

Research Article

Genetic analysis of oleic acid and linoleic acid content in relation to oil quality in groundnut

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Abstract

The present study was conducted to estimate the heritability as well as relationship of oleic acid and linoleic acid with oil quality parameters among the backcross progenies in BC_1F_4 and BC_1F_5 generation of groundnut. The linoleic acid, oleic to linoleic acid ratio and polyunsaturated to saturated fatty acid ratio exhibited high PCV and PCV estimates as well as high heritability coupled with high genetic advance as per cent of mean in both generations. The strong negative relationship between oleic acid and linoleic acid was observed in virtual elimination of linoleic acid from seed lipids is accompanied by an equivalent increase in the content of oleic acid, the proportion of other fatty acids remained unchanged, indicate that the mutation blocks the desaturation of oleic to linoleic acid in high oleate advance backcross progenies.

Keywords

Groundnut, backcross progenies, fatty acid, oil quality

Introduction

Groundnut is the main oilseed legume crop grown mainly in arid and semi arid tropics of the world and its kernels contain good quantities of oil (44 - 56%), protein (22 - 30%), minerals (phosphorous, calcium, magnesium and potassium) and vitamins (E, K and B groups). Fatty acid profiles are more useful in deciding nutritional properties as well as end use functionality of edible plant oils. For both nutritional and industrial purposes, the composition of fatty acids determines the economic value of seed oil (Sanyal and Randal Linder, 2012). For food or feed, oil that is high in the level of beneficial oleic acid (C18:1) is most preferred. Main fatty acids present in groundnut are classified into two groups namely saturated fatty acids (palmitic acid, stearic acid, behenic acid and lignoceric acid) and unsaturated fatty acids (oleic acid, linoleic acid and eicosenoic acid). Oleic acid, linoleic acid and palmitic acid are three major fatty acids in groundnut which constitutes about 90% of fatty acid composition of groundnut oil and remaining fatty acids includes stearic acid, arachidic acid, eicosenoic acid and lignoceric acid.

Even though linoleic acid (C18:2) is an essential fatty acid, it is not suitable for cooking purpose because of its oxidative instability leading to formation of trans fatty acids which is linked to increased risk of cardiovascular diseases (De Souza, 2015). Vegetable oils with high levels of oleic acid (18:1) are preferred for food and industrial purposes. Simultaneous increase of oleic acid and decrease of linoleic acid content, leads to high O/L ratio which in turn imparts higher oxidative stability and reduced risk of cardiovascular diseases and cancer (Wilson *et al.*, 2006). The relative proportion of oleic acid and linoleic acid in groundnut oil determines oil quality and storage life (Worthington and Hammons, 1977).

Fatty acids synthesized through cycle of biochemical reactions involving multienzyme fatty acid synthases. Fatty acid desaturases are enzymes that introduce double bonds into the hydrocarbon chains of fatty acids and play an essential role in fatty acid maintenance metabolism and of biological membranes in living organisms (Singh et al., 2000). Biochemical comparison of developing seeds from mutant and parental varieties of groundnut showed that high oleate phenotype was correlated with reduced activity of the microsomal oleoyl - PC desaturase (Ray et al., 1993). A spontaneous groundnut mutant with 80 per cent oleic and 2 per cent linoleic acid was first isolated by Norden et al. (1987). F435-derived high oleate groundnut cultivars contain two key mutations within the Δ^{12} fatty acid desaturase gene coding region which include a 1bp substitution of G:C \rightarrow A:T at 448 bp in the A genome and a 1bp insertion of A:T at 441_442 bp in the B genome position respectively. Both of these mutations contribute to decreased activity of desaturase leading to accumulation of oleate versus



linoleate (Chen *et al.*, 2010). Inheritance of high oleic acid is reported to be governed by duplicate recessive genes (Moore and Knauft., 1989; Gangadhara and Nadaf, 2016) but influence of modifiers and additional epistatic interactions (Isleib *et al.*, 1996) are also found in some crosses. Generation mean analysis for oil quality traits by Aruna and Nigam (2009) and Singkham *et al.* (2012) revealed the predominant role of additive and additive \times additive gene action in genetic control of the fatty acid content and quality parameters.

