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VALIDATION OF MOLECULAR MARKERS LINKED TO QUALITY TRAITS IN INDIAN MUSTARD (*Brassica juncea* L)

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Glucosinolate content

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ABSTRACT

Present study consisted of parents and their generations viz; P1 (NRCHB-101, DRMR 150-35), P2 (PDZ-1, Heera and RLC-3), F_1 s (NRCHB-101xPDZ-1, NRCHB-101xHeera, NRCHB-101xRLC-3, DRMR-150-35xPDZ-1, DRMR-150-35xHeera and DRMR150-35xRLC-3), and their back crosses (BC_1F_1 and BC_2F_1) with a view to validate molecular markers linked to quality traits (low glucosinolate and erucic acid) in Indian mustard. The phenotyping of parents and backcross progenies was performed for estimation of total seed glucosinolate content and erucic acid content using UV-Vis spectrophotometer and Gas chromatography respectively. Parents were also validated for low erucic acid trait using set of two CAPS markers for *FAE1.1* gene and all parental genotypes under study generated fragments of expected size for each CAPS maker. The CAPS markers for *FAE1.1* gene amplified 432 bp and 427 bp fragments for E1 marker and E2 marker respectively. Markers linked to low glucosinolate showed polymorphism among donor and recipient parents. The genotyping among backcross progenies revealed heterozygous banding pattern with markers linked to low glucosinolate and erucic acid traits. It was concluded that total glucosinolate content of most of the samples of backcross generations were in the range of 50-85 $\mu\text{mol/g}$ defatted seed meal whereas erucic acid content ranged 10.75% to 33.18%. Validation of these molecular markers linked to quality traits can be used efficiently for MAS by mustard breeders for improvement of oil quality in breeding programme.

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1 Introduction

Indian mustard (*Brassica juncea* (L.) Czern and Coss) is one of the most important oilseed among rapeseed-mustard group of crops. It is cultivated along with four other closely related oilseed species viz. *B. rapa*, *B. napus*, *B. carinata* and *Eruca sativa*. Over the past couple of decades, these crops have become one of the most important sources of vegetable oil in the world. Brassicas oilseed share second (25%) after soybean (38%) in total oilseeds production, however, it stands first (23%) in edible oil production in the country (Jat et al., 2019). Continuous improvement in rapeseed-mustard has resulted in nutritionally superior edible oil and meal as an important source of protein in animal feed (Jesch & Carr, 2017). Mustard oil is an important dietary component and its quality is defined by improved fatty acid profile of oil with low erucic acid content (Pham & Pham, 2012). Quality of its seed meal is determined by low glucosinolate content and a large group of secondary plant metabolites derived from different amino acids such as methionine, phenylalanine and tryptophan (Priyamedha et al., 2015). The oil extracted from Indian mustard has lowest concentration (approx. 7%) of saturated fatty acids with higher proportion of unsaturated fatty acids viz. oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid. Of the total fatty acids, there is predominance of erucic acid (C22:1) fraction (35.7-51.4%) along with higher glucosinolate content in seed meal in traditional varieties of Indian mustard (Chauhan et al., 2007). Consumption of higher amount of mustard oil having high erucic acid (>20%), causes myocardial fibrosis, impaired myocardial conductance, lipidosis etc. and also increases blood cholesterol (Ackman et al., 1977; Chien et al., 1983). Therefore, reduction in erucic acid is one of the important objectives in quality amelioration of Indian mustard seed oil. It is desirable to increase oleic acid content in oil as it is less vulnerable to oxidations and also it is very much effective in reducing total cholesterol level of blood (Mattson & Grundy, 1985; Liu et al., 2003).

Efforts are underway towards development of low erucic and/or low glucosinolate and high yielding varieties through deployment of genes responsible for quality traits into high yielding varieties. The most convenient and authentic way for pyramiding genes responsible for low erucic and low glucosinolate is through marker assisted breeding (MAS). MAS is the selection for a trait based on genotype using associated markers rather than the phenotype of the trait (Foolad & Sharma, 2005). MAS is highly beneficial in achieving the same breeding progress in a much shorter time than through conventional breeding as well as pyramiding combinations of genes that could not be readily combined through other means (Xu & Crouch, 2008).

