GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF *BRASSICA* GENOTYPE(S) FOR LOW AND HIGH ERUCIC ACID CONTENT USING SSR MARKERS

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ABSTRACT
In conventional *Brassica* oilseeds, the occurrence of erucic acid is considered as anti-nutritional factor for human consumption as this causes toxic effects on the heart at higher enough doses. Per se there is an urgent need to curtail the erucic acid content and breed varieties having low erucic acid through breeding and biotechnological means. The present study was conducted on 48 *Brassica* genotype (s) with the aim to identify genotypes with low and high erucic acid content on the basis of SSR markers. For the study a total of 50 SSR markers were selected for the amplification of genomic DNA extracted. Out of these, only 23 SSR markers were found to be polymorphic. A total of 109 alleles were identified 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. The dendrogram was prepared and major three clusters were obtained. Most of the genotypes were grouped according to the sites they developed. Genotypes reported with low erucic acid content showed high similarity and grouped together. Mustard genotypes identified with higher genetic variability with useful traits may be used for crop improvement programmes in upcoming days.

KEYWORDS: Indian mustard, fatty acid, oil quality, erucic acid, SSR markers.

INTRODUCTION

India is the third largest mustard seed producer in the world. Indian mustard or brown mustard [*Brassica juncea* (Linn.) Czern & Coss] is a natural amphidiploids (2n=36), derived from interspecific cross of *Brassica campestris* (2n=20) and *Brassica nigra* (2n=16) followed by natural chromosome doubling. It is mainly grown for oil-seed usage in India. Among the major oilseed producing countries, India contributes about 7% at the global level. Oilseed crops hold an important position in Indian economy also. Major rapeseed mustard producing countries of the world are Canada, China, France, Germany, Poland, UK, India, Australia, Russia and Ukraine (Anonymous, 2015). In India, the total area, production and productivity under cultivation of rapeseed mustard in 2016-17 were 6.65 million hectare, 7.10 million tones and 1069 $kgha^{-1}$, respectively. In M.P., it was sown in 0.723 million hectare and produced 0.607 million ton with 840 kgh^{-1} productivity in 2016-2017 (Anonymous, 2016).

Genetic improvement of crops can be accelerated when there is a broad genetic diversity and information on these genetic resources is available. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. The collection of these genetic resources and the assessment of genetic diversity within and between these resources should be given priority for varietal improvement. Molecular markers are useful contrivances for detecting dissimilarity within different cultivars. There are several advantages of the DNA based diversity analysis techniques; highly reproducible result, can be done at any stage of growth, only a small amount of plant tissue is needed, trait such as insect and disease resistance can be selected. DNA based diversity analysis permit the reliable tracking of beneficial trait during varietal selection, DNA based diversity analysis is the only practical or available technique for ensuring the presence of multiple beneficial genes in a single variety.

In traditional *Brassica* oilseeds, the occurrence of erucic acid is considered as anti-nutritional factor for human consumption that causes toxic effects on the heart at higher enough doses. As such there is need to minimize the erucic acid content and have low erucic acid genotypes through breeding and biotechnological tools. On the basis of above background the present study was undertaken with the intend to identify *Brassica* genotype(s) for low and high erucic acid content with the help of SSR molecular markers.

MATERIALS AND METHODS

Experimental material

Experimental material consists of forty -eight Indian mustard genotypes acquired from the Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior M.P. (AICRP on Rapeseed and Mustard) and IARI, New Delhi. These were collected from different parts of India and abroad (Table 1).These genotypes were selected on the basis of their high and low erucic acid possessing abilities from 196 genotypes on the basis of biochemical analysis work carried out by Shyam and Tripathi (2019).

TABLE 1: List of mustard genotypes along with source used in present experimentation

