PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.10177

eISSN 2348-1900

RESEARCH ARTICLE

Genetic analysis of Indian x East European $F_{2:3}$ cross for yield traits and erucic acid in *Brassica juncea* (L.) Czern. & Coss.

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Received: 22 June 2025; Accepted: 30 September 2025; Available online: Version 1.0: 12 December 2025

Cite this article: Tushadri S, Gupta SK, Ravinder S, Rubby S, Nashra A, Akriti S, Preety R. Genetic analysis of Indian x East European F2:3 cross for yield traits and erucic acid in *Brassica juncea* (L.) Czern. & Coss. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.10177

Abstract

The quality of oil in *Brassica juncea* is significantly influenced by its erucic acid content, highlighting the need for developing low-erucic acid genotypes through Marker-Assisted Selection (MAS). This study aimed to characterize 175 segregating F_3 genotypes derived from Indian genetic backgrounds from cross between Indian (Pusa Mehak) and East European (Primus) parents using Cleaved Amplified Polymorphic Sequence (CAPS) markers followed by restriction digestion for Fatty Acid Synthesase (*FAE*) gene's two paralogs- *FAE1.1* and *FAE1.2* genes, which govern erucic acid biosynthesis. The genotypic classification based on amplification and restriction digestion revealed six distinct allelic configurations, with *FAE1.1* exhibiting a stronger correlation with erucic acid content than *FAE1.2*. To validate these genetic findings, the biochemical profiling of oil samples by gas chromatography confirmed a bimodal distribution of erucic acid (17.45–50.92 %), with homozygous recessive e_1e_1 genotypes displaying significantly lower erucic acid (22.26 % and 24.44 %) compared to heterozygous and dominant homozygous genotypes (27.29–45.20 %). However, *FAE1.2* did not show a clear association with erucic acid content, indicating its limited contribution to the trait. Gene *FAE1.2*'s limited role in determining erucic acid levels compared to *FAE1.1*, could shift selection priorities towards focusing on *FAE1.1*, making it the primary target for MAS in breeding programs. The study validated MAS as an efficient strategy for early-stage selection of low-erucic acid genotypes, potentially reducing breeding cycles and accelerating the development of superior Indian mustard varieties. The identified seven low-erucic acid genotypes hold promise for breeding programs aimed at improving oil quality. The integration of MAS with morphological and physiological selection criteria could further enhance breeding efficiency and facilitate development of high-yielding, nutritionally improved *B. juncea* varieties.

Keywords: *Brassica juncea*; CAPS markers; erucic acid; *FAE1.1*; *FAE1.2*; gas chromatography; MAS

Introduction

The genus *Brassica* has a long history of domestication, dating back to approximately 500 BCE. Multiple independent domestication events have been recorded, originating from diverse progenitors across distinct geographical regions, including China, Eastern India and the Caucasus-currently recognized as major centres of genetic diversity (1-2). Molecular and biochemical analyses corroborate this, delineating two geographically distinct gene pools: the Chinese and the Indian (3-4). Among *Brassica* species, Indian mustard (*Brassica juncea* L. Czern. & Coss.) exhibits the highest acreage and production in India. The Ministry of Agriculture and Farmers' Welfare reported a record production of rapeseed-mustard at 12.873 million metric tonnes for the 2024-25 period (5).

Despite its agronomic significance, *B. juncea* oil in India is characterized by an inherently high content of erucic acid (C22:1), constituting 34-50 % of its total fatty acid profile. Elevated erucic acid levels pose health risks, including hypercholesterolemia and myocardial infarction (6-7). Given global regulatory benchmarks, reducing erucic acid to below 2 % of the total fatty acid content is imperative to align with international dietary standards.

The development of Low-erucic acid (LEA) mustard necessitates the introgression of recessive alleles from donor genotypes such as Zero Erucic Mutant1 i.e. Zem 1 and its derivative Heera through backcross breeding, employing an elite Indian mustard variety as the recurrent parent (8). However, this conventional breeding approach is labour-intensive, requiring successive backcrossing, selfing in each generation to isolate homozygous zero-erucic acid plants.

Recent advancements in molecular breeding have enabled the deployment of markers closely linked to the erucic acid trait, expediting selection efficiency. The erucic acid content in *Brassica* species is primarily regulated by the Fatty Acid Elongase 1 (*FAE1*) gene, which encodes the enzyme β-ketoacyl-CoA synthase. This enzyme plays a crucial role in the initial step of a four-step enzymatic process that leads to the synthesis of Very Long-Chain Monounsaturated Fatty Acids (VLCMFAs), a key component in the biosynthesis of erucic acid (9–11). Mutations in the coding sequences or regulatory regions, such as Single Nucleotide Polymorphisms (SNPs), InDels (Insertion Deletions) and transposable element insertions, are the primary causes of *FAE1* gene dysfunction, which leads to a reduction in VLCMFA levels in seeds. The identification of polymorphisms within candidate genes controlling erucic acid biosynthesis facilitates early selection of heterozygous carriers in backcross generations (11).

The inheritance of LEA content follows an additive genetic model, controlled by two major genes, making the introgression process relatively straightforward through backcrossing (11–12). However, the precise selection of high-erucic acid homozygotes remains challenging due to phenotypic overlaps with heterozygous intermediates. Molecular mapping of *FAE1* gene responsible for the elongation of C18:1 to C22:1 has enabled the identification of SNPs (variations in a single nucleotide base pair in the DNA sequence, which can be used as markers to identify genetic differences between individuals or populations) in genes *FAE1.1* and *FAE1.2*, which co-segregate with quantitative trait loci governing erucic acid content (13). These SNP-based markers have been validated for MAS in Indian and East European mustard gene pools.

Two SNPs in the coding DNA sequence of *FAE1.1* gene at 591 bp and 1265 bp position were identified to be polymorphic between high erucic acid and low erucic acid genotypes while one SNP was found to be polymorphic between different genotypes at *FAE1.2* locus identified at position 237. The presence of these SNPs leads to alteration in the recognition site of restriction enzymes either by changing it or creating it. In this case, the SNPs at 591 bp and 1265 bp in *FAE1.1* gene and at 237 bp position upstream of promotor region in *FAE1.2*, create/change the restriction site for *Hpy99I*, *BglII* and *MnlI* restriction enzymes, respectively (13). This helped to differentiate between the genotypes with low and high erucic acid content thereby facilitating in the early identification of LEA genotypes at seedling stage itself.

