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Deciphering the Information on Inheritance Mode of Erucic Acid Trait in Indian Mustard (*Brassica juncea* L.)

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ABSTRACT

Background: Indian mustard [*Brassica juncea* (L.) Czern and Coss] is most important oilseed crop grown in *Rabi* season and have been used as a source of edible oil in the Indian subcontinent. Low use of mustard oil is basically due to the presence of high content of erucic acid which not only deteriorate the quality of oil but also have harmful effects on human body.

Methods: Knowledge of erucic acid trait inheritance is crucial so as to define the breeding strategies for erucic acid trait in Indian mustard. In present study the experiment to study mode of inheritance of erucic acid trait was carried out among progeny of crosses involving high erucic acid (NRCHB-101) and low erucic acid (Heera and PDZ-1) cultivars of *Brassica juncea*. The study was conducted using six generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 derived from the crosses, NRCHB-101 × Heera and NRCHB-101 × PDZ-1.

Result: The content of erucic acid of F_1's was found intermediate between the parents while in F_2 seeds itwas segregated into 5 classes *i.e.*, <2%, 2-10%, 11-20%, 21-30% and >30% with a genotypic ratio of 1:4:6:4:1. Backcross seeds (B_1 and B_2) derived from the F_1 backcrossing with high erucic acid and low erucic acid parents were classified into three classes with a ratio of 1:2:1. The segregation patterns confirmed that in case of *B. juncea* two genes are responsible for governance of erucic acid trait and these genes are inherited in additive manner. The results of this investigation also showed that inheritance of erucic acid content trait is under embryonic control with absence of maternal effect in *B. juncea*. This understanding of *B. juncea's* erucic acid inheritance will help breeders create *B. juncea* cultivars with zero erucic acid.

Key words: *Brassica juncea,* Digenic, Erucic acid, Inheritance.

INTRODUCTION

Rapeseed-mustard is India's second most important edible oilseed crop, contributing around 5% of gross national product and 10% of the value of agricultural products and also among seven other crops cultivated in India it contributes to nearly 25% of total oilseed production (Anonymous, 2015; Jat *et al.*, 2019). In India demand of edible oil is increasing day by day with increasing population and living standards of the people which results in short supply of edible oil which is being met by importing oil from other countries. People nowadays are concerned not only with what they eat, but also with how the ingredients they ingest affect their health. Oil, protein, fiber, moisture and extractable compounds are all present in mustard seed in varying proportions: 34-45%; 17-25%; 8-10%; 6-10%; and 10%-12% respectively (Pandey *et al.*, 2013). Mustard oil's nutritional value is governed by the amount of oil and the proportions of saturated, mono-saturated and polyun saturated fatty acids (Friedt and Snowdon, 2009). Erucic acid, which makes up nearly 40% to 60% of the total fat in Indian rapeseed-mustard oil, is both excessive and undesirable in edible oil because it has long-term side effects (Mandal *et al.*, 2002; Chauhan *et al.*, 2007).

On the other hand, it is also seen that a very high percentage of unsaturated fatty acid is found in Rapeseed mustard oil whereas saturated fatty acid contributes to only 7% of total fatty acid. In conventional Indian mustard cultivars, the most abundant fatty acid found to be around 50% is Erucic acid (C22:1), whereas percentage of oleic acid found is nearly 15 to 20% which is lower than the Erucic acid percentage. Among the oilseed crops, unsaturated fatty acids contribute to only 5-7% in Indian mustard oil whereas substantial amounts of essential fatty acids such as linolenic and linoleic acid are also found. Long term use of edible oil containing high percentage of erucic acid pose serious health risks such as triglycerides accumulation in heart which results in lowering of myocardial conductivity and it also affects the blood cholesterol level in the body and also results in lipid deposition and malnutrition among humans (Gopalan *et al.*, 1974; Renard and McGregor, 1976; Ackman *et al.*, 1977). Basically, erucic acid content in the genus *Brassica* depends on various factors and varies accordingly. It varies with the allelic composition, genetic background; genotypes with differences in their level of ploidy and regardless of these factors environmental impact also has decisive role

