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COMBINING ABILITY FOR OLEIC ACID IN PEANUT (*Arachis hypogaea* L.)

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SUMMARY

Elevated oleic acid concentration increases the quality and shelf-life of peanut and their derived products. Incorporation of this character is an important objective of most peanut breeding programs. The objectives of this study were to examine the general combining ability (GCA) and specific combining ability (SCA) effects for oleic acid concentration and identify promising crosses for developing high oleic peanut varieties. Twenty crosses in the F_2 and F_3 generations from a full diallel matting of five parental genotypes and their parents were evaluated under field conditions in a randomized complete block design with four replications for two seasons along with the original parental lines. Seed samples were analyzed for fatty acid compositions by gas liquid chromatography. The GCA effects were significant for oleic acid and O/L ratio in both F_2 and F_3 generations. The SCA and reciprocal effects were also significant, but their relative contributions to variation among crosses were much smaller than those of the GCA effects. The results suggested that additive gene action was important in the inheritance of oleic acid concentration, and selection for high-oleic acid should be effective. The crosses of the line [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$ with SunOleic 97R and Georgia-02C are most promising to exploit genetic variation for oleic acid, which in this population appears to be beyond the genetic control of the *ol* genes.

Key Words: Peanut breeding, fatty acids, general combining ability, inheritance, seed quality, specific combining ability

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important oil-bearing crop and the kernels contain 50% oil. Oleic and linoleic acids are the primary fatty acids in peanut oil accounting for 80% of fatty acid composition (Andersen and Gorbet, 2002). Oleic acid was positively correlated with the ratio of oleic to linoleic acids (O/L ratio), and it was negatively associated with linoleic acid (Dwivedi *et al.*, 1993).

Maintaining quality is a concern for peanut products worldwide. Low quality and short storability are generally associated with rancidity of the kernels. High-oleic acid peanut has much longer shelf life and flavor stability than normal-oleic peanut (O'Keefe *et al.*, 1993; Mugendi *et al.*, 1998). In addition, iodine value (IV) provides a good measurement of the degree of oil unsaturation and stability (Andersen and Gorbet, 2002). High oleic oil may provide health benefits since it has been associated with lowered blood serum cholesterol especially with low density lipoproteins (LDL) in humans (O'Byrne *et al.*, 1997).

Most studies supported a two-gene model of qualitative inheritance of oleic acid controlled by two recessive genes (ol_1 and ol_2) (Moore and Knauft, 1989; Isleib *et al.*, 1996; López *et al.*, 2001). In the case of qualitative inheritance, breeding for high oleic acid would be relatively easy.

However, quantitative inheritance of this character has also been reported in normal oleic acid peanut germplasm (Upadhyaya and Nigam, 1999). Upadhyaya and Nigam (1999) reported that additive × additive epistasis was detected for oleic acid concentration in peanut. Mercer *et al.* (1990) found that general combining ability (GCA) was detected for oleic acid in the F_1 and F_2 generations and maternal effects were significant in the F_1 generation but dissipated in the F_2 generation. In the case of quantitative inheritance, breeding for this character would be complex and difficult, particularly, if the character has low heritability.

The previous results were dependent on materials used (with or without high oleic acid *ol* genes) and methods of studies (qualitative or quantitative), and genetic control of oleic acid may be beyond the two major genes with large effect (Isleib *et al.*, 2006a). The O/L ratio in a segregating population with high oleic acid showed continuous variation ranging from 2.2 to 25.4 (López *et al.*, 2001). In addition to ol_1 and ol_2, oleic acid concentration may be influenced by environmental and additional genetic factors might have modified the expression of this character (Isleib *et al.*, 2006a), causing the progenies described by López *et al.* (2001) to segregated continuously.

High oleic acid can improve peanut oil quality similar to that of olive oil, and is an important objective of most breeding programs. Since the discovery of high oleic acid in peanut, Georgia-02C (Branch, 2003) and SunOleic 97R (Gorbet and Knauft, 2000) were developed and used as parents in many peanut breeding programs to transfer the high oleic character. However, a breeding line ([(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$) developed at Khon Kaen University,has intermediate oleic acid concentration, and it may carry either ol_1 or ol_2 or another oleic modifying gene.

The objectives of this study were to determine GCA and SCA effects for oleic acid concentration in peanut crosses, and to determine the effect of an intermediate oleic genotype when crossed with high oleic genotypes and normal oleic genotypes.

MATERIALS AND METHODS

Plant materials

Five peanut genotypes differing in oleic acid concentration were used in the crossing program to produce F_1 hybrids. They included SunOleic 97R, Georgia-02C, [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$, KKU 1 and KK 5. SunOleic 97R and Georgia-02C are high oleic varieties which are known to carry two recessive genes, ol_1 and ol_2 (Chu *et al.,* 2009). [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$ is a breeding line developed at Khon Kaen University and genetic control of oleic acid in this peanut genotype has not been studied. KKU 1 and KK 5 are local varieties with normal oleic acid concentration. These five genotypes were crossed in a full diallel mating design to produce 20 F_1 hybrids.

