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Use of biochemical indices as selection criteria for improvement in quality traits of Indian mustard (*Brassica juncea* l.)

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Abstract
Oilseed crops occupy a place of prime importance in Indian economy. Rapeseed-mustard is a major oilseed crop grown in India which contains higher fraction of nutritionally undesirable long chain fatty acids especially Erucic acid in oil and Glucosinolate in defatted meal. Development of genotypes with improved quality of oil and defatted meal is of paramount importance and getting top priority worldwide. With this objective 30 genotypes were investigated to estimate the extent and nature of variability (GCV,and PCV), broad sense heritability and genetic advance for eight quality components of oil in Indian mustard (*Brassica juncea* L.). Variability study revealed that the phenotypic coefficient of variations was invariably higher than their corresponding genotypic coefficient of variations. High phenotypic and genotypic coefficient of variation for all the fatty acids including Oleic, Erucic, Eicosenoic, Palmitic, Linolenic and Linoleic acids, whereas the same were low for oil content and Glucosinolate. All the quality contributing characters *viz*., Glucosinolate, Oil content, Palmitic, Oleic, Linoleic, Linolenic, Eicosenoic and Erucic acids exhibited high broad sense heritability. Except Glucosinolate and Oil content, all the fatty acids especially Oleic, Erucic, Eicosenoic, Palmitic and Linolenic acids have recorded high heritability coupled with high genetic advance indicating that the control of additive gene effects over these traits. Furthermore, association and path analysis signifies that indirect selection of high amount of Oleic acid, Linoleic acid and higher oil content would certainly reduced the proportion of Glucosinolate, Erucic acid and Eicosenoic acid (undesirable quality components) resulting into improvement in the quality parameters of Indian mustard genotype(s).

Keywords: Indian mustard, quality improvement, fatty acid profile, glucosinolate, genetic estimates, indirect selection criteria, association and path analysis, biochemical analysis**.**

Introduction
Indian sub-continent is the natural repository of the oilseed crops, yet is importing about 40% of the edible oil in the country. The rise in the population growth at alarming rate and living standard of people makes India a chronically deficient country with respect to production of edible oil. Its vegetable oil import further raised by 14% and a sum of Rs. 32,000 crore was spent on this import during the oil year 2009-10 (Yadava *et al*. 2012) [29]. This makes India the world's largest oil importer. Thus, oilseed crop occupy a place of prime importance in Indian economy. Since, oils extracted from oilseed crops have been used predominantly as edible oil in several parts of the world, it plays an important role as energy rich source of our dietary system. Dietary fat, a concentrated source of energy, supplies about half of the calories and carries fat soluble vitamins. It's by-products are major source of cattle feed and concentrates besides being used as manures and find uses in many other industrial and domestic purposes. Among the different oilseeds, the species belonging to *Brassicaceae* family are third major source of edible oil used globally next to palm and soya oil (Oil World, 2012). The oil extracted from *Brassica juncea* (Czern, and Coss.), the Indian mustard is being consumed by the people in most of the parts of country and the production ranks second among all oilseeds (Chopra and Prakash, 1991; DRMR Vision 2050) [7]. But, due to 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) [23]. European economic committee has restricted cultivation of Brassica crop that contains more than 10% Erucic acid in their oil (Dhillon *et al*., 1992) [9]. Linolenic acid is also one of the few polyunsaturated fatty acid present in rapeseed-mustard oil which disturbs the oxidation stability of oil as it gets readily oxidized, reducing shelf life and frying stability of the oil (Ahmed *et al.* 2013). Glucosinolates, is an another undesirable organic compounds that contain

sulfur and nitrogen, are found in most of the Indian cultivars of rapeseed and mustard to the tune of 80-160µm g^{-1} defatted seed meal (Agnihitri and Kaushik, 1999) [3]. They produce characteristic pungent smell in the meal which reduces palatability of feed. Glucosinolates also produces toxic effects because they contain goitrogenic and other anti-nutritional properties that restrict its use for animal nutrition. Therefore, it is of great need to develop genotypes of Indian mustard having lower level of Erucic acid (<2%) and Glucosinolate (<30 µm g^{-1}) with higher yield potential (Kaushik, 1998) [16]

