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Estimation of Heritability by Parent-offspring Regression for High-oleic Acid in Peanut

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Abstract: Oleic acid in peanut oil increases shelf-life and kernel quality. Heritability for oleic acid is important for predicting selection progress. The aims of this study were to estimate the heritability of oleic acid content by parent-offspring regression and to determine the correlation among oil characters. The data were collected in the F_2 and F_3 generations derived from the crosses between two high-oleic peanuts (SunOleic 97R and Georgia-02C) and a low oleic peanut (KKU 1). The F_2 populations were planted in the rainy season (2008) and the F_3 populations derived from the F_2 populations were planted in the dry season (2008/09) at Khon Kean University. Heritability estimates in narrow sense were intermediate to high for oleic and linoleic acids (0.63-0.72 and 0.57-0.72, respectively). Heritability estimates for % oil were low in all populations. Significant and negative correlation was observed between oleic and linoleic acids (r = -0.98). The high heritability for oleic acid in this study indicated that selection for high oleic acid in peanut was effective in the F_2 generation.

Key words: *Arachis hypogaea* L., correlation, inheritance, narrow sense, oleic acid, selection progress

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an economically important oil crop. There is approximately 45-50% of oil in the kernels and the main compositions of the oil consist of oleic (C18:1), linoleic (C18:2), palmitic acid (C16:0), stearic (C18:0), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0) and lignoceric (C24:0) acids (Dwivedi *et al*., 1993). The major fatty acids in peanut oil are oleic and linoleic acids accounting for 80% of total fatty acid (Jonnala *et al*., 2005).

A high-oleic acid and a high ratio of oleic to linoleic acid (O/L ratio) are associated with longer shelf-life and lower rancidity. High-oleic peanut has maintained more desirable flavor quality during storage and longer stability of kernel quality compared to normal-oleic peanut (O'Keefe *et al*., 1993; Braddock *et al*., 1995; Mugendi *et al*., 1998). Regular peanut consumption reduces cardiovascular disease risk (Alper and Mattes, 2003). Eating high oleic peanut diet can reduce low density lipoprotein (LDL) in human (O'Byrne *et al*., 1997).

Oleic acid in peanut is controlled by two recessive genes (ol_1 and ol_2) (Moore and Knauft, 1989). The inheritance of oleic acid in Virginia and Spanish-type peanut is controlled by two loci, but modifiers and additional epistatic interactions may be occurring (Isleib *et al*., 1996; Lopez *et al*., 2001). Oleic acid in peanut is not completely controlled by recessive genes and *ol* genes exhibited pleiotropism influencing oleic, linoleic, palmitic, total C_{18} fatty acids and total saturated fatty acids (Isleib *et al*., 2006). Mercer *et al*. (1990) found that additive effects are more important than non-additive effects in the inheritance of oleic, linoleic acids and O/L ratio in peanut and maternal effects were also significant for these characters. Additive×additive epistasis was also detected for oleic acid in peanut (Upadhyaya and Nigam, 1999). Genotype, genotype×year and genotype×planting date interactions were significant for oleic and linoleic acid content in peanut (Andersen and Gorbet, 2002). Maturity effect also influences oleic, linoleic acid content in peanut seeds (Hinds, 1995). High soil temperature increases oleic acid in peanut seed, but it reduces linoleic acid content (Golombek *et al*., 1995).

The information on the heritability of oleic acid and other fatty acid compositions and the phenotypic correlations among these characters will be profitable for planning suitable breeding strategies for improving high oleic acid in peanut seeds. The effective selection for characters under improvement depends on sufficient additive genetic variation of the characters that are expressed as heritability. Therefore, the objectives of this

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study were to determine the heritability of oleic acid and other fatty acid compositions in peanut and the relationship among oil characters.

MATERIALS AND METHODS

Plant materials: Two peanut genotypes (SunOleic 97R and Georgia-02C) with high oleic acid (Gorbet and Knauft, 2000; Branch, 2003) and one genotype (KKU 1) having low oleic acid were used as parents to generate three F_1 crosses including Georgia-02C×KKU 1, SunOleic 97R× KKU 1 and SunOleic 97R × Georgia-02C.

Field experiment in F_2 generation: The F_1 hybrids were further grown in small plots with spacing of 50 cm between rows and 20 cm between plants within row for producing F_2 seeds for evaluation and generation advance. The experiment was conducted at the Field Crop Research Station of Khon Kaen University (KKU) in the Northeast of Thailand (16°26'N, 102°50'E, 190 masl) in the rainy season during June-October 2008.

