PLANT SCIENCE TODAY
Vol 12(3): 1-8
https://doi.org/10.14719/pst.6528

eISSN 2348-1900

RESEARCH ARTICLE

Inheritance of low erucic acid content in Indian mustard *Brassica juncea* (L.)

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Received: 04 December 2024; Accepted: 11 June 2025; Available online: Version 1.0: 08 August 2025; Version 2.0: 22 August 2025

Cite this article: Kavita R, Sunil KR, Kamilla P, Aastha S, Rajeev B, Bupesh K, Ravinder SS. Inheritance of low erucic acid content in Indian mustard *Brassica juncea* (L.). Plant Science Today. 2025; 12(3): 1-8. https://doi.org/10.14719/pst.6528

Abstract

The high erucic acid content of *Brassica juncea* oil is associated with a number of negative effects in mammals, including humans. Therefore, the objective of this study was to investigate the inheritance of erucic acid content across F_1, BC_1F_1 and BC_1F_2 generations derived from crosses between high erucic acid genotypes (RSPR-03 and RH-749) and low erucic acid genotypes (PM-21 and Pusa Karishma). The erucic acid content in selfed seeds, F_1, BC_1F_1 progenies and parental lines was quantified using gas-liquid chromatography (GLC). Ten plants from each parent, twenty F_1s plants, one hundred BC_1F_1 plants and two hundred BC_1F_2 plants were phenotyped. The range of the erucic acid content was 17.12 % to 41.94 %. The backcrosses' BC_1F_2 generation has a lower erucic acid level than its parents. The BC_1F_2 generation of backcross [RSPR-03 × (RSPR-03 × Pusa Karishma)] had the lowest erucic acid content (17.12 %), followed by the BC_1F_2 generation of backcross [RH-749 × (RH-749 × PM-21)], which had an 18.48 % erucic acid content. The population data conformed well to the expected segregation ratio of 1:4:6:4:1, suggesting that low erucic acid is governed by recessive inheritance and that the trait is digenically inherited with an additive gene effect. Among the studied genotypes, the BC_1F_2 generation from the cross [RSPR-03 × (RSPR-03 × Pusa Karishma)] exhibited superior performance for more than 50 % of the evaluated morphological and biochemical traits.

Keywords: back cross; erucic acid; gas liquid chromatography; inheritance; recessive

Introduction

Indian mustard (*Brassica juncea* (L.) Czern and Coss.) is one of the most significant species within the Brassica genus of the Brassicaceae family. It accounts for approximately 80 % of the total area under rapeseed-mustard cultivation and is predominantly grown in the Indian subcontinent. India is one of the leading producers of rapeseed-mustard, cultivating it over the largest area (19.8 million ha) and contributing 9.8 % to global production (1).

After soybeans, rapeseed-mustard is the second most valuable oilseed crop in India. *Brassica juncea* is particularly well-suited to semi-arid conditions due to its superior drought-tolerant and shatter-resistant than *Brassica napus* and *Brassica rapa*. With a total production and productivity of 10.1 million t and 1511 kg/ha, it is grown on 6.7 million ha in India (2). With the growing population and rising living standards, the demand for edible oils is expected to increase substantially, necessitating enhanced oilseed production.

Although mustard oil is rich in energy and contains low level of saturated fatty acids, it is also high in erucic acid. However, it is valued for its significant amount of unsaturated fatty acids, omega-3 fatty acids, vitamins E, A and K. Research indicates that high levels of erucic acid in edible oils are associated with adult heart disease and delayed childhood brain development (3).

Because *Brassica* oil contains more than 2 % erucic acid, the European Economic Union (EEU) has banned the production of *Brassica* crops (4, 5). The concentration of glucosinolate and erucic acid are significant factors in determining the quality of seed meal and mustard oil respectively. The term "Canola" has been registered by the Western Canadian Oilseed Crushers' Association to describe rapeseed-mustard types that satisfy the requirements for low erucic acid (< 2.0 %) and low glucosinolate (< 30 µmol/g of defatted cake). Double zero ("00") variants are the name given to these types. Single zero ("0") types are those that include either low levels of glucosinolate (30 moles/g defatted seed meal) or erucic acid (< 2.0 %). International initiatives are taken to genetically eradicate these unwanted components from oilseed *Brassicas* which was sparked by nutritional and end-user needs.

The successful commercial release of "double zero," (canola-type quality), *Brassica napus* and *Brassica rapa* varieties in the 1970s marked a significant milestone. The biosynthetic pathway for fatty acid desaturation produces both linoleic and linolenic acids, which are necessary for human health. Selection of cultivars with high linoleic acid tends to raise linolenic acid levels, whilst selection for varieties with low linolenic acid levels tends to lower them. Therefore, it

may be concluded that genotypes with zero erucic, low linolenic, high oleic and high lenoleic fatty acids as well as double zero are available and that some double zero varieties are also being grown in western nations like India (6).