Given the undesirable effects of high erucic acid in human diet, breeding programs have prioritized the development of LEA varieties (13,14). Two major genetic pools of *B. juncea* are identified: the East-European pool, characterized by low erucic acid content and the Indian pool, which possesses a higher erucic acid profile but superior yield potential under sub-continental conditions. Breeding objectives necessitate the simultaneous improvement of oil quality and yield performance. To address these challenges, strategic hybridization programs incorporating parental genotypes from both the Indian and East European gene pools were deployed in the current study. The present study evaluates $F_{2:3}$ segregating populations derived from interspecific crosses to identify high-yielding, low-erucic-acid genotypes suitable for Indian context. MAS utilizing CAPS markers- CAPS591, CAPS1265, CAPS237 and restriction digestion enzymes *Hpy99I*, *BglII* and *MnlI*, respectively was employed using gene-specific primers targeting polymorphic coding regions governing erucic acid biosynthesis. Molecular screening was complemented by biochemical profiling to validate genetic selection and ensure phenotypic accuracy. The study addresses gaps by integrating phenotypic data and genotypic data and validating the results by biochemical analysis for providing a robust framework to develop superior mustard cultivars with optimized oil quality and yield attributes specially suited to Indian context.

Materials and Methods

Experimental material

The experimental material comprised F_2 population derived from a cross between Pusa Mehak (Indian genotype) x Primus (European genotype). The F_2 population derived from the above cross contained a total of 359 plants in 2019-20. This F_2 population was used for the development of F_3 plants. The individual F_3 plants of each genotype were sown in 4 rows, making a plot of 2.70 sq. m (3 m x 0.9 m) with row-to-row distance of 30 cm and plant to plant distance of 10 cm. The standard dose of fertilizer (80 kg N + 40 kg P_2O_5 + 30 kg K_2O per hectare) and plant protection measures were adopted for raising a good crop. The sowing of segregating populations was done during winter seasons of 2019-20 and 2020-21.

Molecular methods

Genomic DNA isolation protocol

The genomic DNA isolation for 175 genotypes was carried out using CTAB method (15) and purified using 2 µL of RNase A (10 mg/mL). Quality of genomic DNA was quantified using gel electrophoresis on a 0.8 % agarose gel (gel intensity).

Primers for PCR amplification

CAPS markers (Table 1) (13) were used for genomic DNA amplification (synthesized by Bioserve Biotechnologies, India).

PCR reaction

Polymerase Chain Reaction (PCR) amplification was carried out as per the conditions outlined in Table 2, using Red Taq Ready Mix (with $MgCl_2$) from Sigma-Aldrich.

PCR amplification profile

PCR tubes containing all the reagents along with template DNA were thoroughly mixed and subjected to the thermal profile given (Table 3) in a gradient Mastercycler. The amplified product was stored at 4 °C.

Table 1. CAPS markers and their respective restriction enzymes

Gene	Primer name	Primer sequence	Restriction enzyme	Amplicon size (bp)
FAE1.1	CAPS591	F- TCGTGGCTTGACTTCTTGAG R- GGACCTATTATCACCAGCGTAAA	*Hpy99I*	432
FAE1.1	CAPS1265	F- ACGTTAGGTCCGTTGATTCTTC R- GGGTATCTGTCGATGCAATGT	*BglII*	427
FAE1.2	CAPS237	F- TAACCATCGCTCCACTCTTTG R- TCAAGAAGTCAAGCCACGAC	*MnlI*	219

Table 2. Components of PCR reaction

Reagents	Concentration	Quantity (µL)
Template DNA	10 ng	2.0
PCR grade sterile water	-	6.0
Forward primer	10 µM	1.0
Reverse primer	10 µM	1.0
Red Taq Ready Mix	-	15.0
Total volume		25.0

Table 3. Thermal profiles for DNA amplification

Steps	Cycles	Temperature (°C)	Duration
Initial Denaturation	1	94	4 min
Denaturation	40	94	30 sec
Annealing	40	55	30 sec
Extension	40	72	30 sec
Final Extension	1	72	5 min

The annealing temperature for all three primers was standardized at 55 °C

A 4 µL aliquot of the 25 µL PCR product was loaded onto 3 % agarose gel mixed with 3 µL DNA loading dye and 3 µL of double distilled water (for balancing volume) to determine the level of amplification of DNA.

Restriction enzyme digestion

The amplified PCR products (after checking for level of amplification) were digested using restriction endonuclease enzyme in a final reaction volume of 15 µL and were incubated in thermocycler after mixing properly (by pipetting and vortexing) at 37 °C for 5-15 min for *BglII* and *MnlI* and 1 hr for *Hpy99I* after which inactivation at 67 °C for 20 min was followed. The components for restriction digestion along with their volumes are given in Table 4.

CAPS PCR banding profile

The restriction digested amplified products were subjected to electrophoresis on 3.5 % agarose gel. In each PCR tube (containing the restriction digested amplified product), 3 µL of DNA loading dye was added and thoroughly mixed and loaded into separate wells in the gel. DNA ladder (100 bp) was used as molecular weight marker for determining the molecular weights of restriction digested amplified PCR products. Electrophoresis was carried out at 120 V for 1.5 hr and then visually examined under UV and documented using the gel documentation system.

Extraction of fatty acids and estimation of erucic acid

The estimation of erucic acid was carried out using Gas Chromatography (GC) based method. For extraction of fatty acids, a total of 20–25 seeds were ground in a 5.00 mL culture tube to a fine powder using pestle and mortar. To it, 0.50 mL of hexane was added and incubated at room temperature for 16 hr (overnight incubation). After incubation, the supernatant hexane layer was transferred to a fresh tube and 0.50 mL of sodium methoxide was added (prepared by adding 80.00 mg of NaOH to 100.00 mL of methanol). The mixture was incubated at room temperature for 45 min. Following incubation, 7.50 mL of NaCl solution was added (prepared by adding 8.00 g of NaCl to 100.00 mL of distilled water) and vortexed. After vortexing, the mixture was left at room temperature for approximately 30 min and the upper phase was collected and used for injection into the GC system. Erucic acid percentage was calculated using the GC system's integrated software.

Results

Characterization of selected Indian type genotypes using CAPS markers

A total of 175 Indian type genotypes selected from a segregating F_3 population were used for molecular characterization using CAPS markers reported earlier for *FAE1.1* and *FAE1.2* genes (Fig. 1). Isolated DNA was quantified using gel electrophoresis (Fig. 2).

Digestion of CAPS591 amplicons with *Hpy99I* enzyme yielded three distinct fragments in high erucic acid genotypes: an uncut 432 bp fragment corresponding to *FAE1.2* locus and two digested fragments of 224 bp and 198 bp derived from *FAE1.1*. In contrast, no cleavage was observed in low erucic acid genotypes (Fig. 3 & 4). Likewise, digestion of CAPS1265 amplicons with *BglII* enzyme produced three fragments in LEA genotypes-an undigested *FAE1.2* fragment and two digested fragments (209 bp and 198 bp) from *FAE1.1* locus. Only a single uncut fragment was detected in HEA genotypes (Fig. 5 & 6).