Attempts have been made for identification of molecular markers linked to the traits like low erucic and low glucosinolate by various scientists (Gupta et al., 2004; Ramchiary et al., 2007; Hasan et al.,

2008; Bisht et al., 2009). Since then various studies have been conducted to validate these markers in Indian mustard. The present study was undertaken to employ the quality trait linked markers in pyramiding of the genes responsible for low erucic and low glucosinolate content in different high yielding varieties through marker assisted backcross breeding approach involving different single/ double low lines/varieties.

2 Materials and Methods

2.1 Genetic material and growth conditions

The field studies were carried out during *rabi* cropping seasons of 2015 to 2017 (October to March) for consecutive three years at ICAR-Directorate of Rapeseed Mustard Research Bharatpur, India (27°11'N Latitude and 77°27'E Longitude and 180 m above sea level).

The materials for the present investigation consisted of parents and their generations viz; P1 (NRCHB 101, DRMR 150-35), P2 (PDZ 1, Heera and RLC-3), F₁s (NRCHB101xPDZ-1, NRCHB101xHeera, NRCHB101xRLC-3, DRMR150-35xPDZ-1, DRMR 150-35xHeera and DRMR150-35xRLC-3), and their back crosses (BC₁F₁ and BC₂F₁). NRCHB101 and DRMR150-35 are high yielding varieties with high glucosinolate and high erucic acid content were taken as recipient parents whereas PDZ-1, Heera and RLC-3 are varieties/genotypes with low glucosinolate and low erucic acid were taken as donor parents.

Backcross progenies i.e. BC₁F₁, BC₂F₁ along with their parents (NRCHB101, DRMR150-35, PDZ-1, RLC-3 and Heera) were sown during *rabi* 2016-17 and 2017-18 respectively. The seed were sown in plot consisting 5 rows of length 5m each. The rows were spaced 30 cm apart with plant to plant spacing of 10 cm. For raising a healthy crop recommended agronomic practices were applied.

2.2 Development of backcross populations

Two high glucosinolate and erucic acid varieties of *B. juncea* (NRCHB101 and DRMR150-35) were used as female parent and crossed with low glucosinolate and erucic acid donors (PDZ-1, RLC-3 and Heera). During Rabi season 2015-16, emasculation of selected buds was performed 24-48 hr before anthesis, and fresh pollen from the donor parent was applied to the stigmas. The flowers were then protected with paper bags and labelled. The resultant F₁ plants thus obtained were backcrossed with recurrent parent to produce BC₁F₁ generations at offseason nursery IARI-regional station, Wellington, Tamil Nadu during *Kharif* 2016. The BC₁F₁ generation plants were raised in *rabi* 2016-17 and were further backcrossed to recipient parent of each cross to produce BC₂F₁ generation. The BC₂F₁ generation plants were raised in *rabi* 2017-18 and were further backcrossed for generation advancement.

A negative selection based on visual observations was conducted among all the BC₁F₁ and BC₂F₁ plant generation in order to eliminate plants or lines resembling morphological characteristics with donor parents that are PDZ-1, RLC-3 and Heera. Each generation plants i.e. F₁, BC₁F₁, BC₂F₁ were validated using molecular markers linked to quality traits in their subsequent sowing seasons.

2.3 Molecular analysis

Genomic DNA was isolated from fresh, young and healthy leaves using the standard cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle., 1990). Purification of DNA was carried out by adding 10g/100ml of RNase to the sample vial containing crude DNA at a proportion of 3µl/100ml. DNA quantification was done by using agrose at a concentration of 0.8%. For amplification of parents and backcross progenies i.e. BC₁F₁ and BC₂F₁, a set of five low glucosinolate trait linked polymorphic markers i.e. (GER1 (Q1); Myb28 (Q2); At5g41 (Q3); At5GAJ67 (Q4) and GER-5 (Q5) (Bisht et al., 2009; Singh