Backcross and introgression of novel traits are useful for genetic improvement in breeding programmes. Genetic variability is a prerequisite for genetic improvement but earlier reports indicated very limited genetic variability for O/L ratio in germplasm collection and advanced breeding lines (Hammond et al., 1997; Asibuo et al., 2008). Genetic variability for economic traits is the pre-requisite for any successful breeding programme as the degree of response to selection depends on the quantum of variability. Breeding programmes depend on the genetic systems controlling their inheritance and influence of genetic as well as environmental factors on their expression. The study of variation for the traits under selection and their heritability along with genetic advancement potentials are necessary to predict the response to selection. The study of components of genetic variance helps in further partitioning of genetic variance into additive and non-additive components for measuring the type of gene action involved in the expression of traits under consideration. Hence, an understanding of heritability and relationship between of oleic and linoleic acid with oil quality parameters are useful for planning effective selection procedure in evolving high O/L groundnut genotypes.

Materials and Methods

The present study was performed with two backcross progenies of BC_1F_4 and BC_1F_5 generations derived from cross between GPBD 4 and GM 4-3. GPBD 4 is high yielding genotype with foliar disease resistance whereas non-recurrent parent, GM 4-3 is high oleate mutant. Two backcross generations, BC_1F_4 and BC_1F_5 of cross (GPBD 4 × GM 4-3)-34 × GPBD 4 consisting of 134 and 249 progenies respectively were evaluated by following augmented design with three checks at the experimental plots of Department of Genetics and Plant Breeding, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during summer 2012 and *kharif* 2012 respectively. Standard agronomic practices were followed to raise a healthy crop.

Near Infrared Reflectance Spectroscopy (NIRS) is a more rapid nondestructive technique for screening of large population of seed for analysis of desirable changes in the fatty acid composition, protein and oil (Velasco and Becker, 1998; Biskupek-Korell and Moschner, 2007). Well matured dried kernels were used for fatty acid estimation using Near Infrared Reflectance Spectroscopy (model 6500). NIR diffuse reflectance spectra were collected by а monochromator NIR spectrometer model 6500 (Foss NIRS systems, France) with the range from 400 to 2500 nm, which consisted of a light source of tungsten halogen lamps of 50W, 12 volts. The spectrometer was equipped with silicon detector. For NIRS analysis, single seed was placed in a special adapter about 3 mm thick, with a diameter of 37 mm and a central hole of 6 mm. Before spectra acquisition, a reference spectrum was collected from a standard check cell (IH-0324A, Infrasoft International, LLC, France).

Before using NIR spectrophotometer, it was calibrated using chemical reference method with the application of multivariate regression models to interpret chemical information encoded in the spectral data. The calibrated equations were developed (Kavera et al., 2014) using principle component regression (PCR), partial least square and modified partial least square (m PLS) regression models. The results were confirmed by GC analysis (Kavera, 2008). In BC_1F_4 generation, matured pods were harvested individually and well matured single seed was scanned five times covering all sides of seeds of each genotype and an average of five consistent scans from each genotype was taken for analysis of fatty acids. The superior progenies with high oleic acid (>70%) were selected and advanced to next generation. Different oil quality parameters were estimated as per the formula by Velasco et al. (1997), Mozingo et al. (1988) and Dwivedi et al. (1998). Fatty acid profiles of recurrent parent, nonreccurrent parent and check are depicted in the pie chart and frequency distribution of BC_1F_4 and BC_1F_5 generations were plotted using SPSS. Scatter diagrams were plotted by R studio. Genotypic and phenotypic coefficients of variation were worked out as per the method suggested by Burton and De Vane (1953), heritability and genetic advance were calculated according to Johnson (1955) and Robinson et al. (1949). Simple correlation coefficient was calculated as per Panse and Sukhatme (1967).