For the *FAE1.2* locus CAPS237 generated a 219 bp amplicon. Upon digestion with *MnlI*, both *FAE1.1* and *FAE1.2* alleles were cleaved in HEA genotypes, resulting in fragments of 112 bp and 87 bp. In LEA genotypes, however, only the *FAE1.1* allele was digested, while the *FAE1.2* allele remained intact (Fig. 7 & 8).

Thus, CAPS591 and CAPS1265 are effective co-dominant markers for distinguishing homozygous and heterozygous genotypes at the *FAE1.1* locus within breeding populations. In contrast, CAPS237 functions as a dominant marker for the *FAE1.2* locus aiding to identify clearly homozygous dominant individuals and not differentiate between heterozygous and recessive homozygous genotypes (Supplementary Table 1).

Based on amplification and restriction digestion (Fig. 1-7), the genotypes of 175 plants were classified into various classes depending upon the allelic configuration at each locus (Table 5). (Supplementary Table 1).

Table 4. Components for restriction enzyme digestion of amplified PCR product

Reagents (µL)	**For digestion with *Hpy99I***	**For digestion with *BglII***	**For digestion with *MnlI***
Amplified DNA product	6.0	6.0	6.0
10X Buffer	1.5	1.5	1.5
Enzyme	5.0	2.0	1.0
Double distilled sterile water	2.5	5.5	6.5
Total volume	15.0	15.0	15.0

Table 5. Allelic configuration of 175 segregating individuals of F_3 population

S. No.	Allelic configuration		No. of plants	Average erucic acid (%)
	FAE 1.1	***FAE 1.2***		
1.	*e1e1*	*E2e2/e2e2*	1	22.26
2.	*e1e1*	*E2E2*	3	24.44
3.	*E1e1*	*E2e2/e2e2*	28	27.29
4.	*E1e1*	*E2E2*	46	34.13
5.	*E1E1*	*E2e2/e2e2*	46	39.66
6.	*E1E1*	*E2E2*	51	45.2

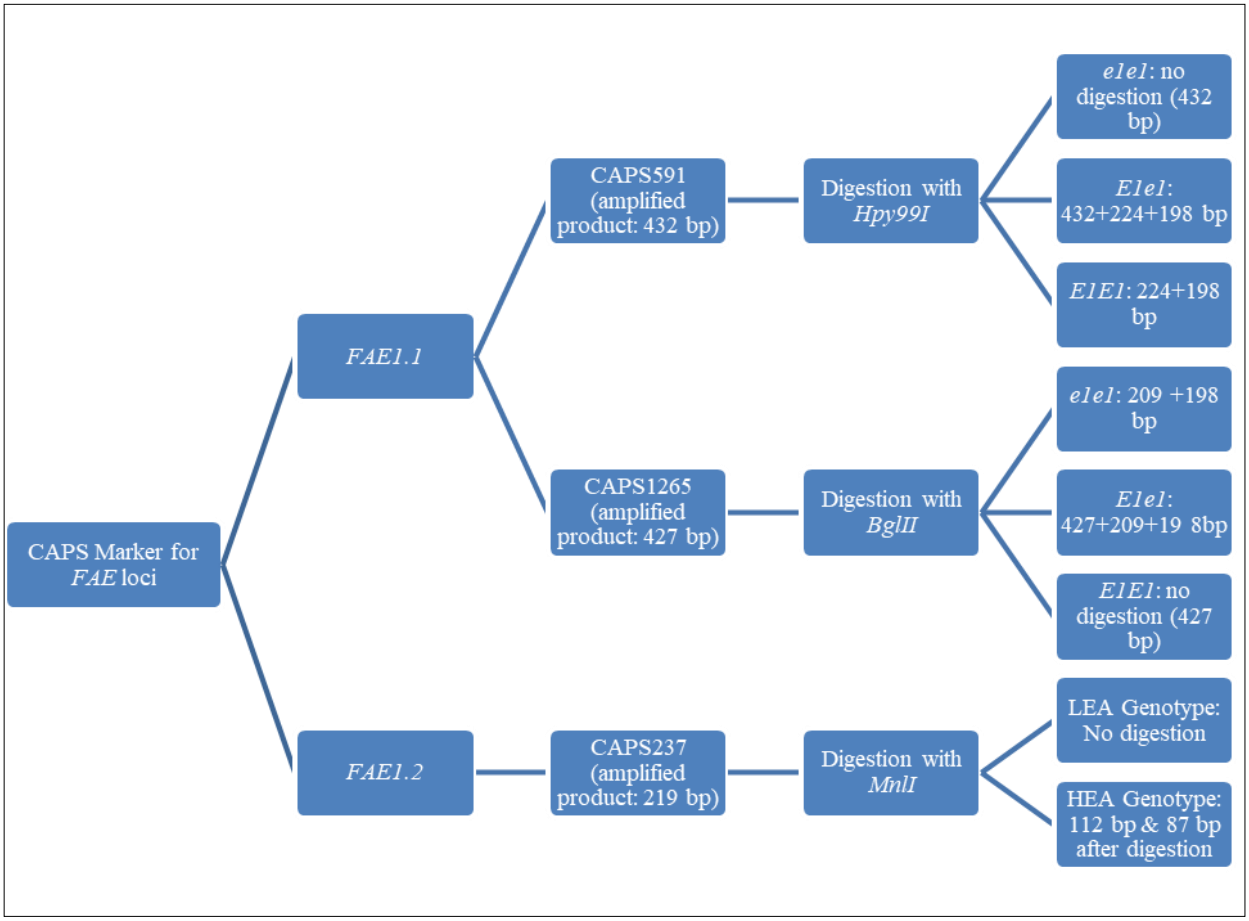

Fig. 1. CAPS marker-based differentiation of *FAE1.1* and *FAE1.2* alleles in HEA and LEA genotypes.