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in erucic acid content. According to previous findings, embryonic genotype controls erucic acid in the oil, which is regulated by one gene in diploid species, *B. rapa* (AA) and two genes in amphidiploids species, *B. napus* (AACC). Kirk and Hurlstone (1983) confirmed that the genes for erucic acid content in *B. juncea* are inherited in additive manner. In *B. napus* and *B. rapa*, a series of alleles have been found, allowing for the breeding of strains with erucic acid levels in seed oil that ranges from less than one percent to nearly 60 per cent of total fatty acids. In recent years, Indian mustard breeding programs have paid more and more attention to the development of low-erucic acid varieties. In order to make Indian varieties meet international quality standards, there is an urgent need to concentrate efforts to obtain varieties with low erucic acid content *i.e.*, below 2%. Priyamedha *et al.* (2021) deployed double zero (less than 2 per cent erucic acid in seed oil and less than 30 micromoles glucosinolate in seed meal) trait and also white rust trait in improved backgrounds in *B. juncea.* For selecting an efficient breeding programme knowing the mechanism of inheritance and the number of genes that influence erucic acid inheritance is critical for generating low erucic acid rapeseed-mustard cultivars. There is a dearth of knowledge about Indian mustard's genetics when it comes to erucic acid (Tiwari, 1995; Potts and Daryl, 1999). However, there are multiple studies on the inheritance of erucic acid in *Brassica napus* and *Brassica rapa* (Fernandez-Escobar *et al.*, 1988; Luhs and Friedt, 1995). In the present study, the genetics of Indian mustard erucic acid content was explored, which is critical for developing appropriate breeding procedures for the enhancement of low erucic acid trait in Indian mustard.

Materials and Methods

Experimental material and sowing conditions

The experiment for present study was carried out at experimental field at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India during *rabi* cropping seasons consecutively for three years (2015-18). Experimental material for present study consisted of one popular variety NRCHB-101 (recipient parent), having high erucic acid content (>30%) and two genotypes/varieties with low erucic acid (<2%) *viz.* Heera and PDZ-1 (donor parents) of *B. juncea*. The crosses *i.e.*, NRCHB-101×Heera and NRCHB-101×PDZ-1 were attempted during *rabi* 2015-16. For generation of backcrosses,resultant F_1 plants were backcrossed with recipient parent to produce B_1 and donor parents of respective cross to produce B_2, also F_1 plants are selfed to produce F_2 seed of respective cross during *rabi* 2016-17. Seeds from parents, F_1 s, F_2, B_1 and B_2were screened with molecular markers linked to erucic acid in their subsequent sowing seasons. To study inheritance pattern of erucic acid, the F_1s, F_2, B_1 and B_2 generations of two crosses (NRCHB-101×Heera and NRCHB-101×PDZ-1) were sown along with their parents (NRCHB101, PDZ-1 and Heera) during *rabi* 2017-18 in randomized complete block design. Each progeny was sown with different number of rows in a plot of 5 m length with 30 cm spacing of among rows and 15 cm between plants. Parents and F_1s were sown in two rows each, backcrosses (B_1 and B_2) in three rows each and F_2 progeny in five rows. Standard agronomic practices for raising healthy crop were followed in each rabi season. For erucic acid analysis thenumber of plants harvested ranged from 10-14 for P_1 and P_2, 15-20 for F_1, 112-144 for F_2 and 52-96 for backcross generations (B_1 and B_2) (Table 1).

Erucic acid content estimation using GLC

The analysis of erucic acid content of parent's seed, F_1s, F_2, B_1 and B_2 generations was performed using a Gas Liquid Chromatography (GLC) (Perkin Elmer Clarus 600). This GLC is based on flame ionization detector (FID) and analysis was carried out according to the method standardized by Goli *et al*. (2008). The Column temperature: 150°C-270°C, Injector temperature: 250°C and Detector temperature: 250°C were all maintained. GLC was programmed in a manner that there will be a rise of 10°C per minute and was finally maintained at fixed temperature of at 270°C. Comparision between the retention time of known standard samples with those samples taken under study under similar condition was done so as to record the peaks of fatty acid methyl esters.