Development of genotypes with improved quality of oil and defatted meal is of paramount importance and getting top priority worldwide. Even a slight superiority of newly evolved variety in yield coupled with quality over the commercial varieties is enough to ensure replacement of the former by the later without much efforts. During the present era of health consciousness, there has been a growing awareness about the nutritional quality of the oil and meal and this has shifted the emphasis towards breeding for high yield coupled with quality traits in rapeseed- mustard in order to bridge the gap between production and consumption (FAO, 2002) [11]. Hence, it remained one of the paramount important breeding objective in the recent years. Low Erucic acid strains were identified for the first time in Canadian varieties of summer rape (Stefansson *et al*., 1961) [26] and summer turnip rape (Downey, 1964) [10]. Later on, similar strains were identified in Indian mustard germplasms by Kirk and Oram (1981) in Australia through repeated selfings followed by selection of individual seeds having reduced level of Erucic acid(<2%) which were named as ZEM 1 and ZEM 2. Since then, several works through selection, mutation as well as conventional breeding and modern biotechnological techniques have been reported for developing mustard variety with low Erucic acid (Anand and Downey, 1981; Chen *et al*., 1988) [5, 6].

However, Oleic (C 18:1)) and Linoleic (C18:2) acids are considered as vital for nutritionally superior oil, are present in low to moderate amount in mustard oil. Oleic acid has been reported to lower cholesterol level, a major component associated with coronary heart diseases (Grunday, 1986) [13]. High Oleic acid proportion would allow the oil to be used even more widely because of thermo stability. Linoleic acid is an another essential fatty acid that is the basis for prostaglandin and other essential body regulator. An increase in these fatty acids in oil would be of great value for people with low- fat intakes. It is therefore, essential to bred mustard varieties with increased proportion of these fatty acids to make the mustard oil more nutritive and safe for human consumption.

The success of any breeding programme in general and improvement of specific traits through selection in particular, totally depends upon the genetic variability present in the breeding population of a particular crop (Yadav *et al*., 2011) [28]. The characters for which variability is present must be highly heritable. Genetic variability provides not only a basis for selection but also some valuable information regarding selection of diverse parents to be used in a hybridization programme. A thorough knowledge of variability due to various genetic causes, actual genetic variation heritable in the progeny and genetic advance that can be achieved through selection is quite essential. The progress due to selection depends on heritability, selection intensity and genetic advance of the characters. Heritability and genetic advance estimates for different targeted traits helps the breeder to apply appropriate breeding methodology in the crop improvement programme. Although,variability estimates provide information on the extent of improvement possible in different characters, but they do not explain about the extent and nature of relationship prevalent between these characters. The value of studies on relationship between various quality characters in addition to yield components which influence quality is very great indeed, as it furnishes to the plant breeder with an easy and fairly reliable means of isolating high yielding with better quality genotypes from the breeding material. Correlation coefficients never reveal the complex interrelationship between various characters which are related to the dependent variable. Hence, it is necessary to discriminate them and study their correlation and causation in order to give proper weightage to the characters while deciding criteria for selection to bring genetic improvement. This could be achieved by path- coefficient analysis as suggested by Dewy and Lu (1959) [8].

Information on the genetic architecture of the quality characters would facilitate breeders to bring up its improvement through conventional breeding methods. Such information for oil and other quality components in mustard varieties grown in India is limited. And vary from material to material. Therefore the present investigation was aimed to illustrate the genetic control of these quality parameters in order to devise an appropriate breeding methodology for improving Indian mustard genotypes with super oil quality.