S. No.	Genotypes	Germplasm/Varieties	Origins/Sources	Country of origin
1	MRNJ-1	Germplasm	ZARS, Morena(M.P.)	India
2	MRNJ-2	Germplasm	ZARS, Morena(M.P.)	India
3	MRNJ-3	Germplasm	ZARS, Morena(M.P.)	India
4	MRNJ-4	Germplasm	ZARS, Morena(M.P.)	India
5	MRNJ-5	Germplasm	ZARS, Morena(M.P.)	India
6	MRNJ-6	Germplasm	ZARS, Morena(M.P.)	India
7	MRNJ-7	Germplasm	ZARS, Morena(M.P.)	India
8	MRNJ-8	Germplasm	ZARS, Morena(M.P.)	India
9	MRNJ-9	Germplasm	ZARS, Morena(M.P.)	India
10	MRNJ-10	Germplasm	ZARS, Morena(M.P.)	India
11	MRNJ-11	Germplasm	ZARS, Morena(M.P.)	India
12	MRNJ-12	Germplasm	ZARS, Morena(M.P.)	India
13	MRNJ-13	Germplasm	ZARS, Morena(M.P.)	India
14	MRNJ-14	Germplasm	ZARS, Morena(M.P.)	India
15	MRNJ-15	Germplasm	ZARS, Morena(M.P.)	India
16	MRNJ-16	Germplasm	ZARS, Morena(M.P.)	India
17	MRNJ-17	Germplasm	ZARS, Morena(M.P.)	India
18	MRNJ-18	Germplasm	ZARS, Morena(M.P.)	India
19	MRNJ-19	Germplasm	ZARS, Morena(M.P.)	India
20	MRNJ-20	Germplasm	ZARS, Morena(M.P.)	India
21	MRNJ-21	Germplasm	ZARS, Morena(M.P.)	India
22	MRNJ-22	Germplasm	ZARS, Morena(M.P.)	India
23	MRNJ-23	Germplasm	ZARS, Morena(M.P.)	India
24	MRNJ-24	Germplasm	ZARS, Morena(M.P.)	India
25	MRNJ-25	Germplasm	ZARS, Morena(M.P.)	India
26	ISC-3	RIL	Rasi seed company, Gururam (Haryana)	India
27	JM-2	Variety	RVSKVV, Gwalior (M.P.)	India
28	RVM-1	Variety	RVSKVV, Gwalior (M.P.)	India
29	RVM-2	Variety	RVSKVV, Gwalior (M.P.)	India
30	Rohini	Variety	CSAUAT, Kanpur (U.P.)	India
31	Maya	Variety	CSAUAT, Kanpur (U.P.)	India
32	NRCDR-2	Variety	DRMR, Bharatpur (Rajasthan)	India
33	DRMRIJ-31	Variety	DRMR, Bharatpur (Rajasthan)	India
34	DRMR-150-35	Variety	DRMR, Bharatpur (Rajasthan)	India
35	GM-2	Variety	SDAU, Banaskantha (Gujarat)	India
36	CS-54	Variety	CSSRI, Karnal (Haryana)	India
37	RB-50	Variety	CCSHAU, Hisar (Haryana)	India
38	RH749	Variety	CCSHAU, Hisar (Haryana)	India
39	JD-6	Variety	IARI, New Delhi	India
40	L-4	Germplasm	Canada	Canada
41	L-6	Germplasm	Canada	Canada
42	PM-25	Variety	IARI, New Delhi	India
43	PM-21	Variety	IARI, New Delhi	India
44	PM-22	Variety	IARI, New Delhi	India
45	PM-24	Variety	IARI, New Delhi	India
46	PM-29	Variety	IARI, New Delhi	India
47	PM-30	Variety	IARI, New Delhi	India
48	LES-39	Variety	IARI, New Delhi	India

Molecular analysis

For genomic DNA isolation, fresh leaf tissue was collected from 3-4 week old plants grown in the field. DNA was isolated using modified CTAB method (Murray and Thompson, 1980). Highly polymorphic random SSR (Table 2), gene based SSR (Table 2) and gene based CAPS molecular markers (Table 2) were used during present investigations. Amplicons were separated on 3% agarose gel through electrophoresis and subsequently gels were subjected to documentation with UV image analyzer and their images were saved for further band scoring. The banding pattern of each set of primer was scored separately. For estimating the size of amplicon of each sample the band position was compared with a base pair of standard weight marker. The base pair position was scored as "A, B, C, D, E *etc*" and absence of band was scored as blank.

The major allelic frequency, polymorphism information content and genetic distance based clustering was performed with Unweighted Pair Group Method for Arithmetic Average (UPGMA) tree using Power Marker v 3.25 and the dendrogram was constructed using MEGA 4.0 software. The population structure was inferred using Structure Harvester. The relation between genetic similarity identified by SSR markers and taxonomic distance measured by mean genetic distance was analyzed using Jaccard's Similarity Index and average taxonomic distance calculated by NTSYS-pc v 2.1.

TABLE 2: List of SSR and CAPS markers with their sequences used in the study

S. No.	Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Random SSR markers			
1	BRMS-093	TCCAAGTAGACCGAATCAAGAGAGT	ATAAATCGAACCTGAAACCATGTCT
2	BRMS- 098	TGCTTGAGACGCTGCCACTTTGTTC	CATTCCTCCCCACCACCTTCACATC
3	BRMS- 240	CAAGAGTATTTGTGTGGGTTGACTC	AAATAACGAACGGAGAGAGAGAGAG
4	BRMS- 324	AACTTAACCGAAACCGAGSTAGGTG	AATCTCGAAATTCATCGACTTCCTC
5	CB -10065	CGGCAATAATGGACCACT	CGGCTTTCACGCAGACTTCG
6	SORF -73	CGTGGGCCAAGCTTAGATTA	CGTTCAAGAAGACACAGATCAAA
7	SR- 7223	AGGACCCGACTTTCCTTGTT	ACCAAACTCGGCGTACAAAT
8	SR- 9222	CACCGAACAAAACTGAGGGT	CGTTTCACTGCGTTCTACCA
9	SR 94102	ATCCCCAAACTACCCTCACC	AGGATGAGCAAAGGAAAGCA
10	SR-9447	AAATTCGAAAATGCAAACGG	CCAATCTTGGAACAATAGAAGATG
11	OI 10-CO 5	GGCTACAAAATGTTTGATAAGCTCT	ACCTGAAAGAGAGGCTACACAT
12	SSR Na10-B08	AGAGAAAAACACTTCCCGCC	GTGAGCTTTGCGAAACACG
13	SSR Na10-B10	GTCGGGTTTGAGTGAGTTGG	CATCGCAGATCCTTCTCTCC
14	SSR Na10-B11	TTTAACAACAACCGTCACGC	CTCCTCCTCCATCAATCTGC
15	SSR Na10-C01	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC
16	SSR Na10-C03	TTGGGTGTCTTTGTTACCCC	ACCGAGAAGACTGATACGGG
17	SSR Na10-C06	TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG
18	SSR Na10-C08	GTTTGGTTCAGAGGCAGAGG	CTATCGCTGCAGAAGAAGGG
19	SSR Na10-D03	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGAGACACAC
20	SSR Na10-D07	CTACTTTGATGGACACTTGCC	TCTGAAGTTGATTAGTCGGTCC
21	SSR Na10-D08	TCCATTCATTAAAATCGGCG	TTCTGATCCCTTTCTCTCCC
22	SSR Na10-D09	AAGAACGTCAAGATCCTCTGC	ACCACCACGGTAGTAGAGCG
23	SSR Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG
Gene specific SSR markers			
24	FAE1	ATGACGTCCGTTAACGTA	AAGACTTGTCGTCAGCTCCA
25	Sal-SRK-I	GATTATCTCGTGTCTGAATG	GGTAATGTCGAATCTCTCCT
CAPS markers			
26	CAPS1265	ACGTTAGGTCCGTTGATTCTTC	GGGTATCTGTCGATGCAATGT
27	CAPS237	TAACCATCGCTCCACTCTTTG	TCAAGAAGTCAAGCCACGAC