The mode of inheritance of any characteristic is a prerequisite for establishing a breeding strategy and creating genotypes with desirable qualities before the beginning of the genetic enhancement of that trait. However, there are yet insufficient reports of investigations on the inheritance of erucic acid content from various foreign sources. Two recessive genes controlling the amount of erucic acid in *B. napus* were revealed (7, 8). Two recessive genes with additive effects were identified to influence the inheritance of the low erucic acid phenotype in *B. juncea* (9). Additionally, it was shown that the Indian geographical group of *B. juncea* (with 50 % erucic acid) includes alleles for high erucic acid levels at two loci, but the Eastern European types (with 25 % erucic acid) possess these alleles at only one locus (10).

As of present, many advanced lines with outstanding agronomic traits have been developed locally. Studying the inheritance of erucic acid content in these lines can help clarify the genetic mechanisms and guide breeding strategies for improving this trait in indigenous germplasm. Traditional breeding techniques for upgrading are diverse and have advanced from simple mass selection to the development of hybrid cultivars. The most popular technique for extracting oil from seed meals is solvent extraction, which involves balancing the solvents and the samples. In sizable segregating populations, the erucic acid concentration of over 300 individual plants can be precisely estimated using gas chromatography (GC). After the crop has been harvested, GC is a destructive method that exploits seeds for measurement.

Molecular markers have been widely utilized in recent years to map and designate genes of agricultural value. The availability of molecular markers tightly linked to the gene(s) of interest enables the indirect selection at the seedling stage without the need to go for the tedious task of large-scale phenotyping, which is more expensive, time-consuming and stage-specific. The erucic acid characteristic (C22:1) in *B. juncea* has been molecularly mapped and tagged using a candidate gene technique (11). By using double haploid mapping populations obtained from a high (Varuna) x low erucic acid (Heera) hybrid, two QTLs driving the variance of seed erucic acid content were assigned to two linkage groups of *B. juncea* map and seven single nucleotide polymorphisms (SNPs) were reported from the candidate genes in this cross. These SNPs were verified in the cross LES 39 x Varuna (12). It was then employed as PCR-based molecular markers for the early detection of low and high erucic acid traits in *B. juncea* genotypes. The only feasible and non-destructive method for monitoring the LEA trait in plant progenies is utilized to produce "0" and "00" variety nucleus seeds for a sustainable and effective seed production chain to fulfil the demand of various seed production agencies by marker-assisted selection.

Therefore, the processes and methodology must be standardized for this technology so that it can be used on a broad scale for the aforementioned goal.

The current study effectively illustrates the digenic recessive inheritance pattern with additive gene effects of low erucic acid content in Indian mustard. The observed segregation pattern in the BC_1F_2 generation confirms that this trait is genetically controlled, offering a strong foundation for molecularly assisted backcross breeding to manipulate it. The BC_1F_2 populations' significantly lower erucic acid content, especially from crosses involving Pusa Karishma and PM-21, not only confirms the efficacy of introgression but also shows these lines' potential for further hybrid development.

Materials and Methods

The present study was undertaken at the Experimental Farm of the Division of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, during the *Rabi* seasons 2019-20, 2020-21 and 2021-22, Additionally, an off-season trail was carried out at the Krishi Vigyan Kendra (KVK), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Ladakh during the *Kharif* season 2021.

Experimental material

The experimental material consisted of two popular varieties RH-749 and RSPR-03 of Indian mustard (*B. juncea*), having high erucic acid content (> 40 %) and two varieties viz., PM 21 and Pusa Karishma with low erucic acid (< 2 %) (Table 1). To study the inheritance pattern of the erucic acid trait, four crosses were attempted during the *Rabi* season 2019-20 and 2020-21. The backcrosses were attempted in both the crosses and the resulting BC_1F_1s progenies were selfed to generate BC_1F_2 seeds during the *Rabi* 2020-21 for studying the mode of inheritance of erucic acid gene(s). For validation of reported markers, the parents and the advanced generation were used.

Biochemical analysis

Oil extraction

Diethyl ether (B.P.37-40 °C) was used as the solvent in Soxhlet extraction to extract the lipids from the sample. Six hr were spent extracting 116 g of the dry milled sample from a flask that had been previously weighed. The flask was dried at 60 °C for 10 min after the solvent evaporated, chilled in a desiccator and then weighed again until it reached a consistent weight.

Table 1. List of experimental material with coding

Parents	Code
RH-749	P1
RSPR-03	P2
Pusa Karishma	P3
PM-21	P4
Cross	
RSPR-03 ×Pusa Karishma	F1
RSPR-03 ×PM-21	F2
RH-749 ×Pusa Karishma	F3
RH-749 ×PM-21	F4
Backcross (BC_1F_1)	
RSPR-03× (RSPR-03×Pusa Karishma)	K1
RSPR-03 × (RSPR-03 ×PM-21)	K2
RH-749 × (RH-749 ×Pusa Karishma)	K3
RH-749 × (RH-749 ×PM-21	K4
BC_1F_2	
Selfing of RSPR-03× (RSPR-03×Pusa Karishma)	S1
Selfing of RSPR-03× (RSPR-03 ×PM-21)	S2
Selfing of RH-749 × (RH-749 ×Pusa Karishma)	S3
Selfing of RH-749 × (RH-749 ×PM-21)	S4

Extraction of fatty acids and estimation of erucic acid

GC based method was employed to estimate erucic acid content. Approximately 20–25 crushed seeds were incubated overnight in 0.5 mL of hexane. Following incubation, 0.5 mL of sodium methoxide was added to the supernatant and the mixture was incubated for 45 min. Subsequently, 7.5 mL of NaCl solution was added and the mixture was vortexed. After 30 min, the upper phase was collected for GC injection. The percentage of erucic acid was quantified using GC software by calculating the relative peak areas.