et al., 2018) and a set of two low erucic acid trait linked CAPS markers that is E1 and E2 for gene *FAE1.1* (Gupta et al., 2004) (Table 1,2) were used. PCR assay was carried out in 96-well Fast Thermal Cycler (PE Applied Biosystems, USA) in a total volume of 10 µl reaction mixture. Each reaction mixture contained genomic DNA of 25 ng as template, 1.0 unit Taq DNA polymerase (Dream Taq), 20 ng each forward and reverse primer, 10X Dream Taq PCR assay buffer with 1.5 mM MgCl₂, 0.2 µl dNTPs mix and reaction volume was made up to 10 µl by adding nuclease free water. Amplification was carried out according to the following set up: The cycle was repeated 35 times after initial denaturation at 94°C for 5 min. Each cycle consists of cyclic denaturation at 94°C for 45 s, annealing temperature at 55-58°C for 50 s and the primer extension at 72°C for 1 min and final extension of 72°C for 7 min followed by incubation at 4°C. The PCR product bands resolution was done electrophoretically on 2.5 % agarose gel containing 0.01% ethidium bromide prepared in 1x TAE (Tris-Acetic acid-EDTA) buffer. 100bp DNA Ladder (Thermo Scientific) was used as standard to compare PCR amplicon band sizes. The gel was run for 2.5 h at 120V. After

Table 1 Details of the molecular markers and restriction enzymes used in validation of erucic acid

S.No.	Gene	Chromosome location of FAE 1.1	Molecular Marker code	Molecular marker name	Nucleotide sequence (5'→3')	T _m (°C)	Restriction enzyme	Size of band (bp)		
								Amplicon size (bp)	High E.A	Low E.A
1	<i>FAE1.1</i>	LG17 in <i>B.juncea</i> (Gupta et al.2004)	E1	FAE II F FAE II R	TCGTGGCTTGACTTCTTGAG GGACCTATTATCACCAGCGTAAA	55	<i>Hpy99I</i>	432	432 224 198	432
2	<i>FAE1.1</i>		E2	FAE III F FAE III R	ACGTTAGGTCCGTTGATTCTTC GGGTATCTGTCTGATGCAATGT	55	<i>BglII</i>	427	427 209 198	427

Table 2 Details of glucosinolate QTLs linked markers used for validation

Molecular Marker Code	Molecular Marker Name	Nucleotide sequence (5'→3')	QTL Name	Linkage group	Co-localized candidate gene	PCR Product	T _m (°C)
(Bisht et al.2009)							
Q1	GER-1MRPR IP3GER-1 F	5'CATGTCGACGACTTCTTCTCTAGTCA 5'GGTTTTCCCTTGGATTGAGTCT	<i>J2Gsl1</i>	A2	<i>BjuA.GSL-ELONG.a</i>	950(H)/650 (L) bp	55°C
Q2	Myb28R Myb28F	5'TATCCTCTTCATTGACAATCTGCTCAG 5'AAGGGGCATGGACCACCGA	<i>J17Gsl5</i>	A3	<i>BjuA.Myb28.a</i> <i>BjuA.GSL-ELONG.c,d</i>	1020(H)/1000 (L)bp	58°C
Q3	At5g41F At5g41R	5'GTTTCAGGGTGACTCTCCTCTTG 5'GCTTGTGCACCTTCATCGTC	<i>J9Gsl3</i>	A9	---	800(H)/770 (L)bp	58°C
Q4	At5GAJ67F At5GAJ67FR	5'CAGCGTAAGGAAGAAGAGAGAC 5'CCATCACTATGTCATTGCCA	<i>J9Gsl3</i>	A9	---	450(H)/- bp	58°C
Q5	GER-5FPF GER-5MRPR	5'GACATCATGGAAGTTGGTTTCCCTGC 5'GGGAGGTAACCTGTTTCTTCATCCA	<i>J3Gsl2</i>	A3	<i>BjuA.GSL-ALK.a</i>	350(H)/310(L) bp	55°C

electrophoresis, the gel picture was visualized using gel documentation system (IG/LHR, Syngene, UK).

2.4 Total seed glucosinolate extraction and quantification

Total glucosinolate was estimated in seeds of parents, BC₁F₁ and BC₂F₁ plants which were found low using molecular markers. Estimation of total glucosinolate content in defatted seed meal was carried out (Mawlong et al., 2017) as per standardized method. The absorbance was taken at 425 nm using a spectrophotometer (Labomed UV-VIS Double beam UVD-3500) after colour development due to complex formation between glucosinolates methanolic extract and sodium tetrachloropalladate solution. The absorbance so obtained was used in the predicted formula (Mawlong et al., 2017) to calculate total glucosinolate content;

$$\text{Total glucosinolate concentration } (\mu\text{mol/g}) = 1.40 + 118.86 \times A_{425}$$

Whereas A_{425} = absorbance at 425.