RESULTS & DISCUSSION

Molecular breeding is one of the noteworthy and fast approaches for crop improvement in mustard (Pushpa *et al*., 2016; Kapadia *et al*., 2019). High quality DNA was used for amplification of highly polymorphic SSR across 48 genotypes of mustard. Working concentration of 25ng/ulDNA was used to amplify all the markers. A total of 50 SSR markers set were amplified with DNA samples of two Indian mustard genotypes in preliminary experiment. Out of 50 SSR markers, a total of twenty-seven were found monomorphic and 23 were polymorphic. These 23 polymorphic markers were amplified in selected 48 Indian mustard genotypes.

The representative gel illustrated amplified monomorphic and polymorphic banding patterns of selected primers. A total 109 alleles were amplified across 23 markers among all the 48 genotypes (Table 3). Diversity analysis of Indian mustard genotypes was done using 23 highly polymorphic SSR markers. All amplified 109 alleles were observed across 23 markers in 48 genotypes. A total of 109 alleles were identified 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. The results were similar with the results of Yadav *et al*. (2012), studied genetic diversity among 30 Indian mustard genotypes using 20 ISSR primers showing 156 amplified bands with an average of 7.8 bands per primer out of which 115 were polymorphic. Size of amplified fragments ranged from 100 bp to 1500 bp and number varied from 4 to 14. The primer BRMS-098 showed highest gene diversity (0.77) and PIC value (0.73) which is representing 77% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.51) was observed for the primer SSR Na10-D07, indicating 51% polymorphism. All 23 primers representing PIC value more than 50%, so these primers can further be used for diversity analysis. Similarly Fayyaz *et al*. (2014) also used 90 genotypes for molecular characterization using 24 microsatellite markers, but only 12 SSR primer combinations generated a total of 33 alleles, of that 32 were polymorphic, whereas only one had monomorphic locus. The average number(s) of polymorphic alleles per locus was 2.66. The PIC values ranged from 0.395 for primer Ra2-E03 to 0.726 for primer BRMS-019 with an average genetic diversity of 0.584 per locus. Recently, Habib *et al*. (2019) presented diversity among 20 *Brassica juncea* genotypes using 15 SSR primer pairs. A total of 79 polymorphic alleles with an average of 5.26 alleles per locus were generated. The polymorphism information content (PIC) values ranged from 0.41 to 0.94 reflecting the presence of high allelic variation. The magnitude of similarity coefficient was found to be the maximum amongst pair-wise combinations of genotypes.

TABLE 3: List of SSR and CAPS markers with their major allele frequency, allele number, gene diversity, heterozygosity and PIC values

Markers	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC value
BRMS-093	0.48	6.00	0.71	0.00	0.68
BRMS- 098	0.33	6.00	0.77	0.00	0.73
BRMS- 240	0.33	4.00	0.74	0.00	0.69
BRMS- 324	0.56	4.00	0.60	0.00	0.55
CB -10065	0.46	5.00	0.69	0.00	0.65
SORF -73	0.42	5.00	0.69	0.00	0.63
SR- 7223	0.44	3.00	0.62	0.00	0.54
SR- 9222	0.52	4.00	0.63	0.00	0.57
SR 94102	0.38	5.00	0.74	0.00	0.70
SR-9447	0.52	6.00	0.64	0.00	0.59
OI 10-CO 5	0.44	4.00	0.62	0.00	0.55
SSR Na10-B08	0.31	6.00	0.75	0.77	0.71
SSR Na10-B10	0.42	4.00	0.71	0.83	0.66
SSR Na10-B11	0.35	5.00	0.73	0.00	0.68
SSR Na10-C01	0.54	4.00	0.60	0.00	0.53
SSR Na10-C03	0.44	5.00	0.65	0.00	0.58
SSR Na10-C06	0.38	6.00	0.75	0.00	0.71
SSR Na10-C08	0.40	4.00	0.69	0.00	0.63
SSR Na10-D03	0.48	4.00	0.63	0.00	0.56
SSR Na10-D07	0.65	5.00	0.55	0.00	0.51
SSR Na10-D08	0.44	5.00	0.68	0.00	0.62
SSR Na10-D09	0.38	4.00	0.68	0.00	0.62
SSR Na10-D11	0.51	5.00	0.65	0.44	0.60
Mean	0.44	4.74	0.68	0.09	0.62
Gene specific SSR markers					
FAE1	0.37	5.00	0.70	0.00	0.64
Sal-SRK-I	0.43	5.00	0.71	0.00	0.66
Mean	0.40	5.00	0.70	0.00	0.65
CAPS markers					
CAPS237	0.50	5.00	0.66	0.00	0.61
CAPS1265	0.31	4.00	0.74	0.00	0.69
Mean	0.41	4.50	0.70	0.00	0.65