Field experiment for the F_2 generation

Twelve seeds for each of 20 F_1 hybrids and five parental lines were planted in the rainy season 2008 at the Field Crop Research Station of Khon Kaen University (KKU) in Northeast Thailand (16°26′N, 102°50′E,190 masl) for producing F_2 seeds for fatty acid evaluation and generation advance. The experiment was arranged in a randomized complete block design with four replications. Plots consisted of two-rows, 1.2 m in length with spacing of 50 cm between rows and 20 cm between plants within a row. The variety Kalasin 2 was planted to provide a border plant at the end of each row to demarcate plots. Routine cultural practices were followed to grow the plots (Singkham *et al*., 2010). Supplementary irrigation was given during the dry periods in the rainy season with an overhead sprinkler system.

The F_1 plants for each cross and the parental lines were harvested at maturity. Border plants in each plot were discarded, and ten F_1 plants form each plot were harvested manually. The seeds of parental lines were bulked for each plot, while the F_2 seeds from each F_1 plant in the plot were bulked and divided into two sets. The first set was analyzed for fatty acids and the other set was used for planting plots to produce the F_3 generation.

Field experiment for the F_3 generation

The remnant F_2 seeds of 20 crosses and seeds of parental lines were planted in the dry season (2008/09) at the Field Crop Research Station of KKU. A randomized complete block design with four replications was used. Seeds were planted in two-row plots 1.2 m long with spacing of 50 cm between rows and 20 cm between plants within a row. The variety Kalasin 2 was used to plant border plants at the ends of the rows. Field crop management was similar to that in the F_2 experiment. Ten plants in each plot were harvested at maturity. The seeds from each plot were bulked for fatty acid analysis.

Fatty acid analysis

Fifty mature kernels for each sample in the F_2 and F_3 generations were bulked for the determination of oil concentration and fatty acid compositions. The samples were ground and oven-dried at 70 °C for about 15 to 20 h. Moisture concentration was then determined by weight difference between sample prior to oven-drying and after oven-drying. Oil was extracted from seeds by the Soxtec extractor (50 mL of petroleum ether was used as a solvent).

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

The extracted oil was analyzed for fatty acid concentration 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 C17:0 an internal standard. The mixture was incubated at 80 °C for 2 h. After incubation, 200 µL of 0.9% (w/v) 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. Hydrogen and air were used at the rate of 30 and 300 ml/min, 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 to 220 °C and held at this temperature for 8 min. The injector temperature and detector temperature were 250 °C and 300 °C, respectively. The standard fatty acids that were used to identify the fatty acid concentration in peanut varieties consisted of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behenic, erucic and lignoceric acids. Fatty acids are reported here in concentration (percentage as related to total fatty acids).

O/L ratio, IV and the ratio of unsaturated to saturated fatty acids (U/S ratio) (Singkham *et al.*, 2010) were computed as below:

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

Analysis of variance for each character was performed according to the procedures by Hoshmand (2006). When the differences were statistically significant, Duncan's multiple range test (DMRT) was used to compare mean differences. Estimates of combining ability in the F_2 and F_3 generations were computed by using Method 1 Model 2 of Griffing (1956). The relative importance of GCA and SCA effects was calculated as GCA/SCA mean squares. Tests for significance of GCA and SCA effects were done using the *t*-test. Simple correlation was used to determine the relationship between parental line performance and their progenies.

RESULTS

Parental means for fatty acid composition and oil characters

Parents were significantly different for most characters in both seasons (rainy 2008 and dry 2008/09) except for % oil in the rainy season (Table 1). The parental means for oleic acid concentration varied from 45.3 to 80.5% of total fatty acid. Georgia-02C and SunOleic 97R had the highest oleic, eicosenoic, lignoceric acid, O/L ratio and U/S ratio in both seasons. Georgia-02C and SunOleic 97R also had low palmitic, stearic, linoleic, arachidic acids and low IV in both seasons. KK 5 and KKU 1 had relatively low oleic acid and O/L ratio, and they had higher linoleic acid in both seasons. [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ had intermediate-oleic acid in both the rainy and dry seasons (71.6 and 67.3%, respectively).

Combining ability for fatty acid composition and other oil characters

GCA was significant for all characters in both the F_2 and F_3 generations (Table 2). SCA was also significant for palmitic, stearic, oleic, and linoleic acids, as well as % oil, O/L ratio, IV and U/S ratio in both F_2 and F_3 generations. The reciprocal effects were significant for palmitic, oleic, linoleic acids, % oil, O/L ratio and IV in both the F_2 and F_3 generations. The ratios of GCA/SCA mean squares for oleic acid and linoleic acid were high in both the F_2 and F_3 generations, and, therefore, GCA contributed greater to the variation in these characters than did SCA.