Results and Discussion

Fatty acid composition of high oleate mutant, GM 4-3, moderate oleate parent, GPBD 4 and low oleate



genotype, TMV 2 are depicted in Fig 1. The fatty acid composition of genotypes differ from each other with respect to three major fatty acids namely oleic acid, linoleic acid and palmitic acid. Three major fatty acids viz. oleic acid, linoleic acid and palmitic acid constitute 90% of the total oil composition in groundnut. The high oleate mutant, GM 4-3 had 74 % oleic acid and 7.5 % linoleic acid content; whereas high yielding foliar disease resistant genotype, GPBD 4 had 51 % oleic acid and 27 % linoleic acid respectively. Low oleate genotype, TMV 2 had 37 % oleic acid and 38 % linoleic acid (Fig.1) respectively. Analysis of variance revealed significant differences among the backcross progenies for fatty acids and oil quality parameters studied. The genetic parameters viz. genotypic and phenotypic coefficients of variation, heritability in broad sense, genetic advance along with mean and range of different characters are presented in Table 1. The existence of high variability in backcross progenies of both generations for oleic acid, linoleic acid and O/L ratio was observed (Fig. 2). Oleic acid ranged from 53.19 to 76.56 with a mean value of 65.72 in BC_1F_4 generation, whereas in BC_1F_5 , it ranged from 52.17 to 74.27 with a mean value of 63.94. Linoleic acid ranged from 5.95 to 25.9 with a mean value of 14.03 in BC_1F_4 generation, whereas in BC_1F_5 , it ranged from 3.91 to 25.18 with a mean value of 14.11. Maximum O/L ratio (18) was observed in progenies of BC1F5 generation compared to 12 in BC1F4 generation. Minimum iodine value (71.23) was observed in BC₁F₅ generation.

The magnitude of phenotypic coefficient of variation (PCV) estimates were higher than genotypic coefficient of variation (GCV) for all fatty acids as well as oil quality parameters and there was narrow differences between PCV and GCV estimates in both backcross generations (Table 1). This narrow difference between PCV and GCV estimates indicated that variability was mainly due to genotypic differences and there was little influence of environment in the expression of these traits. Oleic to linoleic acid ratio and polyunsaturated to saturated fatty acid ratio exhibited high PCV and GCV estimates whereas palmitic to stearic acid ratio showed moderate estimates of PCV and GCV. Low PCV and GCV estimates were observed for oleic acid, behenic acid, total saturated fatty acids, unsaturated to saturated fatty acid ratio, iodine value and oleic acid desaturation ratio in both generations. The linoleic acid, oleic to linoleic acid ratio and polyunsaturated to saturated fatty acid ratio exhibited high heritability coupled with high genetic advance as per cent of mean in both generations (Table 1).

High heritability coupled with high genetic advance as per cent mean for these traits indicated greater scope for successful selection as these traits as they could be under the influence of additive gene action. High heritability coupled with moderate genetic advance as per cent of mean was observed for oleic acid, palmitic to stearic acid ratio and oleic acid desaturation ratio. Moderate heritability and low genetic advance as per cent of mean was observed for total saturated fatty acids and unsaturated to saturated fatty acid ratio. Similar result was obtained by Kavera (2008).

Correlation coefficients between different fatty acids and oil quality parameters are presented in the Table 2. Oleic acid is a monounsaturated fatty acid; about 38% of oleic acid is found in normal groundnut genotypes and 74% in high oleate groundnut genotypes. Oleic acid is associated with several health benefits such as reduced risk of cardiovascular diseases, cancer and amelioration of inflammatory diseases. Oleic acid content exerted positive association with O/L ratio. arachidic acid. unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio. Oleic acid exhibited negative correlation with palmitic acid, linoleic acid, behenic acid, total saturated fatty acids, total long chain saturated fatty acids, polyunsaturated to saturated fatty acids, iodine value and palmitic to stearic acid (Fig 4 and 5). Linoleic acid is one kind of polyunsaturated fatty acid and its acyl residues are susceptible to oxidation and rancidity of oil (Patel et al., 2004). Linoleic acid was positively correlated with total saturated fatty acids (palmitic acid, behenic acid, lignoceric acid), total long chain saturated fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio. Linoleic acid was negatively correlated negatively with oleic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio (Fig 3 and 4).