2.5 Estimation of erucic acid content in seed oil

Erucic acid estimation in seed oil of parents and backcross (BC₁F₁ and BC₂F₁) generations was carried out by conducting fatty acid profiling using Gas Liquid Chromatography (GLC) according to the FAME (fatty acid methyl esters) method developed by Goli et al. (2008). Perkin Elmer Clarus 600 equipped with flame ionization detector (FID) was used for analysis of samples. Nitrogen gas was used as a carrier and the standardized temperature conditions of oven injector and detector were maintained at 230 to 250°C respectively. The peaks identification of FAME was conducted by comparing the retention time of the samples under analysis to that of control sample which were used as standards of erucic acid content and were subjected to similar separation conditions as that of samples under study. The erucic acid content obtained was expressed in form of percent total fatty acids.

3 Results and Discussion

3.1 Validation for glucosinolate and erucic acid content using linked markers

3.1.1 BC₁F₁ Generation

Six BC₁F₁ populations, developed from the cross NRCHB101×PDZ-1, NRCHB101×RLC-3, NRCHB101×Heera, DRMR150-35×PDZ-1, DRMR150-35×RLC-3, DRMR150-35×Heera, comprising of 76, 52, 80, 84, 100 and 80 plants respectively, were genotyped for glucosinolate content. Out of these 472 plants, 75 plants were found to exhibit heterozygous banding pattern for all the three markers i.e. GER-1 (Q1); At5g41 (Q3); and GER-5 (Q5) (Figure 2 and 3). These 75 screened plants were further subjected to erucic acid validation using CAPS markers available for this study.

Out of these 75 plants, 64 plants were found with E1E1 genotype i.e. for high erucic acid (three fragments with E1 marker while single fragment for E2 marker), none of the individual plant showed e1e1 genotype i.e. for low erucic acid (single fragment with E1 marker while three fragments for E2 marker) and 11 were having E1e1 genotype i.e. intermediate ER (single fragment with E1 and E2 both the markers or three fragments with E1 marker and E2 marker both) (Table 3). These 11 plants which were exhibiting heterozygote banding pattern for *FAE1.1* genes were selected for further backcrossing with recurrent parent. These 11 plants obtained after erucic acid validation were derived from the crosses DRMR150-35×PDZ-1, DRMR150-35×RLC-3, NRCHB101×PDZ-1 and NRCHB101×Heera. BC₂F₁ generation derived from these four crosses were screened for quality traits (low glucosinolate and low erucic acid) using molecular markers as mentioned above.

Table 3 Genotyping of BC₁F₁, BC₂F₁ for glucosinolate trait and erucic acid.

S.No	Cross name	No. of plants screened (BC ₁ F ₁)	No. of plants obtained (using low glucosinolate markers)	No. of plants obtained (using Erucic acid markers)	No. of plants screened (BC ₂ F ₁)	No. of plants obtained (using low glucosinolate markers)	No. of plants obtained (using Erucic acid markers)
1	DRMR150-35XPDZ-1	84	20	4	105	15	1
2	DRMR150-35XRLC-3	100	16	3	30	8	5
3	DRMR150-35X Heera	80	8	-	-	-	-
4	NRCHB101XPDZ-1	76	15	2	52	8	4
5	NRCHB101XHeera	80	9	1	171	22	8
6	NRCHB101XRLC-3	52	7	1	-	-	-
Total no. of plants		472	75	11	358	53	18