SunOleic 97R, Georgia-02C and [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ had high GCA effects for oleic acid (6.41, 7.20 and 3.16, respectively) in the F_2 generation and also in the F_3 generation (8.02, 7.72 and 2.16, respectively) (Table 3). In contrast to oleic acid, these genotypes had low GCA effect for linoleic acid (Table 3). For O/L ratio, GCA effects were high for SunOleic 97R and Georgia-02C in both the F_2 and F_3 generations. The correlation coefficients between fatty acid composition of the parents and their progenies were positive and significant, ranging from 0.39 to 0.59 except % oil in both the F_2 and F_3 generations.

For SCA effects in the F_2 generation, SunOleic 97R × Georgia-02C had the highest SCA effects for oleic acid (2.16) and O/L ratio (5.30), whereas KKU 1 × Georgia-02C had the lowest SCA effect for oleic acid (–4.13) but it had the highest SCA effects for linoleic acid (3.75) and IV (2.80) (Table 4). For SCA effects in the F_3 generation, SunOleic 97R × Georgia-02C had the highest for oleic acid (3.47) and for O/L ratio (8.40), but it had the lowest SCA for linoleic acid (–3.68) and IV (–3.39) (Table 5). KK 5 × SunOleic 97R had the highest SCA effect for linoleic acid (8.54) but it had the lowest SCA effect for oleic acid (–3.68).

The GCA effect of intermediate oleic genotype,((NC 17090 x B1)-9-1 x KK 60-3)F6-8-3 was positive and significant ranging from 2.16 to 3.16 for olelic acid in F2 and F3(Table 3). The SCA effects of intermediate oleic genotype ([(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$) with high oleic genotypes (SunOleic 97R and Georgia-02C) were both negative for oleic acid concentration, and the crosses with normal oleic genotypes (KK 5 and KKU 1) also exhibited negative for oleic acid concentration in both F_2 and F_3 generations (Table 4 and 5).

Table 1. Mean of fatty acid concentration (% of total fatty acid), % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) of parental lines in the rainy season 2008 and the dry season 2008/09 in Thailand.

Parental line	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
Rainy season 2008												
SunOleic 97R	6.3 d	3.1 d	78.7 a	4.5 d	1.4 d	1.8 a	2.8 b	1.7 a	47.2	18.0 b	76.8 d	5.5 a
Georgia-02C	6.5 d	2.8 d	79.8 a	3.1 e	1.3 d	1.8 a	2.7 b	1.7 a	46.5	27.1 a	75.4 d	5.7 a
F6-8-3[a]	7.3 c	4. 5 b	71.6 b	11.0 c	1.9 b	1.0 b	2.7 b	1.1 b	45.0	6.6 c	81.3 c	4.8 b
KK 5	11.5 a	4.0 c	46.9 c	31.0 a	1.7 c	0.7 c	3.0 ab	1.3 b	47.6	1.5 c	94.6 a	3.7 c
KKU 1	10.6 b	5.4 a	47.5 c	28.4 b	2.2 a	0.6 d	3.2 a	1.2 b	50.3	1.7 c	90.5 b	3.4 d
Mean	8.6	4.0	63.8	16.4	1.7	1.2	2.9	1.4	10.5	47.3	84.1	4.5
F-test	**	**	**	**	**	**	**	**		**	**	**
Dry season 2008/09												
SunOleic 97R	6.1 e	2.3 c	80.5 a	3.8 d	1.3 c	1.8 a	2.5 b	1.7 a	47.8 ab	21.8 a	77.1 c	6.2 a
Georgia-02C	6.9 d	2.6 c	78.3 a	4.8 d	1.3 c	1.8 a	2.7 b	1.6 a	51.4 a	17.1 b	77.2 c	5.6 b
F6-8-3[a]	8.6 c	4.1 b	67.3 b	14.4 c	1.8 b	1.0 b	2.6 b	1.2 b	45.8 b	4.7 c	83.5 b	4.5 c
KK 5	11.9 a	4.1 b	45.3 c	30.2 a	1.7 b	0.8 bc	3.2 a	1.3 b	43.6 b	1.5 c	91.8 a	3.4 d
KKU 1	10.7 b	5.2 a	49.2 c	27.0 b	2.1 a	0.7 c	3.3 a	1.3 b	45.4 b	1.9 c	89.7 a	3.4 d
Mean	8.8	3.6	64.1	16.0	1.6	1.2	2.9	1.4	9.4	46.8	83.8	4.7
F-test	**	**	**	**	**	**	**	**	**	*	**	**

[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$

* and ** significant at the 5% and 1% probability levels, respectively

Means in the same column followed by the same letter (s) are not significantly different (at $P < 0.05$) by DMRT

Table 2. Mean squares for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) in the F_2 and F_3 generations.

