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Molecular Characterization of Candidate Gene in Glucosinolate and Erucic Acid Using SSR Markers

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ABSTRACT

Indian mustard is the second most important oilseed crops of India, next to groundnut sharing 27.8% in India's oilseed economy. The improved mustard seeds contain 39-44% oil. Oil quality is determined by fatty acid profile, whereas, level of erucic acid predicts the quality of seed oil. Breeding of oilseed has evoked a strong bottleneck selection towards double-low (00) seed quality with zero euroic acid and low seed Glucosinolate content. DNA based molecular markers are important tools in breeding programmes for crop improvement. The main role of these makers to detect the polymorphism. The experimental material comprised 71 genotypes including parents, half diallel crosses and simplified triple test crosses for study. In the molecular characterization of erucic acid and glucosinolate using 4 SSR markers was clustered into three groups. Molecular analysis was done using four markers and a total of 56 amplified bands were obtained, out of which 17 were polymorphic. All the genotypes were clustered in three groups by using DARwin software. Cluster I had seven genotypes *viz.*, RSPR-01, PM-21, PM-22, RB-50, Urvashi, Nov. Gold and Pusa-Bold followed by cluster II (five genotypes *viz.*, RSPR-03, Varuna, Pusa-Karishma, RL-1359 and Kranti and cluster III (two genotypes *viz.*, PM-24 and NRCDR-2).

INTRODUCTION

Rapeseed and mustard crops belong to genus *Brassica* and family cruciferae. *Brassica* is the economically most important genus consisting of oilseeds, vegetables and forage crops. Therefore, these are grown for vegetable, oil, fodder, condiment and green manure purposes. *Brassica juncea* commonly known as Indian mustard, is the second most important oilseed crops of India, next to groundnut sharing 27.8% in India's oilseed economy.

Now-a-days molecular markers are used efficiently for exact estimation of genetic diversity and determination of uniqueness of crop genotype (Kondic-Spika *et al.*, 2008 and Nyende 2008) and enhance traditional breeding programs to improve crops (Frey *et al.*, 2004 and Smith and Hlntjaris, 1996). A number of molecular markers have been developed to assess genetic diversity and discriminate between genotypes of different crops. The study of genetic variation, genetic mapping and marker assisted breeding have previously utilized many molecular markers such as RFLP (Restriction Fragment Length Polymorphism) (Pradhan 2003), RAPD (Random Amplified Polymorphic DNA) (Divaret, 1999), AFLP (Amplified Fragment Length Polymorphism (AFLP) (Lombard *et al.*, 2002) and SSR (Simple Sequence Repeats) (Plieske, 2001).

The development and implementation of molecular marker technology have given rise to a greater understanding of genome complexity and provide vital tools for mapping genes of agronomic importance with the goal of implementing marker-assisted breeding of elite crop cultivars. The plummeting cost of genome sequencing combined with significant advances in molecular biology and the rapid rise of novel bioinformatics approaches have made marker technology an invaluable and easily accessible tool in genome analyses (Pradhan 2003).

Breeding of oilseed has evoked a strong bottleneck selection towards double-low (00) seed quality with zero euroic acid and low seed glucosinolate content. In *B. juncea*, L. genotypes containing high levels of erucic acid and seed glucosinolates (++ quality) represent a comparatively genetically divergent source of germplasm. Glucosinolate content is a complex quantitative trait which means the introgression of novel germplasm from this gene pool requires recurrent backcrossing to avoid linkage drag for high glucosinolate content. Molecular markers for key low-glucosinolate alleles could potentially improve the selection process.

There are various studies related to the development of molecular marker, heterosis, and a marker associated with the important physiological characters of *Brassica juncea*, L. Becker *et al.*, (1995) compared the genetic diversity in rapeseed cultivars with resynthesized lines using allozyme and RFLP markers. Ecke *et al.*, (1995) studied that QTLs for oil content showed a close association in location to the two erucic acid genes, indicating a direct effect of the erucic acid genes on oil content in *Brassica napus*, L.

