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HERITABILITY AND GENETIC ADVANCEMENT OF OIL QUALITY TRAITS IN GROUNDNUT (*Arachis hypogaea* L.)

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ABSTRACT

Two backcross generations (BC_2F_2 and BC_2F_3) derived from cross(GPBD 4 × GM 4-3)-38 × GPBD 4) between GPBD 4 and GM 4-3 were studied for heritability and genetic advancement of oil quality traits in groundnut (*Arachishypogaea* L.).Oleic acid rich oils are favourable over saturated or polyunsaturated fatty acids due to their industrial properties and edible market benefits. Two backcross generations showed significant variation for oleic acid, linoleic acid,O/L ratio and other quality traits. Oil stability indicator, O/L ratio improved from 2.96 (BC_2F_2) to 5.853(BC_2F_3) indicating the considerable genetic advance and response to selection. The negative correlation between oleic acid and linoleic acid, suggests that increased the levels of oleic acid at the cost linoleic acid and similar interrelationships among fatty acids and oil quality parameters should be considered for breeding for desired oil quality traits in groundnut.

INTRODUCTION

The groundnut is an important oilseed legume crop originating from South America and contains a good source of edible oil, protein and nutrients. The groundnut seed contains 48-50 per cent of oil content and both oleic acid (36-67%) and linoleic content (15-43%) together contribute 80 per cent of oil respectively (Moore and Knauft, 1989, Wang *et al*., 2015). Other fatty acids consists of palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0). The linoleic acid is polyunsaturated fatty acid (PUFA), it adversely affects the oil stability due to its susceptibility to oxidation leading development of off-flavours and rancidity in stored oil and its products (Patel *et al.,* 2004). Consumption of saturated fatty acids with a chain length of 8 to 16 has been related to increased blood low density lipoprotein (LDL) cholesterol content that has been identified as one of the major causes of coronary heart diseases in western countries (Scarth and Tang, 2006). Vegetable oils with increased levels of monounsaturated fatty acid, oleic acid in combination with reduced levels of the polyunsaturated fatty acid, linoleic acid show a higher oxidative stability as well as lower oxidative products (McVetty and Scarth, 2002). Oleic acid is monounsaturated fatty acid in groundnut oil imparting many health benefits and extended shelf life for long term storage. The relative proportion of oleic acid and linoleic acid in groundnut oil determines the oil quality and storage life (Worthington and Hammons, 1977). Groundnut oil with high proportion of O/L ratio is most preferable, as it reduces the risk of cardiovascular diseases by balancing the low density lipoproteins levels in the blood (Vassiliou *et al.,* 2009).

In cultivated tetraploid groundnut (2n = 4x = 40, AABB), the conversion of oleic acid to linoleic acid is catalyzed by the Δ^{12} fatty acid desaturase (FAD).Two homoeologous genes (FAD2A and FAD2B) encoding for the desaturase are located on A and B genomes, respectively. Decreasing the desaturase activity by gene mutation can significantly increase the oleic acid to linoleic acid ratio. A spontaneous groundnut mutant with 80 per cent oleic and 2 per cent linoleic acid was isolated by Norden *et al*. (1987). F435-1 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'→A:T at 448 in the A genome and a 1bp insertion of A:T at 441_442 in the B genome. Both of these mutations contribute to abolishing or reducing the desaturase activity, leading to accumulation of oleate versus linoleate (Chen *et al*., 2010). Inheritance studies of high oleic acid content in groundnut was reported to be due to two recessive mutations (mutant loci designated as ol_1 and ol_2) and probably controlled by two major genes (Moore and Knauft,1989, Gangadhara and Nadaf, 2016).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.Estimation of heritability is useful for response of population to selection (Falconer, 1981) as well as choosing of suitable breeding programmes (Robertson 1957; Hill 1971). Hence, an understanding of heritability, expected genetic advance and relationship among oil quality traits is necessary for planning effective selection procedure in evolving high O/L groundnut genotypes.