Material and Methods

The experimental material for the present study consist of 30 genotypes of Indian Mustard (*Brassica juncea* L.) including 21 established advanced breeding lines generated at the present research station through hybridization; six genotypes from different AICRP centers and three checks (Vardan as national check, Pusa Bold as zonal check and Shivani as local check). Field experiment was laid out in a Randomized Block Design with three replications at the experimental area of Birsa Agricultural University, Ranchi during *rabi* 2012-13. Each genotype was sown in three rows per plot per replication. The plot size was kept as 0.9 x 4m. The spacing of 30cm between rows and 10cm between plants were maintained. Recommended agronomic practices were followed to raise the crop. Replicated samples of each treatments on plot basis were subjected to bio-chemical analysis for oil content, Glucosinolate content and fatty acids profile estimation at Division of Genetics, IARI, New Delhi. Oil content of seed sample was analyzed using Zeltex ZX-800 an NIR-emitting diode based near-infrared transmittance analyzer. Glucosinolate Content in seeds was estimated by NIRS (Near Infrared Reflectance spectroscopy) following the method of Font *et al.* (1998). Fatty acid profile was analysed by Gas Liquid Chromatography (Mucon Model 5765) using SP (2300+2310 SS columns) also at Division of Genetics, IARI, New Delhi. The replicated mean values were subjected for various statistical analysis including analysis of variance as per the method suggested by Singh and Chaudhary (1979) [24]. The estimates of different genetic parameters viz., genotypic (GCV) and Phenotypic coefficient of variation (PCV), heritability in broad sense and genetic advance were computed as per the method suggested by Lush, 1949 followed by Johnson *et al.* (1955) [15]. Genotypic and phenotypic correlations coefficients were estimated according to the formula suggested by Miller *et al.* (1958) [21]. Path analysis was carried out using the genotypic correlation coefficients to know the direct and indirect effects of the components as suggested by Wright (1921) [27] and illustrated

by Dewey and Lu (1959) [8].

Results and Discussion

Variance is the measure of variation. In the present study, thirty genotypes of Indian mustard were evaluated to assess their potential in respect eight quality traits. Analysis of variance revealed highly significant differences among genotypes for all the eight quality characters investigated indicating thereby presence of sufficient genetic variability in the genotypes selected for this study (Table-1). High magnitude of variability has been reported in Indian mustard germplasms and varieties for various quality characters by Kumar (2013) [18]. The reason for high magnitude of variability in the present study may be due to the fact that the genotypes selected for the present study were evolved involving different pedigree and developed at different regions representing different agro-climatic conditions of the country. The effectiveness of selection largely depends on two main factors *viz*., the extent of genetic variability present in the base population in which selection has to be practiced, and secondly the heritability of the character. Among the eight quality characters, wide range of variability was observed for Linolenic acid(7.4-13.7).Eicosenoic acid(3.1-5.3), Erucic acid(21.6-38.6), Oleic acid(15.6-28.5) and Palmitic acid (7.4-11.9) whereas, for Linoleic acid(18.6-26.9), Glucosinolate(130.8-144.9) and Oil content(37.8-44.0), it was moderate to narrow (Table-2).The character showing wide range of variability provide greater opportunities for selection as compared to those having narrow range of variability. All the characters showed very low coefficient of variation. The lower values of coefficient of variation (≤ 20) shows the best genetic potential and its genetic influence while the higher shows more influence of environmental fluctuations. Khan *et al*. (2008) [17] strengthen our results and statement who reported lower values of coefficient of variation for all the quality traits in *Brassica* population.

The phenotypic coefficient of variations was invariably higher than their corresponding genotypic coefficient of variations for all the traits studied. This is mainly due to the fact that the phenotypic variance also consists of error variance in addition to genotypic variance. Among eight quality characters studied under present investigation revealed the presence of high phenotypic and genotypic coefficient of variation for all the fatty acids including Oleic, Erucic, Eicosenoic, Palmitic, Linolenic and Linoleic acids, whereas these estimates were low for oil content and Glucosinolate (Table-2). The difference between the magnitude of phenotypic and genotypic coefficient of variation for these quality characters were minimal which indicated that the variability for these traits is mainly due to the genetic factor and their expression is least affected by environmental fluctuations.

Khan *et al.* (2008) [17] have also reported presence of high phenotypic and genotypic variance for all the fatty acids, except, oil and protein content in *Brassica* population. Kumar (2013) [18] has also found high PCV and GCV for all the fatty acids except for oil content which is in support of our present findings. Thus, the results revealed the presence of high amount of genetic variability in the evaluated genotypes for the major quality contributing characters which indicated that further improvement in these traits would be possible through selection.

