

Full Length Research Paper

Genetic analysis of oil content and quality parameters in Indian mustard (*Brassica juncea* (L.) Czern and Coss)

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A two-year study was carried out during *rabi* 2006 to 2007 and 2007 to 2008 with 24 parents and 80 F₁ crosses of Indian mustard to assess the nature of variability and association for fatty acid profile and oil content. Analysis of variance indicated significant differences for all the quality characters investigated. The environmental effects were significant for erucic and oleic acid content and the influence of environmental factors appeared to be less on other characters. The genotype × environment interactions were non-significant for all the characters, hence, the data were pooled over the years and discussed on the basis of mean of two years. The coefficients of variation at phenotypic level varied from 4.6% for oil content to 50.9% for oleic acid. The genotypic coefficients of variability were high for oleic, palmitic + stearic, erucic and linolenic acid, erucic acid and palmitic acid + stearic acid had the least genotypic variation (GCV: 16.3 to 16.9%). The heritability in broad-sense was relatively high for oleic (61.5%) and erucic acid (56.3%). The high heritability was associated with high genetic advance only for oleic acid suggesting the role of additive gene action in the inheritance of this character. Erucic acid negatively and significantly correlated with the rest of the fatty acids except linolenic acid and significant correlation with oleic ($r = -0.536$) and eicosenoic acid ($r = -0.260$). Although, oil content had very low direct effect (-0.011) on erucic acid but its positive association was the result of its strong positive indirect effect through oleic acid (0.435), which was partially neutralized by negative indirect effects (-0.112) through linolenic acid. The implications of these results in the quality-breeding programme were discussed in this paper.

Key words: Indian mustard, correlation coefficients, path analysis, fatty acid content, oil content.

INTRODUCTION

Indian mustard is extensively grown in India and the major source of edible oil. Oil quality was determined by fatty acid profile, whereas, level of erucic acid predicts the quality of seed oil. Oil of *Brassica juncea* (Czern and Coss), the Indian mustard is consumed in large quantity and the production ranks second among all oil seed in India (Chopra and Prakash, 1991). But due to the presence of undesirable long chain fatty acids like eicosenoic acid (10%) and erucic acid (50%) in the seed oil, it becomes detrimental to human health.

Erucic acid increases blood cholesterol, interferes in myocardial conductance and shortens coagulation time (Renard and McGregor, 1992). In the present era of health consciousness, the quality of oil remains a cause of concern among consumers and hence, its improvement. It gained the paramount importance among the breeding objectives in recent years.

The success of any crop improvement programme relies upon the extent of variability for a character to be improved. Correlation studies among various characters help breeders to formulate the appropriate breeding strategy to improve a number of characters simultaneously. Path analysis provides an insight into the causes of correlations between two characters. Such information for oil and erucic acid quality characters is limited in the Indian-mustard varieties grown in India.

Therefore, the present investigation attempts to assess the extent of genetic variation and nature of associations among quality characters in Indian mustard. Furthermore, the correlation coefficients of erucic acid with other characters were partitioned into direct and indirect effects by path analysis to precisely elucidate the nature of association. It contains a low amount of saturated fatty acids and an appreciable amount of unsaturated fatty

acids. However, oleic (C18:1) and linoleic (C18:2) acids, which are vital for nutritionally superior oil, are low to moderate in mustard.

Oleic acid has been reported to lower cholesterol levels, a major component associated with coronary heart disease (Grundty, 1986). High oleic acid content would allow the oil to be used even more widely because of increased thermo stability. Linoleic acid is an essential fatty acid that is the basis for prostaglandin and other essential body regulators. An increase of this fatty acid in oil would be of great value for people with low-fat intakes. It is, therefore, imperative to breed mustard varieties with increased levels of both of these fatty acids to enhance the nutritional quality as well as versatility of mustard oil.

Canola quality Indian mustard oil in the international market has high oleic acid (60%) and 20 to 30% linoleic acid and is considered highly desirable. Low erucic acid strains were initially identified for the first time in Canadian varieties of summer rape (Stefansson et al., 1961) and summer turnip rape (Downey, 1964). Later on, similar strains were identified in Indian mustard (*B. juncea*) germplasm by Kirk and Oram (1981) in Australia. They attempted repeated selfings followed by selection of individual seeds having much reduced levels (<2%) of erucic acid. These genotypes were named as ZEM 1 and 2. Besides the native variability, mutagenesis was used successfully to induce zero erucic acid mutants in *B. rapa* L. var. *annua* (Laakso et al., 1986).