MATERIALS AND METHODS

The material for the present study consists of two backcross generations (BC_2F_2 and BC_2F_3) derived from cross between GPBD 4 and GM 4-3. GPBD 4 is high yielding genotype with foliar disease resistance, where as non-recurrent parent GM 4-3, is high oleate mutant. Two backcross generations, BC_2F_2 and $BC_2F_3$of cross (GPBD 4 × GM 4-3)-38 × GPBD 4 consists of 215 and 51 respectively were evaluated by following the 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 *kharif* 2011 and 2012 respectively. The standard agronomic practices were followed to raise the healthy crop. Well matured dried kernels were used for fatty acid estimation using Near Infrared Reflectance Spectroscopy (model 6500). NIR diffuse reflectance spectra were collected by a 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 50 W 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 (mPLS) regression models. The results were confirmed by GC analysis (Kavera, 2008). In BC_2F_2 generation, the matured pod were harvested individually and well matured single seed was scanned for 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 segregants with oleic acid more than 70 per cent were selected and advanced to next generation. Different oil quality parameters estimated as per the formula by Velasco *et al.* (1997); Mozingo *et al.* (1988) and Dwivedi *et al.*(1998). Frequency distribution of BC_2F_2 and BC_2F_3 generations was plotted using SPSS 16.0 and scatter diagrams was plotted by Rstudio. Genotypic and phenotypic coefficient 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). The simple correlation coefficient was calculated as per Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among the backcross population for oil quality traits studied. The genetic parameters *viz.*, genotypic and phenotypic coefficients of variation, heritability in broad sense and genetic advance along with mean and range of different characters are presented in Table 1. The existence of high variability for oleic acid, linoleic acid and other oil quality parameters was observed in

Table 1: Estimates of genetic parameters for oil quality traits in BC_2F_2 and BC_2F_3 generation of (GPBD 4 × GM 4-3)-38 × GPBD 4 cross of ground nut

Parameters	Generation	PAL (%)	STE (%)	OLE (%)	LIN(%)	BEH (%)	O/L ratio	TSFA (%)	P/S ratio	U/S ratio	CIV	Pm/St ratio	ODR
Minimum	BC_2F_2	7.029	3.405	42.705	5.699	3.356	1.313	18.512	0.300	3.311	72.768	1.506	0.568
	BC_2F_3	6.634	3.559	57.339	7.495	3.398	2.878	17.792	0.415	3.866	75.285	1.242	0.742
Maximum	BC_2F_2	11.228	5.098	73.145	32.521	4.472	12.835	22.938	1.418	4.358	93.634	3.018	0.928
	BC_2F_3	8.989	5.340	73.082	20.171	4.219	9.751	20.164	1.010	4.584	85.536	2.236	0.907
Mean	BC_2F_2	9.670	4.172	56.418	20.650	3.831	2.966	20.809	0.990	3.758	85.068	2.337	0.732
	BC_2F_3	7.787	4.257	66.465	12.248	3.767	5.853	19.074	0.638	4.179	79.083	1.843	0.844
δ² g	BC_2F_2	0.158	0.036	12.911	11.105	0.028	1.461	0.179	0.022	0.013	8.966	0.045	0.002
	BC_2F_3	0.084	0.023	8.376	5.482	0.013	1.685	0.016	0.011	0.005	2.491	0.021	0.001
δ² p	BC_2F_2	0.395	0.106	14.145	12.034	0.038	1.550	0.501	0.025	0.034	10.194	0.070	0.002
	BC_2F_3	0.274	0.091	9.678	6.313	0.020	1.758	0.287	0.014	0.024	3.429	0.041	0.001
GCV (%)	BC_2F_2	4.105	4.553	6.37	16.127	4.378	41.289	2.033	14.912	3.078	3.519	9.027	6.03
	BC_2F_3	3.729	3.599	4.351	19.211	2.998	22.246	0.652	16.79	1.721	1.997	7.75	3.721
PCV (%)	BC_2F_2	6.493	7.792	6.668	16.787	5.117	42.531	3.401	15.878	4.912	3.753	11.339	6.186
	BC_2F_3	6.728	7.098	4.677	20.617	3.792	22.721	2.808	18.556	3.706	2.343	10.995	3.897
h² (BS)	BC_2F_2	0.400	0.341	0.913	0.923	0.732	0.942	0.357	0.882	0.393	0.880	0.634	0.950
	BC_2F_3	0.307	0.257	0.866	0.868	0.625	0.959	0.054	0.819	0.216	0.726	0.497	0.912
GA	BC_2F_2	0.517	0.229	7.072	6.595	0.296	2.417	0.521	0.286	0.149	5.785	0.346	0.089
	BC_2F_3	0.332	0.160	5.547	4.494	0.184	2.618	0.060	0.199	0.069	2.771	0.208	0.062
GAM (%)	BC_2F_2	5.346	5.480	12.538	31.914	7.716	82.569	2.504	28.850	3.973	6.799	14.803	12.110
	BC_2F_3	4.258	3.759	8.339	36.875	4.882	44.869	0.312	31.296	1.646	3.506	11.253	7.322