Statistical analysis

Chi-square (χ^2) test wasemployed to test goodness of fit of observed and expected frequency in segregating generations.

Results and Discussion

The present study involved NRCHB101 as high erucic acid (>30%) parent (P_1) as well as Heera and PDZ-1 as low erucic acid (<2%) parent (P_2). The erucic acid content of F_1s from the crosses, NRCHB-101× Heera and NRCHB-101× PDZ-1 was found intermediate between their respective high erucic acid (NRCHB101) and low erucic acid (Heera and PDZ-1) parents but with higher values, which indicate that erucic acid might be controlled by recessive genes and also the genotype of the developing embryo, rather than the female sporophyte, determined the erucic acid content of F1 embryos. Embryonic control of erucic acid synthesis was also observed in case of *B. carinata* (Gentinet, 1996), *B. napus* (Harvey and Downey, 1964; Kondra and Stefansson, 1965), *B. rapa* (Dorrell and Downey, 1964) and *B. juncea* (Kirk and Hurlstone, 1983; Meena and Sachan, 2009). The frequency distribution of segregating generation (F_2, B_1 and B_2) for both crosses (NRCHB101×Heera and NRCHB101×PDZ-1) were separated into five classes consisting of seeds with varying erucic acid content with the range of <2%, 2-10%, 11-20%, 21-30% and >30% (Fig 1). Chi-square (χ^2) test was done to assess the goodness of fit of observed and expected frequency in segregating generations. F_2 segregants of both the crosses had erucic acid content within the parental range with no transgressive segregant for the trait. The segregation pattern of 144,112 seed samples in F_2

Table 1: Chi-square test and probabilities of goodness of fit for erucic acid traitin *Brassica juncea.*

Crosses	Generations	Total no. of plants	No. of plant with% erucic acid					Expected ratio	Chi square value	P value
			<2	2-10	11-20	21-30	>30			
NRCHB101(P1) × HEERA(P2)	P_1	10	-	-	-	-	10	-	-	-
	P_2	14	14	-	-	-	-	-	-	-
	F_1	15	-	-	-	15	-	-	-	-
	F_2	144	6	38	60	31	9	1:4:6: 4:1	2.46	0.65
	B_1	80	-	-	14	41	25	1:2:1	3.07	0.21
	B_2	96	19	57	20	-	-	1:2:1	3.38	0.18
NRCHB101(P1) × PDZ-1(P2)	P_1	10	-	-	-	-	10	-	-	-
	P_2	12	12	-	-	-	-	-	-	-
	F_1	20	-	-	-	20	-	-	-	-
	F_2	112	5	25	39	34	9	1:4:6: 4:1	2.95	0.56
	B_1	76	-	-	13	41	22	1:2:1	2.59	0.27
	B_2	52	19	55	30	-	-	1:2:1	2.66	0.26

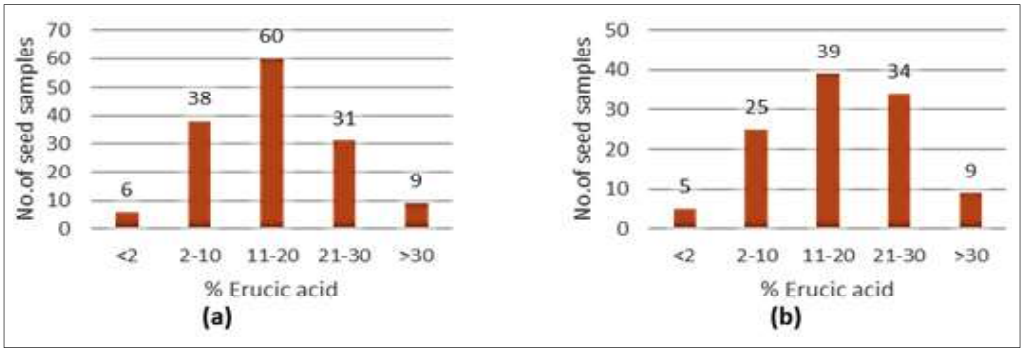

Fig 1: Frequency distribution of Eruric and content in F2 population of (a) NRCHB101×HEERA (b) NRCH101×PDZ-1.