Monounsaturated oleic acid is significantly more stable to oxidation than polyunsaturated linoleic acid, thus the ratio of oleic to linoleic acid determines the susceptibility of lipids to oxidation and consequently determine the oil quality. O/L ratio was positively associated with stearic acid, oleic acid, arachidic acid, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio, whereas O/L ratio was associated negatively with palmitic acid, linoleic acid, behenic acid, total saturated fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio. Similar results were obtained by Gangadhara *et al.*, (2015). Both



oleic acid and linoleic acid are produced along the desaturation pathway, which starts with desaturation of stearic acid to oleic acid and further on to linoleic acid (Voelker and Kinney, 2001). Accumulation of oleic acid in plants can therefore be attributed to either increased activity of stearoyl-ACP deasaturation catalyzing the desaturation of stearic acid to oleic acid or reduced activity of oleoyl-phosphatidyl (PC) desaturase that is responsible for desaturation of oleic acid to linoleic acid (Lacombe *et al.*, 2001).

Fatty acid composition can be expressed as different quality parameters of oil. The magnitude of these parameters are directly proportional to the activity of individual enzyme systems (Cherif et al., 1975) believed to be responsible for desaturation of oleic acid and linoleic acid respectively. Stearic acid is one of the component fatty acids ranging from 2 to 4% of the total oil fraction of groundnut. Stearic acid has a neutral effect on blood serum LDL cholesterol concentration and is therefore a desirable constituent of oils for food use (Byfield et al., 2006). Stearic acid was associated positively with behenic acid and O/L ratio whereas it was negatively correlated with eicosenoic acid, lignoceric acid, polyunsaturated to saturated fatty acid ratio and palmitic to stearic acid ratio in both generations. Consumption of saturated fatty acids with a chain length of 8 to 16 has been related to increased blood low density lipoprotein cholesterol content, which is main cause of coronary heart diseases (Scarth and Tang, 2006). Total saturated fatty acids was associated positively with palmitic acid, linoelic acid, behenic acid, lignoceric acid, total long chain fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio and it exhibited negatively association with oleic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio.

Unsaturated to saturated fatty acid ratio was correlated positively with unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio, whereas it was correlated negatively with palmitic acid, linoelic acid, behenic acid, lignoceric acid, total long chain fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio Similar results were reported by Gangadhara *et al.*, (2015). Iodine value is measure of unsaturation of fats and oils and is widely used in the groundnut industry as an indicator of relative storage life of groundnut products (Holley *et al.*, 1968). Iodine value was associated positively with palmitic acid, linoleic acid, behenic acid, total long chain fatty

acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio. Iodine value was associated negatively with stearic acid, oleic acid, arachidic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio. The proportion of palmitic to stearic acid is an indicator of efficiency of oil biosynthesis in the seeds (Harwood, 1996). Low palmitic to stearic acid ratio would be an indicative for efficient oil biosynthesis in seeds (Mollera and Schierholt, 2002). Palmitic to stearic acid ratio was correlated positively with palmitic acid, linoleic acid, eicosenoic acid, lignoceric acid, total saturated fatty acids, polyunsaturated to saturated fatty acid ratio and iodine value, whereas it was correlated negatively with stearic acid, oleic acid, arachidic acid, O/L ratio and oleic acid desaturation ratio. Oleic acid desaturation ratio is a measure of activity of desaturation enzymes. Oleic acid desaturation ratio was associated negatively with palmitic acid, linoleic acid, behenic acid, ligoceric acid, total saturated fatty acids, total long chain saturated fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio.

Linoleic acid, oleic to linoleic acid ratio and polyunsaturated to saturated fatty acid ratio exhibited high PCV and GCV estimates, whereas palmitic to stearic acid ratio showed moderate estimates. Linoleic acid, oleic to linoleic acid ratio and polyunsaturated to saturated fatty acid ratio exhibited high heritability coupled with high genetic advance as per cent of mean in both generations which indicated greater scope for successful selection as these traits which could be under the influence of additive gene action. The virtual elimination of linoleic acid from seed lipids is accompanied by an equivalent increase in content of oleic acid. The proportion of other fatty acids remained unchanged which indicates the mutation block in desaturation of oleic to linoleic acid in high oleate advance backcross progenies. The strong negative relationship between oleic acid and linoleic acid was mainly due to the influence of desaturation of oleic acid to linoleic acid in the fatty acid biosynthesis.