Table 2: Phenotypic correlation coefficients among oil quality traits in BC_2F_2 and BC_2F_3 generations of (GPBD 4 x GM 4-3)-38 × GPBD 4 cross of groundnut

	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	-.305**	-.865**	.859**	-.307**	-.421**	.357**	-.415**	-.769**	.690**	-0.067	.831**	-.685**	.798**	.734**	-.863**
STE (%)	-.268*	1	0.087	-.136**	.188**	-.288**	.376**	-.104*	.159**	.302**	.314**	-.260**	-.260**	-.194**	-.856**	.113*
OLE (%)	-.877**	0.062	1	-.978**	.258**	.524**	-.676**	.322**	.822**	-.796**	-.194**	-.927**	.794**	-.891**	-.512**	.990**
LIN (%)	.855**	-0.061	-.993**	1	-.356**	-.495**	.599**	-.338**	-.864**	.715**	0.084	.976**	-.681**	.966**	.546**	-.997**
ARA (%)	-.445**	-0.206	.498**	-.541**	1	-0.088	0.003	.617**	.588**	.196**	.745**	-.483**	-.216**	-.461**	-.318**	.322**
EICO (%)	-0.225	-.679**	.381**	-.387**	.295*	1	-.459**	.378**	.226**	-.588**	-.229**	-.393**	.550**	-.417**	-0.039	.515**
BEH (%)	.656**	0.235	-.873**	.848**	-.431**	-.531**	1	-0.068	-.412**	.771**	.624**	.463**	-.758**	.464**	-0.08	-.636**
LIG (%)	-.303*	-.399**	0.191	-0.189	.626**	.452**	-0.16	1	.552**	-0.083	.604**	-.395**	.110*	-.334**	-.172**	.333**
O/L ratio	-.803**	-0.025	.881**	-.882**	.579**	.423**	-.694**	.520**	1	-.470**	.230**	-.909**	.466**	-.866**	-.509**	.848**
TSFA (%)	.853**	0.013	-.891**	.850**	-0.195	-.424**	.858**	-0.069	-.699**	1	.580**	.552**	-.976**	.567**	.137**	-.750**
TLCSFA (%)	0.11	-0.109	-.293*	0.247	.560**	-0.047	.451**	.686**	0.036	.527**	1	-0.09	-.574**	-0.059	-.280**	-.129**
P/S ratio	.830**	-0.082	-.974**	.989**	-.593**	-.354**	.791**	-0.244	-.916**	.775**	0.146	1	-.525**	.978**	.621**	-.961**
U/S ratio	-.847**	0.014	.873**	-.819**	0.148	.379**	-.829**	0.106	.723**	-.984**	-.519**	-.752**	1	-.496**	-.159**	.725**
IV	.814**	-0.065	-.965**	.989**	-.584**	-.379**	.799**	-0.178	-.866**	.784**	0.185	.989**	-.736**	1	.560**	-.944**
Ps/St ratio	.842**	-.731**	-.639**	.625**	-0.234	0.217	.319*	-0.021	-.548**	.567**	0.094	.623**	-.579**	.600**	1	-.532**
ODR	-.862**	0.059	.996**	-.999**	.530**	.388**	-.862**	0.185	.876**	-.866**	-.266*	-.984**	.836**	-.983**	-.629**	1