Molecular analysis

Genomic DNA was extracted from 20-25 day-old mustard leaves using the modified CTAB method (13). It was followed by RNase treatment and purification with a phenol: chloroform: isoamyl alcohol mixture. DNA quality was assessed on 0.8 % agarose gel electrophoresis. For PCR analysis, the DNA was diluted to a working concentration of 25 ng/µl.

PCR amplification was performed using 20 SSR markers in a 25 µL reaction volume with Red Taq Mix. The thermal cycling conditions included an initial denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The amplified products were visualized on a 3 % agarose gel using a gel documentation system.

All necessary reagents, including electrophoresis buffers, RNase, TE buffer and CTAB buffer, were prepared following standard protocols.

Results

The fatty acid profiling for all the parents viz. RH-749, RSPR-03, Pusa Karishma and PM-21 and four backcross F_2s' population RSPR-03 × (RSPR-03 × Pusa Karishma), RSPR-03 × (RSPR-03 × PM-21), RH-749 × (RH-749 × Pusa Karishma) and RH-749 (RH-749 × PM-21) (Fig. 1) was carried out to determine individual fatty acid concentrations in each sample.

Erucic acid

Erucic acid was estimated in four parent lines, with the highest content present in RSPR-03 (41.94 %), followed by RH-749 (40.53 %) (Fig. 2). The lowest content was found in PM-21 (1.11 %), followed by Pusa Karishma (1.69 %). In the BC_1F_2s generations of the four crosses, the erucic acid content ranged from 17.12 % to 28.10 %. The erucic acid content in different backcrosses was as follows: RSPR-03 × (RSPR-03 × Pusa Karishma) had 17.12 %, RSPR-03 × (RSPR-03 × PM-21) had 25.02 %, RH-749 × (RH-749 × Pusa Karishma) had 28.10 % and RH-749 × (RH-749 × PM-21) had 18.48 %. Each backcross exhibited a different range of erucic acid content (Table 2).

Palmitic acid

The average palmitic acid content across all parents and BC_1F_2 population was 2.54 %. The highest palmitic acid content was observed in RSPR-03 × (RSPR-03 × Pusa Karishma) (3.79 %) and lowest palmitic acid PM-21 (1.89 %).

Stearic acid

The average stearic acid content was 1.24 %. The highest content was found in RH-749 × (RH-749 × PM-21) (2.73 %) and RH-749 having the lowest (0.63 %).

Oleic acid

The average oleic acid content was 20.29 %, with RSPR-03 × (RSPR-03 × Pusa Karishma) (24.85 %) having the highest oleic acid and RH-749 × (RH-749 × Pusa Karishma) having the lowest (11.80 %) (Table 2).

Linoleic acid

The average linoleic acid content was 21.85 %, with RH-749 × (RH-749 × Pusa Karishma) (27.65 %) having the highest linoleic and PM-21 having the lowest (15.04 %). For linolenic acid RSPR-03 × (RSPR-03 × PM-21) (24.94 %) recorded the highest linolenic acid and PM-21 having the lowest (10.55 %) (Table 2).

Oil content

The oil content ranges from 35.5 % (RSPR-03) to 42.65 % (Pusa Karishma) in the case of parents. In the case of BC_1F_2 population, RSPR-03 × (RSPR-03 × PM-21) recorded lowest oil content i.e., 37.55 % and RH-749 × (RH-749 × Pusa Karishma) recorded highest oil content i.e. 40.55 % (Table 2).