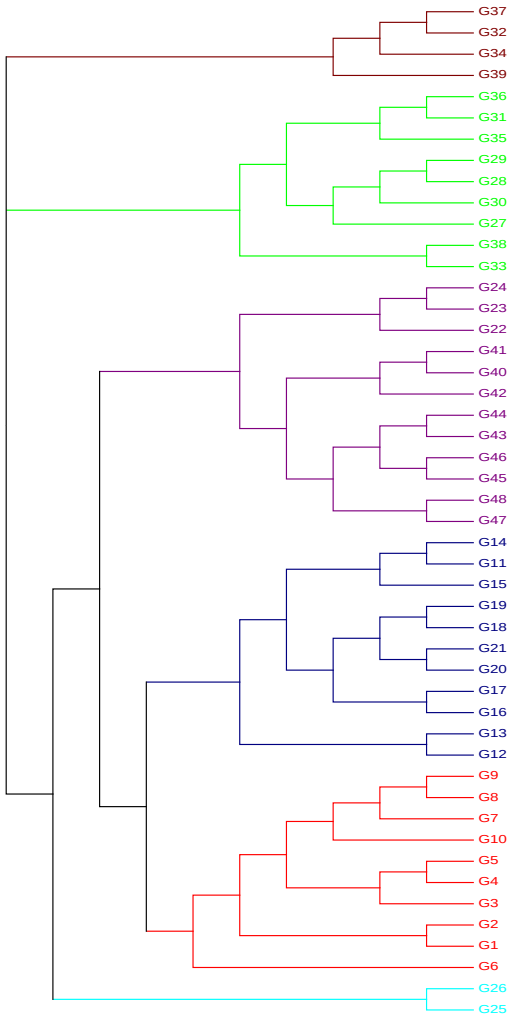

FIGURE 1. Dendrogram of highly polymorphic SSR markers using power marker v3.25 software

The dendrogram was prepared based on scoring done for the entire polymorphic SSR markers. Major three clusters were obtained *i.e*., C-I, C-II and C-III, in which cluster C-I consisted of 4 genotypes (Fig.1; Table 4). Cluster C-II had 9 genotypes. Among these nine genotypes three *namely*: JM-2, RVM-1 and RVM-2 have been developed at Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh. Cluster C-III contained 35 genotypes. Most important cluster C-III contained 35 genotypes including MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6 germplasm lines representing similar place of their origin (Zonal Agriculture Research Station, Morena, Madhya Pradesh, India) except L-4 and L-6. Further PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30 are notified varieties and grouped together with higher similarity index. These all varieties have been developed at Indian Agricultural Research Institute, New Delhi having low erucic acid content. These all varieties are preferred for timely sown under rainfed conditions. In these varieties, Pusabold, Pusa Agrani, Pusa Barani, PusaJagannath are involved as one of the parents. Likewise, in a previous study conducted by Vinu *et al*. (2013) with 44 mustard genotypes, four clusters were formed. Among them first cluster comprised eight varieties developed at IARI, New Delhi. In six of these varieties, except two early maturing varieties *i.e*., Pusa Agrani and Pusa Tarak, Varuna is involved as one of the parents directly or through the ancestry. The result of the present investigation shows the usefulness of SSR markers in identification of the close pedigree association in the studied genotypes. Previously, a similar result concerning efficiency of SSR markers in determination of genetic variability have also been reported by Plieske and Struss (2001). Related types of studies on application of SSR markers have also been performed in *B. napus* (Hopkins *et al*., 2006). In the present study, good quality polymorphic markers were found that can be utilized fr the identification of genotypes with useful traits for future breeding purposes. With the help of these markers, genes introgression can be done to desirable genetic backgrounds for the improvement of Indian mustard.

TABLE 4: Cluster groups of Indian mustard genotypes and their characteristics using highly polymorphic SSR markers

Major clusters	Sub clusters	No. of genotypes	Name of genotypes	Remark
C-I,		4	RB-50, NRCDR-2, DRMR150-35 and JD6	Suitable for rainfed and salinity condition (RB50), Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew & *A. blight* (DRMR150-35), Drought (rainfed), suitable for early (September) and late (November) sowing under irrigated situations (JD6)
C-II	C-II (a)	7	CS-54, Maya, GM-2, RVM-2, RVM-1, Rohini and JM-2	Salinity tolerant (CS54), Suitable for irrigated condition and white rust resistance (Maya), Nontraditional areas (GM2), Early maturity and high oil content (RVM1), Suitable for rainfed and late sown conditions (RVM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Suitable for rainfed condition and white rust resistance (JM2)
	C-II (b)	2	RH749 and DRMRIJ-31	Suitable for irrigated condition (RH749), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-III	C-III (a)	33	MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6	Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39)
	C-III (b)	2	MRNJ-26 and MRNJ-25	

The population structure of the 48 Indian mustard genotypes was estimated using STRUCTURE v2.3.3 software based on SSR. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 3. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, with into three groups (Fig. 2 and Fig.3) using a membership probability threshold of 0.80. First population represented by green colour contained 10 genotypes. Second population (red) represented 8 genotypes and third (blue) 11genotypes. Admixture genome was represented by subpopulation of 5 genotypes green and red, 5 other genotypes represented green and blue, 3 genotypes represented all three population *i.e.*, green, red and blue and 4 genotypes red and blue subpopulation. Molecular assessment of crop genetic variability using SSR markers is important due to their co-dominance nature and capability to disclose a high number of alleles per locus. It is extremely valuable for modern-day plant breeding (Ghosh *et al.*, 2019). The identified diverse genotypes can be utilized for future breeding programmes in mustard.