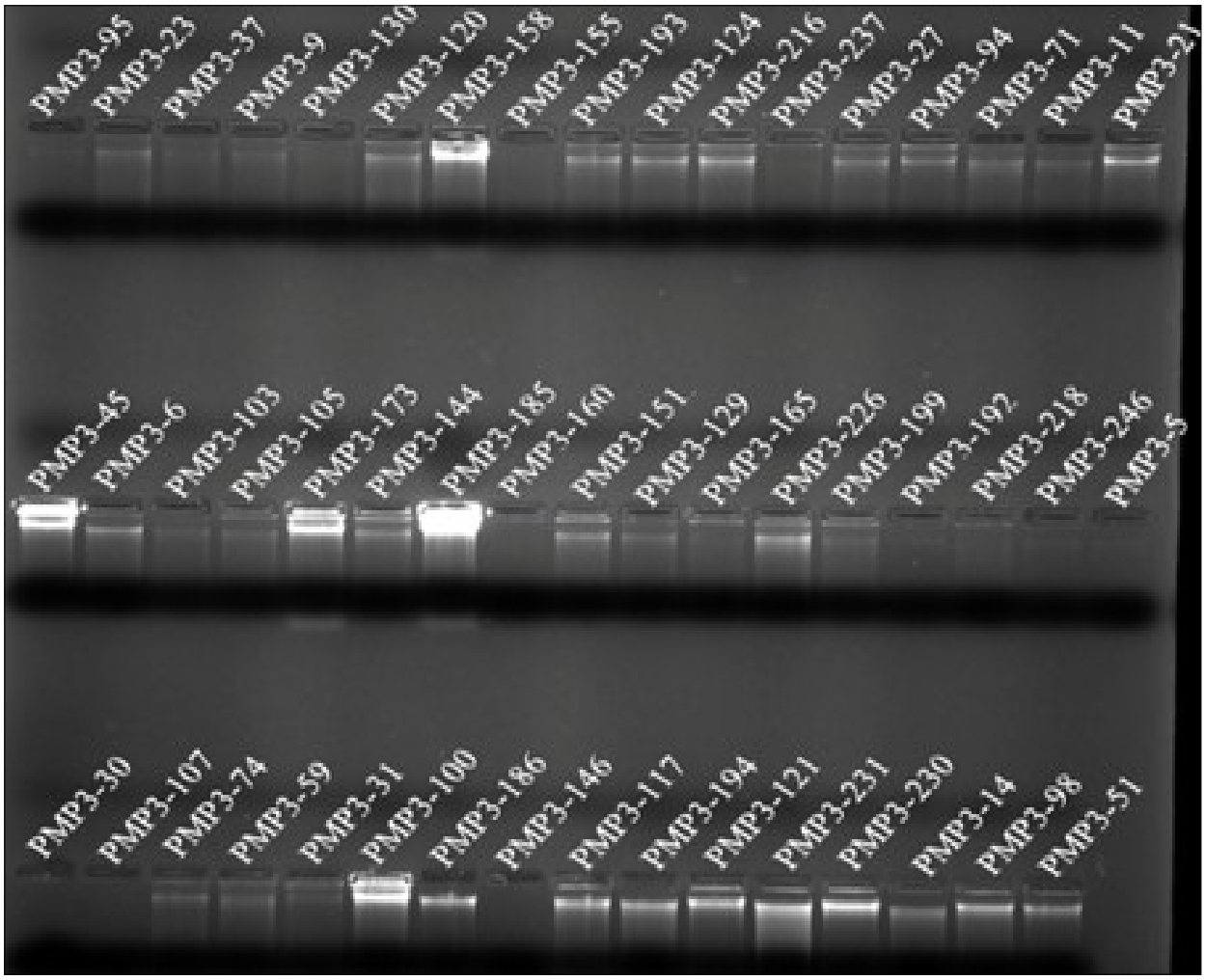

Fig. 2. Isolated DNA as seen on 0.8 % agarose gel. PMP3-1 - PMP3-244: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus).

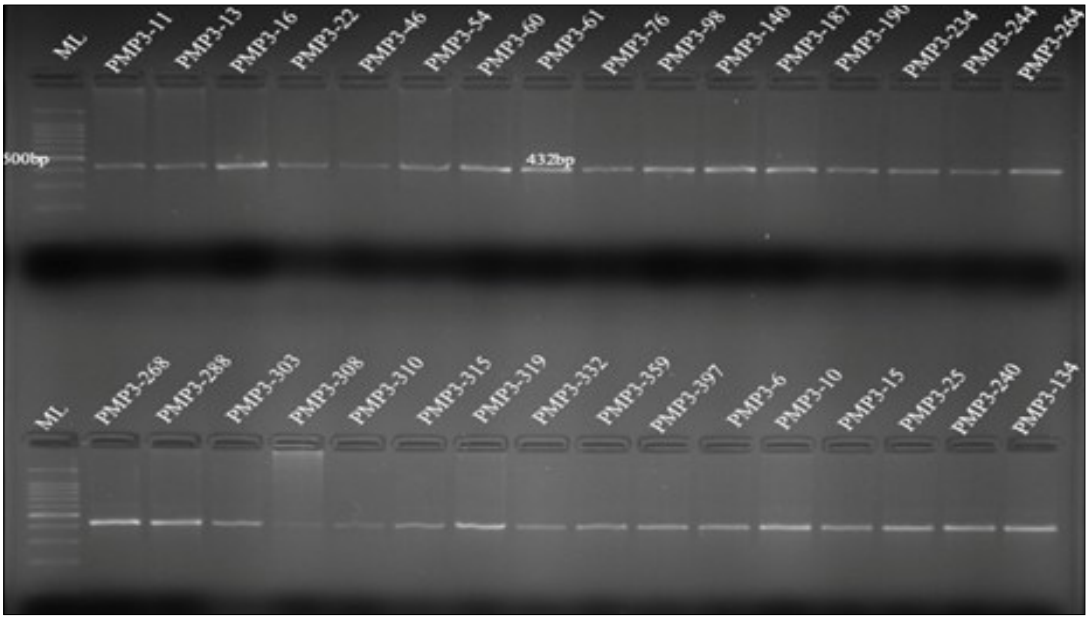

Fig. 3. Amplified PCR product of CAPS591 marker on 3 % agarose gel with amplicon size of 432 bp.