Source	d.f.	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
F_2 generation													
GCA[a]	4	13.60**	1.96**	609.50**	393.69**	0.21**	0.67**	0.16**	0.14**	1.15**	219.78**	158.31**	2.51**
SCA[b]	10	0.26**	0.14**	5.75**	4.23**	0.01	0.02	0.03	0.01	7.22**	37.86**	2.46**	0.10*
Reciprocals	10	0.09*	0.06	8.58**	5.60**	0.01	0.01	0.01	0.01	4.93**	0.62**	2.94**	0.02
GCA/SCA[c]		52.26	14.07	105.98	93.1	21.47	34.5	5.72	22.62	0.16	5.8	64.38	24.97
Error	48	0.04	0.02	0.89	0.58	0.003	0.005	0.01	0.004	1.17	1.05	0.61	0.01
F_3 generation													
GCA	4	15.91**	3.34**	723.20**	434.55**	0.31**	0.64**	0.28**	0.15*	11.48**	217.39**	155.70**	3.64**
SCA	10	0.80**	0.10*	17.10**	19.44**	0.01	0.03	0.05	0.01	6.14**	41.63**	17.93**	0.16**
Reciprocals	10	0.50**	0.09	22.53**	17.71**	0.02	0.01	0.05	0.02	3.32**	7.33**	12.42**	0.05
GCA/SCA		19.86	31.93	42.28	22.35	44.26	21.88	5.96	15.25	1.87	5.22	8.68	23.43
Error	48	0.08	0.04	6.19	3.59	0.01	0.004	0.01	0.01	1.54	1.54	4.11	0.02

* and ** significant at the 5% and 1% probability levels, respectively

[a] General combining ability

[b] Specific combining ability

[c] The ratio of general combining ability mean squares and specific combining ability mean squares

Table 3. General combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) and correlation between fatty acid compositions of parental lines and their progenies in the F_2 and F_3 generations.

Parental line	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
						F_2 generation						
SunOleic 97R	–0.98**	–0.41**	6.41**	–5.01**	–0.15**	0.24**	–0.09**	0.11**	0.14	3.85**	–2.97**	0.47**
Georgia-02C	–0.98**	–0.52**	7.20**	–5.97**	–0.15**	0.30**	0.01	0.14**	–0.28	5.94**	–3.91**	0.46**
F6-8-3[a]	–0.56**	0.22*	3.16**	–2.46**	0.06**	–0.07**	–0.13**	–0.15**	0.01	–1.31**	–1.60**	0.13**
KK 5	1.41**	0.22*	–8.57**	7.14**	0.06**	–0.24**	0.01	–0.06**	–0.36	–4.30**	4.82**	–0.49**
KKU 1	1.11**	0.50**	–8.21**	6.30**	0.18**	–0.24**	0.20**	–0.05**	0.48	–4.19**	3.66**	–0.57**
r[b]	0.59**	0.47**	0.59**	0.58**	0.50**	0.56**	0.39*	0.47**	–0.09	0.47**	0.57**	0.56**
						F_3 generation						
SunOleic 97R	–1.12**	–0.64**	8.02**	–5.75**	–0.21**	0.26**	–0.16**	0.11**	0.41	5.58**	–2.87**	0.69**
Georgia-02C	–1.11**	–0.54**	7.72**	–6.33**	–0.14**	0.28**	–0.02	0.14**	1.30**	4.41**	–4.10**	0.50**
F6-8-3[a]	–0.43**	0.32**	2.16**	–1.85**	0.10**	–0.10**	–0.15**	–0.11**	0.52	–1.93**	–1.41*	0.02
KK 5	1.59**	0.15**	-9.48**	7.28**	0.04	–0.19**	0.13**	–0.11**	–1.11**	–3.86**	4.31**	–0.59**
KKU 1	1.07**	0.71**	-8.42**	6.65**	0.21**	–0.25**	0.21**	–0.04	–1.12**	–4.21**	4.07**	–0.62**
r[b]	0.53**	0.54**	0.56**	0.54**	0.52**	0.55**	0.36*	0.44**	0.16	0.45**	0.47**	0.56**

* and ** significant different from zero at the 5% and 1% probability levels, respectively

[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$

[b] Correlation coefficient between fatty acid contents of parents and their progenies

Table 4. Specific combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) in the F_2 generation.