3.1.2 BC₂F₁ generation

Four BC₂F₁ population developed from the cross DRMR150-35×PDZ-1, DRMR150-35×RLC-3, NRCHB101×PDZ-1 and NRCHB101×Heera comprise of 105, 30, 52 and 171 individual plants respectively, these plants were genotyped for glucosinolate content (Table 3). Out of these 358 plants, 53 plants were found to exhibit heterozygous banding pattern among all the three markers i.e. GER-1 (Q1); At5g41 (Q3); and GER-5 (Q5)(Figure 2 and 3). These 53 plants exhibiting heterozygous banding pattern were further screened for low erucic acid trait using CAPS markers. Out of these 53 plants, 35 were having E1E1 genotype i.e. for high ER (three fragments with E1 marker while single fragment for E2 marker), none of the individual plant showed e1e1 genotype i.e. for low ER (single fragment with E1 marker while three fragments for E2 marker) and 18 were E1e1 genotype i.e. intermediate ER (single fragment with E1 and E2 both the markers or three bands with E1 marker and E2 marker both)(Figure 4,5). These 18 plants which were found to exhibit heterozygotes banding pattern for *FAEI.1* gene and were selected for further backcrossing with recurrent parent for generation advancement. Qu et al. (2015) performed qRT-PCR for validation of two genes(BnGRT2 and BnMYB28) associated with glucosinolate content and also identified associated SNP marker which could be useful in marker assisted breeding of low seed glucosinolate in *B.napus*.

The present study, aimed at validation of earlier reported markers that are PCR based and can be utilized routinely to detect the presence of high and low glucosinolate genotypes (Bisht et al., 2009) and erucic acid. These markers were used on parents, BC₁F₁ and BC₂F₁ progenies. The set of five markers linked to low glucosinolate trait (GER-1 (Q1); Myb28 (Q2); At5g41 (Q3); At5GAJ67 (Q4) and GER-5 (Q5) and CAPS markers (E1 and E2) got amplified in all the parents.

In present investigation as a faster screening procedure, only three molecular markers GER-1 (Q1); At5g41 (Q3); and GER-5 (Q5) were taken to screen the BC₁F₁ and BC₂F₁ population for low glucosinolate trait. Markers GER-1 (Q1) and GER-5 (Q5) have been already reported for screening of low glucosinolate lines in *B.juncea* (Singh 2010) and were validated in RIL population derived from cross between low erucic acid (Mustard-21) and a double low line(EC-597325) and also suggested their 71 percent involvement in phenotypic variance (Pushpa et al. 2015).

Markers linked to low glucosinolate taken under this study showed polymorphism among the donor and recipient parents. The amplicon size of 650 bp was observed in case of donor genotypes namely PDZ-1, RLC-3 and Heera whereas amplicon size of 950 bp was observed in recipient genotypes NRCHB101 and DRMR150-35 with marker GER-1 linked to *J2Gsl1* (*BjuA. Gsl-ELONG.a*)(Figure 2). Pushpa et al. (2015) also reported that low glucosinolate content

lines showed amplicon size of 650 bp and lines with high glucosinolate content show amplicon size of 950 bp. In case of marker At5g41 (Q3) linked to *J9Gsl3* amplified product size of 800 bp in case of NRCHB101, DRMR150-35 whereas in case of PDZ-1, RLC-3 and Heera it was 770 bp. The third marker GER-5 linked to *J3Gsl2* (*BjuA. Gsl-ELONG.c, d*) gave amplicon size of 310 bp in PDZ-1, RLC-3 and Heera and 350 bp in NRCHB101 and DRMR150-35 (Figure 3). Present results are in agreement with earlier report of Pushpa et al. (2015). Ripley & Roslinsky (2005) identified and validated molecular marker for 2-propenyl glucosinolate in *B.juncea*. In another study molecular evaluation for low glucosinolate content was conducted with the aid of 80 SSR markers in *B.carinata* (Marquez-Lema et al. 2008). Gene-linked markers for seed glucosinolate loci were identified in *B.napus* genotypes by Hasan et al (2008). To predict homozygous and heterozygous individuals for *FAEI.1* gene in backcross populations E1 and E2 CAPS molecular markers were used. Parents were also validated for low erucic acid trait using CAPS markers for *FAE 1.1* gene namely E1 and E2 where all parental genotypes under study generated fragments of expected size for each CAPS maker and showed clear cut differentiation between low and high erucic acid genotypes. The E1 and E2 CAPS markers for *FAEI.1* gene amplified 432 bp and 427 bp fragments respectively. Gupta et al. (2004) reported that *FAEI.1* gene contributes more to the phenotype as compared to *FAEI.2* and also verified the applicability of SNP's in marker assisted manipulation of erucic acid trait among a set of contrasting germplasm of *B.juncea*. Digestion of PCR amplified product of E1 molecular marker with *Hpy99I* enzyme resulted three fragments (one undigested fragment and two digested fragments) of 432 bp, 224 bp and 198 bp size for high erucic acid genotypes i.e.NRCHB101, DRMR150-35 whereas in case of low erucic acid genotypes i.e. PDZ-1, RLC-3 and Heera only single undigested fragment of 432 bp was visible (Figure 1 a). Reverse is the case with second CAPS molecular marker i.e.E2 under study. After digestion with *BglIII* enzyme the low erucic acid genotypes i.e. PDZ-1, RLC-3 and Heera resulted in three fragments (one undigested fragment and two digested fragments) 427 bp, 209bp and 198 bp whereas only single undigested fragment of 427 bp was visible in case of high erucic acid genotypes i.e. NRCHB101 and DRMR150-35 (Figure1 b).Confirmation of these markers linked to quality trait (low glucosinolate and low erucic acid) in all the parents leads us to further screening of BC₁F₁ and BC₂F₁ populations. A similar pattern of these CAPS marker upon restriction digestion was reported in a study conducted on 21 diverse genotypes of *B.juncea*, *B.nigra* and backcross population and also reported efficiency of these CAPS markers (Saini et al. 2016). The selection of CAPS was done because they are highly polymorphic between high erucic acid and low erucic acid genotypes. Bharti et al (2018) also characterized candidate genes of glucosinolate and erucic acid using SSR markers,correlated heterosis with quality traits and selected heterozygous crosses for breeding programmes.