MATERIAL AND METHODS

The present study was carried out at the research farm and molecular laboratory of the Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu, F.O.A Chatha, during Rabi season 2013-2016. Two sets of experimental designs were generated in the present investigation in which seven genotypes (RSPR-01, RSPR-03, PM-21, PM-22, PM-24, Varuna and Pusa Karishma) were sown during the Rabi 2013-2014 and crossed in a diallel mating design for generating experimental material for half diallel while all fourteen genotypes (RSPR-01, RSPR-03, PM-21, PM-22, PM-24, RB-50, Urvashi, Nov-Gold, NRCDR-2, Kranti, RL-1359, Pusa-Bold, Varuna and Pusa Karishma) of Indian mustard were sown for raising triple test cross combinations during Rabi 2014-15. Of these, 12 entries were used as a female parent and two agronomically quality wise superior and genetically diverse varieties namely Varuna, Pusa-Kraishma and their F_1 as male testers (L_1, L_2, and L_3 respectively).

Molecular characterization of a candidate gene in glucosinolate and erucic acids was attempted using a set of 4 SSR primer pairs, every 2 primers for glucosinolate and erucic acids respectively (Table No. 1). Four Brassica SSR primer combinations selected from the collection available in the public domain (Lowe et al. 2002, 2004; see www.brassica.info/ssr/ SSRinfo.htm). Cetyltrimethyl ammonium bromide (CTAB) was commonly used for this purpose. Plant DNA was isolated using CTAB (CetylTrimethyl Ammonium Bromide) method as modified by Saghai-Maroof *et al.* (1984).

Table No. 1: SSR Markers selected for the Present Study

S. No.	Primer/ markers	Sequence (Forward/ reverse)	Primer sequence	Annealing temp (ºC)
1	sR7178	F	5′CTGATGAGGTGACGCAGTGT3′	48ºC
		R	3′GGCTTGAGTAAAGCGACCTG5′	
2	sS1702	F	5′CAGATGAGACAACACAGGAAACA3′	53ºC
		R	3′ACTCAATACGTTTTTCGCGG 5′	
3	Na10C01	F	5′TTTTGTCCCACTGGGTTTTC3′	53ºC
		R	3′GGAAACTAGGGTTTTCCCTTC5′	
4	Ra3E05	F	5′TTCTCATGCTCCAACCACAG3′	48ºC
		R	3′GTTTCTTCCAAGCCAAGCTG5′	

DNA was amplified using SSR primers in Polymerase chain reaction (PCR) machine following standard protocols given by Malone and Oliver (2011).

Table No. 2: Compositions of various PCR reaction mixture and PCR Cycling profile used in various PCR amplification reactions.

Reaction component	
Total reaction volume	10 µl
Template DNA (ng)	1 µl
Primer(M) fp	0.75 µl
Primer(M) rp	0.75 µl
dNTP(mM)	0.3 µl
Mgcl2(mM)	1 µl
Taq polymerase (U)	0.2 µl
Double distilled water up to	6 µl
PCR profile	
No. of cycles	35
Initial denaturation(°C)	94°C/4min
Denaturation(°C)	94°C/1min
Annealing(°C)	Tm°C/1min
Extension(°C)	72°C/1min
Final extension (°C)	72°C/5min
Store temperature(°C)	4°C

The SSR allele sizes were determined by the position of bands relative to the DNA ladder. PIC values for each primer were estimated using formula given by Powell *et al.* (1996) PIC= 2fi (1-fi); Where fi is the frequency of a j^{th} allele in i^{th} primer and summation extends over 'n' patterns. PIC is synonymous with the term 'gene diversity' as described by Weir (1990). A total number of alleles were recorded for each microsatellite marker in all the genotypes under study by giving the number to amplified alleles as 1, 2 and so on. The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix. The amplicon size was made out of 100 bp ladder runned along with the PCR products on the gel. The genetic diversity among the germplasm lines was computed using Computer Software Programme – DARwin (Perrier *et al*., 2006). The genetic diversity among the germplasm lines was computed using Computer Software Programme – DARwin (Perrier *et al.*, 2006).

