Correlation analysis and path coefficient studies for fatty acid profile in Indian mustard (*Brassica juncea* L)

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Abstract

In the present study, one hundred seventy-five genotypes of Indian mustard (*Brassica juncea*) were analyzed for its major fatty acid traits using correlation coefficient and path coefficient to examine the cause and consequences relationship with direct and indirect components. 175 $F_{2:3}$ plants formed by crossing Pusa mehak (Indian genotype) × Primus (East-European genotype) and selfing the F_1s and F_2s were utilized for this study. The correlation coefficient of the major fatty acids revealed that there was positive correlation and significance for stearic acid and oleic acid (0.67), palmitic acid and linoleic acid (0.60), linolenic and oleic acid (0.50). While significant negative correlations existed between erucic acid and all other fatty acids except eicosenoic acid (0.41). It was also established that a reduction in erucic acid exhibits an elevation in both oleic and linoleic acids and a simultaneous decrease in eicosenoic. In path coefficient studies, when oil content as dependent character and all other residual 7 fatty acids percentage was considered as independent contributing characters, eicosenoic acid (G= 0.76 ,P=0.73) and oleic acid (G= 0.72, P=0.71) displayed high order of direct effect on oil content and it is followed by linolenic (G= 0.55, P= 0.53), erucic acid (G= 0.47, P= 0.46), linoleic acid (G= 0.35, P= 0.33). These characters also been identified as major direct and indirect contributors towards oil contents and fatty acid profile in Indian mustard. The variation in grain yield unexplained by the seven causes was considered to be due to residual effect (G= 0.28, P= 0.31), which is uncorrelated with other related factors.

Keywords: Indian mustard, correlation coefficient, fatty acid, path coefficient analysis

Introduction

Brassica, encompassing rapeseed-mustard, stands as a prominent oilseed crop, ranking second only to soybean within the oilseed hierarchy. Among the Brassica group, Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] holds particular significance, primarily cultivated during the *Rabi* season. Despite its relatively lower levels of polyunsaturated fatty acids and higher saturated fatty acid content compared to other edible oils, Indian mustard oil contains a substantial amount (34–50% of total fatty acids) of erucic acid (C22:1), a major drawback due to its potential health implications, such as myocardial infarction and elevated blood cholesterol (Mortuza *et al.,* 2006). Reducing erucic acid content becomes imperative to enhance the nutritional profile of Indian mustard oil, aligning it with international standards that prescribe erucic acid content to be below 2% of total fatty acids. The pursuit of low erucic acid (LEA) varieties in *Brassica juncea* led to the identification of the zero erucic mustard (ZEM) as the initial source (Kirk and Oram, 1978). This genotype and its derivatives have played a pivotal role in breeding programs, facilitating the development of LEA varieties

through phenotypic selection involving intricate biochemical assays.

Brassica juncea comprises two distinct gene pools: the East European gene pool, characterized by low erucic acid, and the Indian gene pool, possessing undesirably high erucic acid content but exhibiting better yield under Indian subcontinent conditions. Consequently, the quest for improved oil quality coupled with high yield stands as a primary objective for Indian mustard. Comparatively, rapeseed/mustard oil boasts minimal harmful saturated fatty acids and contains essential fatty acids, namely linoleic and linolenic acid, in adequate proportions. Existing studies (Genet *et al.,* 2004; Sial *et al.,* 2004; Patel *et al.,* 2003; Chauhan *et al.,* 2002; Rahman *et al.,* 1999; Rudloff *et al.,* 1999) highlight the interrelationships between oil and various fatty acid traits. Reduction in erucic acid correlates with elevated levels of oleic and linoleic acids. Chauhan *et al.* (2012) reported a negative and significant relationship between erucic acid and oleic, and linoleic acid. Earlier emphasis primarily focused on indicating the relative importance of different component characters concerning plant selection based on variabilities and interrelationships

among genotypes' quantitative characters. In such scenarios, path coefficient analysis emerges as a valuable tool for discerning the direct and indirect associations among diverse characters. The term- path coefficient was coined by Wright in 1921 to denote the direct influence of one variable (cause) on another variable (effect), quantified by the standard deviation remaining in the effect after estimating the influence of all other possible paths except that of the cause.For the present study, $F_{2:3}$ segregating generation of crosses of Indian and East European mustard was subjected to biochemical profiling for correlation analysis and path coefficient analysis.

Materials and Methods

The experimental design incorporated F_3 individuals originating from a controlled cross between Pusa Mehak (Indian genotype) and Primus (European genotype). The initial F_2 population resulting from this crossbreeding effort comprised a total of 359 plants during the 2019-20 *Rabi* agricultural season. F_2 population was selfed to obtain the F_3 population. The individual F_3 plants of each genotype were systematically sown in a designated plot consisting of four rows, totalling 2.70 m^2 (3 m × 0.9 m). The spatial arrangement involved maintaining a row-to-row distance of 30 cm, with a plant-to-plant distance of 10 cm, achieved through thinning 30 days post-sowing. In adherence to recommended agricultural practices, the experimental field received a prescribed fertilizer dosage (120 kg N + 40 kg P_2O_5 + 40 kg K_2O per hectare). Additionally, irrigation was consistently applied at critical stages to optimize crop growth.