ML: Molecular Ladder of 100 bp; PMP3-11 – PMP3-134: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

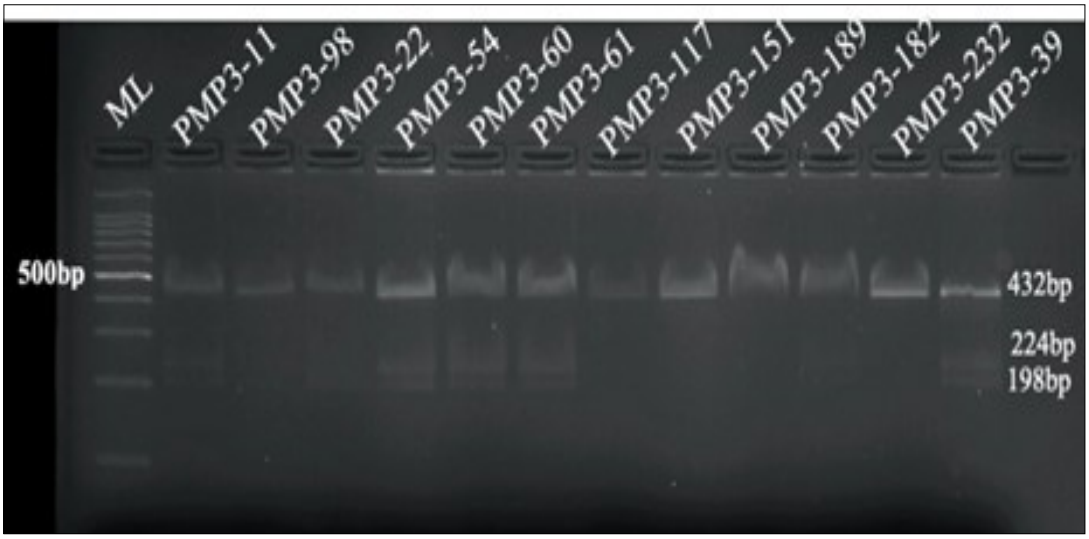

Fig. 4. *Hpy99I* restriction digested PCR amplified product of CAPS591 marker on 3.5 % agarose gel.

ML: Molecular Ladder of 100 bp; PMP3-11 - PMP3-39: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

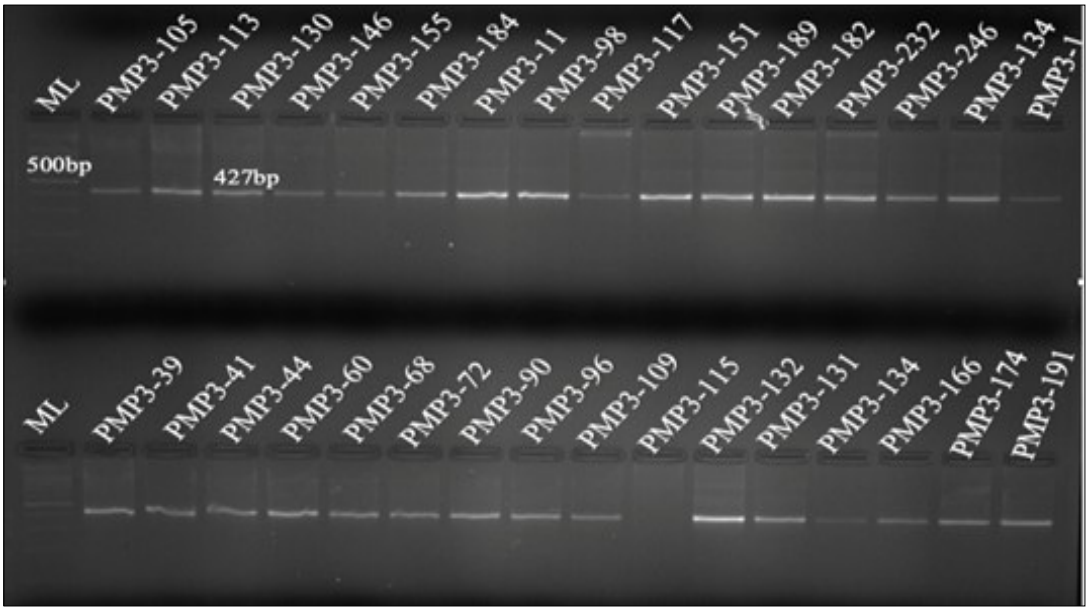

Fig. 5. Amplified PCR product of CAPS1265 marker on 3 % agarose gel with amplicon size of 427 bp.

ML: Molecular Ladder of 100 bp; PMP3-105– PMP3-191: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

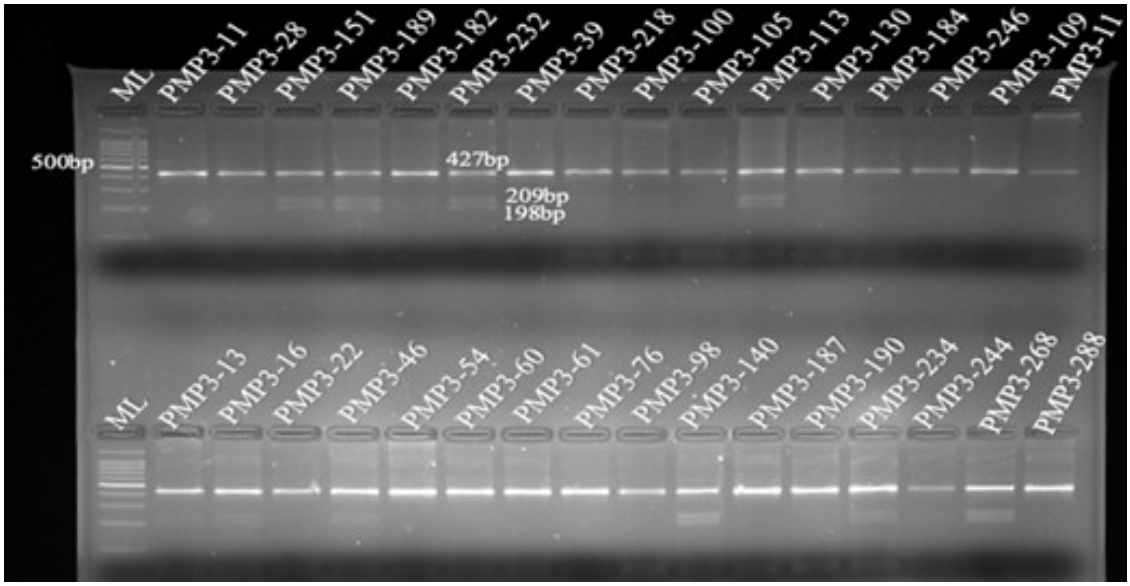

Fig. 6. *BglII* restriction digested PCR amplified product of CAPS1265 marker on 3.5 % agarose gel.