RESULTS

The present investigation was undertaken to standardize the DNA isolation methods and to optimize the PCR reaction conditions to study polymorphism among 71 genotypes (14 parents, 21 half diallel crosses and 36 TTC crosses) of Indian mustard using SSR markers. The polymorphic data was used me) molecular characterization of a candidate gene in glucosinolate and erucic acids among parents, and (ii) to find heterozygous among hybrids.

Polymorphism among fourteen parent genotypes of Indian mustard was detected using SSR primers for the present investigation (Plate No. 1-4). Using a total of four SSR primers, 56 amplified bands were obtained of which 17 were polymorphic. DNA amplification profile and polymorphism generated in fourteen parent genotypes of Indian mustard four SSR primers are presented in Table No. 3. The total number of amplified bands varied between 3 (primer Na10C01) and 5 (primers Ra3E05) with an average of 4 bands per primer. The polymorphism percentage ranged from as low as 21.43% (primer sR7178) to as high as 42.85% (primer Na10C01). Average polymorphism across all the fourteen parent genotypes was found to be 30.35% Table No.3.

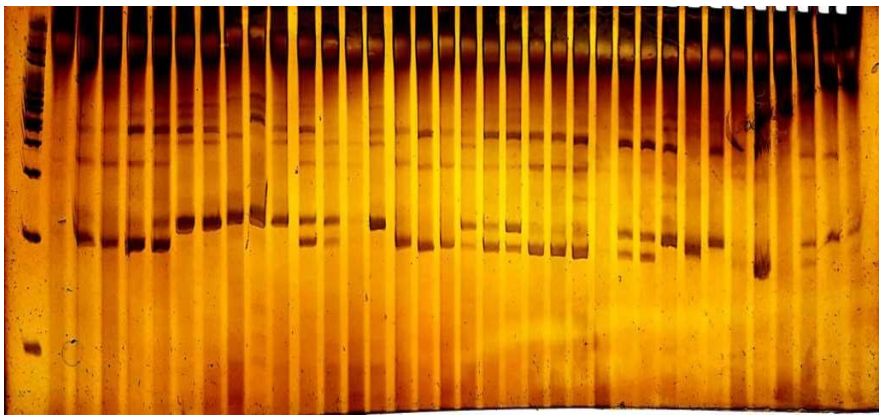

Plate 1: A Representative Sample of pcr Amplification Profile of 71 Genotypes obtained Using ssr primer sr7178 on Page

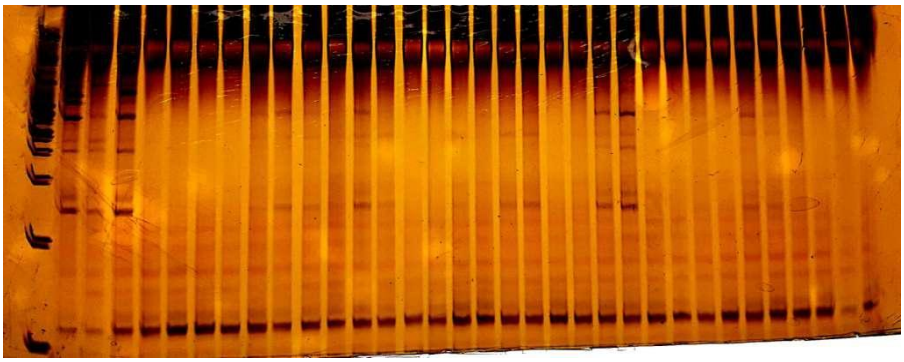

Plate 2: A Representative Sample of PCR Amplification Profile of 71 Genotypes obtained Using SSR Primer Na10C01 on PAGE