Figure 1 Agarose gel showing validation of markers linked to quality trait (Erucic acid); (a) E1 (b) E2. M- 50bp Ladder, 1-PDZ-1 (low), 2-Heera (low), 3-RLC-3 (low), 4- NRCHB101 (high), 5-DRMR 150-35 (high).

Figure 2 Agarose gel showing Validation of markers linked to quality (Glucosinolate); GER-1 (Q1) M-100bp ladder Plant No.1-6 BC₂ (DRMR150-35XPZ-1), Plant No. 7-12 BC₂ (DRMR150-35xRLC-3), Plant No. 13-18 BC₂ (NRCHB101xPDZ-1), Plant No. 19-24 BC₂ (NRCHB101xHeera), P1-DRMR150-35, P2-HEERA, P3-PDZ-1, P4-NRCHB101 and P5-RLC-3.

Figure 3 Agarose gel showing Validation of markers linked to quality (Glucosinolate); GER-5 (Q5) M-50bp ladder Plant No.1-6 BC₂ (DRMR150-35XPZ-1), Plant No. 7-12 BC₂ (DRMR150-35xRLC-3), Plant No. 13-18 BC₂ (NRCHB101xPDZ-1) and Plant No. 19-24 BC₂ (NRCHB101xHeera), P1-DRMR150-35, P2-HEERA, P3-PDZ-1, P4-RLC-3 and P5-NRCHB101.

Figure 4 Agarose gel showing validation of markers linked to quality trait (Erucic acid); E1. M- 50bp Ladder Plant No.1-9 BC₂ (DRMR150-35XPZ-1), Plant No. 10-19 BC₂ (DRMR150-35xRLC-3), Plant No. 20-23 BC₂ (NRCHB101xPDZ-1), Plant No. 24-26 BC₂ (NRCHB101xHeera).

Figure 5 Agarose gel showing validation of markers linked to quality trait (Erucic acid); E2. M-50bp Ladder Plant No.1-9 BC₂ (DRMR150-35XPZ-1), Plant No. 10-19 BC₂ (DRMR150-35xRLC-3), Plant No. 20-23 BC₂ (NRCHB101xPDZ-1), Plant No. 24-26 BC₂ (NRCHB101xHeera).

3.2 Biochemical analysis for glucosinolate and erucic acid content

The parental lines, NRCHB101, DRMR150-35, PDZ-1, RLC-3 and Heera have total glucosinolate content 115.56 $\mu\text{mol/g}$, 120.86 $\mu\text{mol/g}$, 19.96 $\mu\text{mol/g}$, 14.07 $\mu\text{mol/g}$ and 33.19 $\mu\text{mol/g}$ seed, respectively. However, the erucic acid content in seed for these parental lines was found to be 34.53%, 38.19%, 4.16%, 0.80% and 0% respectively.