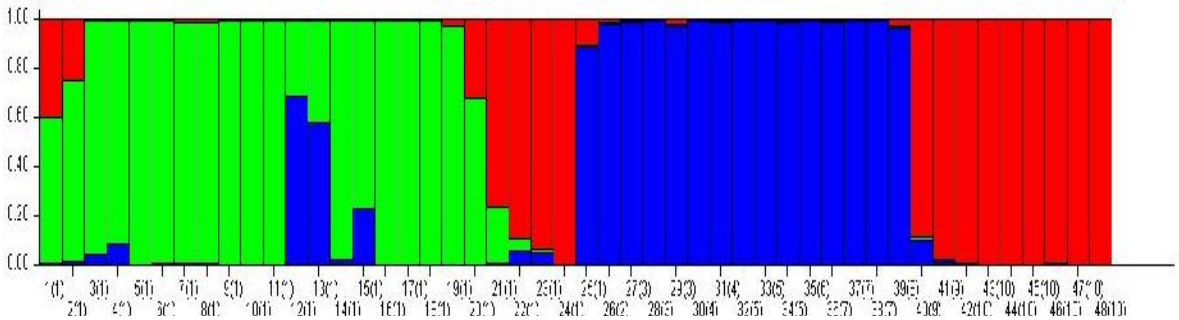

FIGURE 2. Model based population structure plot with K = 3, using 28 Primers

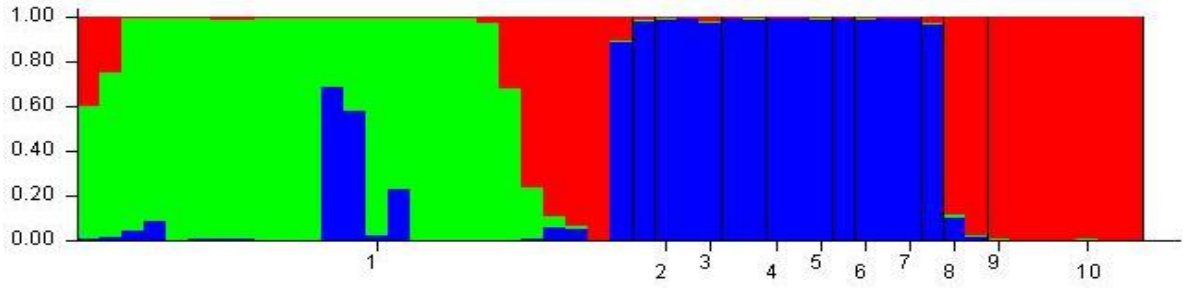

FIGURE 3. Single line analysis based on population structure plot with K=3, using 28 Primers

Screening for erucic acid content in Indian mustard genotypes using gene based SSR markers

Erucic acid is one of the promising traits having multipurpose use and there are several reports where molecular markers have been used for screening and characterization (Bharti *et al*., 2018 and Saini *et al*., 2016). Gene based SSR markers have been used for erucic acid and glucosinolate screening (Farzad *et al*., 2013; Bharti *et al*., 2018). In the present study, two gene based SSR markers for erucic acid were used to screen mustard germplasm lines. Maximum major allele frequency was 0.43 of Sal-SRK-I and minimum 0.37of FAE1 with an average of 0.40. A total of 10 alleles were identified with an average of 5 alleles per locus for polymorphic SSR markers. Allele number of both markers were 5.00 and also mean was 5.00. Highest gene diversity was documented 0.71 of Sal-SRK-I and minimum 0.70 of FAE1 with an average of 0.70. Maximum polymorphic information content was 0.66 of Sal-SRK-I and minimum 0.64 of FAE1 with mean value of 0.65. The primer which showed highest gene diversity (0.71) and PIC value (0.66) was observed in Sal-SRK-I, which is representing 71% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.64) was observed for the primer FAE1, indicating 64% polymorphic nature of the marker. Both primers representing PIC value more than 50%, so these primers can further be used. The genetic relationships among Indian mustard genotypes were characterized. Major three clusters were formed *i.e*., C-I, C-II and C-III. Cluster I makes a group of 4 diverse genotypes *namely*: CS-54, GM-2, DRMR-150-35 and DRMRIJ-31. Cluster II was also having 4 genotypes including MRNJ-6, MRNJ-5, RH-74.9 and RB-50. Cluster III had 40 genotypes (Fig. 4, Table 5).