Above diagonal – BC_2F_2 generation Below diagonal – BC_2F_3 generation ; PAL- Palmiticacid ; STE- Stearic acid OLE- Oleic LIN- Linoleic acid ARA- Arachidic acid EICO- Eicosenoic acid; BEH- Behenic acid LIG- Lignoceric acid IV- Iodine value O /L ratio- Oleic to linoleic acid ratio TSFA- Total Saturated fatty acids; TLCSFA- Total long chain saturated fatty acids P/S ratio- Polyunsaturated to saturated fatty acid ratio Ps/St ratio- Palmitic to stearic acid ratio; U/S ratio- Unsaturated to saturated fatty acid ratio; ODR- Oleic acid desaturation ratio

both backcross generation (Fig. 1 and 2)

PCV and GCV estimates

The phenotypic coefficient of variation (PCV) estimates were higher than genotypic coefficient of variation (GCV) for all oil quality parameters and there were narrow differences between PCV and GCV estimates in both backcross generations (Table 1). This narrow differences between PCV and GCV estimates indicated that variability was mainly due to genotypic differences and little influence of environment in the expression of these traits. Low PCV and GCV estimates were observed for palmitic acid, stearic acid, oleic acid, behenic acid, total saturated fatty acids, unsaturated to saturated fatty acid ratio, iodine value and oleic acid desaturation ratio in both generations. Moderate PCV and GCV estimates were observed for linoleic acid and polyunsaturated to saturated fatty acid ratio. These results are in accordance with Kavera (2008), Channayya (2008) and Gangadharaet *al.*(2015) for PCV and GCV estimates of fatty acids and quality parameters.

Heritability and genetic advance estimates

Linoleic acid,oleic to linoleic acid ratio and polyunsaturated to saturated fatty acids ratio were exhibited high heritability coupled with high genetic advance as per cent of mean in both generations. Similar high heritability and genetic advance as percent of mean of linoleic acid was also observed byKavera (2008), Channaya (2009) and Sarvamangala(2009) in groundnut and Kumar (2013) in Indian mustard. High heritability coupled with high genetic advance as per cent of mean indicates the possibility of improvement through selection of these traits. Palmitic acid and stearic acid exhibited moderate heritability couple with low genetic advance as per cent mean, whereas,palmitic to stearic acid ratio showed moderate estimates in both generations (Azharudheen, 2010). High heritability coupled with low genetic advance as per cent of mean was observed for oleic acid, behenic acid and iodine value in both backcross generations (Sarvamangala, 2009, Azharudheen, 2010 and Kavera, 2008).

Response to selection for oleic acid and O/L ratio

The frequency distribution of oil quality traits in BC_2F_2 and BC_2F_3 generation are presented in Figure 1 and 2. The mean value of monounsaturated fatty acid oleic acid content in BC_2F_3 generation was 66.46% compared to 56.41% in BC_2F_2 generation, whereas as polyunsaturated fatty acid linoleic acid content declined to 12.24% (BC_2F_3) from 20.65% in BC_2F_2 generation (Table 1). Oil stability indicator O/L ratio also improved from 2.96(BC_2F_2) to 5.853(BC_2F_3). This indicates accumulation of oleic acid and declined conversion of oleic acid to linoleic acid content as decline in the activity of fatty acid desaturase. Unsaturated to saturated fatty acid ratio improved from 3.75 to 4.17 from $BC_2F_2$to BC_2F_3 generation, whereas polyunsaturated to saturated fatty acid ratio decreased from 0.99 to 0.63 in one generation selection.