Eleven plants of BC₁F₁ which were found low for quality traits with molecular markers, their biochemical analysis was performed and total glucosinolate content in defatted seed meal was found in range of 68.31 to 92.36 $\mu\text{mol/g}$ while the erucic acid content ranged from 16.67% to 30.26 %. Result of study revealed that there was no line in <30-60 μmole glucosinolate/g defatted seed meal content and below 2% erucic acid content. Among the studied 11 plants, most of the plants were in range 65-85 μmole glucosinolate/g defatted seed meal. The wide range in total glucosinolate concentrations found in this study indicates that there is potential for further reduction of glucosinolate levels by screening further advanced generations. Variation in seed glucosinolate concentrations of Indian mustard have also earlier been reported by various researchers (Kumar et al., 2004; Padilla et al., 2007; Bellostas et al., 2007; Verkerk et al., 2009; Yang & Quiros, 2010). In case of BC₂F₁ generation samples, the total glucosinolate content was found in the range of 52.43-119.4 $\mu\text{mol/g}$ defatted seed meal while the erucic acid content ranged from 10.75 % to 33.18%. Most of the samples of backcross generations were in the range of 50-85 $\mu\text{mol/g}$ defatted seed meal. Similar study on biochemical analysis for total glucosinolate content was conducted by Singh et al. (2018) in F₂ Generation of *B. juncea* and also validated using molecular markers.

Conclusion

Present study indicated effectiveness of marker assisted selection for quality traits in Indian mustard. Reported molecular markers can save time and improve the efficiency of selection. Once the nuclear background of the recurrent parent is determined, marker-facilitated progeny evaluation in successive generations will help in identification of non-segregating progenies carrying homozygous recessive alleles and thus will lead to development of high yielding double low genotypes.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

- Ackman RG, Eaton CA, Sipos JC, Loew FM, Hancock D (1977) Comparison of fatty acids from high levels of erucic acid of RSO and partially hydrogenated fish oil in non-human primate species in a short-term exploratory study. *Bibliography Nutrition and Dietetics* 25: 170-85.
- Bellostas N, Sorensen AD, Sorensen JC, Sorensen H (2007) Genetic variation and metabolism of glucosinolates. *Advances in Botanical Research* 45: 369- 415.
- Bharti R, Chaudhary N, Sandhu R, Gupta SK, Sharma M (2018) Molecular Characterization of Candidate Gene in Glucosinolate and Erucic Acid Using SSR Markers. *International Journal of Advanced Research Ideas and Innovations in Technology* 4(1) : 400-409.
- Bisht NC, Gupta V, Ramchiary N, Sodhi YS, Mukhopadhyay A, Arumugam N, Pental D, Pradhan AK (2009) Fine mapping of loci involved with glucosinolate biosynthesis in oilseed mustard (*Brassica juncea*) using genomic information from allied species; *Theoretical and Applied Genetics* 11: 413-421.
- Chauhan JS, Singh M, Bhadauria VPS, Kumar A, Meena ML (2007) Genetic analysis of glucosinolate content in Indian mustard (*Brassica juncea* L.). *Indian Journal of Genetics* 67 (4): 411-413.
- Chien K, Bellary A, Nicar M, Mukherjee A, Buja L (1983) Induction of a reversible cardiac lipidosis by a dietary long-chain fatty acid (erucic acid). Relationship to lipid accumulation in border zones of myocardial infarcts. *The American Journal of Pathology* 112:68-77
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- Foolad MR, Sharma A (2005) Molecular Markers as Selection Tools in Tomato Breeding. *Acta Horticulturae* 695: 225-240.
- Goli SAH, Sahri MM, Kadivar M (2008) Enzymatic inter esterification of structured lipids containing conjugated linoleic acid with palm stearin for possible margarine production. *European Journal of Lipid Science and Technology* 110:1102-1108
- Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK (2004) Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and single nucleotide polymorphisms in *FAEI* gene. *Theoretical and Applied Genetics* 108: 743-749.