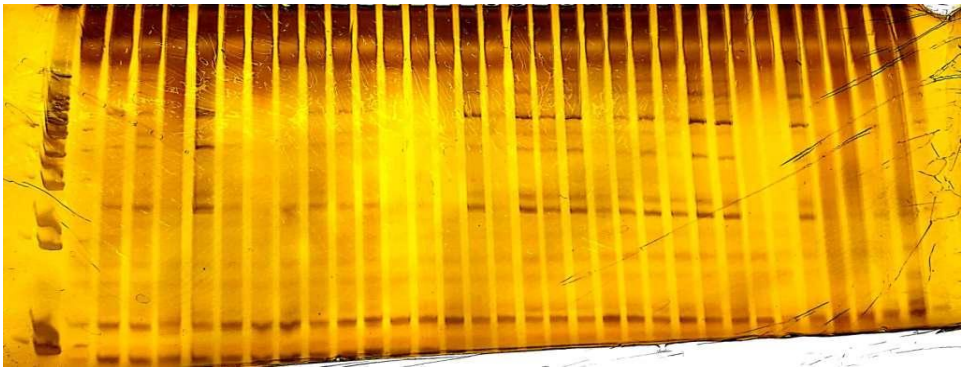

Plate 3: A Representative Sample of PCR Amplification Profile of 71 Genotypes Obtained Using SSR Primer Ra3E05 on PAGE

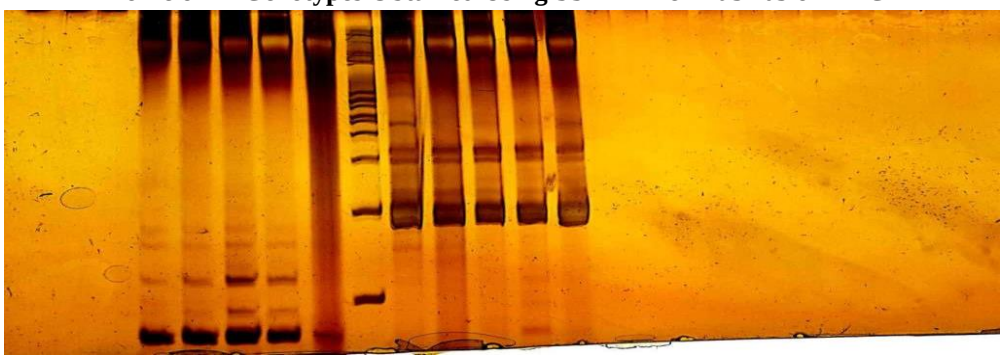

Plate 4: A Representative Sample of PCR Amplification Profile of 71 Genotypes obtained using SSR Primer Na10C01 and on Ra3E05 PAGE

NOTE: No. 1-14 indicates Parents viz., RSPR-01, RSPR-03, PM-21, PM-22, PM-24, RB-50, Urvashi, Nov-Gold, NRCDR-2, Kranti, RL-1359, Pusa-Bold, Varuna and Pusa- Karishma. No. 15-35 indicates half diallel crosses viz., RSPR-01 ×RSPR-03, RSPR-01 ×PM-21, RSPR-01 ×PM-22, RSPR-01 ×PM-24, RSPR-01 ×Varuna, RSPR-01 ×Pusa-Karishma, RSPR-03 ×PM-21, RSPR-03 ×PM-22, RSPR-03 ×PM-24, RSPR-03 ×Varuna, RSPR-03 ×Pusa-Karishma, PM-21 ×PM-22, PM-21 ×PM-24, PM-21 ×Varuna, PM-21 ×Pusa-Karishma, PM-22 ×PM-24, PM-22 ×Varuna, PM-22 ×Pusa-Karishma, PM-24 ×Varuna, PM-24 ×Pusa-Karishma and Varuna ×Pusa-Karishma. No. 36-71 indicates simplified TTC viz., RSPR-01 × Varuna, RSPR-01 × Pusa-Karishma, RSPR-01 × F_1, RSPR-03 × Varuna, RSPR-03 × Pusa-Karishma, RSPR-03 × F_1, PM-21 × Varuna, PM-21 × Pusa-Karishma, PM-21 × F_1, PM-22 × Varuna, PM-22 × Pusa-Karishma, PM-22 × F_1, PM-24 × Varuna, PM-24 × Pusa-Karishma, PM-24 × F_1, RB-50 × Varuna, RB-50 × Pusa-Karishma, RB-50 × F_1, Urvashi × Varuna, Urvashi × Pusa-Karishma, Urvashi × F_1, Nov. Gold × Varuna, Nov.Gold × Pusa-Karishma, Nov.Gold × F_1, NRCDR × Varuna, NRCDR × Pusa-Karishma, NRCDR × F_1, Kranti × Varuna, Kranti × Pusa-Karishma, Kranti × F_1, RL-1359 × Varuna, RL-1359 × Pusa-Karishma, RL-1359 × F_1, Pusa-Bold × Varuna, Pusa-Bold × Pusa-Karishma and Pusa-Bold × F_1.