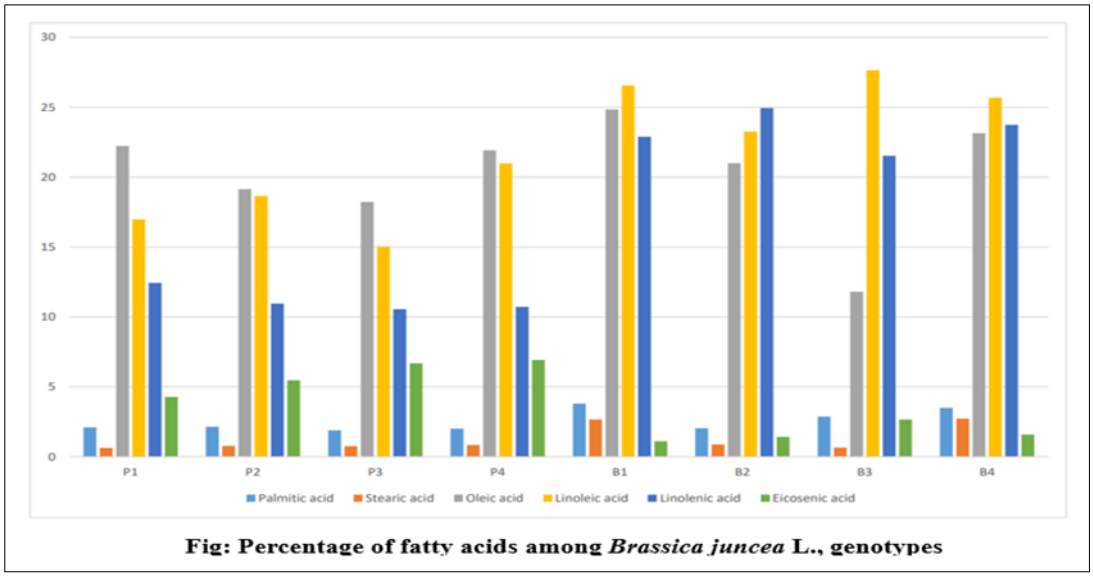

Fig. 1. Percentage of fatty acids among *B. juncea* L. genotypes.

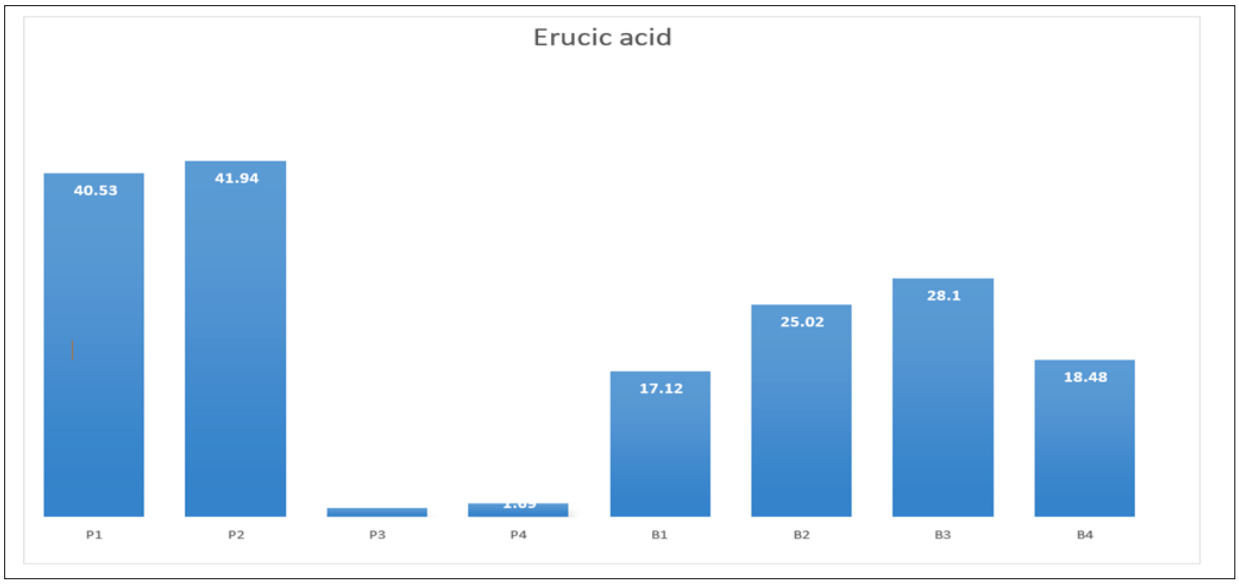

Fig. 2. Erucic acid profiling among *B. juncea* L. genotypes.

Table 2. The erucic acid and fatty acid content in parents and crosses of high × low erucic acid

	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenic acid (%)	Erucic acid (%)	Oil content (%)
RH-749	2.09	0.63	22.23	16.98	12.44	4.27	40.53	35.05
RSPR-03	2.14	0.77	19.14	18.65	10.95	5.46	41.94	37.3
PM-21	1.89	0.75	18.23	15.04	10.55	6.68	1.11	41.45
Pusa Karishma	2.01	0.84	21.92	20.98	10.72	6.92	1.69	42.65
K1	3.79	2.66	24.85	26.55	22.89	1.10	17.12	38.15
K2	2.04	0.88	20.99	23.25	24.94	1.41	25.02	40.55
K3	2.87	0.65	11.80	27.65	21.53	2.66	28.10	37.55
K4	3.49	2.73	23.15	25.68	23.74	1.58	18.48	38.25

Inheritance of low erucic acid trait in Indian mustard

Based upon the recorded data for all plants in the segregating generations, three classes were made in each backcross and five classes for the F_2 generations of the four crosses. The chi-square (X^2) test was employed to assess the goodness of fit between the observed and expected frequencies in the segregating generations. Observations and subsequent analysis for the inheritance of the low erucic acid trait were conducted using data from the parental lines, F_1, BC_1 and BC_1F_2 generations derived from four crosses involving four genetically distinct parents-RH-749, RSPR-03, Pusa Karishma and PM-21-differing in their erucic acid content.