Relationship between oleic acid and O/L ratio with other fatty acids and quality traits

The simultaneous increase and decrease in fatty acids and oil quality parameters after one generation of selection indicates the interdependency and mutual relationship between them due to involvement of fatty acid synthesis chain level and decline in the activity of the enzyme responsible for conversion

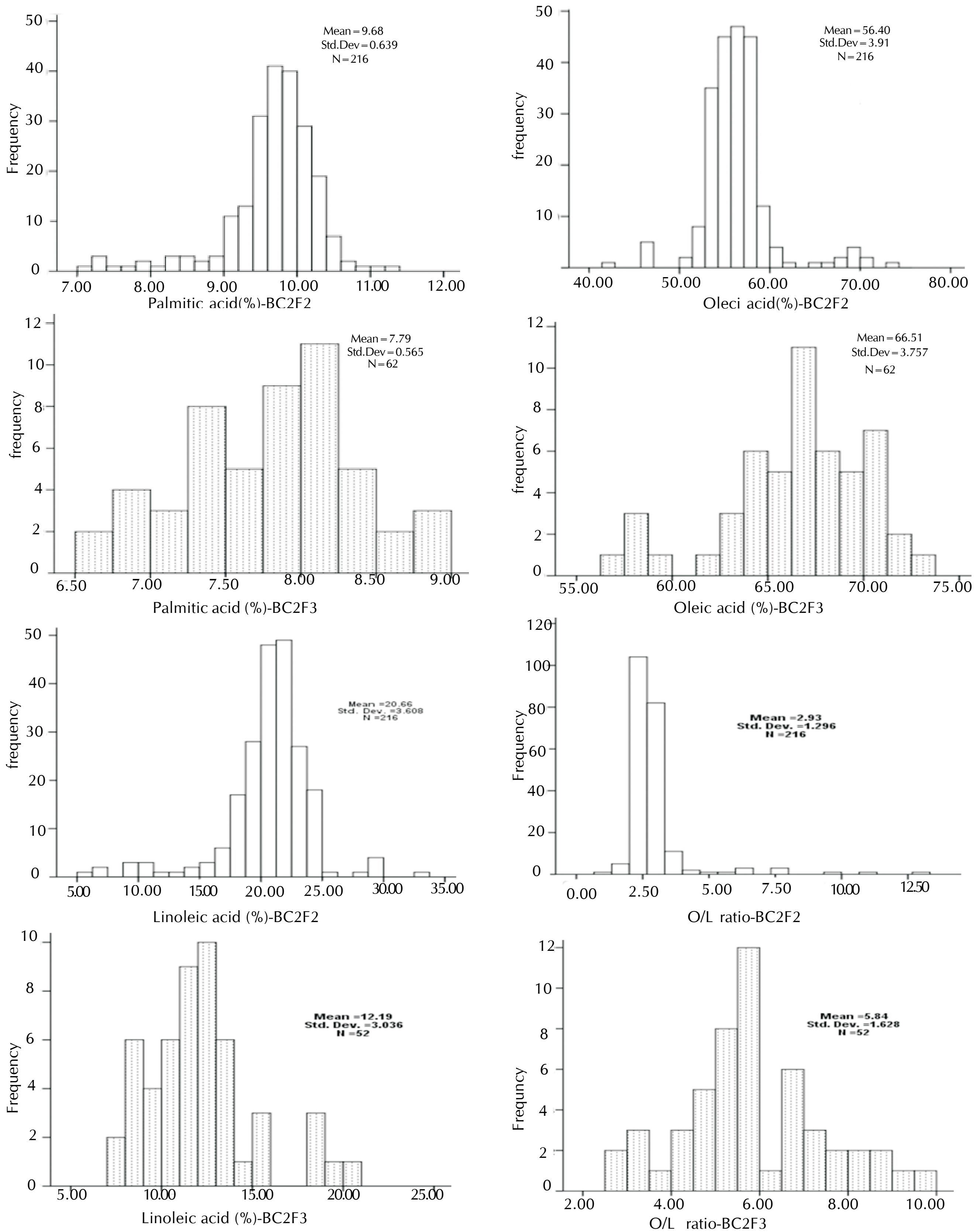

Figure 1: Frequency distribution of fatty acids in BC_2F_2 and BC_2F_3 generations of groundnut