Heritability estimate of quality traits revealed that all the quality contributing characters *viz*., Glucosinolate, Oil content, Palmitic, Oleic, Linoleic, Linolenic, Eicosenoic and Erucic acids also exhibited high heritability due to which these traits are supposed to be transmitted to the progeny in next generation easily and to a great extent (Table-2 and Fig-1). But, heritability itself does not provide any indication towards the amount of genetic progress that would result in selecting best individuals rather it depends upon the amount of genetic advance (Kumar and Sasivannan, 2006) [19]. When heritability and genetic advance as expressed in percent of mean were considered together, it was observed that except Glucosinolate and Oil content, all the fatty acids (especially Oleic, Erucic, Eicosenoic, Palmitic and Linolenic acids) have recorded high heritability coupled with high genetic advance. This indicated that the quality contributing traits (especially fatty acids) are mostly governed by additive gene effects and selection for these traits would be effective for quality improvement. Glucosinolate and Oil content showing high heritability but, coupled with low genetic advance are supposed to be controlled by non-additive gene effects indicating thereby improvement in these characters only through selection could be limited.

High heritability for different fatty acid components except oil content and Glucosinolate have also been reported in *Brassica* populations by Khan *et al*. 2008 [17]. Kumar (2013) [18] recorded high heritability coupled with high genetic advance for Oleic, Erucic Linolenic and Palmitic acid but high heritability associated with low genetic advance for oil content which are in agreement ith the present finding.

Correlation analysis of important plant characters leads to a directional model for quality response. The quality of *Brassica* refers to presence of higher fraction of desirable fatty acids, like, Palmitic, Oleic and Linoleic acids along with high oil content *vis-a-vis* low proportion of undesirable fatty acids mainly long chain unsaturated fatty acids *viz*., Erucic acid, Eicosenoic acid and Linolenic acid coupled with low Glucosinolate content in oil and /or defatted cake of mustard. In the present investigation, the correlation studies among various quality characters at phenotypic and genotypic levels revealed that all the fatty acids *viz*., Palmitic, Oleic, Linoleic, Linolenic, Eicosenoic and Erucic acids as well as Glucosinolate and oil content showed differential relationship among themselves with respect to magnitude and direction. All the desirable quality components *viz*., Palmitic acid, Oleic acid, Linoleic acid and oil content had strong positive associationship among themselves. Whereas, these desirable quality traits were found to be significantly and negatively associated with undesirable quality components, like, Erucic acid, Linolenic acid, Eicosenoic acid and Glucosinolate. The association among undesirable quality components were positive mostly with significant value (Table-3 and Fig.-2).

This indicated that slight improvement in desirable quality components will automatically reduce the proportion of undesirable quality characters. Hence, indirect selection based on higher values of desirable quality components especially Oil content, Oleic acid, Linoleic acid and Palmitic acid will certainly improve the quality of oil and defatted cake of Indian mustard. This might be due to the facts that the synthesis of long chain unsaturated fatty acids Linolenic, Eicosenoic and Erucic acids which are also considered as undesirable fatty acids, start after synthesis of Oleic acid in the biosynthetic pathways. Higher synthesis of Oleic acid or Linoleic acid may stop further chain elongation and instauration of fatty acid in the bio-synthesis pathways result in to better quality of oil. Rahman *et al.* (1999) [22] observed positive and significant correlation between Palmitic and Oleic acids; Oleic and Linoleic acids; between Oleic acid and Linolenic acid; between Linoleic acid and Linolenic acid and

between Eicosenoic and Erucic acids. This result is in agreement with the present findings. Similar results indicating presence of negative but significant correlation between Oleic acid and Eicosenoic acid, Erucic acid and between Erucic acid and all other fatty acids except Eicosenoic were also observed by Singh *et al.* (2001) [25] and Islam *et al*. (2009) [14] and Kumar (2013) [18]. Khan *et al*. (2008) [17] have also reported that oil content had negative association with Glucosinolate; Erucic acid and Eicosenoic acid whereas positive correlation of Oleic acid with oil content and Linoleic which supports our results.