Inter-specific hybridization has also been used to transfer/combine desirable fatty acid composition. Shopta and Podkolzine (1982) were successful in combining three Swede rape (*Brassica napus*) varieties free of erucic acid with low linolenic acid (<4%) through hybridization between zero erucic acid *B. napus* and *B. juncea*. Roy (1984) selected a line Onap J, with erucic acid content of less than 0.5%, from a cross between the Indian mustard (*B. juncea*) variety BJ 168 and Swede rape (*B. napus*) variety Cresus-O-precose. Inter-specific hybridization between *B. juncea* cv. RLM 198 × *B. napus* cv. Oro and *B. juncea* cv. RLM 619 × *B. rapa* cv. *Tobin* was also used in India.

Information on the genetic architecture of the characters would enable breeders to take up its improvement by conventional breeding methods. Such information for erucic acid, oleic acid and linoleic acid contents in Indian mustard is limited. Therefore, the present investigation was undertaken to illustrate the genetic control of these fatty acids in the Indian mustard in order to devise an appropriate breeding methodology for improving these fatty acids.

MATERIALS AND METHODS

The experimental material consisted of 80 F₁ and 20 lines and 4 tester parents released varieties of mustard (Indian mustard 104; these were grown in Randomized Complete

Block design (RCBD) during 2006 to 2007 and 2007 to 2008 *rabi* seasons with three replications in 5-row plot of 5 m length, keeping 45 cm row-to-row and 15 cm plant-to-plant spacing. The experiment was conducted at 80:40:40 kg/ha of N: P₂O₅: K₂O. Half the dose of nitrogen and full doses of P₂O₅ and K₂O were applied basally at the time of sowing and the remaining dose of nitrogen was top dressed after first irrigation (35 days after sowing). The crop was also irrigated at 60 days after sowing. The observations were recorded on composite sample from central three rows.

The oil contents were analyzed using NIR (Dicky John Insta Lab 600). Fatty acid profile was analyzed by gas liquid chromatography (Nucon Model 5765) using SP 2300 + 2310 SS columns. Varuna, a non-canola variety of Indian mustard (*B. juncea*) were used as checks. The detailed method for fatty acid analysis has been described earlier (Chauhan et al., 2002a). The mean values were used for analysis of variance using multiple randomized complete block design by Indostat software.

The genotypic (GCV) and phenotypic coefficients of variation (PCV), heritability in broad-sense and genetic advance were calculated by the formulae given by Johnson et al. (1955). The genotypic and phenotypic correlations were calculated as per the method of Al-Jibouri et al. (1958). Path coefficient analysis was done according to the method of Dewey and Lu (1959).

RESULTS AND DISCUSSION

Analysis of variance indicated significant differences for all the quality characters investigated. The environmental effects were significant for erucic, oleic acid and oil content but the environment influence was less on the other characters. Interactions between genotype × environments were non-significant for all the characters; hence, the data were pooled over the years and discussed on the basis of mean of two years. All the genotype showed same pattern of variability for both years for linoleic, linolenic, eicosenoic acid and oil content. Erucic acid ranged from 30.7 to 52.2% and oil content from 35.7 to 41.6%. The range for linolenic acid was 5.7 to 19.6%. The range for palmitic + stearic and oleic acid was 2.3 to 6.0% and 8.9 to 22.7% respectively.

Linoleic acid was the highest in 14.3 to 25.7%. In general, all the characters had higher magnitude of PCV than GCV (Table 1) indicating that environment influenced the expression of these characters but to a varying extent, however, the trend of PCV and GCV was similar. The coefficients of variation at phenotypic and genotypic level varied from 4.6% (oil content) to 50.9% (oleic acid) and 2.6% (oil content) to 39.9% (oleic acid) respectively.

The genotypic coefficients of variation were high for oleic, palmitic + stearic, erucic and linolenic acid, which provide good opportunity for selection for desirable levels

Table 1. Mean, standard error, range, coefficients of phenotypic (PCV) and genotypic variance (GCV), heritability in broad-sense (h^2_b) and genetic advance for quality characters Indian mustard.