Cross	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
SunOleic 97R × Georgia-02C	–0.27	–0.14	2.16**	–1.87**	–0.05	0.11*	–0.09	–0.03	–1.68	5.30**	–1.29*	–0.06
SunOleic 97R × F6-8-3[a]	0.05	0.01	–1.18*	0.96	0.01	–0.01	–0.05	–0.07	1.87*	–2.12*	0.64	–0.20**
SunOleic 97R × KK 5	0.47**	0.49**	–2.48**	1.98**	0.12**	–0.18**	0.07	–0.03	1.54	–3.43**	1.15	–0.13*
SunOleic 97R × KKU 1	0.33	–0.35**	–0.75	0.97	–0.07	–0.07	0.03	0.02	–0.72	–3.22**	0.99	–0.02
Georgia-02C × SunOleic 97R	0.07	0.10	–0.09	–0.21	0.05	–0.01	0.002	–0.05	1.26	1.09	–0.45	0.24**
Georgia-02C × F6-8-3[a]	0.18	–0.01	–1.55*	1.44*	0.03	–0.04	0.23**	–0.01	2.84**	–3.63**	1.11	–0.09
Georgia-02C × KK 5	0.11	0.04	–0.99	0.73	0.01	–0.08	0.002	–0.03	–2.05*	–5.06**	0.36	–0.15*
Georgia-02C × KKU 1	0.39*	0.17	–1.40	1.18	0.08*	–0.07	0.12	0.07	1.79*	–4.94**	0.78	–0.10
F6-8-3[a] × SunOleic 97R	0.08	0.26*	–0.79	0.34	0.10*	0.08	0.11	0.09	1.08	–0.30	–0.04	0.01
F6-8-3[a] × Georgia-02C	0.02	0.20	–0.16	0.19	0.08*	–0.03	–0.02	0.12*	–1.70	–0.38	0.15	–0.14*
F6-8-3[a] × KK 5	0.06	0.003	1.13	–1.62*	–0.02	0.06	–0.08	0.03	0.46	1.84*	–1.80**	0.05
F6-8-3[a] × KKU 1	0.17	–0.13	–0.08	–0.16	–0.08*	0.03	–0.05	0.04	–2.17**	1.57	–0.31	–0.05
KK 5 × SunOleic 97R	0.26	–0.08	–3.48**	2.70**	0.07	–0.01	0.17*	0.05	–0.88	–0.56	1.68**	–0.35**
KK 5 × Georgia-02C	0.39*	0.14	–2.92**	2.31**	0.04	–0.06	0.07	–0.002	0.40	–0.58	1.43*	–0.14*
KK 5 × F6-8-3[a]	–0.05	–0.05	–0.28	0.75	0.004	–0.003	–0.05	0.01	–0.35	–0.13	1.08	–0.01
KK 5 × KKU 1	–0.43*	–0.24*	1.91*	–1.28*	–0.02	0.11*	0.05	0.02	–0.27	3.38**	–0.49	–0.13*
KKU 1 × SunOleic 97R	–0.16	0.16	–0.44	0.41	0.04	–0.02	0.02	–0.01	1.58	–0.09	0.32	–0.10
KKU 1 × Georgia-02C	0.39*	0.06	–4.13**	3.75**	–0.02	–0.18**	0.03	–0.02	–2.25*	–0.99	2.80**	–0.29**
KKU 1 × F6-8-3[a]	0.12	–0.17	–1.70*	0.57	–0.02	0.02	0.04	0.04	2.48**	–0.18	–0.48	–0.01
KKU 1 × KK 5	0.16	0.34**	–1.21	0.31	0.12**	–0.07	0.02	–0.04	–2.06*	–0.06	–0.54	0.26**

* and ** significant different from zero at the 5% and 1% probability levels, respectively

[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$

Table 5. Specific combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) in the F_3 generation.

