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Research Article

Study of Inheritance of Erucic acid in Indian Mustard (*Brassica juncea L. Czern & Coss*)

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ABSTRACT

Abstract: Rapeseed-mustard is second most important group of oilseed crops next to groundnut. Among these, Indian mustard [Brassica juncea (L.) Czern and Coss] is the most important crop grown during rabi season. Though the productivity of Indian mustard in India has considerably improved in last decade but it is still very low in comparison to many European countries. On the other hand, Rapeseed – mustard have been used as a source of oil since long time. The reason for low use of the oil is the presence of high percentage of erucic acid and linolenic acid contents in the oil, which not only deteriorate the oil quality but are also known to produce harmful effects on the body. In the present study one zero erucic acid line PRQ-9701-46 was crossed with JM-1 (46.29%), a high erucic acid cultivar including reciprocals to find out the number of genes controlling inheritance of erucic acid in Brassica juncea. The erucic acid content of F_1's and their reciprocals was intermediate between the parents thus indicating embryonic control of erucic acid and absence of maternal effect in the inheritance of erucic acid in B. juncea. Erucic acid content of F_2 seeds segregated into 5 classes (<2%, 10-22%, 22-34%, 34-46% and 46% erucic acid) with a ratio of 1:4:6:4:1 Backcross seeds of BC_1 generation segregated into three classes (<2%, 10-22%, 22-34% erucic acid) with a ratio of 1:2:1 and backcross seeds of BC_2 generation segregated into three classes 22-34%, 34-46% and >46% erucic acid) with a ratio of 1:2:1. The segregation patterns confirmed that inheritance of erucic acid content in B. juncea was governed by two genes with additive effects.

Key words: Indian mustard, oil, erucic acid, inheritance

INTRODUCTION

Oilseeds play a vital role in Indian agricultural economy and form the second largest agricultural commodity after cereals. Oilseed crops in India account for almost 5% of Gross National Product (GNP) and10% of the value of agricultural products. Rapeseed mustard (*Brassica) contributes* 32% of the total oilseed production in India, and it is the second largest indigenous oilseed crop. The other oilseed crops include groundnut, castor, sesame, sunflower and soybean. The table shows the production (*in million tonnes*) of major oilseed crops in India (*Source : IOPEPC*).

Indian mustard (*B. juncea*) is the predominant crop (about 90%) of rapeseed mustard group of crops in India. Mustard oil is an important component of the Indian diet. Rapeseed-mustard seed, in general, consist of 35-45 % oil, 17-25 % proteins, 8-10 % fibers, 6-10 % moisture and 10-12 % extractable substances. At global level, rapeseed-mustard crops are grown in 53 countries spreading over the 6 continents, covering an area of 22.33 million hectare with on average production of 33.17 million tonnes and an average yield of 1468 kg/hectare ranging from 333 kg/hectare (Tazikistan) to 6667 kg/hectare (Algeria). China, India and Canada are the major rapeseed-mustard growing countries of world. The major rapeseed mustard growing states in India are Rajasthan, Uttar Pradesh, Assam, Bihar, Orissa, West Bengal, Madhya Pradesh, Gujrat, Haryana and Punjab. India holds a premier position in terms of largest area under oilseed in the world, but the yield of the most of the oilseed crops is less than the world average. On the other hand, the demand of edible oils has been estimated at 10 million tonnes for the year 2015 and 11.72 million tonnes by 2030 (Paroda, 2000). Oil quality is determined by relative amounts of saturated, monosaturated and polyunsaturated fatty acids. The fatty acids are long chain single carboxyl group containing organic acids. Conventional varieties of rapeseed-mustard contain about 50 per cent erucic acid in the oil. However, nutritional and end use requirements spawned international efforts to genetically eliminate this undesirable constituent from oilseed *Brassica*. This culminated in the commercialization of '00' or canola quality varieties of oilseed rape and turnip rape during 1970's. In the biosynthetic pathway, erucic acid is the end product, derived through the chain elongation of oleic acid (Jonsson, 1977). It is synthesized by chain elongation of oleoyl-CoA via eicosenoic acid (Pollard and Stumpf, 1980). The enzyme oleoyl elongase is present in high erucic acid strains but is absent in low erucic acid strains (Stumpf and Pollard, 1983). The conversion of oleic acid to erucic acid occurs in a particular fraction containing chloroplasts and plastids in the developing seeds. Low erucic acid strains having genetic blocks in the biosynthetic pathway towards eicosenoic acid and erucic acid were initially identified for the first time in Canadian varieties of summer rape (Stefansson *et al.*, 1961) and summer turnip rape (Downey and Craig, 1964). Later on similar strains were identified in Indian mustard (*B. juncea*) germplasm by Kirk and Oram (1981) in Australia. Rapeseed-mustard oil has substantial amount of unsaturated fatty acids and has around 7 per cent saturated fatty acids, the lowest among the oilseed crop (Chauhan *et al.*, 2002). Rapeseed-mustard consists of saturated fatty acid such as palmitic (C16:0), stearic (C18:0) and monounsaturated fatty acids such as oleic (18:1) eicosenoic (C20:1) and erucic acid (C 22:1) and polyunsaturated fatty acids such as linoleic (C 18:2) and linolenic acid (C 18:3), known as essential fatty acids. Of the total fatty acids, erucic acid (C 22:1) is predominant in Indian cultivars (about 50 per cent). The presence of high erucic acid in oil is considered anti-nutritional, as it has been reported to cause lipidosis in children and myocardial fibrosis in monkeys (Ackman *et al.*, 1977). Thus, there is an urgent need to make concerted efforts for breeding varieties with low levels of erucic acid (<2%) so as to bring the Indian cultivar at par with international quality norms. The knowledge of mode of inheritance and number of genes that control inheritance of erucic acid is essential for the choice of efficient breeding programme for developing low erucic cid varieties of rapeseed-mustard. Two genes, showing dominance and acting in additive manner were previously demonstrated in *B. juncea* (Kirk and Hurlstone,1983).