Soil preparation was done by ploughing three times. Lime at 625 kg ha^{-1} was incorporated into the soil during soil preparation. The seeds were treated with captan (3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1, 3 (2H)-dione) at the rate of 5 g kg^{-1} of seeds before planting to prevent stem rot (caused by *Aspergillus niger*) and also treated with ethrel (2-chloroethylphosphonic acid) 48% at the rate of 2 ml L^{-1} water to break seed dormancy. Pre-emergence herbicide, Alachlor (2-cholro-2', 6'-diethyl-N-(methoxymethyl) acetanilide 48%, w v^{-1}, emulsifiable concentrate) at the rate of 3.75 L ha^{-1}, was applied just after planting. A seed was planted for each hill Chemical fertilizers of N-P-K at the rate of 23.4 N kg ha^{-1}, 10.2 P kg ha^{-1} and 19.4 K kg ha^{-1} were applied at 14 days after emergence (DAE). Gypsum ($CaSO_4$) at the rate of 312 kg ha^{-1} was applied at 45 DAE. Carbofuran (2,3-dihydro-2, 2-dimethylbenzofuran-7-ylmethylcabamate 3% granular) was applied during the early pod forming stage to control subterranean ants (*Dorylus orientalis*). Manual weeding was done once to keep the experimental plots free from weeds. During 15 to 70 DAE, pests and diseases were controlled by weekly applications of carbosulfan [2-3-dihydro-2, 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% w v^{-1}, water soluble concentrate] at 2.5 L ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1}. Supplementary irrigation was given during the dry periods in the rainy season while the crop was fully irrigated at weekly intervals in the dry season with an overhead sprinkler system.

Thirty-five F_1 plants of Georgia-02C×KKU 1, 18 F_1 plants of SunOleic 97R×KKU 1 and 32 F_1 plants of SunOleic 97R×Georgia-02C were harvested manually. The F_2 seeds of individual F_1 plants were divided into two groups. The first group of the F_2 seeds was evaluated for fatty acids and another group of the F_2 seeds was set aside for evaluation in the F_3 generation. Therefore, there were 35 F_2 progenies, 18 F_2 progenies and 32 F_2 progenies for Georgia-02C×KKU 1, SunOleic 97R×KKU 1 and SunOleic 97R×Georgia-02C, respectively, available for evaluation and next experiments.

Field experiment in F_3 generation: Three experiments were conducted separately for each cross because the differences in numbers of progenies within the crosses. The experiments were set up at the Field Crop Research Station of KKU in the dry season during December 2008-March 2009. A randomized complete block design with two replications was used for each experiment. The experiment for Georgia-02C×KKU 1 cross consisted of 35 families, the experiment for SunOleic 97R×KKU 1 cross had 18 families and the experiment for SunOleic 97R× Georgia-02C cross had 32 families. Ten seeds of each family were planted in one-row plot with 1.2 m long and 20 cm between plants within row. The variety Kalasin 2 was used as border plants at the ends of the rows and border rows around the experiments. Crop management was practiced similarly to that mentioned previously in the experiment in the F_2 generation. Ten plants of each plot were harvested at maturity. The seeds of each family of three crosses were bulked and prepare for fatty acid analysis.

Oil preparation and fatty acid analysis: Twenty mature kernels for each plot were used for determination of oil content and fatty acid compositions. Ground seed sample was dried at 70°C about 15-20 h. Moisture content was measured by weight difference. Two grams of dried seeds were extracted for oil by the Soxtec extractor (50 mL of petroleum ether was used as a solvent):

$$\text{Percentage of oil} = \frac{\text{Oil weight (g)}}{\text{Ground seed weight (g)}} \times 100$$

The extracted oil was determined for fatty acid content by gas liquid chromatography (GLC). The protocol of fatty acid analysis was modified from Bannon *et al.* (1982). Fatty acid methyl esters (FAME) were prepared by adding 1 mL of 2.5% H_2SO_4/MeOH in 10 mg of oil sample and 100 μL of 0.01 g mL^{-1} C17:0 an internal standard. The mixture was incubated at 80°C for 2 h. After incubation, 200 μL of 0.9% (w v^{-1}) NaCl and

200 μL heptane were added to the mixture and mixed well. The FAME was extracted into heptane. The concentration of oil sample was 33 μg, which was dissolved in a 1 μL of FAME. The FAME sample (2 μL) was injected to GLC (with Flame Ionization Detector: FID) for fatty acids analysis. Fatty acid analysis was conducted on Shimadzu Gas Chromatograph GC-14B-CR7A and SGE fort GC capillary column (30 m×0.25 mm ID BPX70 0.25 μm) was used. Helium was the carrier gas at a flow rate of 30 mL min^{-1}. Hydrogen and air were used at the rate of 30 and 300 mL min^{-1}, respectively for the ignition of the FID. Oven temperature was maintained at 130°C for 2 min. Then it was programmed at 5°C min^{-1} to 220°C and held at this temperature for 8 min. The injector temperature and detector temperature were 250 and 300°C, respectively. The standard fatty acids that were used to identify the fatty acid content in peanut varieties consisted of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behenic, erucic and lignoceric acids.