Cross	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
SunOleic 97R × Georgia-02C	–0.53**	–0.14	3.47	–3.68*	–0.05	0.15**	–0.07	–0.01	–2.57*	8.40**	–3.29*	0.32**
SunOleic 97R × F6-8-3[a]	0.03	–0.08	–1.58	0.92	–0.02	–0.10*	0.09	–0.09	–0.12	–4.08**	0.18	–0.07
SunOleic 97R × KK 5	0.47**	0.08	–1.92	1.46	–0.03	–0.07	0.18*	0.03	2.94**	–3.16**	0.81	–0.33**
SunOleic 97R × KKU 1	1.16	0.23	–3.38	5.24**	0.05	–0.12*	–0.16	–0.04	–0.55	–5.41**	6.10**	–0.34**
Georgia-02C × SunOleic 97R	0.00	–0.10	–0.54	0.69	–0.06	0.04	0.01	–0.05	–0.41	–4.85**	0.76	0.07
Georgia-02C × F6-8-3[a]	–0.01	0.08	1.14	–0.38	0.04	–0.03	0.04	0.00	1.10	–1.94	0.30	–0.03
Georgia-02C × KK 5	0.47**	0.31**	–2.89	3.31*	0.11	–0.18**	0.08	–0.02	0.44	–4.50**	3.12	–0.27**
Georgia-02C × KKU 1	0.44**	–0.28	–3.50	2.46	–0.04	–0.12*	0.08	0.09	–1.11	–3.87**	1.13	–0.24*
F6-8-3[a] × SunOleic 97R	0.46**	0.02	–2.59	2.10	0.01	–0.04	0.06	–0.04	–2.13*	–1.20	1.38	–0.18
F6-8-3[a] × Georgia-02C	–0.11	0.21	–0.17	–1.13	0.08	–0.05	0.00	–0.01	–0.36	0.65	–2.14	–0.13
F6-8-3[a] × KK 5	0.28	–0.12	–0.88	0.82	–0.02	0.04	–0.32**	–0.01	–1.18	1.62	0.72	0.05
F6-8-3[a] × KKU 1	–0.23	0.31*	–0.57	–0.23	0.05	0.04	0.14	0.05	2.08*	2.18*	–0.89	–0.05
KK 5 × SunOleic 97R	1.14	–0.04	–9.38**	8.54**	–0.16*	–0.20**	0.01	–0.21**	–0.89	–3.33**	6.58**	–0.21*
KK 5 × Georgia-02C	0.26	–0.13	–2.24	1.16	–0.03	0.01	0.03	0.08	0.07	–0.21	0.05	–0.10
KK 5 × F6-8-3[a]	0.85**	–0.43**	–1.74	1.35	–0.09	0.10*	0.11	0.02	0.25	–0.29	0.95	–0.10
KK 5 × KKU 1	–0.47*	–0.34*	2.47	–1.98	–0.07	0.11*	–0.05	–0.11	–1.38	3.16**	–1.22	0.35**
KKU 1 × SunOleic 97R	0.23	–0.05	–0.83	1.84	0.06	–0.01	0.23*	0.06	1.24	–0.19	2.45*	-0.06
KKU 1 × Georgia-02C	–0.09	0.11	1.23	0.69	0.03	–0.10*	0.08	0.05	–0.91	0.06	2.15	0.05
KKU 1 × F6-8-3[a]	0.38	0.41	–2.73	1.50	0.19**	–0.01	0.34**	0.08	–2.93**	–0.25	0.20	–0.31*
KKU 1 × KK 5	–0.09	0.08	–0.32	0.48	–0.05	–0.01	–0.20*	–0.18*	0.04	–0.03	0.51	0.08

* and ** significant different from zero at the 5% and 1% probability levels, respectively

[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]$_{F6\text{-}8\text{-}3}$

DISCUSSION

The parental lines could be classified into three groups based on their impact on oleic acid concentration (Table 1). SunOleic 97R and Georgia-02C were classified as genotypes with high oleic acid. [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ was an intermediate genotype, whereas KKU 1 and KK 5 were classified as the normal oleic group.

The difference in the parental lines for oleic acid was not surprising as SunOleic 97R and Georgia-02C are known to carry *ol*$_1$ and *ol*$_2$ genes inhibiting linoleic biosynthesis in peanut (Yu *et al.*, 2008), and, thus, these two recessive genes increase oleic acid in these two genotypes. These genotypes have been used in peanut breeding programs to improve oleic acid concentration. SunOleic 97R was developed from the naturally occurring mutants for *ol*$_1$ and *ol*$_2$ (Gorbet and Knauft, 2000), whereas Georgia-02C was developed from artificial mutants induced by gamma irradiation (Branch, 2003).

The surprising genotype was the line [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ which was intermediate between high group and normal group. It showed consistently higher oleic acid concentration than did KKU 1 and KK 5. [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ was derived from KK 60-3 which was selected from a blend population of NC 7. NC 7 also showed high combining ability for oleic acid (Mercer *et al.*, 1990).

It is unlikely that NC 7 transmission of any *ol* gene to [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ because oleic acid of NC 7 was in a range of the normal oleic group (Isleib *et al.*, 2006b). Therefore, it would carry neither *ol*$_1$ nor *ol*$_2$. If one of the *ol* genes exist in [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$, it should be from the other parents, NC 17090 or B1, but their pedigree is not known. The probability of finding a natural mutant of the *ol*$_1$ allele is quite high with 31.6% in mini-core accessions in the United States (Chu *et al.*, 2007). The cross NC 17090 × B1, was a segregating population from the NCSU peanut breeding program (Jogloy, personal communication). Therefore, one of its progenitors may carry a mutant gene. If so the line [(NC17090 × B1)-9-1 × KK 60-3]$_{F6-8-3}$ should be normal instead of intermediate because the dominance expression of the mutant *ol* gene. The expression of *ahFAD2A* transcript in transgenic high oleic peanut genotype was sufficient to alter high oleic to normal oleic (Jung *et al.*, 2000). The most plausible explanation is that it carries other genetic factors that modify the oleic acid concentration. The presence of an intermediate phenotype indicated that the inheritance of oleic acid concentration might modified beyond the genetic control of just the *ol* genes.