Mode of inheritance

The erucic acid content of parental lines indicated that RH-749 and RSPR-03 had high to very high erucic acid content ranging from (38-43 %) while Pusa Karishma and PM-21 had very low (< 2 %) to zero erucic acid content. The F_1 plants of four crosses viz., RSPR-03 × Pusa Karishma, RSPR-03 × PM-21, RH-749 × Pusa Karishma and RH-749 × PM-21 had medium to high erucic acid content comparable to RSPR-03 and RH-749. Since most of the previous studies with different materials support the digenic recessive inheritance, the hypothesis was thus formulated with the same assumption (14-16). It was also demonstrated that the Indian geographical group of *B. juncea* (with~50 % erucic acid) contains alleles for high erucic acid at two loci, whereas the Eastern European group (with <25 % erucic acid) possesses alleles for high erucic acid levels at one locus only (17).

The segregation pattern of the erucic acid trait in the BC_1F_2 generation of four backcrosses RSPR-03 × (RSPR-03 × Pusa Karishma) RSPR-03 × (RSPR-03 × PM-21) RH-749 × (RH-749 × Pusa Karishma) RH-749 × (RH-749 × PM-21) fits well in 1:4:6:4:1 ratio (X^2= 3.97, 3.26, 3.86 and 3.26 respectively) indicating digenic inheritance of erucic acid trait with additive gene action.

Characterization of low erucic acid in *B. juncea* (L.) selected type parents, F_1 and BC_1F_2 genotypes using SSR markers

The allelic configuration at both loci was used to classify the parents, F_1 and BC_1F_2 genotypes into two major classes, viz. low and high erucic acid-containing genotypes. FAE1 (gene-based marker) showed polymorphism for all the parents when amplification was done at 55 °C. The high erucic acid genotypes showed polymorphic bands at 210 and 182 bp whereas the low erucic acid genotypes showed polymorphic bands at 135 bp, 153 bp, 164 bp and 177 bp (Table 3).

Table 3. Average erucic acid content for each allele of both loci

Crosses	Erucic acid classes	Erucic acid (%)	PIC value	H value
RSPR-03×Pusa Karishma	HEA-182bp & LEA- 135 bp	17.12	0.3698	0.4898
RSPR-03 × PM-21	HEA-182bp & LEA-153 bp	25.02	0.3249	0.4082
RH-749 ×Pusa Karishma	HEA-210bp & LEA-164 bp	28.10	0.3457	0.444
RH-749 × PM-21	HEA-210bp & LEA-177 bp	18.48	0.3698	0.4898

hea= high erucic acid; lea= low erucic acid

The purity of the F_1s was checked by confirming the presence of bands corresponding to both parents using PCR product images obtained from gel electrophoresis. Then, 10 plants of each BC_1F_2 population were used to check the inheritance of low erucic acid in the selfed generation.

The genotypes amplifying alleles at 135 bp, 153 bp, 164 bp and 177 bp (FAE1) were classified as low erucic acid (LEA) genotypes, while those amplifying either the 210 bp or 182 bp (FAE1) were classified as high erucic acid genotypes (Table 4).

The average erucic acid content in LEA and high erucic genotypes of *B. juncea* was compared using the Student's t-test to assess the statistical significance between the two groups. A significant difference in mean erucic acid content was observed, with P-value of 0.368, 0.3249, 0.3457 and 0.3698 respectively. The H values represent the heterogenic among these genotypes.

The Student's T-test was also used to determine the differences in the average erucic acid among the parents, F_1 and BC_1F_2 genotypes based on their FAE1 alleles. The high erucic acid among all the genotypes is the gene pool amplifying 210 bp and 182 bp alleles at FAE1. It was observed that two contrasting alleles at FAE1 had a significant difference in average erucic acid, as evidenced by the P-value obtained from the T-test.

The range of bands with low and high erucic acid content [RSPR-03 × (RSPR-03 ×Pusa Karishma)] showed 6 bands with high erucic acid content and 4 bands with low erucic acid content ranging from 35.4-40.0 % and 1.0-1.7 % respectively. Similarly, in the other 3 crosses, the number of bands corresponding to high erucic acid ranged from 6-8, with content varying between 38.24 and 41.32 %. In contrast, 2 to 4 bands were observed for low erucic acid, with content ranging from 1.24 % to 1.9 % (Fig. 3–6).

Table 4. Range of bands with high and low erucic acid content

Crosses	Erucic acid content	No. of bands	Range of erucic acid (%)
[RSPR-03 × (RSPR-03 ×Pusa Karishma)]	HEA	6	35.4-40.0
	LEA	4	1.0-1.7
[RSPR-03 × (RSPR-03 × PM-21)]	HEA	8	38.24- 41.32
	LEA	2	1.5-1.8
[RH-749 × (RH-749 ×Pusa Karishma)]	HEA	6	37.57 - 41.26
	LEA	2	1.24- 1.85
[RH-749 × (RH-749 × PM-21)]	HEA	6	38.5- 40.1
	LEA	4	1.7- 1.9

Discussion

Inheritance of low erucic acid trait in Indian mustard

Table 2 lists the observations for the parental lines (P_1, P_2, P_3, P_4), F_1s, four BC_1 and four BC_1F_2 generations derived from the respective crosses. The F_1 plants of the four crosses-RSPR-03 × Pusa Karishma, RSPR-03 × PM-21, RH-749 × Pusa Karishma and RH-749 × PM-21-exhibited medium to high erucic acid concentrations, comparable to their high erucic acid parents RSPR-03 and RH-749. This pattern suggests that low erucic acid is governed by recessive genes.