ML: Molecular Ladder of 100 bp; PMP3-11 - PMP3-288: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

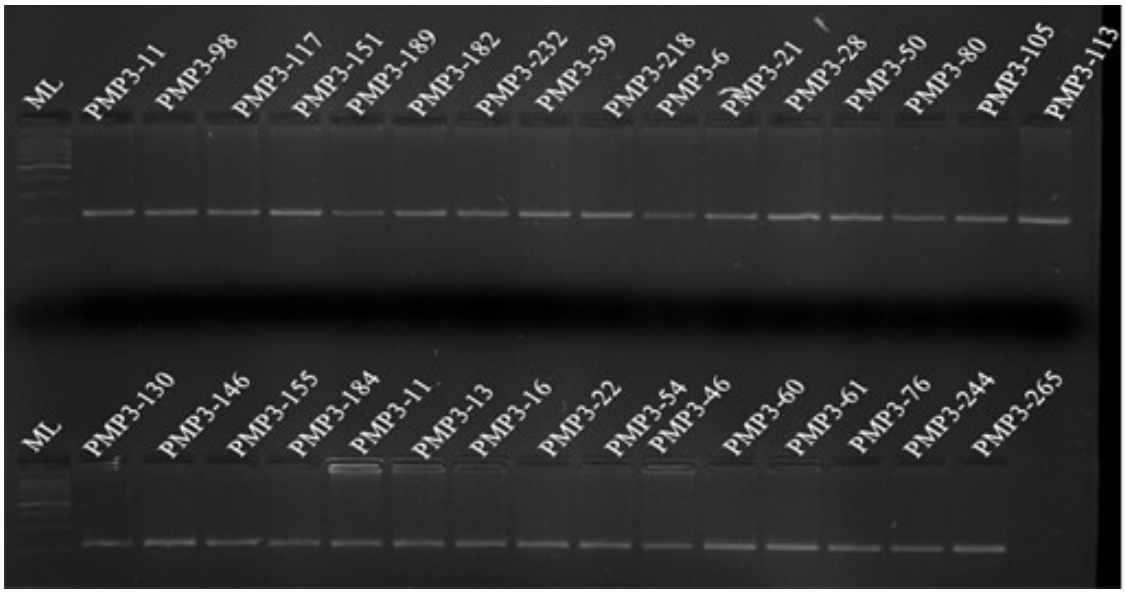

Fig. 7. Amplified PCR product of CAPS237 marker on 3 % agarose gel with amplicon size of 219bp.

ML: Molecular Ladder of 100 bp; PMP3-11 – PMP3-265: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

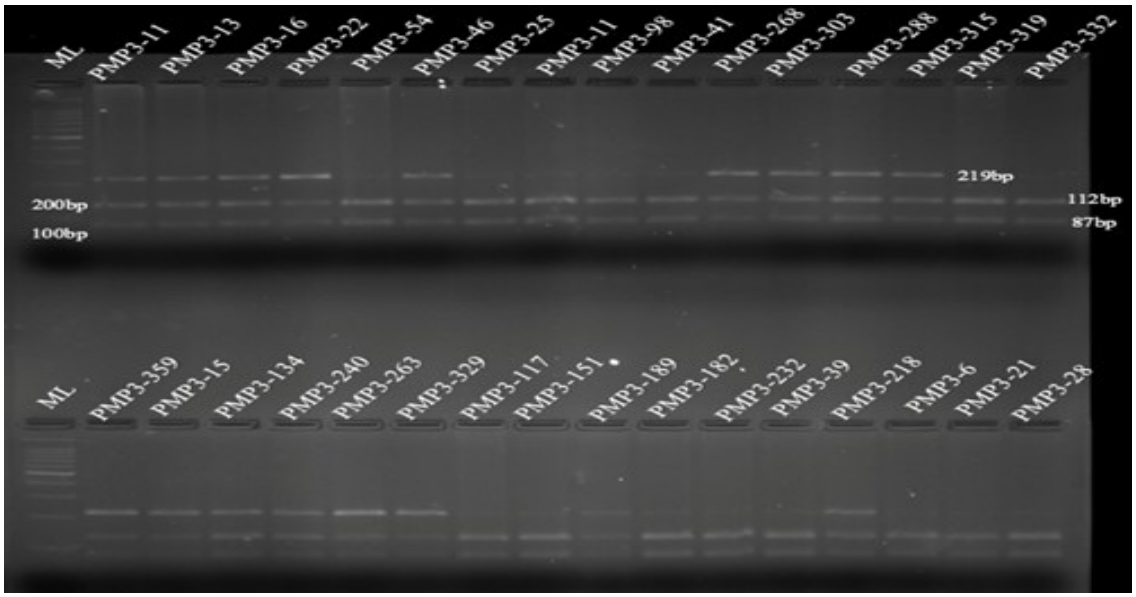

Fig. 8. *MnlI* restriction digested amplified PCR product of CAPS237 marker on 3.5 % agarose gel.

ML: Molecular Ladder of 100 bp; PMP3-11 – PMP3-28: *Brassica juncea* F_3 genotypes (PMP: Pusa mehak X Primus)

Based on the data for erucic acid, genotypes with recessive alleles at the *FAE1.1* locus were found to have, on average, lower erucic acid content (22.26 % and 24.44 %) compared to individuals with either heterozygous or dominant homozygous genotypes, which showed intermediate values of 27.29 % and 34.13 % and higher values (39.66 % and 45.20 %), respectively. On the contrary, the dominant alleles for *FAE1.2* did not show any specific association with individuals from the lower, intermediate or higher percent erucic acid groups.

Characterization of erucic acid in F_3 population

The genotypes with Indian type genetic make-up were selected from the segregating F_3 population based on early flowering. A total of 175 genotypes were selected whose average number of days to 50 % flowering was 80.38, which was closer to the value of Indian parent (58 days) as compared to European parent (137 days). For erucic acid estimation, the retention time (time taken for a compound to pass through the chromatography column) for different fractions of other oils including erucic acid was extrapolated into percent erucic acid present in the oil sample as others. The erucic acid content of 175 F_3 individuals ranged between 17.45 % and 50.92 % with an average of 37.59 %, giving a bimodal distribution (Fig. 9).

Based on biochemical profiling, the erucic acid content of seven F_3 plants selected based on molecular characterization was found to be on the lesser side of the spectrum of erucic acid content (Table 6).

Table 6. Genotypes with lowest erucic acid in F_3 population

S. No.	Genotype No.	Erucic acid content (%)
1.	PMP3-152	17.45
2.	PMP3-160	18.49
3.	PMP3-218	20.76
4.	PMP3-50	22.11
5.	PMP3-101	22.26
6.	PMP3-240	22.40
7.	PMP3-51	22.59

Discussion

Segregation of traits in a cross between Indian and European genotypes

The selection for any trait can be carried out only when there is diversity for that trait (16,17). Therefore, a segregating population with a variety of trait variation would be important for any breeding programme. The F_3 generation obtained from the selfing of F_2 individuals provides a variety of possible and desirable combinations to make selection from (16,17).