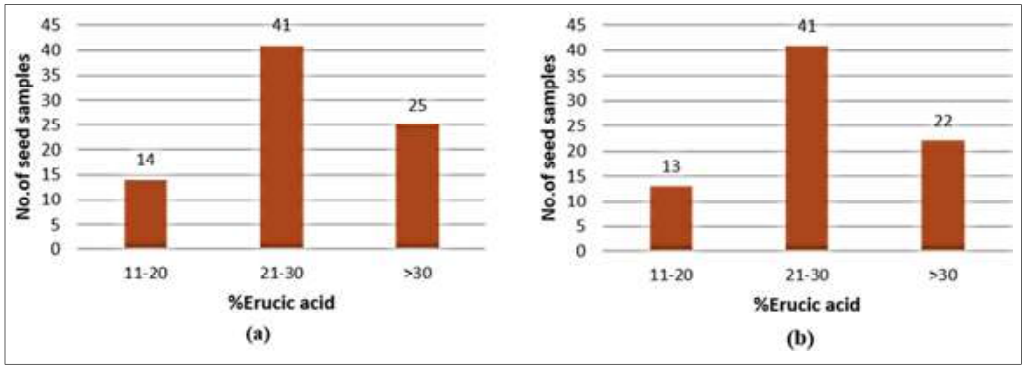

Fig 2: Frequency distribution of Eruric and content in B1 population of (a) [NRCHB101× (NRCHB10×HEERA)] and (b) NRCH101× (NRCHB101×PDZ-1)].

seed, fits well in 1:4:6:4:1 theoretical ratio (χ^2 =2.46, 2.95, 4 df, P > 0.50) which indicated digenic inheritance of erucic acid trait (Table 1). Two B_1 populations, developed from the cross [NRCHB101×(NRCHB101×Heera)] and [NRCHB101 ×(NRCHB101×PDZ-1)], comprising of 80 and 76 individual seed samples respectively, were phenotyped for erucic acid content. B_1 plants separated into three classes *i.e.*,11-20%, 21-30% and >30% based on erucic acid content (Fig 2). The erucic acid trait segregation pattern in both the B_1 populations fit well in 1:2:1 ratio (χ^2 = 3.07 and 2.59 respectively) (Table 1). B_2 population developed from the cross [Heera×(NRCHB101×Heera)] and [PDZ1×(NRCH B101×PDZ-1] comprising of 96 and 52 individual seed samples respectively, were phenotyped for erucic acid content. The B_2 population of both the crosses also segregated into three classes *i.e.*, <2%, 2-10% and 11-20% (Fig 3) with genotypic ratio of 1:2:1 (Table 1). These results of segregation pattern support the similar study conducted by Saini *et al.* (2016) on two B_1 populations, developed from the crossing Pusa Vijay and Pusa Bold as high erucic parent and PM24 and PM30 as low erucic parent. The genetic ratio of 1:4:6: 4:1 and 1:2:1 represented genes model with additive