Since most of the previous studies with different materials pport the digenic recessive inheritance, the

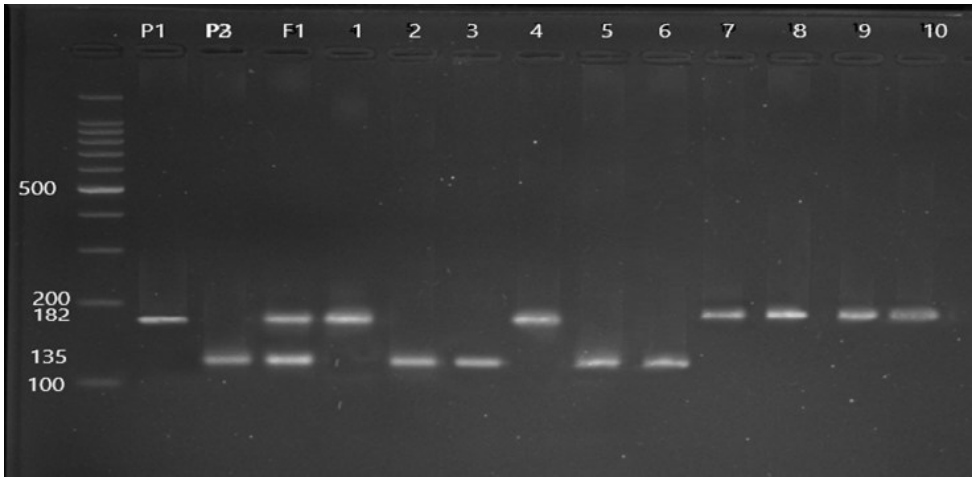

Fig. 3. Amplified PCR product of FAE1 in RSPR-03 × Pusa Karishma.

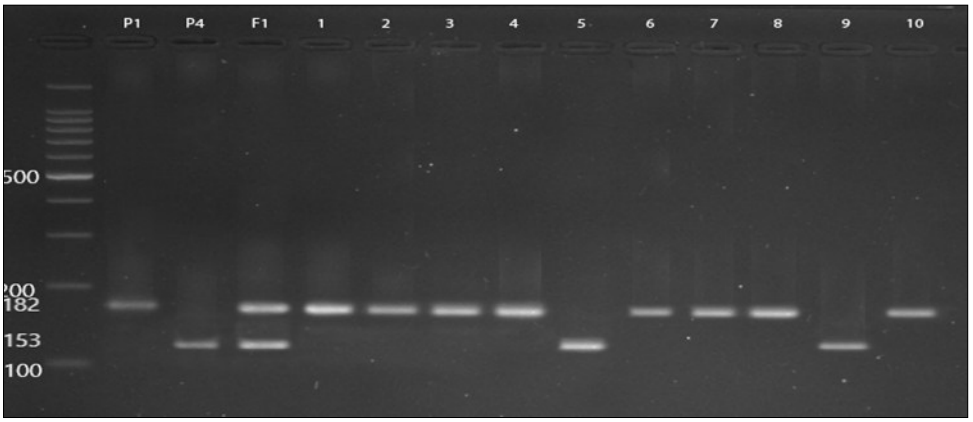

Fig. 4. Amplified PCR product of FAE1 in RSPR-03 × PM-21.

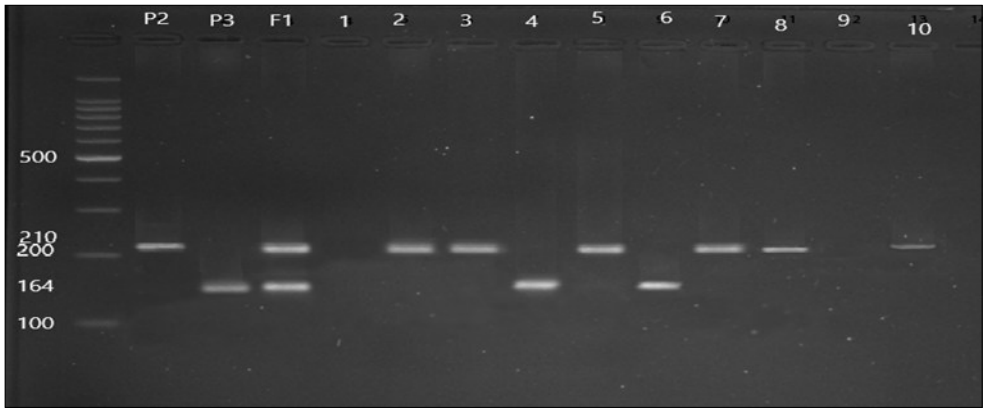

Fig. 5. Amplified PCR product of FAE1 in RH-749 × Pusa Karishma.