Normal-oleic peanut genotypes have 36 to 70% oleic acid and 15 to 43% linoleic acid, whereas high oleate peanut genotypes have approximately 80% oleic acid and approximately 2% linoleic acid. The first report of high oleic peanut genotypes found only two genotypes with elevated oleic acid concentration out of 494 accessions (Norden *et al.*, 1987) that were high-oleic. Segregation patterns in the F$_2$ population indicated that high oleic acid concentration was digenically inherited in Spanish-type peanut, but there seems to be more allelic variation both within and among them. In addition, variation within the high and normal oleate classes indicated that other factors may be involved in determining the precise O/L ratio (Lopez *et al.*, 2001).

In the absence of *ol* mutant genes in normal oleate peanut genotypes, there is genetic variation for oleic characters. In the presence of two *ol* mutant genes, there is also genetic variation in oleic acid concentration. This type of variation should not exist if only two *ol* genes control this character because all peanut genotypes are homologous for these genes. The results might indicate that oleic acid concentration in peanut is modified beyond the control of these two genes although with relatively small effect (Lopez *et al*., 2001; Moore and Knauft, 1989; Norden *et al*., 1987).

GCA and SCA effects were significant for oleic, linoleic acids, % oil, O/L ratio, IV and U/S ratio in both the F_2 and F_3 generations. The results suggested that additive and nonadditive gene action contributed to the variation in these characters. However, GCA effects contributed greater than the SCA effects for these characters. The comparative magnitude of GCA and SCA mean squares is an indicator of the relative importance of the additive and nonadditive gene action in the inheritance of character (Kornegay and Temple, 1986). The results of this study showed high ratios of GCA and SCA mean squares for oleic and linoleic acids suggesting that additive gene action was more important than nonadditive gene action for these characters. The magnitude of GCA variance for % oil was smaller than SCA in the F_2 generation indicating a preponderance of nonadditive gene action for this character.

Reciprocal effects were also detected for oleic, linoleic acids, % oil, O/L ratio and IV in both the F_2 and F_3 generations, suggesting that cytoplasmic factors were also important for these characters. Similar results have been previously reported by Mercer *et al.* (1990) that GCA effects and reciprocal effects were significant for oleic acid, linoleic acid, O/L ratio and IV in the F_1 and F_2 generations, but SCA effects were not significant for oleic and linoleic acid. In addition, they found that maternal effects were significant only in the F_1 generation for these characters.

SunOleic 97R and Georgia-02C were the best parents for high oleic acid concentration and O/L ratio because they had high GCA effects for these characters. This was not unexpected because they are high oleic genotypes. The crosses with SunOleic 97R and Georgia-02C are expected to give high oleic progenies to exploit the merit of *ol* genes from their parents.

The crosses with $[(\text{NC17090} \times \text{B1})\text{-9-1} \times \text{KK 60-3}]_{\text{F6-8-3}}$ as a parent are also interesting especially those with SunOleic 97R and Georgia-02C because $[(\text{NC17090} \times \text{B1})\text{-9-1} \times \text{KK 60-3}]_{\text{F6-8-3}}$ also had significant and positive combining ability, whereas KK 5 and KKU 1 had significant and negative combining ability for oleic acid concentration and O/L ratio.

Moreover, the correlation between the performance per se of parental lines and their progenies was positive and significant for oleic, linoleic acids and O/L ratio, indicating that performance per se can be used as a predictor to select good combining parents for these characters.

In conclusion, additive gene action was more important than nonadditive gene action in determining fatty acid concentration. Genotypes with high oleic concentration can be selected in early generations especially in crosses with SunOleic 97R or Georgia-02C as parents because they carry the two recessive mutant genes (ol_1 and ol_2) for high oleic acid. The crosses SunOleic 97R × $[(\text{NC17090} \times \text{B1})\text{-9-1} \times \text{KK 60-3}]_{\text{F6-8-3}}$ and Georgia-02C × $[(\text{NC17090} \times \text{B1})\text{-9-1} \times \text{KK 60-3}]_{\text{F6-8-3}}$ might hold promise in identifying genetic control of oleic acid concentration beyond two major *ol* genes.

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REFERENCES

Andersen, P.C., and D.W. Gorbet. 2002. Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. *J. Agric. Food Chem.* 50: 1298-1305.

Bannon, C.D., G.J. Breen, J.D. Craske, N. Hai, N.L. Harper, and K.L. O'Rourke. 1982. Analysis of fatty acid methyl esters with high accuracy and reliability. III. Literature review of an investigation into the development of rapid procedures for the methoxide-catalysed methanolysis of fats and oils. *J. Chromatogr.* 247: 71-89.