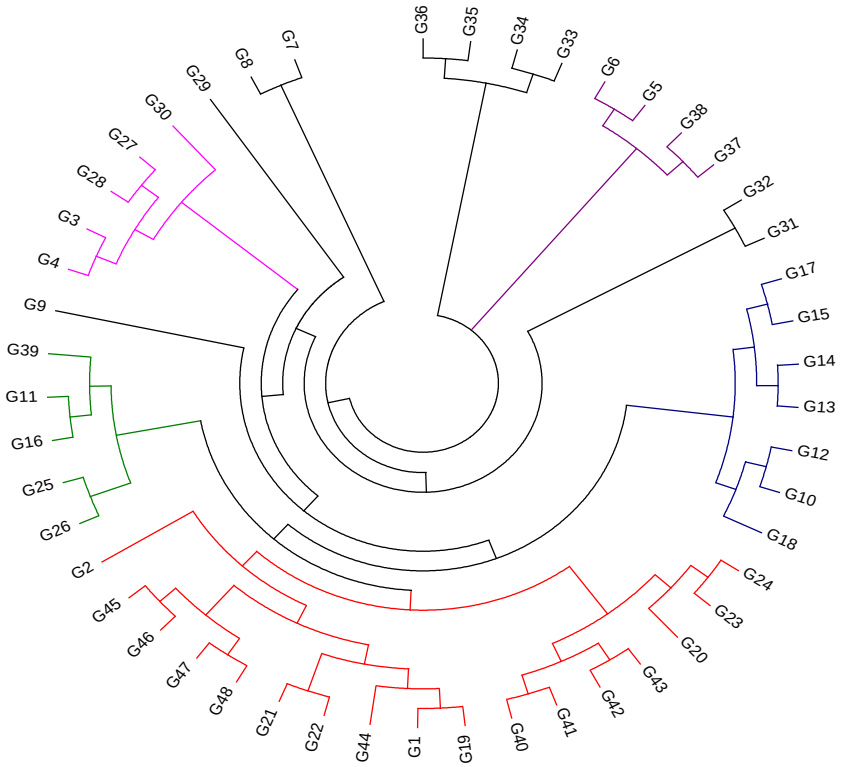

FIGURE 4. Phylogenetic tree analysis of gene based SSR marker data analysis using power marker v3.25 software

TABLE 5: Tabular representation of major and sub clusters formed based on gene based SSR markers

Major clusters	Sub clusters	No. of germplasm lines and varieties	Name of germplasm lines and varieties	Remark
C-I,		4	CS-54, GM-2, DRMR-150-35 and DRMRIJ-31	Salinity tolerant (CS54), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew &*A. blight (*DRMR-150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-II		4	MRNJ-6, MRNJ-5, RH-749 and RB-50	Suitable for irrigated condition (RH749), Suitable for rainfed and salinity condition (RB50)
C-III	C-III (a)	38	NRCDR-2, Maya, MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, PM-21, PM-25, L-6, L-4, MRNJ-19, MRNJ-1, PM-22, MRNJ-22, MRNJ-21, LES-39, PM-30, PM-29, PM-24 MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, JD-6, MRNJ-9, MRNJ-4, MRNJ-3, RVM-1, JM-2, Rohini and RVM-2	Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for irrigated condition and white rust resistance (Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed condition and white rust resistance (JM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Early maturity and high oil content (RVM1), Suitable for rainfed and late sown conditions (RVM2)
	C-III (b)	2	MRNJ-8 and MRNJ-7	

In the present experimentation, gene specific markers were not able to differentiate all mustard genotypes according to their erucic acid content. Contrary, Sarikamis *et al*. (2010) have got successful amplifications with genomic DNA from kale genotypes with the SSR marker Ol12FO2. It was determined that Ol12FO2 was polymorphic among kale genotypes. It was successful amplification of markers within different vegetable *Brassica*s. Further, Chandra *et al*. (2013) estimated 37 diverse Indian mustard genotypes using 10 A and C genome specific SSR markers for the development of molecular profile. Singh *et al*. (2017) used 453 SSRs on mustard genotypes to identify white rust resistance genotypes. The cluster analysis gave three major groups where white rust resistant genotypes were grouped in cluster 1, low quality genotypes in cluster 2 while the recipients were grouped in the cluster 3 indicating that grouping of genotypes based on SSRs corresponded. Similarly, Bharti *et al*. (2018) used four SSR markers to characterize mustard genotypes for erucic acid and glucosinolate contents. All the genotypes were clustered in three groups. Cluster I had seven genotypes followed by cluster II five and cluster III two genotypes. Recently, Kapadia *et al*. (2019) screened molecular markers for powdery mildew resistance in Indian mustard. The molecular analysis for powdery mildew resistance in *Brassica spp.* was carried out with four females, three males, F_{1S} and F_{2S} generation. The SSR markers *viz,* OI10-B12 and OI10-C01 clearly distinguished between susceptible and resistant bulks of interspecific cross GM-3 x Pusa Swarnim. Monika *et al*. (2019) screened out 200 SSR markers for polymorphism in two parental *Brassica juncea* genotypes *i.e*., RB 50, drought tolerant and Kranti, drought susceptible. Markers were able to differentiate drought tolerant and susceptible genotypes with low similarity coefficient. Bhatia and Alok (2014), Gupta *et al*. (2004) and Yan *et al*.(2015) documented that erucic acid trait is governed by two independent genes FAE1.1 and FAE1.2 in allotetraploids *B. napus and B. juncea*. Several earlier studies including Pandey *et al*. (2013), Singh *et al*. (2015), Cao *et al*. (2010) and Lühs *et al*. (1999) have also reported the presence of continuous variation in erucic acid

content in segregating populations of *B. juncea* and *B. napus*, respectively. Related to our study, Saini *et al*. (2016) have also reported that two SNPs in *FAE1.1* at position 591 and 1265 and one in *FAE1.2* at 237 were found polymorphic among low and high erucic acid containing genotypes.

Gene based CAPS markers for screening of erucic acid contents of Indian mustard

Two gene based CAPS markers for erucic acid were used to screen mustard germplasm lines. Maximum major allele frequency was found 0.50 for CAPS237 and minimum 0.31 for CAPS1265 with an average of 0.41. A total of 9 alleles were identified with an average of 4.5 alleles per locus for polymorphic SSR markers. Maximum gene diversity was consisted 0.74 of CAPS1265 and minimum was 0.66 of CAPS237 with an average of 0.70. Highest polymorphic information content was found 0.69 of CAPS1265 and lowest 0.61 of CAPS237 with mean value of 0.65. The primer which showed highest gene diversity (0.74) and PIC value (0.69) was observed in CAPS1265, which is representing 74% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.61) was observed for the primer CAPS237, indicating 61% polymorphic nature of the marker.