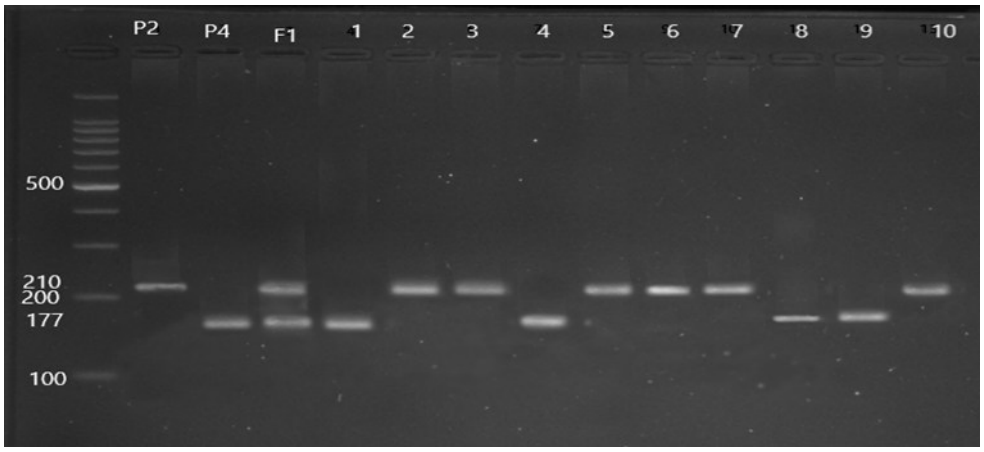

Fig. 6. Amplified PCR product of FAE1 in RH-749 × PM-21.

hypothesis was thus formulated with the same assumption (16, 17). Plants in the segregating generations were divided into five classes based on their different levels of erucic acid (<2 %, 2-14 %, 15-27 %, 28-40 % and > 40 %) (Fig. 7 & 8). To assess the goodness of fit between observed and expected frequency in separating generations, the chi-square (X^2) test was utilized.

The segregation pattern of the erucic acid trait in the BC_1F_2 generation of four backcrosses, RSPR-03 × (RSPR-03 × PM-21), RSPR-03 × (RSPR-03 × Pusa Karishma), RH-749 × (RH-749 × Pusa Karishma) and RH-749 × (RH-749 × PM-21), fit well to a 1:4:6:4:1 ratio (X^2 = 3.97, 3.26, 3.86 and 3.26, respectively), indicating digenic inheritance of the trait with additive gene action. These findings corroborate earlier reports of the digenic recessive inheritance of low erucic acid content in *B. juncea* (16, 17).

Characterization of low erucic acid in *B. juncea* (L.) selected type parents, F_1 and BC_1F_2 genotypes using SSR markers

A single set of primers targeting the FAE1 gene was shown to be polymorphic between high and low erucic acid genotypes. High erucic acid genotype amplified alleles at 210 bp and 182 bp, whereas low erucic acid geneotypes (LEA) exhibited polymorphic bands at135 bp, 153 bp, 164 bp and 177 bp (Table 4). The identification of low erucic acid genotypes in *B. juncea* have been identified with the aid of allelic variation based on the length polymorphism in the area. Marker-assisted selection revealed that the FAE1.1 allele was transmitted in the advanced generation. These results of digenic recessive nature of low erucic acid content are in agreement with the earlier findings

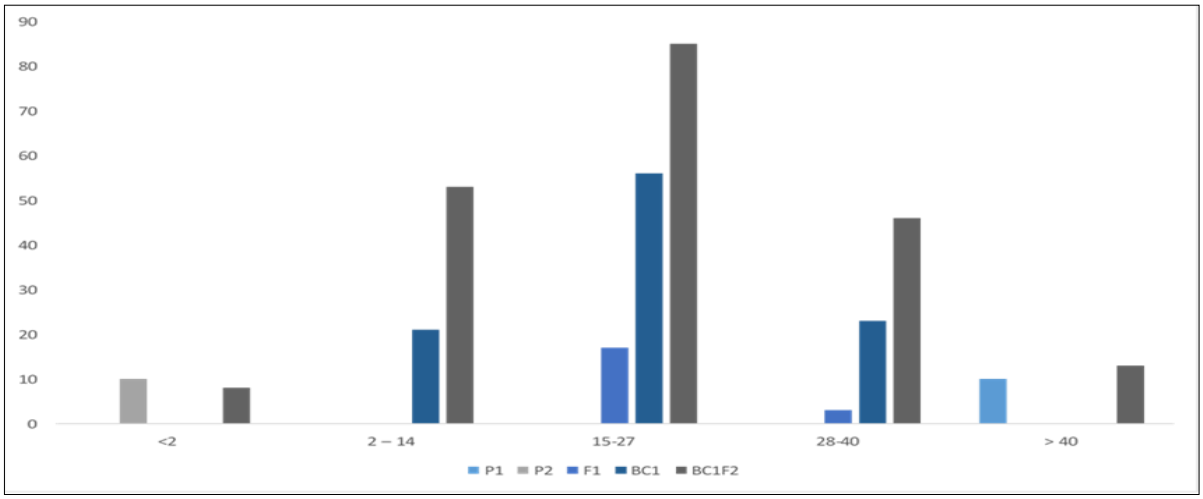

Fig. 7. Frequency distribution of erucic acid in BC_1F_2 population of RH-749 x PM-21.