Characters	Mean \pm SEM	Range	PCV (%)	GCV (%)	h^2 (%)	Genetic advance (%)
Palmitic acid + stearic acid (%)	3.6 \pm 0.6	2.3-6.0	36.4	16.9	21.6	16.2
Oleic acid (%)	15.8 \pm 2.5	8.9-22.7	50.9	39.9	61.5	64.5
Linoleic acid (%)	19.6 \pm 2.0	-14.3-25.7	22.1	7.6	11.9	5.4
Linolenic acid (%)	13.4 \pm 1.5	5.7-19.6	27.6	15.9	33.0	18.8
Eicosenoic acid (%)	7.0 \pm 1.2	4.6-10.4	35.8	12.6	12.4	9.2
Erucic acid (%)	40.3 \pm 2.9	30.7-52.2	21.7	16.3	56.3	25.1
Oil content (%)	39.1 \pm 0.7	35.7-41.6	4.6	2.6	32.2	3.0

Table 2. Correlation coefficient at phenotypic (P) and genotypic (G) levels among quality characters.

Character	Parameter	Oleic acid	Linoleic acid	Linoleic acid	Eicosenoic acid	Erucic acid	Oil content
Palmitic + stearic acid	P	0.184*	0.146	0.208**	0.165*	0.208*	-0.111
	G	0.539	-0.065	-0.319	0.821	-0.546	-0.556
Oleic acid	P		0.001	-0.646**	0.453**	-0.678**	0.201*
	G		0.426	-0.929	1.105	-0.766	-0.127
Linoleic acid	P			-0.084	-0.287**	-0.193*	0.084
	G			4.664	0.436	-0.319	0.099
Linolenic acid	P				-0.303**	0.265**	0.293**
	G				-0.687	0.600	0.280
Eicosenoic acid	P					-0.407**	0.041
	G					-0.936	-0.129
Erucic acid	P						0.068
	G						0.227

*, ** Significant at 5 and 1% level of probability.

of these fatty acids and oil content had the least variation (Table 1). Heritability values ranged from 11.9% (linoleic acid) to 61.5% (oleic acid). The heritability was relatively high for oleic (61.5%) and erucic acid (56.3%). The characters like linolenic acid, and oil content showed moderate heritability while, the remaining characters showed low heritability. However, in earlier studies, (Chauhan et al., 2002a, b) high heritability estimates were reported for erucic, oleic and linoleic acid. The high genetic advance was observed only for oleic acid (64.5%) while the other characters showed low to moderate genetic advance. The high heritability was associated with high genetic advance only for oleic acid suggesting the role of additive gene action in the inheritance of this character. The results were in agreement with earlier reports (Chauhan et al., 2002b).

The extent of variation for oil content was found to be low, accompanied by low to moderate estimates of heritability and low genetic advance, medicating thereby,

the chances of improvement of these characters by simple selection were limited.

Correlations

Phenotypic level

Erucic acid is an undesirable fatty acid found in Indian mustard oil of prevalent Indian cultivars. It had negative and significant relationships with most of the other fatty acids but showed positively significant correlation with linolenic acid and oil content (Table 2). The results implied that an increase in the level of other fatty acids, particularly that of oleic acid, which had the highest negative direct effect (Table 3), would result in the reduction of erucic acid content.

The negative relationships of erucic with oleic and linoleic acid have been reported earlier also (Zhou and

Table 3. Path analysis at phenotypic (P) and genotypic (G) levels taking erucic acid as dependent.

Character	Parameter	Palmitic + stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Oil content	Erucic acid
Palmitic + stearic acid	P	-0.127	-0.150	0.047	0.080	-0.040	-0.011	0.005
	G	-0.364	-0.094	0.004	-0.115	-0.089	-0.083	-0.005
Oleic acid	P		-0.812**	-0.646**	0.453**	-0.678**	-0.536**	-0.533**
	G		-0.175	-0.929	1.105*	-0.766	-0.127	-0.708
Linoleic acid	P			-0.318	-0.287*	-0.193*	0.084	0.056
	G			-0.664	0.436	-0.319	0.099	-1.105
Linolenic acid	P				-0.303	0.265**	-0.030	0.293**
	G				-0.687	0.600	0.280	0.890
Eicosenoic acid	P					-0.407	0.041	-0.260**
	G					-0.936	-0.129	-0.587
Oil content	P						0.101	0.265
	G						0.102	-0.593

Liu, 1987; Singh et al., 2001; Chauhan et al., 2002a; Meena, 2006). Increase in the level of oleic acid might also result in the high oil content as both characters were positively correlated and reduction in the erucic acid because of negative relationship between oleic acid and erucic acid (Table 2). Erucic acid had negative and significant correlations with oil content.