Table No. 3: DNA amplification profile and polymorphism generated in fourteen parent genotypes of Indian mustard four SSR primers

S.NO	Primer	Base pair (bp) size	No of allele/locus	No. of polymorphic bands	Percentage polymorphism
1	sR7178	190	4	3	21.43
2	sS1702	280	4	4	28.57
3	Na10C01	130	3	6	42.85
4	Ra3E05	190	5	4	28.57

The PIC values provide an estimate of the prejudiced power of a locus by taking into account the number of alleles that are expressed as well as the relative frequencies of the alleles in question. PIC values ranged from 0.34 to 0.49 with an average of 0.41. The highest PIC value was recorded by primer Na10C01 followed by primer sS1702 and primer Ra3E05 (Table 4).

Table No. 4: Alleles Amplified and PIC Values of Primers

S.NO	Primer	No of allele/locus	PIC = 2fi(1-fi)
1	sR7178	4	0.34
2	sS1702	4	0.40
3	Na10C01	3	0.49
4	Ra3E05	5	0.40

Clustering was done using using DARwin 5.0 (Perrier *et al* 2006). The 14 *Brassica juncea* entries were broadly divided into three clusters (Fig. 1). The individuals within any one cluster are more closely related than individuals in different clusters. The cluster I consisting of seven genotypes was the largest among all and was closely followed by cluster II having five genotypes. While cluster III was the smallest and consisting of two genotypes. The cluster I further divided into two sub-clusters consisting five and two genotypes, respectively. The check variety 'Varuna' used in the present study fell in cluster II. The clustering pattern thus obtained in the study confirmed the discriminating power and reliability on the SSR markers for genetic diversity studies (Table No.5).

Table No. 5: Clustering Pattern Obtained by SSR Analysis

Cluster	No. of Genotypes	Genotypes
I	7	RSPR-01, PM-21, PM-22, RB-50, Nov-Gold, Pusa Bold, Urvashi
II	5	RSPR-03, Varuna, Pusa-Karishma, RL-1359 and Karnti
III	2	PM-24 and NRCDR-2

Testing of Heterozygocity

Heterozygous is a combination of the dominant and recessive allele. It is a hybrid of two different alleles. Scoring was obtained from PAGE gel. Whereas, 'A' denoted as a dominant parent, 'B' as a recessive parent and 'H' as heterozygous.

Percentage of Heterozygous in Half Diallel Design

Seven genotypes were used in half diallel design. From which twenty-one crosses were obtained. Percentage of heterozygous obtained in half diallel design using SSR primer presented in the following Table No.6. The percentage ranged from heterozygous from 19.04 to 33.33. Primer sR7178 and primer Ra3E05 showed highest heterozygous percentage while as primer sS1702 showed least heterozygous percentage among the primers. Seven crosses viz., RSPR-01×PM-21, RSPR-01 ×PM-22, RSPR-03 ×PM-21, RSPR-03 ×PM-22, RSPR-03 ×PM-24, RSPR-03 × Varuna and PM-24 × Pusa-Karishma exhibited heterozygous for primer sR7178. Primer sS1702 exhibited four heterozygous viz., RSPR-03 ×PM-22, RSPR-03 ×PM-24, PM-21 ×PM-24 and PM-22 ×Pusa-Karishma whereas, primer Na10C01 revealed six heterozygous viz., RSPR-01 ×PM-22, RSPR-01 ×PM-24, RSPR-01 ×Pusa-Karishma, RSPR-03 ×PM-24, PM-22 ×Varuna and PM-22 ×Pusa-Karishma. Seven crosses viz., RSPR-01×RSPR-03, RSPR-01 ×PM-21, RSPR-01 ×Varuna, RSPR-03 ×PM-21, RSPR-03 ×PM-22, RSPR-03 ×Varuna and PM-24 ×Pusa-Karishma exhibited heterozygous for primer Ra3E05.