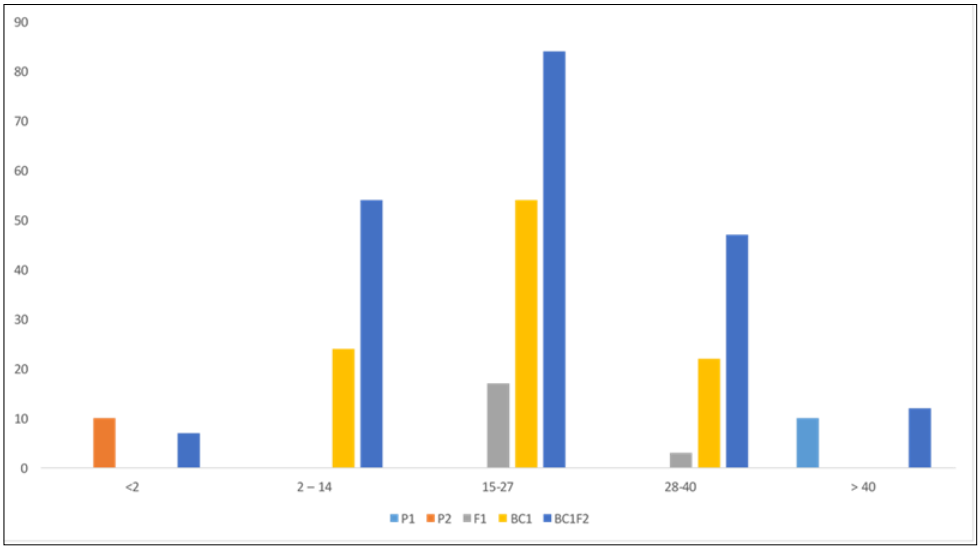

Fig. 8. Frequency distribution of erucic acid in BC_1F_2 population of RH-749 x Pusa Karishma.

(18).

Conclusion

The BC_1 and BC_1F_2 population were derived from four backcrosses involving the following parental combinations: RSPR-03 × (RSPR-03 ×Pusa Karishma), RSPR-03 × (RSPR-03 × PM-21), RH-749 × (RH-749 ×Pusa Karishma) and RH-749 × (RH-749 × PM-21). Each cross consisted of 100 and 200 plants respectively. The medium to high erucic acid concentration of the F1 plants of the four crosses-RSPR-03 ×Pusa Karishma, RSPR-03 × PM-21, RH-749 ×Pusa Karishma and RH-749 × PM-21-was similar to those of their high erucic acid parents RSPR-03 and RH-749, suggesting that recessive gene(s) regulate low erucic acid.

The genetic composition of the parental lines and the BC_1F_2 populations was determined through molecular analysis using Simple Sequence Repeat (SSR) markers targeting the FAE1.1 locus, known to regulate erucic acid content in *B. juncea.*

Co-dominant SSR markers (FAE1) were used to determine the genetic makeup of all parents and the BC_1F_2 population for the FAE1 locus; however, the markers distinguish between heterozygous and recessive homozygous genotypes.

Molecular data revealed a significant association between erucic acid content and allelic variation at the FAE1.1 locus, with FAE1.1 contributing more substantially to erucic acid accumulation than FAE1.2. However, the current dataset did not provide sufficient evidence to confirm a definitive correlation between erucic acid levels and allelic configuration at FAE1.2. To validate the molecular findings, biochemical analysis of erucic acid content was conducted using gas chromatography in the selected parental lines, BC_1 and BC_1F_2 plants.

Combining molecular data with biochemical investigations confirmed that the parents, RH-749 (40.53 %) and RSPR-03 (41.94 %) had the highest erucic acid values, while PM-21 (1.11 %) and Pusa Karishma (1.69 %) had the lowest levels. Among the BC_1F_2 populations, erucic acid content ranged from 17.12 % to 28.10 %. Specifically, the SSR marker data corresponds to the following values: RSPR-03 × (RSPR-03 ×Pusa Karishma) (17.12 %), RSPR-03 × (RSPR-03 × PM -21) (25.02 %), RH-749 × (RH-749 ×Pusa Karishma) (28.10 %) and RH-749 × (RH-749 × PM-21) (18.48 %).

Low-erucic acid genotypes that will be helpful in future mustard breeding programs can be developed from the two selfed generations of (RSPR-03 × (RSPR-03 × Pusa Karishma)) and (RH-749 × (RH-749 × PM-21)). The medium to high erucic acid concentration of the four F_1 plant crosses-RSPR-03 ×Pusa Karishma, RSPR-03 × PM-21, RH-749 × Pusa Karishma and RH-749 × PM-21-was similar to that of RSPR-03 and RH-749, suggesting that a recessive gene or genes regulate low erucic acid.

Acknowledgements

Authors wish to thank Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Authors' contributions

SK guided the overall research and participated in the design of the study. KR carried out the molecular genetic studies and field experiments drafted the manuscript. KP carried out the field experiments and drafted the manuscript. RB participated in conducting agronomical trials in the field. BK participated in the design of the study and performed the statistical analysis. RS conceived of the study and participated in its design and coordination. AS helped in designing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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