The ratio of oleic to linoleic acids (O/L ratio), iodine value (IV) and the ratio of unsaturated to saturated fatty acids (U/S ratio) (Singkham *et al*., 2010) were computed:

- O/L ratio = % oleic acid/% linoleic acid
- IV = (% oleic acid×0.8601) + (% linoleic acid×1.7321) + (% eicosenoic acid×0.7854)
- U/S ratio = (% oleic acid + % linoleic acid + % eicosenoic acid)/(% palmitic acid + % stearic acid + % arachidic acid + % behenic acid + % lignoceric acid)

Statistical analysis: Heritability estimates in narrow sense (h_{rg}^2) were calculated by parent offspring method using the data of F_3 on F_2 families as suggested in Smith and Kinman (1965):

$$h_{rg}^2 = b/2r_{op}$$

where, b is regression coefficient or slope and r_{op} is relationship of parents-offspring.

Linear regression coefficients (b) were calculated by regressing of F_3 progeny means (Y_i) on F_2 plants (X_i). Standard errors (SE) for the slope of each regression were calculated as follows (Ibrahim and Quick, 2001);

$$SE = [(Y_i^2-(X_iY_i)2/X_i^2)/(n-2)X_i^2]/X_i^2$$

where, n is the number of families.

Simple correlation coefficients were calculated to determine the relationship between the fatty acid compositions of each cross in the F_2 and F_3 generations.

RESULTS

Variation in fatty acid compositions and oil characters: Oleic acid, % oil, the ratio of oleic to linoleic acids (O/L ratio) and the ratio of unsaturated to saturated fatty acids (U/S ratio) were higher in SunOleic 97R and Georgia-02C than in KKU 1, but palmitic, stearic and linoleic acids and iodine value (IV) in SunOleic 97R and Georgia-02C were lower than in KKU 1 (Table 1). Oleic acid contents in the three crosses ranged from 60.5-78.7% of total fatty acid. Linoleic acid contents varied from 4.0-18.1% and % oil were in a range of 45.8-47.8%. Palmitic acid content of three crosses varied from 6.7-10.3%, whereas stearic, arachidic, eicosenoic, behenic and lignoceric acid contents were presented in small amounts, ranging between 1.1-3.9%. Iodine value (IV) and U/S ratio in the three crosses were in the ranges of 75.9-84.8 and 4.2-5.7, respectively. The two crosses of high-oleic parents and low-oleic parent (SunOleic 97R×KKU 1 and Georgia-02C× KKU 1) were lower in oleic acid and O/L ratio than the cross of high-oleic parents (SunOleic 97R×Georgia-02C), but they were higher in linoleic acid than the cross of high-oleic parents in both the F_2 and F_3 generations. The standard deviations for oleic, linoleic acids and IV in the two crosses between high-oleic parents and low-oleic parent were higher than in the cross of high-oleic parents in both generations.

Heritability estimates for fatty acid compositions and oil characters: Heritability estimates in narrow sense of three peanut crosses were calculated for eight fatty acids, % oil, O/L ratio, IV and U/S ratio (Table 2). Heritability estimates for oleic acid varied from 0.63-0.72 and linoleic acid varied from 0.57-0.72. Heritability estimates for palmitic acid, O/L ratio and U/S ratio were high in the two crosses of high-oleic parents and low-oleic parent (SunOleic 97R×KKU 1 and Georgia-02C×KKU 1) ranging from 0.60-0.82, whereas the heritability estimates for these characters in the cross of high-oleic parents (SunOleic 97R×Georgia-02C) were low ranging from 0.12-0.31. Heritability estimates for % oil and lignoceric acid were low in all crosses ranging from 0.00-0.28.

Phenotypic correlation among fatty acid compositions and oil characters: The correlation coefficients between oleic acid and linoleic acid were significant and negative ($r = -0.98$, $p \leq 0.01$) in both the F_2 and F_3 generations (Table 3). The O/L ratio had positive and significant correlations with oleic, eicosenoic, lignoceric acids and U/S ratio, but it had negative and significant correlations with palmitic, stearic, linoleic acids and IV in both the F_2 and F_3 generations. Percentage of oil was positively correlated with oleic acid ($r = 0.30$, $p \leq 0.01$) in the F_3 generation, but not significant in the F_2 generation.