Branch, W.D. 2003. Registration of 'Georgia-02C' peanut. *Crop Sci.* 43:1883-1884.

Chu, Y., C.C. Holbrook, and P. Ozias-Akins. 2009. Two alleles of *ahFAD2B* control the high oleic acid trait in cultivated peanut. *Crop Sci.* 49: 2029-2036.

Chu, Y., L. Ramos, C.C. Holbrook, and P. Ozias-Akins. 2007. Frequency of a loss-of-function mutation in oleoyl-PC desaturase (*ahFAD2A*) in the mini-core of the U.S. peanut germplasm collection. *Crop* Sci. 47: 2372-2378.

Dwivedi, S.L., S.N. Nigam, R. Jambunathan, K.L. Sahrawat, G.V.S. Nagabhushanam and K. Raghunath. 1993. Effect of genotypes and environments on oil content and oil quality parameters and their correlation in peanut (*Arachis hypogaea.* L.). *Peanut Sci.* 20: 84-89.

Gorbet, D.W., and D.A. Knauft. 2000. Registration of 'SunOleic 97R' peanut. *Crop Sci.* 40: 1190-1191.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9: 463-493.

Hoshmand, A.R. 2006. *Design of experiments for agriculture and the natural sciences*. 2nd edn. Chapman & Hall/CRC, Boca Raton, FL.

Isleib, T.G., C.T. Young, and D.A. Knauft. 1996. Fatty acid genotypes of five Virginia-type peanut cultivars. *Crop Sci.* 36: 556-558.

Isleib, T.G., R.F. Wilson, and W.P. Novitzky. 2006a. Partial dominant, pleiotropism, and epistasis in the inheritance of the high-oleate trait in peanut. *Crop Sci.* 46: 1331-1335.

Isleib, T.G., H.E. Pattee, T.H. Sanders, K.W. Hendrix, and L.O. Dean. 2006b. Compositional and sensory comparisons between normal- and high-oleic peanuts. *J. Agric. Food Chem.* 54: 1759-1763.

Jung, S., G. Powell, K. Moore, and A. Abbott. 2000. The high oleate trait in the cultivated peanut (*Arachis hypogaea* L.). II. Molecular basis and genetics of the trait. *Mol. Gen. Genet.* 263: 806-811.

Kornegay, J.L., and S.R. Temple. 1986. Inheritance and combing ability of leaf hopper defense mechanism in common bean. *Crop Sci.* 26: 1153-1158.

López, Y., O.D. Smith, S.A. Senseman, and W.L. Rooney. 2001. Genetic factor influencing high oleic acid content in Spanish market-type peanut cultivars. *Crop Sci.* 41: 51-56.

Mercer, L.C., J.C. Wynne, and C.T. Young. 1990. Inheritance of fatty acid content in peanut oil. *Peanut Sci.* 17: 17-21.

Moore, K.M., and D.A. Knauft. 1989. The inheritance of high oleic acid in peanut. *J. Hered.* 80: 252-253.

Mugendi, J.B., C.A. Sims, D.W. Gorbet, and S.F. O'Keefe. 1998. Flavor stability of high-oleic peanut stored at low humidity. *J. Am. Oil Chem. Soc.* 75: 21-25.

Norden, A.J., D.W. Gorbet, D.A. Knauft, and C.T. Young. 1987. Variability in oil quality among peanut genotypes in Florida breeding program. *Peanut Sci.* 14: 7-11.

O'Byrne, D.J., D.A. Knauft, and R.B. Shireman. 1997. Low fat-monounsaturated rich diets containing high-oleic peanuts improve serum lipoprotein profiles. *Lipids.* 32: 687-695.

O'Keefe, S.F., V.A. Wiley, and D.A. Knauft. 1993. Comparison of oxidative stability of high- and normal-oleic peanut oils. *J. Am. Oil Chem. Soc.* 70: 489-492.

Singkham, N., S. Jogloy, T. Kesmala, P. Swatsitang, P. Jaisil, and N. Puppala. 2010. Genotypic variability and genotype by environment interactions in oil and fatty acids in high, intermediate and low oleic acid peanut genotypes. *J. Agric. Food Chem.* 58: 6257-6263.

Upadhyaya, H.D., and S.N. Nigam. 1999. Detection of epistasis for protein and oil contents and oil quality parameters in peanut. *Crop Sci.* 39: 115-118.

Yu, S., L. Pan, Q. Yang, P. Min, Z. Ren, and H.Zhang. 2008. Comparison of the Δ12 fatty acid desaturase gene between high-oleic and normal-oleic peanut genotypes. *J. Genet. Genomics.* 35: 679-685.