Extraction of fatty acids

For fatty acid extraction, 20-25 seeds were finely powdered in a 5 mL culture tube with 0.5 mL hexane, followed by overnight incubation. The supernatant was transferred to a new tube, and 0.5 mL sodium methoxide (prepared with 80 mg NaOH in 100 mL methanol) was added, with a subsequent 45-minute incubation. After adding 750 mL NaCl solution (prepared with 8 g NaCl in 100 mL distilled water) and vortexing, the upper phase was collected for GC injection. Percentages of different fatty acids were calculated using the GC system's software based on peak area.

Correlation analysis

The significance of the phenotypic correlation coefficient was tried out against 'r' values in the 'r' table of Fisher and Yates (1938) for (n2) (DF) degree of freedom, where 'n' is the no. of treatment. The sign of phenotypic correlation was tested using t test (two-tail). The degree of freedom used is (genotypes*replication) – 2.

Path coefficient analysis

Path coefficient analysis was one according to Dewey and Lu (1959). The grain yield per plant served as the dependent variable, influenced directly and indirectly by all seven characters considered as independent variables.

Results and Discussion

Correlation analysis

To explore the correlation and assess the relative importance of various fatty acid characters in the selection program, correlation coefficients were calculated at both genotypic and phenotypic levels for all possible pairs among the seven characters (Table 1 and Table 2). The genotypic correlation, on the whole, exhibited similar signs and slightly higher magnitudes than their phenotypic counterparts. In phenotypic correlation (Table 1), erucic acid was negatively and significantly correlated with oleic acid, linoleic acid, palmitic acid, stearic acid, linolenic acid. It was positively and significantly correlated with eicosenoic acid. Eicosenoic acid exhibit highly significant and positively correlated with palmitic acid and oleic acid. Linolenic acid exhibit highly significant and negatively correlated with linoleic acid. Linoleic acid exhibit highly significant and positively correlated with Stearic acid.

In genotypic correlation (Table 2) the values obtained were generally larger than phenotypic correlation. Erucic acid was negatively and significantly correlated with oleic aicd, linoleic acid, palmitic acid, stearic acid, linolenic acid as above while it was positively and significantly correlated with eicosenoic acid. Basudev *et al*. (2001) documented noteworthy and positive correlations, as follows: between palmitic acid and oleic, linoleic, linolenic, and eicosenoic acids; between stearic acid and oleic acid; between oleic acid and linoleic acid; between linoleic acid and linolenic acid. Additionally, significant and negative correlations were found between stearic acid and eiconesic and erucic acids, as well as between erucic acid and all other fatty acids except eicosenoic acid. Rahman *et al*. (1999) observed positive and significant correlations: between palmitic acid and oleic and linoleic acids; between oleic acid and linolenic acid; between linoleic acid and linolenic acid; and between eicosenoic acid and erucic acid.

Path coefficient analysis

Conducting a path coefficient analysis involved designating oil content as the dependent character and the remaining seven fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, and erucic acid) as independent contributing characters. The direct and indirect effects of different fatty acids is given in Table 3 and Table 4. The minimal direct effects observed for the remaining characters suggest that their contributions to oil content were

Table 1: Phenotypic correlation for 175 F_3 plants

Character	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)	Erucic acid (%)
Palmitic acid (%)	1	0.15**	0.18**	0.60**	0.16**	0.17**	-0.50**
Stearic acid (%)		1	0.67**	0.38**	0.37**	-0.29**	-0.62**
Oleic acid (%)			1	0.39**	0.50**	0.46**	-0.84**
Linoleic acid (%)				1	0.24**	-0.19**	-0.68**
Linolenic acid (%)					1	-0.30**	-0.63**
Eicosenoic acid (%)						1	0.41**
Erucic acid (%)							1

Table 2: Genotypic correlation for 175 F_3 plants

Character	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)	Erucic acid (%)
Palmitic acid (%)	1	0.16*	0.19**	0.66**	0.20**	0.18**	-0.53**
Stearic acid (%)		1	0.70**	0.42**	0.40**	-0.31**	-0.65**
Oleic acid (%)			1	0.42**	0.55**	0.48**	-0.85**
Linoleic acid (%)				1	0.27**	-0.21**	-0.71**
Linolenic acid (%)					1	-0.33**	-0.68**
Eicosenoic acid (%)						1	0.41**
Erucic acid (%)							1

negligible.

Palmitic acid: The direct effect of palmitic acid on oil content was negative (G= -0.40, P= -0.43). Palmitic acid contributed indirectly through oleic acid (G=0.35, P=0.37) and eicosenoic acid (G=0.29, P=28). Palmitic acid contributed indirectly through all other fatty acids positively except erucic acid (G= -0.33, P= -0.34).