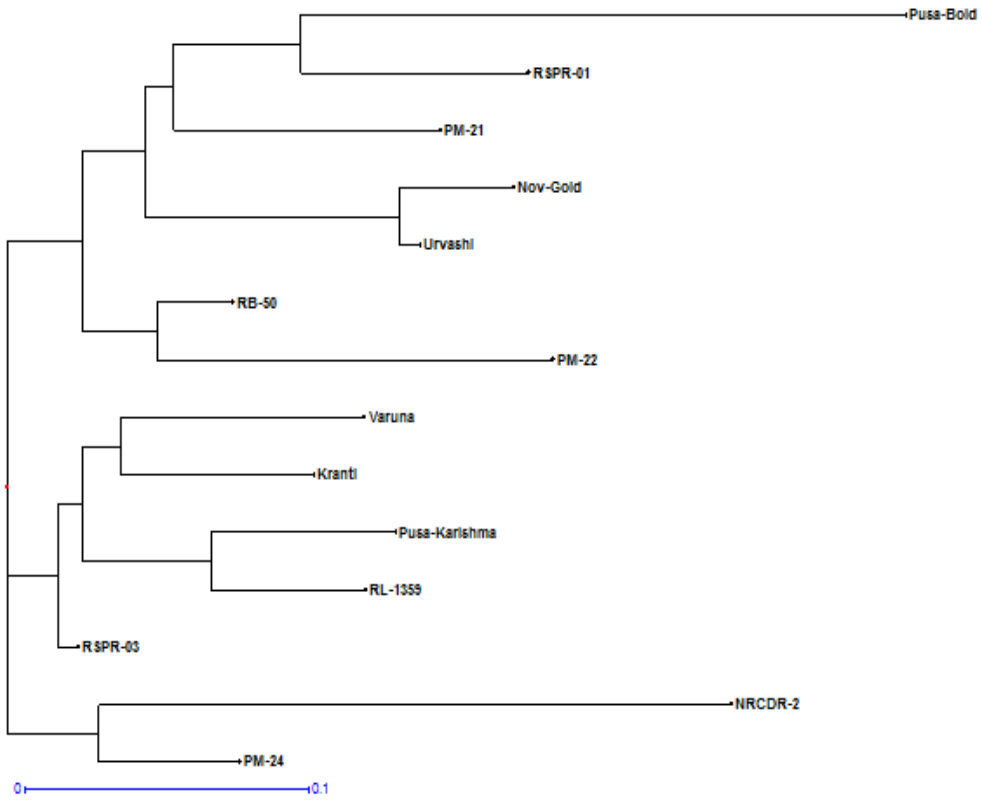

Fig. 1: Dendrogram Obtained by SSR Marker Analysis Using DARwin 5

Percentage of heterozygous in simplified TTC

In triple test cross, twelve genotypes were used as a female parent and two genetically diverse varieties and their F_1 as male testers (L_1, L_2, and L_3 respectively). Thirty- six crosses were obtained through TTC design. Percentage of heterozygous obtained in TTC design using SSR primer presented in the Table No. 7. The percentage ranged from heterozygous from 30.55 to 41.66. Primer Ra3E05 showed highest heterozygous percentage while as primer sS1702 showed least heterozygous percentage among the primers. Thirteen crosses viz., RSPR-03 × Pusa-Karishma, RSPR-03 × F1, PM-22 × F1, PM-24 × Varuna, Urvashi × Varuna, Urvashi × Pusa-Karishma, Urvashi × F1, Nov. Gold × Varuna, Nov-Gold × Pusa-Karishma, Nov-Gold × F1, NRCDR × Pusa-Karishma, NRCDR × F1 and Pusa-Bold × Varuna exhibited heterozygous for primer sR7178. Primer sS1702 exhibited eleven heterozygous viz., RSPR-03 × F1, PM-24 × Pusa-Karishma, PM-24 × F1, RB-50 × Varuna, Urvashi × Varuna, Urvashi × Pusa-Karishma, NRCDR × Varuna, NRCDR × Pusa-Karishma, NRCDR × F1, Kranti × Pusa-Karishma and Pusa-Bold × Pusa-Karishma whereas, primer Na10C01 revealed twelve heterozygous viz., RSPR-01 × Pusa-Karishma, RSPR-01 × F1, RSPR-03 × Varuna, RSPR-03 × F1, PM-24 × Pusa-Karishma, PM-24 × F1, RB-50 × F1, Nov. Gold × Varuna, NRCDR × Varuna, Kranti × Varuna, Kranti × Pusa-Karishma and Pusa-Bold × Varuna. Fifteen crosses viz., RSPR-01 × Pusa-Karishma, RSPR-03 × Pusa-Karishma, PM-21 × Varuna, PM-21 × Pusa-Karishma, Urvashi × F1, Nov. Gold × Varuna, Nov.Gold × Pusa-Karishma, Nov.Gold × F1, NRCDR × Varuna, NRCDR × Pusa-Karishma, NRCDR × F1, Kranti × Pusa-Karishma, RL-1359 × Varuna, RL-1359 × F1 and Pusa-Bold × Varuna exhibited heterozygous for primer Ra3E05.