Table 1: Mean and standard deviation of the parental lines and three peanut crosses for fatty acid compositions, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV) and the ratio of unsaturated to saturated fatty acid (U/S ratio) in F_2 and F_3 generations

	Parental line			SunOleic 97R×Georgia-02C		SunOleic 97R×KKU 1		Georgia-02C×KKU 1	
Fatty acid composition	KKU 1	SunOleic 97R	Georgia-02C	F_2	F_3	F_2	F_3	F_2	F_3
Palmitic acid	10.4±1.0	6.3±0.3	6.7±0.3	6.7±0.5	6.7±0.6	8.9±1.2	9.2±1.8	9.1±1.2	10.3±4.3
Stearic acid	6.0±1.1	2.4±0.9	2.9±0.4	3.1±0.4	2.3±0.3	3.9±0.5	3.1±0.5	3.8±0.4	3.4±0.9
Oleic acid	47.3±3.6	80.4±2.0	79.5±1.0	78.7±2.2	78.4±3.8	64.8±7.5	62.8±11.5	63.7±7.6	60.5±10.4
Linoleic acid	28.7±2.8	3.7±1.0	3.2±0.4	4.0±1.7	4.7±3.2	15.6±6.2	17.2±9.5	15.8±6.2	18.1±7.7
Arachidic acid	2.3±0.2	1.3±0.3	1.4±0.1	1.4±0.1	1.3±0.1	1.6±0.2	1.6±0.2	2.1±2.7	1.6±0.3
Eicosenoic acid	0.6±0.1	1.7±0.1	1.8±0.1	1.7±0.1	1.9±0.2	1.1±0.2	1.3±0.3	1.2±0.3	1.2±0.4
Behenic acid	3.3±0.3	2.6±0.1	2.8±0.2	2.7±0.4	3.0±0.2	2.8±0.2	3.2±0.4	2.9±0.3	3.3±0.3
Lignoceric acid	1.3±0.2	1.6±0.1	1.6±0.2	1.7±0.5	1.7±0.2	1.3±0.1	1.6±0.1	1.4±0.3	1.5±0.2
% oil	43.5±1.8	47.5±1.8	50.5±1.6	47.0±2.6	47.7±1.9	46.5±2.5	45.8±2.6	47.3±2.6	47.8±2.6
O/L ratio	1.7±0.3	23.3±7.0	25.2±3.2	22.6±7.2	21.8±6.5	6.0±5.0	7.3±7.0	6.3±6.4	6.9±7.6
IV	90.8±1.9	76.9±0.8	75.4±0.7	75.9±1.5	77.0±2.3	83.6±4.1	84.8±6.3	83.1±4.7	84.4±5.6
U/S ratio	3.3±0.2	6.1±0.6	5.5±0.3	5.4±0.4	5.7±0.4	4.5±0.5	4.5±0.8	4.2±0.6	4.2±0.8

Table 2: Heritability by parent-offspring regression and standard errors of three crosses for fatty acid compositions, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV) and the ratio of unsaturated to saturated fatty acid (U/S ratio)

	Narrow sense heritability		
Fatty acid composition	SunOleic 97R×Georgia-02C	SunOleic 97R×KKU 1	Georgia-02C×KKU 1
Palmitic acid	0.31±0.00	0.61±0.00	0.82±0.00
Stearic acid	0.03±0.00	0.34±0.00	0.66±0.00
Oleic acid	0.68±0.00	0.63±0.00	0.72±0.00
Linoleic acid	0.70±0.00	0.57±0.00	0.72±0.00
Arachidic acid	0.01±0.00	0.57±0.00	0.01±0.00
Eicosenoic acid	0.17±0.01	0.45±0.00	0.67±0.00
Behenic acid	0.02±0.00	0.43±0.00	0.00
Lignoceric acid	0.00	0.28±0.00	0.18±0.00
% oil	0.16±0.00	0.00	0.05±0.00
O/L ratio	0.27±0.00	0.81±0.00	0.61±0.00
IV	0.27±0.00	0.45±0.00	0.49±0.00
U/S ratio	0.12±0.00	0.79±0.00	0.60±0.00

Table 3: Correlation coefficients between fatty acid composition, % oil, the ratio of oleic to linoleic acid (O/L ratio), iodine value (IV), the ratio of unsaturated to saturated fatty acid (U/S ratio) for three crosses in the F_2 generation (above diagonal) and the F_3 generation (below diagonal)