Based on dendrogram, genetic relationships among Indian mustard genotypes were documented. Major three clusters *viz.*, C-I, C-II and C-III were formed. Cluster I had 2 genotypes. Cluster II contained 42 genotypes. While C-III consisted of 4 genotypes (Fig. 5, Table 6).

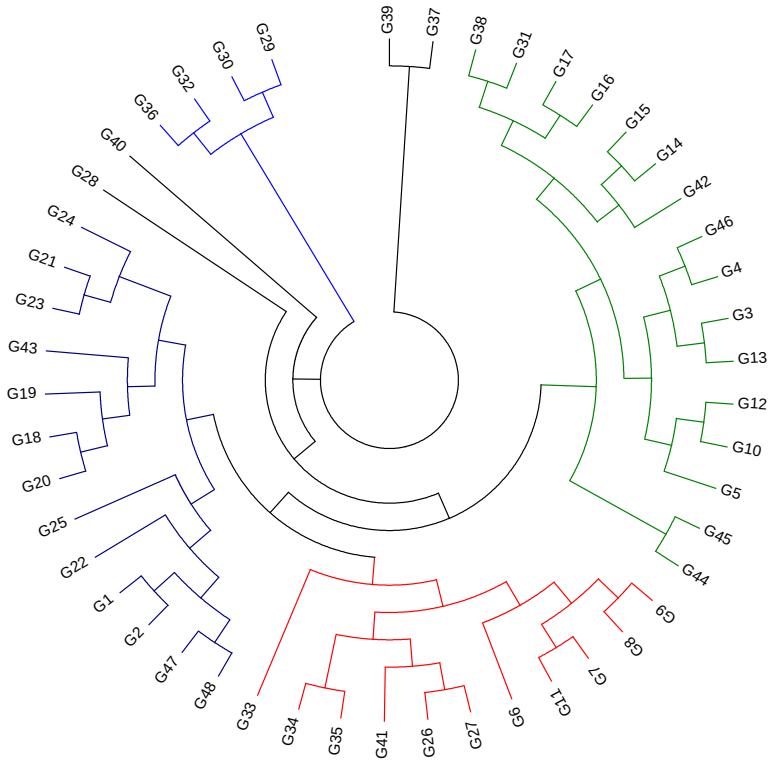

FIGURE 5. Dendrogram of gene based CAPS marker data analysis using power marker v3.25 software

The most interesting group was formed in cluster II (a), including 41 indigenous and exotic genotypes including RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1. RH749, Maya, PM25, PM29, PM24, PM22, JM-2, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30 and RVM-1 that are released varieties suitable for irrigated and rainfed condition, white rust resistance, high temperature tolerant, suitable for early sown, low erucic acid, timely sown, non-traditional areas, bold seeded, high oil content and high yielding, having early maturity and tolerant to powdery mildew and *A. blight*, so that these germplasm lines falling along with these varieties may be contained these characteristics features as well.

On the basis of the results of the present study, it can be concluded that SSR markers are the better tools to discriminate *B. juncea* genotypes based genetic variability present at molecular level. Information obtained on genetic variation based on microsatellite markers can be used to select diverse genotypes for future breeding purposes to minimize erucic acid content in Indian mustard.

TABLE 6: Tabular representation of major and sub clusters formed based on CAPS markers

Major clusters	Sub clusters	No. of germplasm and varieties	Name of germplasm and varieties	Remarks
C-I,		2	JD6 and RB50	Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed and salinity condition (RB50)
C-II	C-II (a)	41	RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1	Suitable for irrigated condition (RH749), Suitable for irrigated condition and white rust resistance (Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Suitable for rainfed condition and white rust resistance (JM2), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew & *A. blight* (DRMR150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31), Early maturity and high oil content (RVM1)
	C-II (b)	1	L-4	
C-III		4	RVM-2, Rohini, NRCDR-2 and CS-54	Suitable for rainfed and late sown conditions (RVM2) High oil content, drought tolerant and suitable for irrigated condition (Rohini), Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Salinity tolerant (CS54)

REFERENCES

Bharti, R., Chaudhary, N., Sandhu, R., Gupta, S.K. and Sharma, M. (2018) Molecular characterization of candidate gene in glucosinolate and erucic acid using ssr markers. *International Journal of Advance Research, Ideas and Innovations in Technology*. 4(1): 400-409.

Bhatia, V. and Alok, A. (2014) Molecular marker analysis of the linkage drag around the FAE1 loci of *Brassica juncea* during conventional backcross breeding. *Journal of Crop Science and Biotechnology*. 17: 147–154.

Cao, Z., Tian, F., Wang, N., Jiang, C., Lin, B., Xia, W., Shi, J., Long, Y., Zhang, C. and Meng, J. (2010) Analysis of QTLs for erucic acid and oil content in seeds on A8 chromosome and the linkage drag between the alleles for the two traits in *Brassica napus*. *Journal of Genetics and Genomics*. 37: 231–240.

Chandra, V., Pant, U., Bhajan, R. and Singh, A.K. (2013) Studies on genetic diversity among *Alternaria blight* tolerant Indian mustard genotypes using SSR markers. *The Bioscan*. 8(4): 1431-1435.

Farzad, J. and Cheng, B. (2013) Construction of a genetic linkage map and QTL analysis of erucic acid content and glucosinolate components in yellow mustard (*Sinapis alba* L.). *BMC Plant Biology*. 13(142): 1-9.

Fayyaz, L., Farhatullah, Rabbani, M.A., Iqbal, S., Kanwal, M. and Nawaz, I. (2014) Genetic diversity analysis of *Brassica napus/brassica campestris* progenies using microsatellite markers. *Pakistan Journal Botany*, 46(3): 779-787.