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Parameters	Generation	PAL (%)	STE (%)	OLE (%)	LIN (%)	BEH (%)	O/L ratio	TSFA (%)	P/S ratio	U/S ratio	CIV (%)	Pm/St ratio	ODR
Minimum	BC_1F_4	6.418	3.139	53.192	5.959	3.324	2.053	16.812	0.320	3.785	73.651	1.305	0.672
	BC ₁ F ₅	6.040	2.807	52.174	3.910	3.136	2.072	17.836	0.212	3.548	71.232	1.153	0.674
Maximum	BC_1F_4	10.290	5.098	76.568	25.908	4.171	12.226	20.995	1.254	5.005	91.489	2.988	0.924
	BC ₁ F ₅	10.696	5.311	74.273	25.184	4.817	18.971	21.484	1.216	4.563	89.413	3.411	0.950
Mean	BC_1F_4	7.982	3.996	65.726	14.032	3.743	5.183	18.891	0.737	4.281	81.553	2.022	0.824
	BC ₁ F ₅	8.490	3.963	63.945	14.113	3.840	5.298	19.635	0.713	4.032	80.203	2.187	0.819
$\sigma^2 g$	BC_1F_4	0.269	0.054	15.823	11.194	0.019	3.128	0.202	0.023	0.021	5.813	0.063	0.002
	BC ₁ F ₅	0.547	0.128	22.165	17.508	0.074	5.797	0.218	0.037	0.016	11.298	0.147	0.003
$\sigma^2 p$	BC ₁ F ₄	0.506	0.124	17.056	12.122	0.030	3.217	0.524	0.026	0.042	7.041	0.089	0.002
	BC ₁ F ₅	0.773	0.197	23.581	18.527	0.084	5.891	0.543	0.040	0.038	12.472	0.170	0.003
GCV (%)	BC_1F_4	6.504	5.827	6.051	23.891	3.711	34.314	2.376	20.717	3.391	2.957	12.41	5.314
	BC ₁ F ₅	8.706	9.011	7.362	29.656	7.096	45.803	2.379	26.967	3.174	4.191	17.523	6.753
PCV (%)	BC ₁ F ₄	8.919	8.808	6.282	24.862	4.596	34.801	3.83	21.978	4.774	3.254	14.733	5.453
	BC ₁ F ₅	10.356	11.2	7.593	30.506	7.566	46.176	3.753	28.045	4.837	4.403	18.875	6.879
h² (BS)	BC_1F_4	0.532	0.438	0.928	0.923	0.652	0.972	0.385	0.889	0.504	0.826	0.710	0.950
	BC ₁ F ₅	0.1280	0.6474	0.9399	0.945	0.8796	0.9839	0.4018	0.9246	0.4304	0.9059	0.8619	0.9638
GA	BC_1F_4	0.779	0.317	7.892	6.623	0.231	3.592	0.574	0.296	0.212	4.513	0.435	0.088
	BC ₁ F ₅	1.2803	0.5919	9.4025	8.3792	0.5262	4.9196	0.6099	0.3809	0.1729	6.5903	0.7328	0.1118
GAM (%)	BC_1F_4	9.77	7.9412	12.0056	47.2949	6.1721	69.6996	3.0372	40.2281	4.9611	5.5344	21.5345	10.6675
	BC ₁ F ₅	15.07	14.9358	14.7027	59.3866	13.7089	93.5923	3.1064	53.4151	4.289	8.2171	33.5124	13.6574

Table 1. Estimates of genetic parameters for different oil quality characters in BC₁F₄ and BC₁F₅ generation of groundnut



Table 2. Phenotypic correlation coefficients among fatty acids and oil quality traits of groundnut genotypes in BC1F4 and BC1F5 generations of (GPBD 4
× GM 4-3)-34 × GPBD 4 cross