- Hasan M, Friedt W, Pons-Kühnemann J, Freitag NM, Link K, Snowdon RJ (2008) Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). *Theoretical and Applied Genetics* 116: 1035-1049.
- Jat RS, Singh VV, Sharma P, Rai PK (2019) Oilseed brassica in India: Demand, supply, policy perspective and supply potential. *Oilseeds & fats, crops & lipids (OCL)* 26: 8.
- Jesch ED, Carr TP (2017) Food ingredients that inhibit cholesterol absorption. *Preventive Nutrition and Food Science* 22:67-80
- Kumar S, Yadav SK, Chauhan JS, Singh AK, Khan NA, Kumar PR (2004) Total glucosinolate estimation by complex formation between glucosinolate and tetrachloropalladate (II) using ELISA reader. *Journal of Food Science and Technology* 41: 63-65.
- Liu D, Veit HP, Wilson JH, Denbow DM (2003) Long-term supplementation of various dietary lipids alters bone mineral content, mechanical properties and histological characteristics of Japanese quail. *Poultry Science* 82: 831-839.
- Marquez-Lema A, Pérez-Vich B, Velasco-Varo L, Fernández-Martínez JM (2008) Development and characterisation of a *Brassica carinata* inbred line incorporating genes for low glucosinolate content from *B. juncea*. *Euphytica* 164: 365-375.
- Mattson FH, Grundy SM (1985) Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research* 26:194-202.
- Mawlong I, Sujith Kumar M, Gurung B, Singh K, Singh D (2017). A simple spectrophotometric method for estimating total glucosinolates in mustard de-oiled cake. *International Journal of Food Properties* 20:3274-3281.
- Padilla G, Cartea ME, Velasco P, De Haro A, Ordas A (2007) Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry* 68: 536-545.
- Pham LJ, Pham PJ (2012) Biocatalysed production of structured olive oil triacylglycerols. In: Boskou D (ed) *Olive oil constituents, quality, health properties and bioconversion*. InTech.
- Priyamedha, Singh BK, Thomas L, Manju Bala, Singh VV, Singh Dhiraj (2015) Status and perspective of canola quality rapeseed-mustard cultivation in India : a review. *Journal of Oilseed Brassica* 6: 142-151.
- Pushpa HD, Yadava DK, Singh N, Vasudev S, Saini N, Muthusamy V, Kumble P (2015) Validation of molecular markers linked to low glucosinolate QTLs for marker assisted selection in Indian mustard (*Brassica juncea* L.). *Indian Journal of Genetics* 76:64-68
- Qu CM, Li SM, Duan XJ, Fan JH, Jia LD, Zhao HY, Lu K, Li JN, Xu XF, Wang R (2015) Identification of Candidate Genes for Seed Glucosinolate Content Using Association Mapping in *Brassica napus* L. *Genes (Basel)* 6(4): 1215-1229.
- Ramchiary N, Bisht NC, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK (2007) QTL analysis reveals context-dependent loci for seed glucosinolate trait in oilseed *Brassica juncea*: importance of recurrent selection backcross scheme for the identification of “true” QTL. *Theoretical and Applied Genetics* 116: 77-85.
- Ripley V, Roslinsky V (2005) Marker for 2-propenyl glucosinolate content in *B. juncea* L. and conversion to a SCAR marker. *Molecular Breeding* 16:57-66.
- Saini N, Singh N, Kumar A, Vihan N, Yadav S, Vasudev S, Yadava DK (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(5):831-837.
- Singh J (2010) Validation and use of molecular markers tagged to low erucic acid trait for marker assisted selection in Indian mustard (*Brassica juncea* L.). M.Sc. thesis submitted to PG School, IARI, New Delhi.
- Singh VV, Mawlong I, Dubey M, Paliwal P, Kumar MS (2018) Variability in total glucosinolate content in F₂ generation of *B. juncea* and their molecular validation. *International Journal of Current Microbiology and Applied Sciences* 7: 3346-3357.
- Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B, Rowland I, De Schrijver R, Hansen M, Gerhäuser C, Mithen R, Dekker M (2009) Glucosinolates in Brassica vegetables: The influence of the food supply chain on intake, bioavailability and human health. *Molecular Nutrition and Food Research* 53: S219.
- Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publication to practice. *Crop Science* 48: 391-407.
- Yang B, Quiros CF (2010) Survey of glucosinolate variation in leaves of *Brassica rapa* crops. *Genetics Resources and Crop Evolution* 57: 1079-1089.