Selection of genotypes with Indian genetic constitution

The two gene pools (Indian and East-European) differ for days to flowering and plant height indicating that the gene pools are likely to have gene pool-specific alleles for the two traits (13,18). Therefore, the early flowering trait was used to select Indian type genotypes from the F_3 segregating population. These selected plants were further subjected to MAS using CAPS markers reported to be linked with erucic acid (13).

MAS for erucic acid and validation using biochemical analysis

Molecular markers are useful for selection of desirable genotypes when marker associated with a gene of interest is used. CAPS markers reported in an earlier study that used for the selection of plants at flowering stage (13). Ideally, MAS can be applied at the seedling stage, provided sufficient resources are available (17). MAS was able to broadly classify the 175 individual genotypes into six major classes. It was evident from the groups of genotypes that *FAE1.1* was clearly associated with erucic acid content. The groups with homozygous recessive alleles (*e1e1*) at *FAE1.1* locus were found to have lesser erucic acid, as compared to groups containing heterozygous (*E1e1*) and dominant homozygous (*E1E1*) allelic configuration. However, such association of erucic acid with allelic configuration at *FAE1.2* was not evident from the current set of data. This validated the previous reported fact that *FAE1.1* is a major contributor of erucic acid in *Brassica juncea* (13).

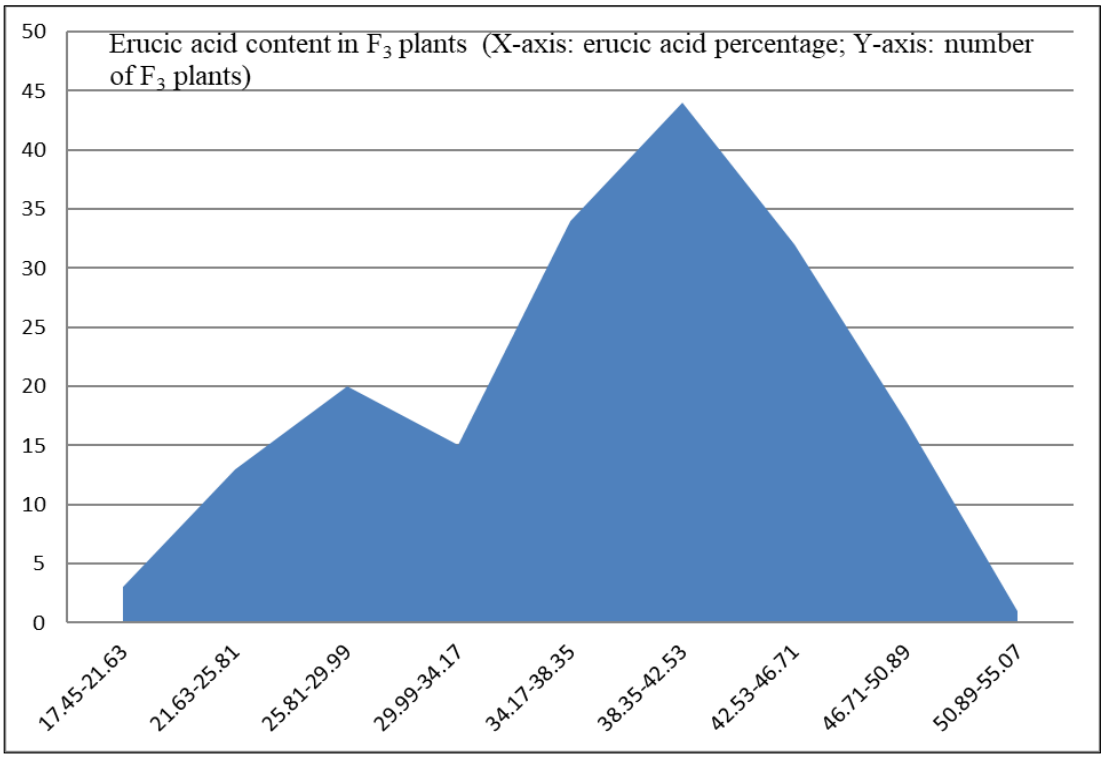

Fig. 9. Erucic acid content distribution in F_3 plants, showing a bimodal distribution.

The results also proved that marker-assisted breeding programme on a large scale can be undertaken for selection of plants with low erucic acid in a segregating population. MAS can significantly reduce the time required to develop low erucic acid varieties. In fact, MAS when combined with other traits including morphological and physiological could prove to be highly beneficial for future Indian mustard breeding programmes.

While MAS has enabled significant progress in breeding LEA mustard lines, its effectiveness is often constrained by the availability of co-dominant markers, linkage drag and incomplete resolution of heterozygotes. A key limitation of the present study lies in the use of the dominant CAPS237 marker for genotyping *FAE1.2* locus, which lacks the ability to distinguish between homozygous recessive (e_2e_2) and heterozygous (E_2e_2) genotypes. This restricts its utility in MAS, particularly when accurate zygosity determination is essential for advancing true-breeding lines. As a result, the application of CAPS237 may lead to overestimation of the dominant allele frequency in segregating populations.

To overcome these limitations, CRISPR/Cas9 genome editing offers a transformative alternative by enabling precise, targeted disruption of the *FAE1* gene family directly in elite genetic backgrounds. In recent studies (19), simultaneous knockout of *FAE1.1* and *FAE1.2* homeoalleles using CRISPR/Cas9 in high erucic acid cultivars (PCR7 and JD6) resulted in near-complete elimination of erucic acid (<0.5 %) and a substantial increase in health-promoting fatty acids like oleic, linoleic and linolenic acids, without compromising agronomic performance. This genome editing approach bypasses the generational time and marker dependency inherent in MAS and allows breeders to generate true -breeding LEA lines rapidly and precisely, thereby accelerating the development of nutritionally superior *B. juncea* cultivars tailored for food-grade oil production.

The recent availability of a Telomere-to-Telomere (T2T), gap-free genome assembly of *B. juncea* 'Wuqi' further amplifies the potential of CRISPR-based approaches in our research (20). The high-quality reference genome constructed using PacBio HiFi, Oxford Nanopore and Hi-C technologies, offers unprecedented resolution for gene annotation, promoter region analysis and off-target prediction, thereby enabling more accurate guide RNA design and editing specificity. Leveraging this T2T assembly, we can now implement precise and efficient genome editing strategies for *FAE1* loci with full confidence in genome context, ultimately facilitating the development of next-generation, true-breeding LEA mustard varieties for safe and nutritionally enriched oil production.

Lowered erucic acid led to a corresponding increase in oleic acid (21) (Supplementary Table 1) a monounsaturated fatty acid known for its cardiovascular and metabolic health benefits.