Genotypic level

Erucic acid showed moderately high positive correlation with linolenic acid. Oleic acid had negative association with linolenic, erucic acid and oil content, while positively related with linoleic, palmitic + stearic and eicosenoic acid (Table 2). Oil content was positively correlated with most of the characters except palmitic + stearic acid ($r = -0.556$), oleic acid ($r = -0.127$), and eicosenoic acid ($r = -0.129$).

On the basis of the present investigation, it would be appropriate to enhance the level of oleic acid which had high extent of variability and negative relationship with erucic acid through hybridization among varieties/donors having high level of oleic acid and low erucic acid as well as, high oil content. However, the pattern of correlations between erucic acid and other quality characters were similar at both genotypic and phenotypic levels except linoleic acid with eicosenoic, oleic, linoleic and eicosenoic acid with oil content (Table 2). These results indicated that environment played an important role in the expression of these relationships.

Path analysis

Phenotypic level

Correlation coefficients only describe the nature and magnitude of association between any two variable but path analysis enables interpretation of cause and effect relationship. Therefore, correlation coefficients of erucic acid (dependent variable) with other quality characters (independent variable) were partitioned into direct and indirect effects. The negative association of palmitic + stearic, oleic, linoleic, and eicosenoic acid with erucic acid was as a result of their high to moderate negative direct effect (-0.127) on erucic acid.

The indirect effects through linoleic, linolenic acid and erucic acid were positive, however, the correlation coefficients remained negative and significant (-0.208) due to major negative indirect effect through oleic acid (Table 3). Oleic acid had the highest direct negative (-0.812) on erucic acid and also had major positive indirect effect through linoleic acid. The indirect effects through other characters were low, hence, the correlation between oleic and erucic acid remained negative but significant.

Linoleic acid also had major negative direct effect (-0.318) though, it had been diluted by positive indirect effects through palmitic + stearic, linolenic, eicosenoic acid and oil content, however, due to negative indirect effects through oleic acid and oil content, the correlation coefficient remained significant and negative. Mainly positive indirect effects through oleic and eicosenoic acid

had nullified the direct negative effect of linolenic acid (-0.303) and resulted into its significant and positive correlation with erucic acid. Looking into positive relationship, it would be desirable to practice selection for low linolenic acid.

Negative direct effect of eicosenoic acid (-0.407) and its negative indirect effect through oleic acid is an indication of reduction in eicosenoic acid by an increase in oleic acid and in turn, the reduction in erucic acid. Oil content had low positive direct effect (0.101) as well as, positive but non-significant correlation with erucic acid. Erucic acid had negative direct effect but it had been neutralized by positive indirect effect through linolenic and oil content, which resulted into positive and significant correlation with erucic acid.

Genotypic level

The highest negative direct effect of palmitic + stearic acid (-0.364) on erucic acid and their negative indirect effects through oleic, linolenic, eicosenoic acid and oil content was the cause of negative association with erucic acid. Besides negative direct effect of oleic acid (-0.175), its negative indirect effects through linoleic, linolenic, eicosenoic acid and erucic acid were responsible for negative association between oleic and erucic acid. It is obvious that direct selection for high oleic content would be desirable to reduce the erucic acid.

Negative direct effects were also showed on erucic acid through linoleic and eicosenoic acid. Its high indirect negative effect through linolenic acid (Table 3) indicated that linoleic acid would reduce with an increase in linolenic acid. The correlation coefficient between eicosenoic and erucic acid was the highest positive relationship; it would be desirable to practice (-0.936) and negative due to its negative direct and indirect effect through other characters except linolenic acid. The high positive association between linolenic and erucic acid was the result of its highest positive direct effect.

Oil content by an increase in oleic acid and in turn, the reduction and oil content showed positive direct effects on erucic acid. Oil content also had (0.102) as well as, positive but non-significant correlation negative association with erucic acid. The results are in conformity with the earlier findings of Shah (1997), Kandil (1994) and Kumar et al. (1999) and Li et al. (1990). The relationship between oil content and erucic acid needs to negative correlation with erucic acid. Hence, a change in be broken because both erucic acid and oil content are considered undesirable.

In the present investigation, the oil content and erucic acid had negative direct effect characters studied and contributed 50% to the variability but had been neutralized by positive indirect effect through the erucic acid content, hence, many other factors, oleic and eicosenoic acid, which resulted into positive responsible for variation in this fatty acid remained unknown, which

need to be further investigated.

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