Under present investigation, the estimates of correlations among various quality contributing traits were partitioned into direct and indirect effects considering Erucic acid as dependent one among the quality attributing variables. This is why because of the fact that Erucic acid is one of the most undesirable long chain unsaturated fatty acid being synthesized at the end of fatty acids biosynthetic pathways. The estimates of path co-efficient analysis at genotypic and phenotypic level considering Erucic acid as dependent variable showed almost similar trend, direction and magnitude of direct and indirect effects (Table-4). From this table is evident that Oleic acid had registered highest negative direct effect followed by Linolenic acid, Palmitic acid and Linoleic acid. The contributions of these traits to Erucic acid were found to be direct but, in negative direction. Positive direct effects were however, recorded for Glucosinolate and Eicosenoic acid but, with lower magnitude. Their indirect effects through Oleic acid were high and positive. Hence, these traits contributed towards Erucic acid indirectly through Oleic acid. Oil content has registered negative direct effect (-0.0719) with lower magnitude however, its indirect effect through Oleic acid was highest but in opposite direction (Table-4 and Fig-3).

This study indicated that among various quality components, Oleic acid was identified as most desirable quality component which affects the most undesirable quality trait *i.e.* Erucic acid followed by Eicosenoic acid and Glucosinolate either directly or indirectly in opposite direction. In addition to Oleic acid, other desirable quality components are Linoleic acid, Plamitic acid and oil contents that also limit the proportion of undesirable quality components to a great extent directly or indirectly. Meaning thereby indirect selection of high amount of Oleic acid, Linoleic acid and higher oil content would certainly reduced the proportion of Glucosinolate, Erucic acid and Eicosenoic acid (undesirable quality components) resulting into improvement in the quality of genotype(s). This may be due to the fact that after Oleic acid synthesis the carbon chain of fatty acid will be elongated in either of the two directions. At the one end, synthesis of Erucic acid takes place while on the other end Linoleic acid followed by Linolenic acid will be synthesized. If carbon chain will be elongated after Oleic acid towards Linolenic acid end, the synthesis of Erucic acid will be reduced at the other end and *vice-versa*. Hence, more attention should be paid on higher proportion of Oleic acid, Linoleic acid and Oil content while practicing indirect selection for quality improvement in Indian mustard.

Kumar (2013) [18] has also reported high to moderate negative direct effects of Oleic, Linoleic and Linolenic acids on Erucic acid while studying genetic analysis of Indian mustard. He also found highest negative direct effect of Oleic acid as well as indirect effect of oil content through Oleic acid on Erucic acid are in agreement with present findings. The results of earlier works of Li *et al*. (1990); Kandil (1994); Shah (1997) and Kumar *et al*. (1999) are also in conformity of the present findings.

Conclusion

Among different biochemical constituents available in Indian mustard, high fraction of Oleic acid, Linoleic acid with higher oil content were proved to be strong biochemical indices as selection criteria which would certainly improve the quality of oil and defatted meal by reducing the proportion of undesirable quality components especially Glucosinolate, Erucic acid and Eicosenoic acid in Indian mustard.

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Table 1: Analysis of variance (mean sum of squares) for eight quality traits

S. No.	Source of variation	D.F	Glucosinolate Content	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid	Oil content
1.	Replication	2	1.57	0.054	0.027	0.071	0.004	0.01	0.084	0.11
2.	Genotype	29	41.55**	3.98**	34.77**	14.1**	5.228**	1.13**	65.34**	7.08**
3.	Error	58	1.38	0.007	0.038	0.028	0.007	0.003	0.081	0.075
4.	Sem±		0.68	0.05	0.11	0.09	0.05	0.03	0.16	0.16
6.	C.D 5%		1.92	0.14	0.31	0.27	0.14	0.09	0.46	0.44

*, ** = significant at p = 0.05 and 0.01 respectively.