Table 1. **Production of different oilseeds in India.**

OILSEEDS	2011-12	2010-11	2009- 10	2008-09	2007-08	2006-07
SOYABEAN	10.65	9.50	8.50	8.90	9.46	7.96
GROUNDNUT	6.02	5.84	5.12	5.92	6.89	5.35
CASTORSEED	1.62	1.19	0.93	0.98	0.91	0.78
SESAMESEED	0.76	0.76	0.76	0.58	0.66	0.61
RAPESEED MUSTARD	6.03	7.10	6.32	6.20	4.59	6.02
LINSEED	0.13	0.16	0.16	0.13	0.18	0.19
NIGERSEED	0.09	0.10	0.08	0.08	0.07	0.07

MATERIAL AND METHODS

The experimental material consisted of six generations of cross between one low erucic acid parent (PRQ-9701-46) and one high erucic parent (JM-1). The parental lines were different from each other in quantitative and qualitative characteristics as indicated below. Six generations namely, P_1, P_2, F1, F_2, BC_1 and BC_2 were evaluated in a compact family block design with three replications in 5m long rows spaced 30 cm, apart with plant to plant distance of 10 cm. The distance of 10 cm between plants was maintained by thinning. One border row on either side of main plots was sown with a variety of mustard (Vardan) and were treated as non-experimental. The numbers of rows were different for different progenies. Each plot thus consisted of different number of rows i.e., single row for parents (P_1 and P_2) and F_1's, three rows for backcrosses (BC_1 and BC_2) and seven rows for F_2 generations. Ten randomly selected plants each of P_1, P_2 and F_1, 40 plants of F_2 and 20 plants each of BC_1 and BC_2 generations, were utilized for recording observations on various characters. The parents, F_1's, F_2's and backcrosses (BC_1 and BC_2) progenies were analyzed for fatty acid composition. Methyl esters for fatty acid analysis were prepared by direct methylating method of seeds. Methyl esters for parents and F_1's were prepared from the bulk seeds whereas, F_2's, BC_1 and BC_2's from single seed.

Table 2. Important characteristics of the parental lines

Genotype/ variety	Maturity	Height	Seed colour	Seed size	Erucic acid content
PRQ-9701-46	Early	Medium	Yellow	Bold	Low
JM-1	Medium	Tall	Brown	Medium	High

RESULTS AND DISCUSSION

The amount of seven important fatty acids in different parents including one zero erucic acid genotypes i.e., PRQ-9701-46 and one high erucic acid parent viz., JM-1 (46.29%) along with the fatty acid composition of F_1's and their reciprocals resulting from the crosses between zero erucic acid and high erucic acid parents are presented in Table 3.

