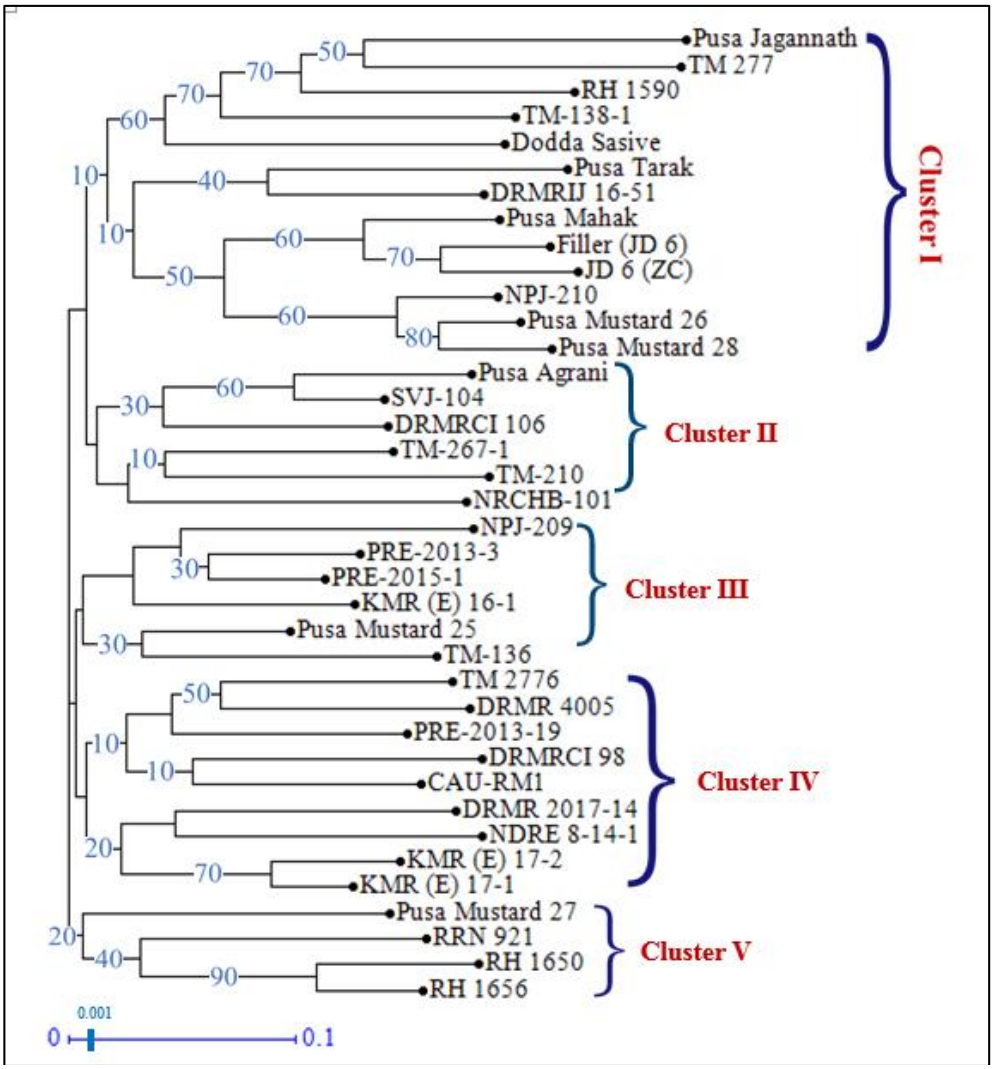

Fig 1: Phylogenetic relationship at RGH loci for Indian mustard genotypes by Sokal-Michener method using neighbour joining algorithm. Numbers at the root level are genotypes and at branch point are bootstrap values

Conclusion

The molecular diversity analysis 38 Indian mustard genotypes employing 18 SSR markers related to RGH loci classified the genotypes into five clusters at similarity coefficient of 0.001. Maximum number of genotypes were grouped under cluster I followed by cluster IV. Cluster I consisted of 13 genotypes, cluster II and cluster II had six genotypes each. While cluster IV consisted of nine genotypes and cluster V with four genotypes. From 18 markers employed for amplification, 130 alleles were amplified of which 128 alleles were scored polymorphic. Jaccard's dissimilarity matrix revealed that genotypes TM 277 and Pusa Jagannath shared maximum dissimilarity with RH 1590, TM 138-1 and Dodda Saasive. Dissimilarity value was lowest between RH 1650 and RH 1656 which shared considerably high bootstrap value of 90. Thus, diverse genotypes can be used for synthesizing mapping populations or segregating materials for the development of varieties and hybrids.

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