Ghosh, S., Mazumder, M., Mondal, B., Mukherjee, A., De, A., Bose, R., Das, S. Bhattacharyya, S., Basu, D. (2019) Morphological and SSR marker-based genetic diversity

analysis of Indian mustard (*Brassica juncea* L.) differing in *Alternaria brassicicola* tolerance. *Euphytica*. 215:206, https:/ /doi.org/10.1007/s10681-019-2523-1(0123456789().,-volV()0123456789().,-volV)

Gupta, V.A., Mukhopadhyay, N., Arumugam, Y.S., Sodhi, D., Pental, A. and Pradhan, A.K. (2004) Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and single nucleotide polymorphisms in *FAE1* gene. *Theoretical and Applied Genetics.* 108: 743–749.

Habib, M.A., Sharma, M., Punya, Salgotra, R.K. and Kiran, U. (2019) Analysis of allelic differentiation and prediction of suitable parents among *Brassica juncea* L. genotypes using Microsatellite Markers. *International Journal of Current Microbiology and Applied Sciences.* 8(6): 3071-3081.

Hopkins, C., Mogg, R., Gororo, N., Salisbury, P., Burton, W., Love, C., Spangenberg, G., Edwards, D. & Batley, J. (2006) An assessment of genetic diversity within and between *Brassica napus* and *Brassica juncea* lines from International Germplasm Collections. Proc. Joint Meeting 14th Crucifer Genetics Workshop 115 & 4th ISHS Symposium on Brassicas. *Acta Horticulture*, *706*, 115-119.

Kapadia, V.N., Sasidharan, N., Patel, D.A. and Rao, K. (2019) Identification of the SSR marker linked to powdery mildew resistant gene(s) in *Brassica* spp. *Journal of Pharmacognosy and Phytochemistry*. 8(3): 388-395.

Lühs, W.W., Voss, A., Seyis, F. and Friedt, W. (1999) Molecular genetics of erucic acid content in the genus Brassica. *In: Proceding 10th International Rapeseed Congress*. 26–29.

Monika, Yadav, R.C., Yadav, N.R., Summy, Avtar, R. and Singh, D. (2019) Genetic diversity analysis for drought tolerance in Indian mustard (*B. juncea* L. Czern & Coss) using Microsatellite Markers. *International Journal of Current Microbiology and Applied Sciences*. 8(1): 2564-2574.

Murray, M.G. and Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*. *8*: 4321-4326.

Pandey, S., Kabdal, M. and. Tripathi, M.K (2013) Study of inheritance of erucic acid in Indian mustard (*Brassica juncea* L. Czern & Coss). *Octa Journal of Biosciences.* 1: 77–84.

Plieske, J. and Struss, D. (2001) Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in Brassicaceae species. *Theoretical and Applied Genetics*, *102*, 689-694.

Pushpa, H.D., Yadava, D.K., Singh, N., Vasudev, S., Saini, N., Muthusamy, V. and Prabhu, K.V. (2016) Validation of molecular markers linked to low glucosinolate QTLs for marker assisted selection in Indian mustard (*Brassica juncea* L. Czern & Coss). *Indian Journal of Genet*ics. 76(1): 64-68.

Rakow, G. & Woods, D.L. (1987) Out crossing in rape and mustard under Saskatchewan prairies conditions. *Canadian Journal of Plant Science*. 67: 147- 151.

Saini, N., Singh, N., Kumar, A., Vihan, N., Yadav, S., Vasudev, S. and Yadava, D.K. (2016) Development and validation of functional CAPS markers for the FAE genes in *Brassica juncea* and their use in marker-assisted selection. *Breeding Science*. 66: 831–837.

Sarikamis, G., Yanmaz, R., Balkaya, A. and Belge, B. (2010) Evaluation of an SSR marker for marker-assisted selection in kale (*Brassica oleracea* var. *acephala*). *Bulgarian Journal of Agricultural Science*, 16(1): 36-41.

Singh, B.K., Nandan, D., Ambawat, S., Ram, B., Kumar, A., Singh, T., Meena, H.S., Kumar, V., Singh, V.V., Rai, P.K. and Singh, D. (2015) Validation of molecular markers for marker-assisted pyramiding of white rust resistance loci in Indian mustard (*Brassica juncea* L.). *Canadian Journal of Plant Science*. 95: 939-945.

Singh, S., Singh, V.V., Ambawat, S., Dubey, M. and Singh, D. (2017) Screening and estimation of allelic differentiation in Indian mustard using SSR markers for background selection. *International Journal of Current Microbiology and Applied Sciences. 6*(9): 2506-2516.

Shyam, C. and Tripathi, M.K. (2019) Biochemical studies in Indian mustard (*Brassica juncea* L.) czern and coss for fatty acid profiling *International Journal of Chemical Studies* 7 (4): 338-343.

Vinu V., Singh, N., Vasudev, S., Yadava, D.K., Kumar, S., Naresh, S., Bhat, S.R. & Prabhu, K.V. (2013) Assessment of genetic diversity in *Brassica juncea* (*Brassicaceae*) genotypes using phenotypic differences and SSR markers. *International Journal of Tropical Biology*. 61(4): 1919-1934.

Yan, G.D., Li, M., Cai, G., Gao, B., Chen, K., Xu, J., Li, F., Li, N., Wang, J. (2015) Characterization of *FAE1* in the zero erucic acid germplasm of *Brassica rapa* L. *Breeding Science*. 65: 257–264.