Table3. The fatty acid composition of parents, F_1's and their reciprocals

Seed genotype	Fatty acid (% of total)						
	Palmitic (16:0)	**Stearic (18:0)**	**Oleic (18:1)**	**Linoleic (18:2)**	**Linolenic (18:3)**	**Eicosenoic (20:0)**	**Erucic (22:1)**
Parent (low erucic acid)							
PRQ-9701-46 (P_1)	4.19	2.20	46.04	31.35	14.55	1.63	0.00
Parents (high erucic acid)							
JM-1 (P_2)	2.76	1.28	14.29	18.26	12.20	4.89	46.29
F_1 (low × high)							
(P_1×P_2)	3.37	1.92	22.08	24.52	13.53	10.57	23.97
F_1 (high × low)							
(P_2×P_1)	4.70	1.63	15.77	26.24	17.54	7.19	25.44

Results revealed that the amount of erucic acid in F_1's and reciprocal F_1's was about intermediate. The calculated t-value (1.224) was less than table t-value. Non-significant t-value at 5 per cent and 1 per cent probability levels revealed non-significant difference for erucic acid content between F_1's and their reciprocals.

The frequency distribution of erucic acid in parents and their F_1, F_2, BC_1, (F_1 × zero erucic acid parent) and BC_2 (F_1 × high erucic acid parent) are presented in Fig. 1 to 3 .

Table 4. Number of F_2 and backcrosses single seeds in different erucic acid classes with chi-square values and probabilities of goodness of fit for three crosses of *B. juncea*

Crosses and generations	No. of seeds with % erucic acid					Expected ratio	$\chi 2$ value	P value
	<2	10-22	22-34	34-36	>36			
PRQ-9701-46 × JM-1								
F_2	7	30	41	25	6	1:4:6:4:1	0.565	0.98-0.95
BC_1	12	27	14	-	-	1:2:1	0.169	0.95-0.90
BC_2	-	-	12	25	10	1:2:1	0.361	0.90-0.80

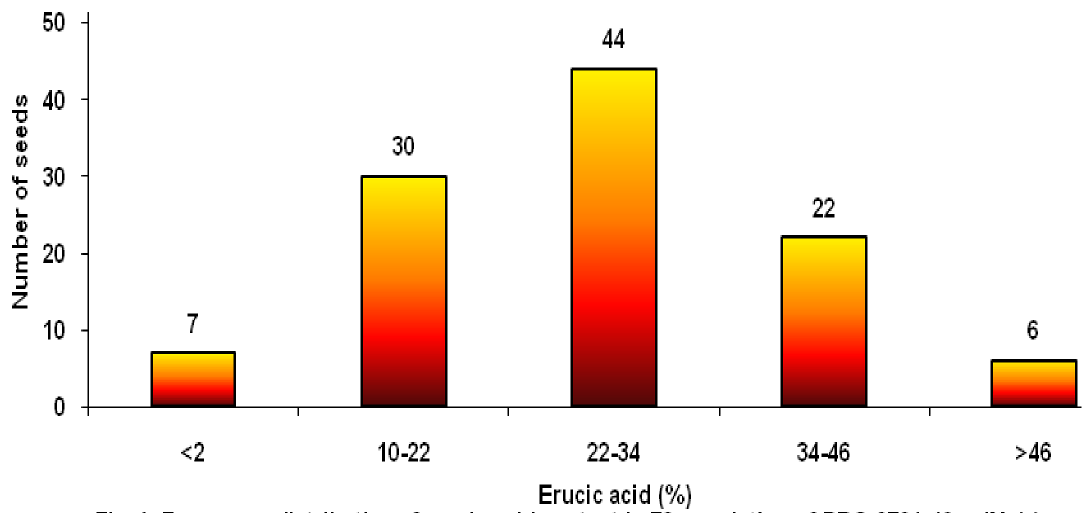

Fig. 1 Frequency distribution of erucic acid content in F2 population of PRQ-9701-46 x JM-1)

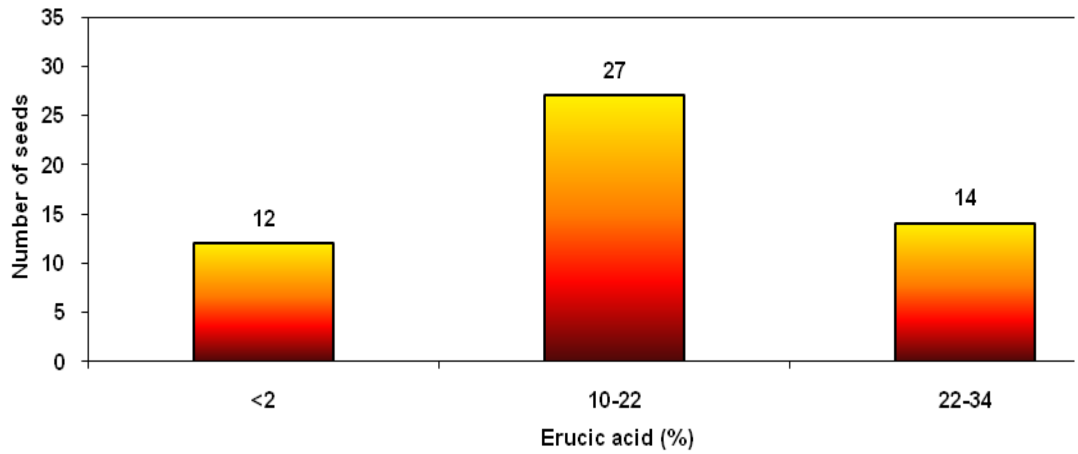

Fig. 2. Frequency distribution of erucic acid content in BC1 population of [(PRQ-9701-46 x JM-1) xPRQ-9701-46]