Conclusion

The molecular analysis of 175 F_3 genotypes using CAPS markers revealed the genetic constitution of the 175 F_3 genotypes at *FAE1.1* and *FAE1.2* loci controlling erucic acid content in *Brassica juncea*. Based on the molecular profiling, six groups based on genotypic configuration at both loci were obtained, corresponding to erucic acid content. The results further validated that the *FAE1.1* gene contributed more to erucic acid content than the *FAE1.2* gene and a clear association was observed between erucic acid content and the *FAE1.1* gene. However, such association of erucic acid with allelic configuration at *FAE1.2* was not evident from the current set of data.

Further, lower erucic acid changes overall oil composition leading to a higher oleic acid (C18:1) and a moderate increase in linoleic acid (C18:2, an omega-6 fatty acid). This altered profile makes the oil composition more similar to canola-quality oil, which is considered healthier for human consumption due to its low saturated fat and favourable unsaturated fat content.

From the results of this study, the seven LEA genotypes identified would form a valuable resource of developing low erucic acid Indian type genotypes in future breeding programmes.

Acknowledgements

Authors thank the Department of Genetics and Plant Breeding, SKUAST Jammu and School of Biotechnology, SKUAST-Jammu.

Authors' contributions

TS, SKG and RS[1] contributed to the conceptualization of the study. TS and RS[1]were responsible for the methodology, validation, formal analysis and investigation. The original draft was prepared by TS, while TS, SKG, RS[1], RS[2], AS and PR contributed to review and editing. Visualization was carried out by TS and supervision was provided by TS, RS[1] and SKG. All authors read and approved the final manuscript. [RS[1]stands for Ravinder Singh and RS[2] stands for Rubby Sandhu].

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process: During the preparation of this work the authors used ChatGPT 4.0 to change references to Vancouver style. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

References

1. Hemingway JS. Mustard: *Brassica* spp. and *Sinapis alba* (Cruciferae). In: Simmonds NW, editor. Evolution of crop plants. London: Longmans; 1976. p. 56-9
2. Prakash S, Hinata K. Taxonomy, cytogenetics and origin of crop Brassicas: a review. Opera Bot. 1980;55:53-7.
3. Vaughan JG, Gordon EI. A taxonomic study of *Brassica juncea* using electrophoresis, gas liquid chromatography and serology. Ann Bot. 1973;37:167-84. https://doi.org/10.1093/oxfordjournals.aob.a084670
4. Song KM, Osborn TC, Williams PH. *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLPs). Theor Appl Genet. 1988;75:784-95. https://doi.org/10.1007/BF00265606
5. India. Ministry of Agriculture & Farmers' Welfare. Second advance estimates of production of major agricultural crops (Kharif & Rabi) for 2024–25. New Delhi: Press Information Bureau; 2025.
6. Renard S, McGregor S. Antithrombogenic effect of erucic acid poor rapeseed oil in rats. Rev Fr Crop Cros. 1992;23:393-6.

7. Mortuza MG, Dutta PC, Das ML. Erucic acid content in some rapeseed/mustard cultivars developed in Bangladesh. J Sci Food Agric. 2006;86:135-9. https://doi.org/10.1002/jsfa.2301
8. Sodhi YS, Mukhopadhyay M, Arumugam N, Verma JK, Gupta V, Pental D, et al. Genetic analysis of total glucosinolate in crosses involving a high glucosinolate Indian variety and a low glucosinolate line of *Brassica juncea*. Plant Breed. 2002;121:508-11. https://doi.org/10.1046/j.1439-0523.2002.00747.x
9. James DW, Lim E, Keller J, Plooy I, Ralston E, Dooner HK. Directed tagging of the *Arabidopsis* fatty acid elongation 1 (*FAE1*) gene with the maize transposon activator. Plant Cell. 1995;7:309-19. https://doi.org/10.1105/tpc.7.3.309
10. Millar AA, Kunst L. Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. Plant J. 1997;12:121-31. https://doi.org/10.1046/j.1365-313X.1997.12010121.x
11. Kirk JTO, Hurlstone CJ. Variation and inheritance of erucic acid content in *Brassica juncea*. Z Pflanzenzücht. 1983;90:331-8.
12. Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK. Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and single nucleotide polymorphisms in *FAE1* gene. Theor Appl Genet. 2004;108:743-9. https://doi.org/10.1007/s00122-003-1481-z
13. Saini N, Singh N, Kumar A, Vihan N, Yadav S, Vasudev S, et al. Development and validation of functional CAPS markers for the *FAE* genes in *Brassica juncea* and their use in marker-assisted selection. Breed Sci. 2016;66(5):831-7. https://doi.org/10.1270/jsbbs.16132
14. Saikia SL, Rai GK, Salgotra RK, Rai RK, Singh M, Rai PK. Erucic acid and glucosinolate variability in *Brassica juncea* L. Int J Chem Stud. 2018;6(6):1223-6.
15. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13-15. https://doi.org/10.2307/2419362
16. Bhandari HR, Bhanu AN, Srivastava K, et al. Assessment of genetic diversity in crop plants - an overview. Adv Plants Agric Res. 2017;7(3):279-86. https://doi.org/10.15406/apar.2017.07.00255
17. Cobb J, Biswas P, Platten J. Back to the future: revisiting MAS as a tool for modern plant breeding. Theor Appl Genet. 2019;132:1-14. https://doi.org/10.1007/s00122-018-3266-4
18. Saini N, Yashpal, Koramutla MK, Singh N, Singh S, Singh R, et al. Promoter polymorphism in *FAE1.1* and *FAE1.2* genes associated with erucic acid content in *Brassica juncea*. Mol Breed. 2019;39:75. https://doi.org/10.1007/s11032-019-0971-x
19. Patra N, Barker GC, Maiti MK. Knockout of fatty acid elongase1 homeoalleles in amphidiploid *Brassica juncea* leads to undetectable erucic acid in seed oil. Plant Physiol Biochem. 2025;222:109679. https://doi.org/10.1016/j.plaphy.2025.109679
20. Zhang R, Dai C, Gong R, Li K, Zhang C, An Z, et al. Gap-free genome assembly and pan-genome of *Brassica juncea* provide insights into seed quality improvement and environmental adaptation. Plant Commun. 2025;6(6):2590-3462. https://doi.org/10.1016/j.xplc.2025.101298
21. Sharma S, Bala M, Kaur G, Tayyab S, Feroz SR. Chemical composition of oil and cake of *Brassica juncea*: Implications on human and animal health. In: Kole C, Mohapatra T, editors. The *Brassica juncea* genome. Compendium of plant genomes. Cham: Springer; 2022. p. 29-55 https://doi.org/10.1007/978-3-030-91507-0_3

Additional information

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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