	PAL (%)	STE (%)	OLE (%)	LIN (%)	ARA (%)	EICO (%)	BEH (%)	LIG (%)	O/L ratio	TSFA (%)	TLCSFA (%)	P/S ratio	U/S ratio	CIV	Pm/St ratio	ODR
PAL (%)	1	332**	911**	.893**	258**	0.099	.528**	.226**	765**	.887**	.319**	.867**	875**	.850**	.892**	898**
STE (%)	487**	1	0.134	-0.147	0.008	573**	.262**	328**	.205**	0.008	0.061	193*	-0.002	167*	710**	0.14
OLE (%)	890**	.290**	1	992**	.290**	0.108	748**	213**	.840**	912**	453**	972**	.896**	958**	730**	.995**
LIN (%)	.868**	336**	987**	1	357**	-0.122	.743**	.177*	858**	.873**	.396**	.989**	845**	.986**	.721**	999**
ARA (%)	354**	.158**	.324**	334**	1	0.042	280**	.521**	.439**	-0.013	.583**	422**	-0.026	439**	200*	.341**
EICO (%)	0.1	474**	.132*	143*	-0.039	1	367**	.404**	0.057	-0.099	-0.077	-0.095	0.056	-0.127	.352**	0.127
BEH (%)	.169**	0.041	435**	.426**	-0.083	366**	1	-0.016	615**	.723**	.547**	.700**	689**	.715**	.255**	751**
LIG (%)	.235**	365**	243**	.258**	.309**	.358**	-0.11	1	0.105	.350**	.687**	0.117	351**	0.128	.313**	187*
O/L ratio	845**	.455**	.834**	846**	.318**	-0.052	233**	137*	1	657**	-0.145	902**	.667**	861**	651**	.846**
TSFA (%)	.818**	-0.07	892**	.847**	-0.051	186**	.503**	.257**	692**	1	.649**	.805**	987**	.799**	.645**	886**
TLCSFA (%)	0.029	-0.006	262**	.254**	.553**	196**	.725**	.401**	-0.068	.462**	1	.304**	647**	.310**	.192*	415**
P/S ratio	.861**	393**	970**	.992**	366**	-0.103	.373**	.241**	877**	.787**	.190**	1	783**	.989**	.724**	984**
U/S ratio	806**	0.043	.860**	786**	0.052	.134*	456**	213**	.672**	976**	410**	729**	1	755**	643**	.857**
IV	.814**	384**	939**	.982**	336**	144*	.399**	.270**	833**	.764**	.236**	.986**	672**	1	.696**	981**
Ps/St ratio	.861**	847**	689**	.700**	308**	.317**	0.087	.328**	737**	.520**	0.019	.729**	500**	.694**	1	721**
ODR	874**	.322**	.992**	999**	.333**	.146*	433**	256**	.839**	861**	260**	987**	.805**	974**	697**	1

Above diagonal - BC_1F_4 Below diagonal - BC_1F_5

PAL - Palmitic acid

ole - oleic acid BEH- Behenic acid

EICO – Eicosenoic acid

IV – Iodine value TSFA – Total saturated fatty acids

O/L ratio - Oleic to linoleic acid ratio

U/S – Unsaturated to saturated fatty acid ratio

STE - Stearic acid

LIG- Lignoceric LIN – Linoleic acid acid

ARA – Arachidic acid

ODR – Oleic acid desaturation ratio

TLCSFA- Total long chain saturated fatty acids ratio

P/S ratio – Polyunsaturated to saturated fatty acid ratio

Ps/St ratio – Palmitic to stearic acid ratio









Fig. 1. Fatty acid composition of TMV 2, GPBD 4 and GM 4-3 groundnut genotypes used in the study







Fig. 2. Frequency distribution of fatty acids in $BC_1F_4(A)$ and $BC_1F_5(B)$ generation





Fig. 3. Frequency distribution of oil quality parameters in $BC_1F_4(A)$ and $BC_1F_5(B)$ generation





Fig. 4. Pairwise association of fatty acids and oil quality parameters in BC1F4 generation



Fig. 5. Pairwise association of fatty acids and oil quality parameters in BC₁F₅ generation