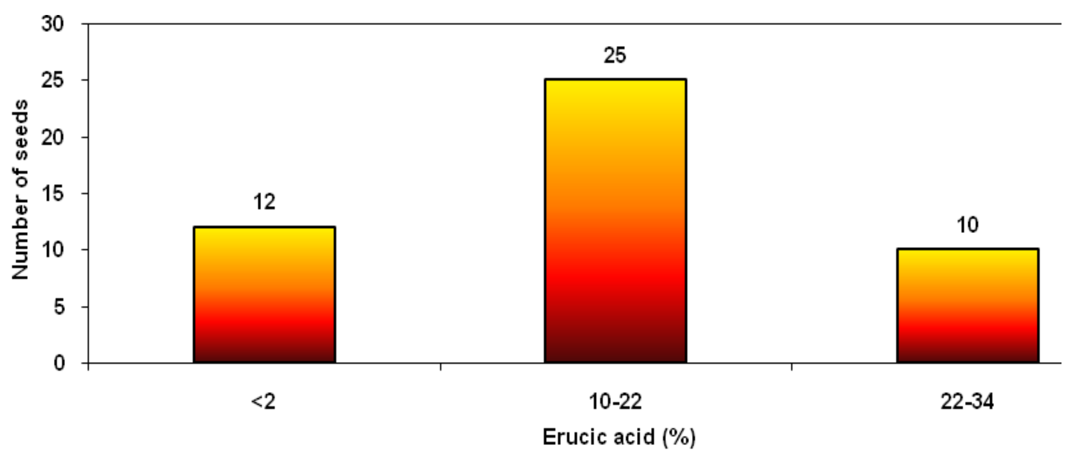

Fig.3 Frequency distribution of erucic acid content in BC2 population of [(PRQ-9701-46 x JM-1) x JM-1]

The number of genes (effective factors) controlling the inheritance of erucic acid content were estimated from actual data. The number of genes for inheritance of erucic acid was estimated following Lande (1981) in cross PRQ-9701-46× JM-1.The minimum numbers of effective factor pairs for erucic acid as estimated by different methods ranged from 1.77 (Lande, 1981) to 2.02 (Burton, 1951) with a mean value of 1.90 in the cross PRQ-9701-46× JM-1. The results indicated that the parents utilized in these crosses differed by at least two pair of major genes for erucic acid. These results revealed that two genes with additive effect control the inheritance of erucic acid in the cross PRQ-9701-46 × JM-1.The expected frequency of various classes in F_2 and backcross populations in digenic inheritance model with additive gene effect are 1:4:6:4:1 and 1:2:1, respectively. Observed frequencies of single seeds of F_2 and backcross population were grouped to five distinct classes. Frequency distribution of these populations obtained for each class separately. The frequency distribution of F_2 generation was separated into five classes consisting in of seeds with erucic acid contents of <2%, 10-22%, 22-34%, 34-46% and >46% Frequency distribution of BC_1 i.e. (F_1×zero erucic acid parent) was separated into three classes including <2%, 10-22% and 22-34% erucic acid content. These three classes for BC_2 (F_1 × high erucic acid parent) were 22-34%, 34-46% and >46%. The genetic ratio of 1:4:6:4:1 and 1:2:1 represented genes model with additive gene effects for F_2 and backcrosses, respectively. The Chi-square test also showed that observed ratios were not significantly different from the expected ratios. So segregation ratio in these generations confirmed digenic hypothesis with additive effect in the inheritance of erucic acid content.

CONCLUSION

Inheritance studies were carried out to find out the number of genes controlling the level of erucic acid in Indian Mustard. Non-significant differences in erucic acid content between F_1's and their reciprocals confirmed that maternal effect was not important in inheritance of this fatty acid. Intermediate 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. This also indicated that genes involving inheritance of erucic acid had little or no dominance effect. The 1:4:6:4:1 segregation ratio in F_2 seeds derived from crosses between zero and high erucic acid parents indicated that erucic acid content was controlled by two genes with additive effects.

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