Table No. 6: Find the Percentage of Heterozygous in Half Diallel Design Using SSR Primer

S.No	Primer	Total no. of crosses	No. of dominant parent	No. of recessive parent	Absence band	No. of heterozyous	%age of heterozyous	Hybrid cross
1	sR7178	21	8	5	1	7	33.33%	RSPR-01×PM-21,RSPR-01 ×PM-22, RSPR-03 ×PM-21, RSPR-03 ×PM-22, RSPR-03 ×PM-24, RSPR-03 ×Varuna and PM-24 ×Pusa-Karishma
2	sS1702	21	10	4	3	4	19.04%	RSPR-03 ×PM-22, RSPR-03 ×PM-24, PM-21 ×PM-24 and PM-22 ×Pusa-Karishma
3	Na10C01	21	7	8	0	6	28.57%	RSPR-01 ×PM-22, RSPR-01 ×PM-24, RSPR-01 ×Pusa-Karishma, RSPR-03 ×PM-24, PM-22 ×Varuna and PM-22 ×Pusa-Karishma
4	Ra3E05	21	8	5	1	7	33.33%	RSPR-01×RSPR-03, RSPR-01×PM-21, RSPR-01×Varuna, RSPR-03×PM-21, RSPR-03×PM-22, RSPR-03×Varuna and PM-24 ×Pusa-Karishma

Table No. 7: Find the Percentage of Heterozygous in TTC Design Using SSR Primer

S.No	**primer**	**Total no. of crosses**	**No. of dominant parent**	**No. of recessive parent**	**Absence band**	**No. of heterozyous**	**% age of heterozyous**	**Hybrid cross**
1	sR7178	36	8	10	5	13	36.11%	RSPR-03 × Pusa-Karishma, RSPR-03 × F1, PM-22 × F1, PM-24 × Varuna, Urvashi × Varuna, Urvashi × Pusa-Karishma, Urvashi × F1, Nov. Gold × Varuna, Nov.Gold × Pusa-Karishma, Nov.Gold × F1, NRCDR × Pusa-Karishma, NRCDR × F1 and Pusa-Bold × Varuna
2	sS1702	36	15	7	3	11	30.55%	RSPR-03 × F1, PM-24 × Pusa-Karishma, PM-24 × F1, RB-50 × Varuna, Urvashi × Varuna, Urvashi × Pusa-Karishma, NRCDR

								× Varuna, NRCDR × Pusa-Karishma, NRCDR × F1, Kranti × Pusa-Karishma and Pusa-Bold × Pusa-Karishma
3	Na10C01	36	10	13	1	12	33.33%	RSPR-01 × Pusa-Karishma, RSPR-01 × F1, RSPR-03 × Varuna, RSPR-03 × F1, PM-24 × Pusa-Karishma, PM-24 × F1, RB-50 × F1, Nov. Gold × Varuna, NRCDR × Varuna, Kranti × Varuna, Kranti × Pusa-Karishma and Pusa-Bold × Varuna
4	Ra3E05	36	13	8	0	15	41.66%	RSPR-01 × Pusa-Karishma, RSPR-03 × Pusa-Karishma, PM-21 × Varuna, PM-21 × Pusa-Karishma, Urvashi × F1, Nov. Gold × Varuna, Nov.Gold × Pusa-Karishma, Nov.Gold × F1, NRCDR × Varuna, NRCDR × Pusa-Karishma, NRCDR × F1, Kranti × Pusa-Karishma, RL-1359 × Varuna, RL-1359 × F1 and Pusa-Bold × Varuna