Stearic acid: The direct effect of stearic acid on oil content was positive (G= 0.24, P=0.23) but minimal. Stearic acid contributed indirectly through all other fatty acids negatively except oleic acid (G=0.43, P=0.41) and eicosenoic acid (G=0.01, P=0.00).

Oleic acid: The direct effect of oleic acid on oil content was highly positive (G=0.72, P=0.71). Oleic acid contributed indirectly through erucic acid (G= -0.77, P= -0.79) highly and negatively, followed by linolenic acid (G= -0.42, P= -0.45). The indirect effect of oleic acid through palmitic acid, linolenic acid, and erucic acid was negative, while through stearic acid, linoleic acid, and eicosenoic acid was positive.

Linoleic acid: The direct effect of linoleic acid on oil content was positive (G= 0.35, P= 0.33). A highly negative indirect effect of linoleic acid through erucic acid (G= -0.55, P= -0.59) was observed. The indirect effect of linoleic acid through palmitic acid, stearic acid,

Table 3: Path coefficient (genotypic) showing direct (bold) and indirect effects of major fatty acids on oil content in 175 $F_{2:3}$ plants

Fatty acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
Palmitic acid	-0.40	0.00	0.35	0.00	0.10	0.29	-0.33
Stearic acid	-0.01	0.24	0.43	-0.01	-0.11	0.01	-0.12
Oleic acid	-0.34	0.05	0.72	0.09	-0.42	0.10	-0.77
Linoleic acid	-0.09	-0.03	0.23	0.35	0.42	0.32	-0.55
Linolenic acid	-0.10	-0.08	-0.44	0.31	0.55	-0.01	-0.27
Eicosenoic acid	-0.31	-0.05	0.18	0.03	-0.35	0.76	-0.53
Erucic acid	0.44	-0.20	-0.40	-0.33	-0.04	-0.79	0.47
Oil content	-0.24	-0.12	0.62*	-0.15	-0.29	0.46	-0.21

*p<0.05

and erucic acid was negative, while oleic acid, linolenic acid, and eicosenoic acid was positive.

Linolenic acid: The direct effect of linolenic acid on oil content was positive (G=0.55, P= 0.53). Linolenic acid contributed indirectly through oleic acid (G= -0.44, P= -0.45) negatively. Linolenic acid contributed indirectly through all other fatty acids negatively except linoleic acid.

Eicosenoic acid: The direct effect of eicosenoic acid on oil content was positive (G= 0.76, P= 0.73). A high indirect effect of eicosenoic acid through erucic acid (G= -0.53, P= -0.56) was observed. Eicosenoic acid contributed indirectly through all other fatty acids negatively except oleic acid and linoleic acid.

Erucic acid: The direct effect of erucic acid on oil content was positive (G= 0.47, P= 0.46). A highly negative indirect effect of erucic acid through eicosenoic acid (G= -0.79, P= -0.80) was observed. Erucic acid contributed indirectly through all other fatty acids negatively except palmitic acid.

Table 4: Path coefficient (phenotypic) showing direct (bold) and indirect effects of major fatty acids on oil content in 175 F$_{2:3}$ plants

Fatty acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
Palmitic acid	-0.43	0.00	0.34	0.00	0.09	0.28	-0.34
Stearic acid	-0.02	0.23	0.41	-0.02	-0.14	0.00	-0.13
Oleic acid	-0.36	0.04	0.71	0.08	-0.45	0.09	-0.79
Linoleic acid	-0.10	-0.04	0.22	0.33	0.41	0.31	-0.59
Linolenic acid	-0.12	-0.09	-0.45	0.30	0.53	-0.02	-0.29
Eicosenoic acid	-0.33	-0.06	0.16	0.02	-0.37	0.73	-0.56
Erucic acid	0.43	-0.23	-0.44	-0.36	-0.06	-0.80	0.46
Oil content	-0.25	-0.13	0.60*	-0.16	-0.30	0.45	-0.22

*$p<0.05$

The variation in grain yield unexplained by the seven causes was considered to be due to residual effect (G= 0.28, P= 0.31), which is uncorrelated with other related factors. Similar results were reported by Islam *et al*. (2009).

Conclusion

In the current investigation, correlation studies indicated that a reduction in erucic acid is associated with an increase in both oleic and linoleic acids, while a reduction in erucic acid leads to a decrease in eicosenoic acid. In the path coefficient studies, where oil content was considered the dependent character and all other residual seven fatty acids percentages were treated as independent contributing characters, eicosenoic acid (G= 0.76, P=0.73) and oleic acid (G= 0.72, P=0.71) exhibited a high direct effect on oil content, followed by linolenic acid (G= 0.55, P= 0.53), erucic acid (G= 0.47, P= 0.46), and linoleic acid (G= 0.35, P= 0.33). These characters were identified as major direct and indirect contributors to oil content and the fatty acid profile in Indian mustard. This study has facilitated the identification of correlations between different fatty acids in a large segregating population and the determination of the contribution of each fatty acid to oil content.

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