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
Palmitic acid		0.73**	-0.96**	0.97**	0.10	-0.92**	0.11	-0.48**	0.11	-0.88**	0.93**	-0.85**
Stearic acid	0.39**		-0.71**	0.72**	-0.03	-0.82**	0.15	-0.40**	0.19	-0.68**	0.68**	-0.72**
Oleic acid	-0.77**	-0.71**		-0.98**	-0.30**	0.92**	-0.17	0.48**	-0.09	0.90**	-0.90**	0.92**
Linoleic acid	0.63**	0.70**	-0.98**		0.15	-0.93**	0.14	-0.52**	0.10	-0.91**	0.96**	-0.85**
Arachidic acid	0.36**	0.86**	-0.74**	0.75**		-0.22*	-0.13	-0.16	-0.03	-0.16	-0.10	-0.47**
Eicosenoic acid	-0.67**	-0.78**	0.92**	-0.91**	-0.80**		0.05	0.56**	-0.19	0.84**	-0.86**	0.85**
Behenic acid	0.09	0.59**	-0.48**	0.50**	0.71**	-0.41**		0.27*	-0.06	-0.19	0.10	-0.29**
Lignoceric acid	-0.53**	-0.45**	0.54**	-0.52**	-0.38**	0.63**	0.17		-0.17	0.54**	-0.55**	0.28*
% oil	-0.33**	-0.23*	0.30**	-0.25*	-0.15	0.23*	-0.16	0.16		-0.08	0.10	-0.08
O/L ratio	-0.60**	-0.72**	0.91**	-0.91**	-0.71**	0.86**	-0.49**	0.52**	0.26*		-0.86**	0.80**
IV	0.34**	0.61**	-0.85**	0.94**	0.69**	-0.80**	0.50**	-0.42**	-0.15	-0.84**		-0.68**
U/S ratio	-0.77**	-0.80**	0.96**	-0.92**	-0.80**	0.91**	-0.58**	0.49**	0.33**	0.89**	-0.74**	

* and ** significant at $p \leq 0.05$ and significant at $p \leq 0.01$, respectively

DISCUSSION

Differences among the crosses for oil compositions might suggest qualitative inheritance of these characters and previous investigations suggested both qualitative inheritance and quantitative inheritance of oleic acid. The two high-oleic parents carried two recessive alleles (ol_1ol_2) and a low-oleic parent carried two dominant alleles (ol_1ol_2) (Moore and Knauft, 1989; Isleib *et al*., 1996; Lopez *et al*., 2001). According to previous investigations, the inheritance of oleic acid could be qualitative. However, modifier genes may be involved in the expression of oleic acid in low to intermediate-oleic acid peanut genotypes (Lopez *et al*., 2001). This can cause the complexity in genetic control of oleic acid.

The heritability estimates in narrow sense were intermediate to high for oleic, linoleic acids and O/L ratio. The results indicated that the selection of peanut genotypes with high oleic acid and high O/L ratio would be achieved in this population because of sufficient additive genes for these traits. Similarly, additive and additive×additive gene effects are involved in the

inheritance of oleic acid (Mercer *et al*., 1990; Upadhyaya and Nigam, 1999).

The results support previous finding and confirm that the inheritance of oleic acid, linoleic acid and O/L ratio is rather strait forward. Whether the inheritance of these characters is qualitative or quantitative, the incorporation of these characters into peanut genotypes with high yield and good agronomic traits will not be difficult and selection can be carried out at early generations.

However, heritability estimates for % oil were low for all peanut crosses, indicating that improvement of % oil in these populations is very difficult. The results are not surprising because peanut is an oil-bearing crop with rather high oil content ranging from 45-50% (Dwivedi *et al*., 1993).

The reverse association between oleic acid and linoleic acid found in this study indicated that the increase in oleic acid is on the expense of linoleic acid. Reverse correlations between oleic acid and linoleic acid were also observed in other studies (Dwivedi *et al*., 1993; Andersen *et al*., 1998). Andersen and Gorbet (2002) pointed out that the relationships among fatty acid compositions in peanut are under genetic control rather than under environmental variations. The results also support previous findings and indicate that linoleic acid will not hamper the progress in selection of oleic acid.

There was low correlation between oleic acid and % oil and the heritability estimates for % oil were also low for all crosses. The result indicated that selection for higher oleic acid and higher % oil at the same time may be difficult.

CONCLUSION

Intermediate and high heritability estimates for oleic, linoleic acids and O/L ratio suggested the high possibility to improve these characters in this peanut population. The negative association between oleic acid and linoleic acid indicated that selection for higher oleic acid will result in lower linoleic acid.

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