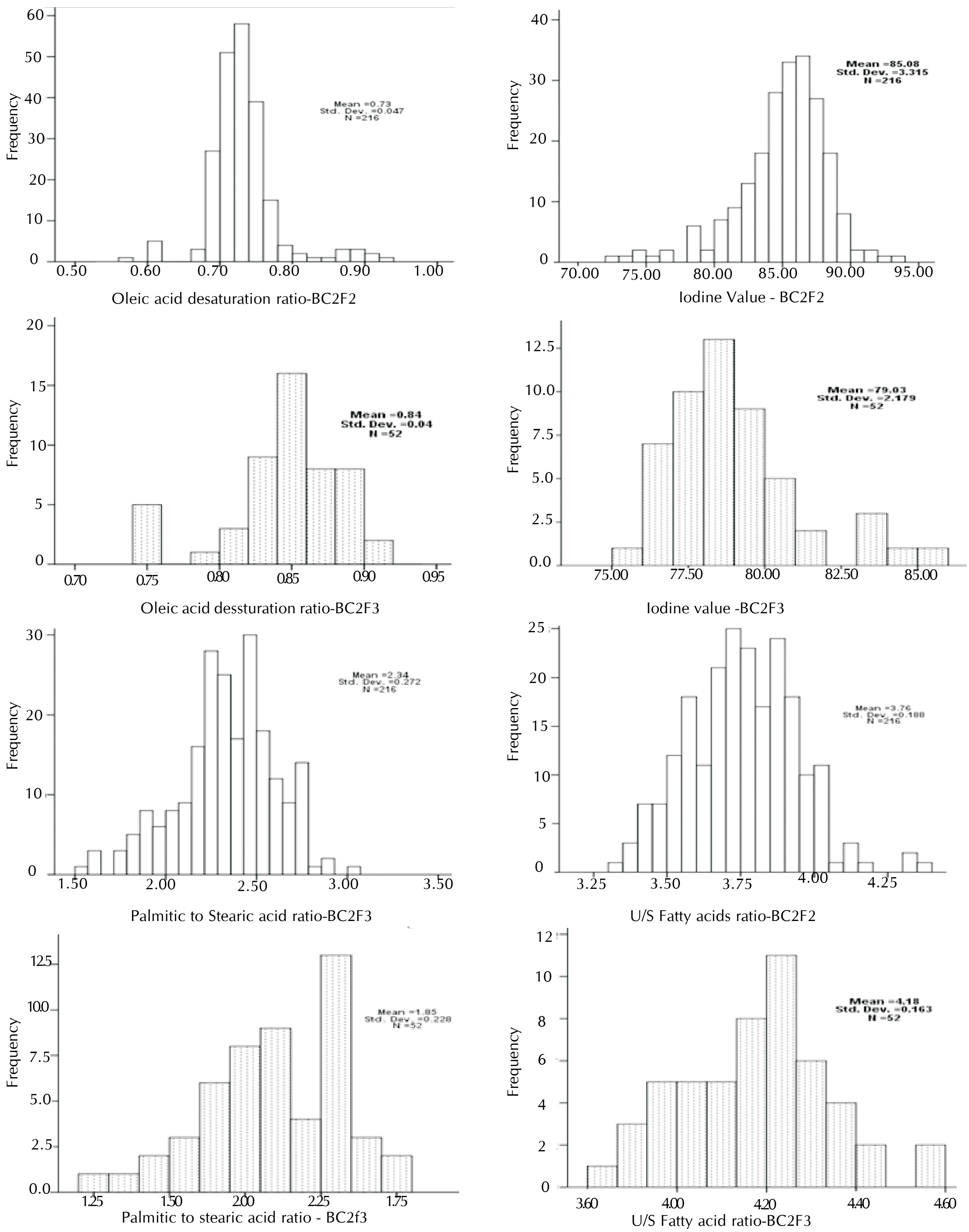

Figure 2: Frequency distribution of oil quality parameters in BC_2F_2 and BC_2F_3 generations of groundnut

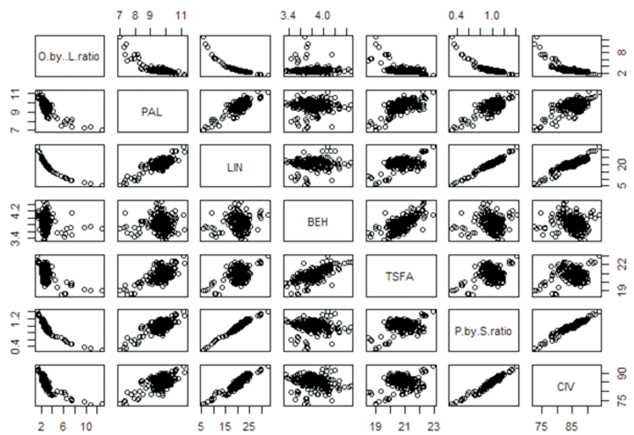

Figure 3: Pair wise correlation (negative) of oil quality traits in BC_2F_2 generation

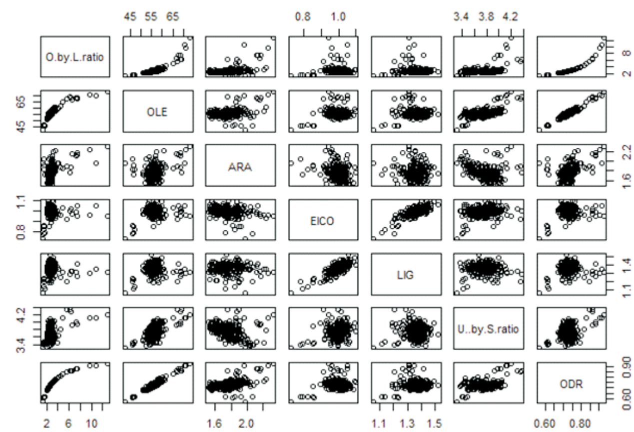

Figure 4: Pair wise correlation (positive)of oil quality traits in BC_2F_2 generation

of fatty acids. The trait high oleic to linoleic acid ratio (high O/L) in groundnut is favoured over low O/L as it confers health benefits and oil stability by extending the shelf life by delaying the development of rancidity (O'Keefe *et al*.,1993).The effective approach to improve oil functionality without hydrogenation is to genetically increase the oleic acid content in groundnut seeds at the expense of linoleic acid. Oleic acid content and O/L ratio associated significant positively with arachidic acid, eicosenoic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio (Gangadhara *et al*., 2015). Oleic acid related strong negatively with palmitic acid, linoleic acid, behenic acid, total saturated fatty acids, total long chain saturated fatty acids, polyunsaturated to saturated fatty acids and iodine value in both generations (Figure 3 and 4).