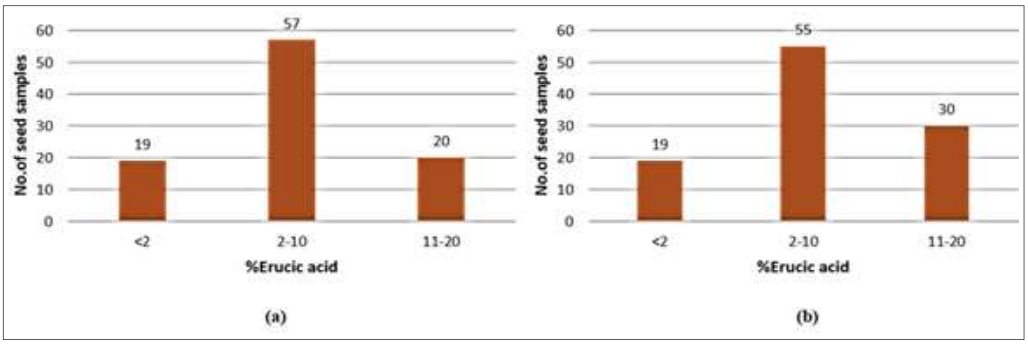

Fig 3: Frequency distribution of Eruric and content in B2 population of [HEERA× (NRCHB101× HEERA)] and [PDZ-1× (NRCHB101× PDZ-1)].

gene effects for F_2 and backcrosses, respectively. These results are in accordance with the study conducted by Singh *et al.* (2015) on genetics of erucic acid using six generations (P_1, P_2, F_1, F_2, B_1 and B_2) derived from the crosses, Varuna × LES-39 and Varuna × LES-1-27as well as with the study conducted by Pandey *et al.,* (2013) on inheritance of erucic acid using genotypes PRQ-9701-46 (low erucic acid) and JM-1 (high erucic acid). Thus the present study concludes that erucic acid content inheritance is governed by two genes in *B. juncea* and these two genes have additive gene effects. Earlier findings by Kirk and Hurlstone (1983), Bhat *et al.* (2002) and Chauhan *et al.* (2003) in *B. juncea* also supported the above cited results of present study. On the other hand, some studies reveal that content of erucic acid in the diploid species like *B. rapa* (AA) is controlled by a single additive gene whereas two genes are responsible for erucic acid synthesis in tetraploid species like *B. napus* (AACC), *B. juncea* (AABB) and *B. carinata* (BBCC) and the two genes are located on one chromosome of each of the A, B and C genomes of these species (Chen and Beversdorf, 1990; Luhs *et al.*, 1999). In present study Chi square test revealed that there was no significant difference in observed ratio and expected ratio in all the generations undertaken under study. As a result,segregation ratio in these generations confirmed that erucic acid content inheritance in *B. juncea* is digenic with additive effect. These results were in agreement with earlier reports by Kirk and Hurlstone (1983); Tiwari (1995) and Potts and Daryl (1999) that two genes with additive effects control this trait. Similarly, two genes were reported to control erucic acid in Brassica napus (Chen and Beversdorf, 1990; Luhs and Friedt, 1995) and *Brassica carinata* (Fernandez-Escobar *et al.*, 1988). However, Qui *et al.* (1993), in Brassica napus,and Dorell and Downey (1964), in *Brassica campestris*, reported that inheritance of erucic acid content was governed by a single major gene.

Conclusion

Present study was carried out to find out the number of genes controlling the level of erucic acid in Indian mustard. Intermediate but high levels of erucic acid in F_1 seeds of crosses indicated that erucic acid was controlled by the genotype of developing embryo and not the female sporophyte whereas in case of F_2 seeds derived from crosses between zero and high erucic acid parents, 1:4:6:4:1 segregation ratio indicated that erucic acid content was controlled by two recessive genes with additive effects. This digenic recessive mode of inheritance of erucic acid with additive gene effect also suggested that low erucic acid cultivars can be developed through introgression of low erucic acid gene in higher yielding cultivars and by identifying transgressive segregants in the early segregating generations in breeding programme. This forms the basis for the formulation and implementation of effective breeding strategies for the development of genotypes with higher qualitative potential.

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Author's contribution

Conceptualization of research (VVS); Designing of the experiments (VVS, PM); Execution of field/lab experiments and data collection (VVS, Monika, IM); Analysis of data and interpretation (VVS, Monika, PM); Preparation of manuscript (VVS, Monika, PM, PKR).

Conflict of interest

The authors declare that they have no conflict of interest.

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