Table 2: Phenotypic variability for quality contributing traits

Sl No.	Characters	Range	Mean ±SEm	PCV	GCV	h^2 (%)	GAM (%)
1.	Glucosinolate Content (µmolg^{-1})	130.8-144.9	139.6 ± 0.68	2.67	2.62	96.68	5.31
2.	Palmitic acid (%)	7.4-11.9	9.6 ± 0.05	11.96	11.95	99.81	24.59
3.	Oleic acid (%)	15.6-28.5	20 ± 0.11	17.02	17.01	99.89	35.03
4.	Linoleic acid (%)	18.6-26.9	22.6 ± 0.09	9.56	9.55	99.80	19.66
5.	Linolenic acid (%)	7.4-13.7	11.2 ± 0.05	11.78	11.77	99.86	24.24
6.	Eicosenoic acid (%)	3.1-5.3	4.5 ± 0.03	13.59	13.57	99.72	27.92
7.	Erucic acid (%)	21.6-38.6	31.5 ± 0.16	14.80	14.79	99.88	30.46
8.	Oil Content (%)	37.8-44.0	40.6 ± 0.16	3.78	3.76	98.94	7.71

Table 3: Genotypic & Phenotypic Correlations among quality traits

Characters	Basis	Glucosinolate content	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Oil content	Erucic acid
Glucosinolate content	G	1	-0.279**	-0.309**	-0.280**	0.231*	0.123	0.073	0.285**
	P	1	-0.272*	-0.304**	-0.271**	0.227*	0.119	0.075	0.280*
Palmitic acid	G		1	0.489**	0.133	0.011	-0.786**	-0.178	-0.658**
	P		1	0.488**	0.132	0.012	-0.783**	-0.177	-0.656**
Oleic acid	G			1	0.368**	-0.154	-0.553**	0.286**	-0.848**
	P			1	0.367**	-0.154	-0.552**	0.284**	-0.847**
Linoleic acid	G				1	-0.357**	-0.154	0.157	-0.439**
	P				1	-0.356**	-0.153	0.156	-0.438**
Linolenic acid	G					1	-0.012	-0.039	-0.095
	P					1	-0.012	-0.038	-0.095
Eicosenoic acid	G						1	0.090	0.666**
	P						1	0.089	0.665**
Oil content	G							1	-0.213*
	P							1	-0.211*
Erucic acid	G								1
	P								1

*, ** = significant at p = 0.05 and 0.01 respectively. G-Genotypic, P-Phenotypic

Table 4: Partitioning of Correlations into direct and indirect effects by path analysis considering Erucic acid as dependent variable

Characters	Basis	Glucosinolate content	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Oil content
Glucosinolate content	G	0.014	-0.004	-0.004	-0.004	0.003	0.002	0.001
	P	0.013	-0.003	-0.004	-0.003	0.003	0.001	0.001
Palmitic acid	G	0.075	-0.273	-0.133	-0.036	-0.003	0.214	0.048
	P	0.074	-0.271	-0.132	-0.035	-0.003	0.212	0.048
Oleic acid	G	0.182	-0.289	-0.590	-0.217	0.091	0.326	-0.168
	P	0.179	-0.288	-0.590	-0.217	0.091	0.326	-0.167
Linoleic acid	G	0.072	-0.034	-0.094	-0.257	0.092	0.039	-0.040
	P	0.071	-0.034	-0.094	-0.257	0.091	0.039	-0.040
Linolenic acid	G	-0.064	-0.003	0.043	0.100	-0.280	0.003	0.011
	P	-0.063	-0.003	0.043	0.099	-0.279	0.003	0.010
Eicosenoic acid	G	0.011	-0.068	-0.048	-0.013	-0.001	0.086	0.007
	P	0.010	-0.069	-0.049	-0.013	-0.001	0.088	0.007
Oil content	G	-0.005	0.013	-0.021	-0.011	0.002	-0.006	-0.071
	P	-0.005	0.012	-0.020	-0.011	0.002	-0.006	-0.070
Erucic acid	G	0.285	-0.658	-0.848	-0.439	-0.095	0.666	-0.212
	P	0.280	-0.657	-0.847	-0.438	-0.095	0.664	-0.210