Relationship among the other fatty acids and oil quality parameters

Pair wise correlation of fatty acids and oil quality parameters in BC_2F_2 and BC_2F_3 generation are presented in Figure 3 and 4. Linoleic acid and polyunsaturated fatty acid are correlated positive significantly with palmitic acid, behenic acid, total saturated fatty acids, polyunsaturated to saturated fatty acid ratio and iodine value. Linoleic acid and polyunsaturated fatty acid are correlated significant negatively with oleic acid, arachidic acid, eicosenoic acid,O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio in both generations(Table 2).Saturated fatty acids are associated with health risks and higher levels of saturated fatty acids are undesirable for oil stability and nutrition .The reduction of the saturated fatty acids of groundnut is also important for the production of biodiesel as reduced saturated fatty acids content would improve winter operability of biodiesel (Korbitz, 2003). Total saturated fatty acids associated positively with palmitic acid, linoleic acid, behenic acid, total long chain fatty acids, iodine value and palmitic to stearic acid ratio and it negatively associated with oleic acid, eicosenoic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio.The higher the proportion of polyunsaturated fatty acid (PUFA), the greater is the oxidation leading to unpleasant odour and tastes, thus limiting the storage quality of the oil (Tatum and Chow, 1992). Polyunsaturated to saturated fatty acid ratio correlated positively with plamitic acid, linoleic acid, behenic acid, total saturated fatty acids, iodine value and palmitic to stearic acid ratio. It negatively correlated with oleic acid, arachidic acid, eicosenoic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation (Figure 3 and 4).

Oil stability and nutritional quality are both dependent on the relative proportions of the saturated and unsaturated fatty acids that constitute the oil. Oxidative rancidity increases with increased levels of polyunsaturated fatty acids. Oxidation of the carbon double bonds of the fatty acids produces acids, aldehydes, ketones and other hydrocarbons that cause unpleasant odour and flavours commonly associated with rancidity, thus limiting the storage quality of the oil (Moore and Knauft, 1989). An overall ratio of 2:1 unsaturated to saturation fatty acid ratio was considered to be best for human diet (Weiss, 2000). Unsaturated to saturated fatty acid ratio correlated positively with oleic acid, eicosenoic acid, O/L ratio and oleic acid desaturation ratio and it correlated negatively with palmitic acid, linoleic acid, behenic acid, total saturated fatty acids, total long chain fatty acids, polyunsaturated to saturated fatty acid ratio, iodine value and palmitic to stearic acid ratio. The iodine value is a measure of the degree of unsaturation in an oil *i.e.* the number of carbon-carbon double bonds in fats or oils. Iodine value is a valuable parameter in understanding oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid. Iodine value associated positively with palmitic acid, linoleic acid, behenic acid, total saturated fatty acids, polyunsaturated to saturated fatty acid ratio and palmitic to stearic acid ratio. It associated negatively with oleic acid, arachidic acid, eicosenoic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio.

Palmitic to stearic acid ratio of storage lipids had been proposed as an indicator for the efficiency of oil biosynthesis in the seeds (Harwood, 1996). A low ratio of this would be an indicator for efficient oil synthesis in the seeds and *vice versa*. (Mollers and Schierholt, 2002). Plamitic to stearic acid ratio correlated positively with palmitic acid, linoleic acid, total saturated fatty acids, polyunsaturated to saturated fatty acid ratio and iodine value. It correlated negatively with stearic acid, oleic acid, arachidic acid, O/L ratio, unsaturated to saturated fatty acid ratio and oleic acid desaturation ratio. Understanding enzymatic and genetic control of denaturation has allowed breeders to select for specific fatty acid profiles in groundnut. Oleic acid desaturation ratio was used to measure the activity of desaturase enzyme (Velasco *et al.,* 1997). Oleic acid desaturation ratio associated negatively with palmitic acid, linoleic acid, behenic 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.

From the present study it can be concluded that the both backcross generations exhibited considerable variation for oil quality traits. From BC_2F_2 to BC_2F_3 generation, oil quality determinant O/L ratio improved from 2.96 to 5.85 after one generation selection process, as it judged by heritability and genetic advance as per cent mean estimates.The positive association of oleic acid with O/L ratio and negative association of oleic acid with linoleic acid implies that simultaneous selection of high oleate and low linoleate will result in enhanced O/L ratio in groundnut.

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