DISCUSSION

DNA based molecular markers are important tools in breeding programmes for crop improvement. The main role of these makers to detected the polymorphism which can be used for qualitative or quantitative traits loci, diversity, pedigree analysis, assess taxonomic and phylogenetic relationships, linkage mapping, etc. SSR is polymerase chain reaction (PCR) based markers help us in the detection of polymorphisms at the molecular level from many individuals or pooled samples at a very fast rate. Good quality DNA free from contaminants and optimization of PCR reaction conditions are certain prerequisites for this technique, which otherwise could result in erratic amplification.

The results of this investigation demonstrated the suitability of SSR data for analysis of genetic diversity in *Brassica juncea* genotypes. The term "canola" was defined as cultivars with the trait of low erucic acid and low glucosinolate content in rapeseed (therefore also termed as "double low" cultivars). The source of low glucosinolate content in seeds was originally from a Polish forage rape cultivar, Bronowski (Krzymanski, 1970). The selection of simple sequence repeat SSR primers was done because they are a polymorphic and suitable marker for the plant genome analysis. Highly polymorphism and abundance make the micro satellites a perfect marker for genetic studies in crop plants, including marker assisted selection genetic mapping and population analysis. The application of primers derived from one species of genus *Brassica spp.* varies in other species as the SSR primers mostly are species specific. Their specificity hinders their applicability between closely related species for comparative studies. Furthermore, these SSRs mostly present in gene-rich genomic regions, which increased their relevance for allele-trait relevance.

The PIC of each *Brassica* primer set was also determined. These values were in the ranged from 0.34 to 0.49 with an average of 0.41. Our findings were further supported by the research work of Yuan and Chao (2007) who detected a total of 21 alleles after using 5 SSR primers. The number of alleles ranged from 2 to 5. The results were also in consistence with Tonguc and Griffith (2004) who detected 1 to 8 alleles. In their study, the PIC value was in the ranged 109 of 0.25-0.86 for thirteen SSR primers. Louarn et al, (2007) observed PIC value of 0.5 or above in 11 SSR primer.

The use microsatellites further strengthened our findings where they tested twenty five microsatellite-specific primer pairs on seventy five *Brassica* species and detected 2 to 7 alleles per microsatellite locus reported by Ofori *et a.l,* (2008).Hasan *et al.,* 2006 describes the molecular genetic characterization of a *B. napus* core collection and the use of the data to describe the molecular genetic diversity.

The molecular diversity analysis was carried out using 4 SSR markers in fourteen parent genotypes. The DARwin 5.0 software was used to generate the dendrogram. All the genotypes were clustered into three clusters of different sizes. Among these, cluster I had maximum genotypes 7 on the other hand one cluster had only two parent genotypes, namely, PM-24 and NRCDR-2. Therefore, these varieties may be used for the development of suitable mapping population for the identification and mapping of quantitative trait loci (QTL) for erucic acid and glucosinolate.

Percentage of heterozygous in half diallel and simplified TTC ranged from 19.04% to 33.33%.and 30.55% to 41.66% respectively. This indicated that heterozygous occurs with 30% average. In mating design, less than 50% crosses were obtained heterozygous which contains both the alleles (dominant and recessive alleles). Correlated the SSR markers and heterosis for the quality traits in *Brassica juncea* and selected the heterozygous crosses for further breeding programmes.

CONCLUSION

The molecular diversity analysis was carried out using 4 SSR markers for erucic acid and glucosinolate. All the fourteen genotypes were clustered into three groups of different sizes by using DARwin 5.0 software. Among these, cluster I had maximum genotypes 7 while cluster III had minimum genotypes viz., PM-24 and NRCDR-2. The varieties may be used for mapping of quantitative trait loci (QTL) for erucic acid and glucosinolate.

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