Residual	G	0.320
	P	0.323

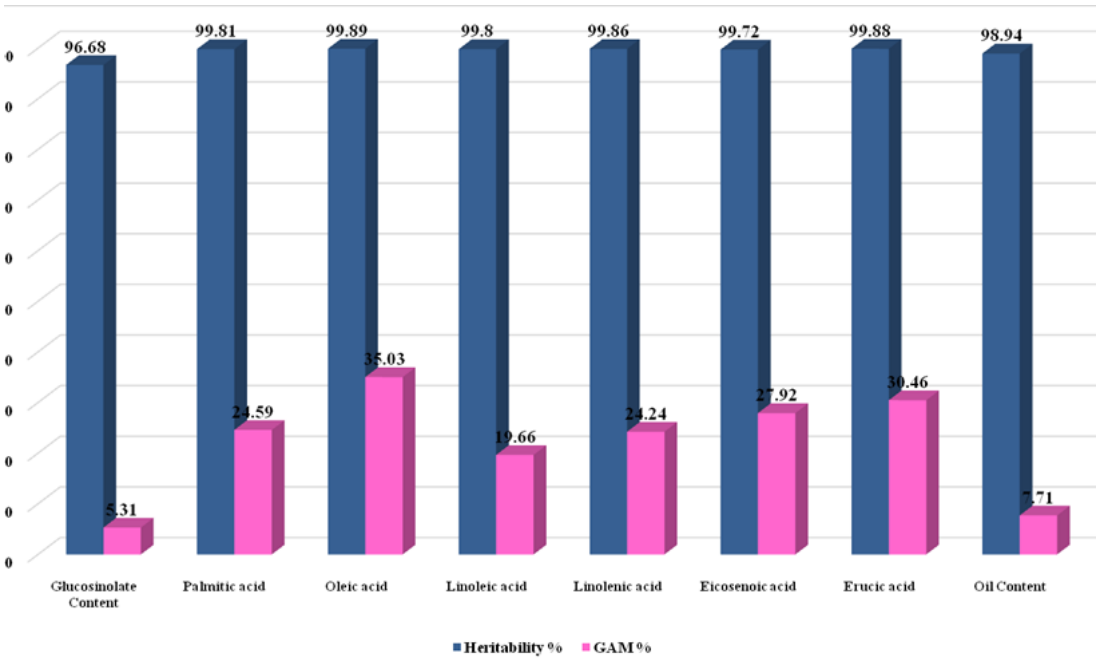

Fig 1: Heritability and genetic advance as expressed in percent of mean for quality traits

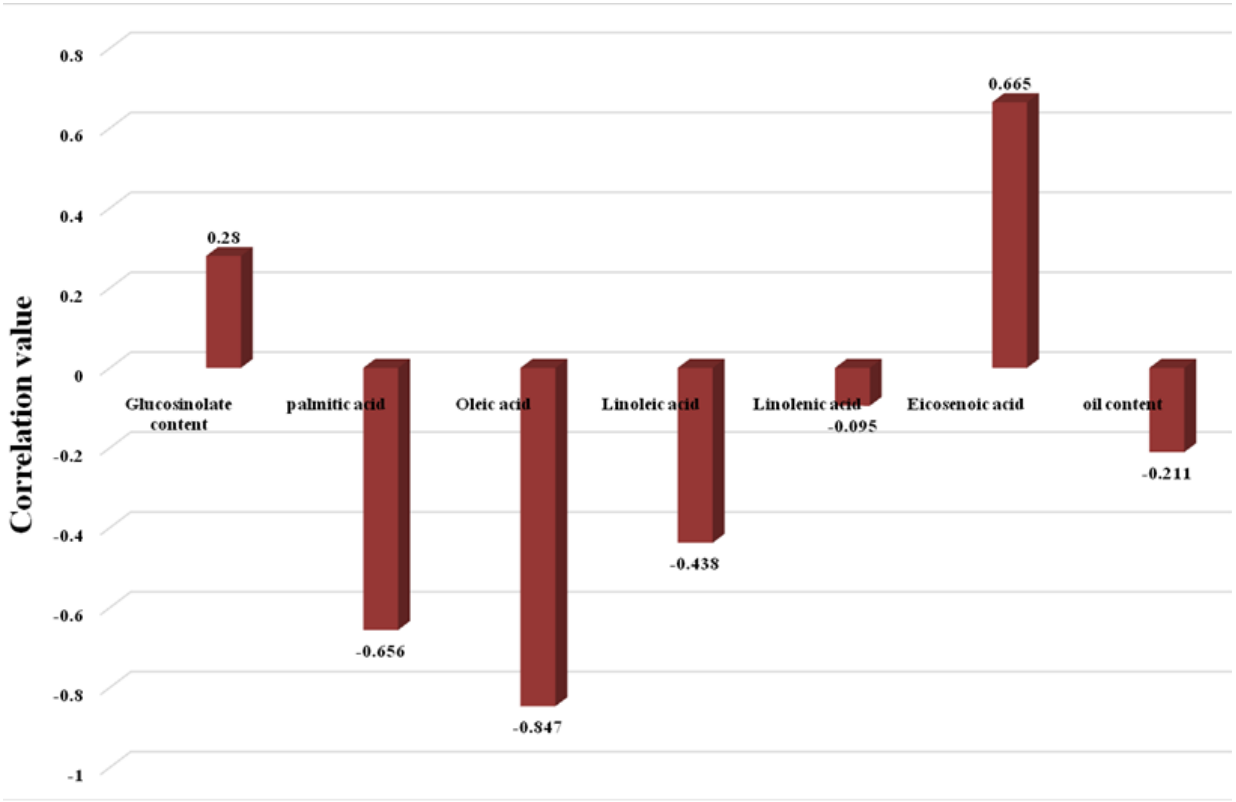

Fig 2: Correlations of Erucic acid with other quality traits

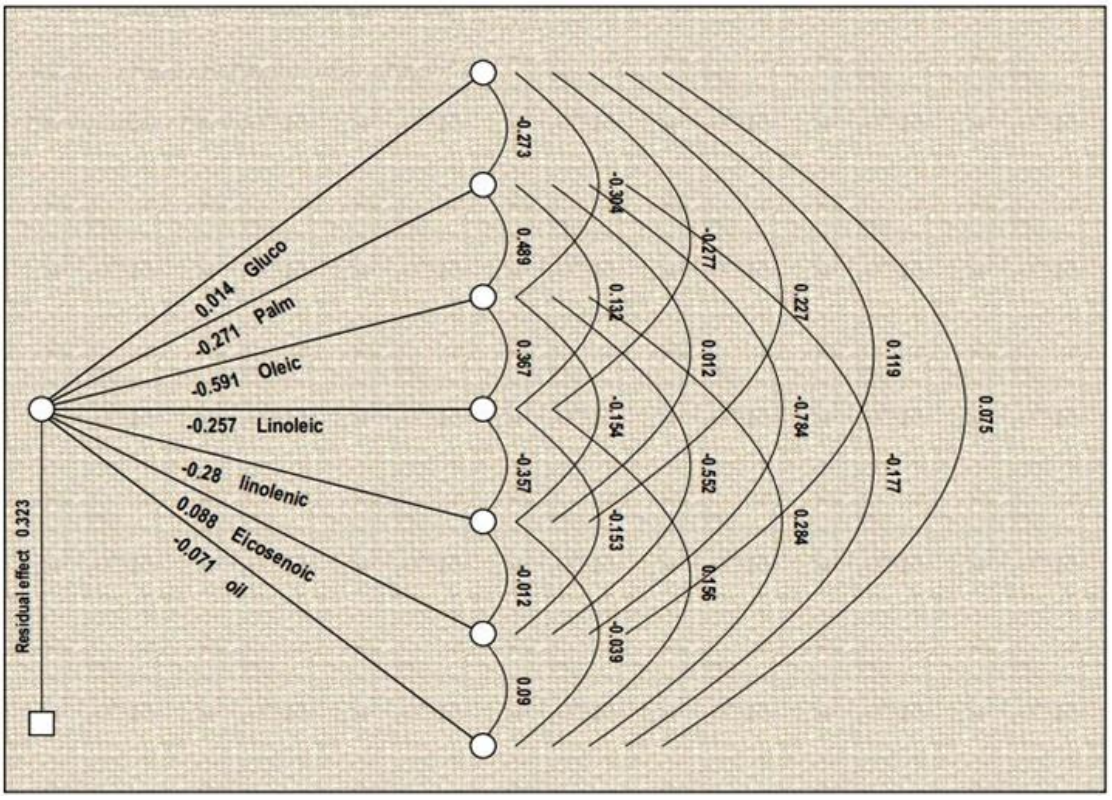

Fig 3: Phenotypic path diagram for Erucic acid

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