Further Studies On The Inheritance Of Fatty Acid Composition In Peanut[1]

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ABSTRACT

Oleic and linoleic acid together constitute about 80% of the fatty acid composition in peanut oil. Increasing the ratio of oleic to linoleic acid will improve the keeping quality of peanut oil. A University of Florida breeding line, designated F435, averages 80% oleic acid and 2% linoleic acid. Initial genetic studies of this fatty acid composition showed that a single recessive gene controlled the trait in two genetic backgrounds and a second recessive gene was necessary for expression in a third background. Further studies have shown monogenic inheritance in 12 parental backgrounds and digenic inheritance in one background. This suggests that either one of the two recessive genes may be common in peanut germplasm, and that crosses could be expected to segregate in simple monogenic ratios. When the proportion of genes from F435 is reduced through backcrossing to less than 0.8%, fatty acid composition remains similar to the original F435 line. Organoleptic and agronomic characteristics do not appear affected by the fatty acid composition change. Given the simple inheritance, lack of background genetic effects, and lack of apparent undesirable linkages, incorporation of high oleic acid into peanut cultivars should be straightforward.

Key Words: *Arachis hypogaea* L., breeding, fatty acids, groundnut, oil quality.

Peanut (*Arachis hypogaea* L.) oil quality improvement is generally associated with an increase in the level of saturation among fatty acids (Knauft *et al.*, 1987). This increased saturation reduces the oxidation tendency of the oil and thus lessens formation of acids, aldehydes, ketones, and other hydrocarbons that cause undesirable odors and flavors (St. Angelo and Ory, 1973).

A peanut breeding line has been identified with 80% oleic acid and 2% linoleic acid (Norden *et al.*, 1987). The line is more saturated than standard cultivars, which average 55% oleic acid and 25% linoleic acid. Inheritance of this high oleic/low linoleic acid line (designated F435) is under simple genetic control (Moore and Knauft, 1989). Duplicate recessive alleles, ol_1 and ol_2, are responsible for this characteristic. F435 and one parent in the original study (Moore and Knauft, 1989) differed by both alleles for the high oleic acid trait, while F435 differed from two other parents by a single allele. This pattern differs from other reports on the genetics of fatty acid composition in peanut, that have identified differences as quantitatively inherited (Khan *et al.*, 1974; Mercer *et al.*, 1990, Tai *et al.*, 1975).

While two of the three lines used in the original genetic studies were homozygous for one of the two recessive alleles necessary for the high oleic acid trait, it is not known whether either recessive allele is common or rare in peanut germplasm. It is also not known whether the genetic background in which the high oleic acid genes are placed will affect the expression of the trait. This study was conducted to answer these questions.

Materials and Methods

Crosses were made using standard artificial hybridization procedures for peanut (Knauft *et al.*, 1987). All segregating generations were grown in greenhouses. At least 11 families were generated for each backcross and a minimum of nine progeny from each family were analyzed for fatty acid composition. Individual BC_nS_1 seed were analyzed using a modification of the method of Young and Waller (1972). Only the distal end of the seed was used for analysis, saving the embryo portion of the seed for subsequent planting. Samples weighing 0.25 g were extracted with 2 mL petroleum ether, vortexed, and centrifuged after 24 h. The petroleum ether was evaporated under a stream of N_2 gas. Two mL NaOH in methanol (0.5N) were added to the remaining oil, the tubes vortexed, and heated in a 100 C water bath for 5 min. After the tubes were cooled, BF_3 in methanol (12%) was added, the tubes vortexed and then heated in a 100 C for 5 min. After tubes were cooled, 2 mL of petroleum ether were added, followed by 2 mL of deionized water. After each addition, the tubes were vortexed. The tubes were then centrifuged for 5 min, and the upper petroleum ether phase from the reaction vials was transferred for fatty acid analysis.

A Hewlett-Packard 5890A gas chromatograph with automatic sample injector was used for analysis of the fatty acid methyl esters. Injector and detector temperatures were set at 250 C. Oven temperature was programmed for an initial temperature setting of 190 C for 3 min, then increasing at a rate of 3 C per min until reaching a final temperature of 220 C. The column was a 2 m glass column packed with 10% cyanosilicone (Supelco SP2330) on 100/120 Chromosorb WAW. A Hewlett-Packard 3392A integrator was used to calculate peak areas. Fatty acid contents are reported as relative proportions of total fatty acids present.

Results and Discussion

To speed the backcrossing process, two crosses were made to the recurrent parent in each cycle prior to self pollination. In each cycle of mating, high-oleic homozygotes ($ol_1ol_1ol_2ol_2$) were crossed to the recurrent parent, F519-9, and the resulting hybrids (BC_nS_0) simultaneously selfed to produce the BC_nS_1 and crossed back to the recurrent parent to produce the $BC_{n+1}S_0$. The $BC_{n+1}S_0$ progeny were selfed to produce the $BC_{n+1}S_1$. All S_1 seed were assayed for fatty acid composition. Because the recurrent parent, F519-9, contains a single recessive gene for high oleic acid (Moore and Knauft, 1989), backcrosses of the S_0 plants with this line produced two types of families after self pollination. Half the families were expected to have no recessive alleles at one locus, while the other half would be heterozygous. Upon self pollination, the heterozygous plants would segregate 3:1 (normal to high oleic) and homozygous plants would not produce high oleic acid seed.

Genetic background effects on the high oleic acid trait

Results from the accelerated backcrossing scheme were consistent with expectations (Table 1). Half the families from each cross segregated for high oleic acid. In those families with segregation, the expected 3 normal oleic acid : 1 high oleic ratio was observed (Table 1).

The six backcrosses of the original high oleic acid line, F435, to the F519-9 breeding line gave lines that contained an average of 25%, 12.5%, 6.25%, 3.13%, 1.56%, and 0.78% F435 genes. The oleic and linoleic acid composition levels remained similar to the original F435 line. The proportion of

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Table 1. Families showing segregation for high oleic acid percentage in the F_2 seed generation and total numbers of normal and high oleic acid seed in families that showed segregation.

Generation	Families with/without segregation	Seeds with normal/high oleic acid	x^2 (3:1)	P
F_2	4/0	161/46	0.770	0.38
BC_1	13/9	204/68	0.005	0.94
BC_2	6/5	111/31	0.660	0.42
BC_3	7/9	89/33	0.116	0.68
BC_4	5/7	84/27	0.003	0.96
BC_5	5/6	111/36	0.002	0.96
BC_6	18/19	94/32	0.009	0.92

several fatty acids appeared to be affected in lines homozygous for the *ol* genes. Palmitic acid content, which was 9% in the original F435 line, dropped to near 6% (Table 2). A small, but statistically significant, increase in lignoceric and behenic acids also was seen in backcross progeny. High oleic acid segregants from the sixth backcross were grown in a greenhouse with extremely high temperatures. This likely explains the slight variation in fatty acid composition in this material.

Thirteen breeding lines were used in further genetic studies. Although we found the duplicate recessive genes ol_1 and ol_2 responsible for the high oleic acid in our original work (Moore and Knauft, 1989), we identified 12 additional lines differing from the original F435 by a single gene and one line that differs by two genes from the original F435 (Table 3). The 91-1718 line is related to F78114, which is the line used in the original genetic studies that contained the two dominant genes for oleic acid content. The other lines used in these studies are not directly related to the lines in the original genetic study. This suggests that one of the two recessive genes may be present in a high proportion in peanut germplasm, while the second gene, contained in F435, is rare. When backcrosses with other breeding lines or more complex crosses are made expected 3:1 ratios still occur (Table 3).

Table 3. Numbers of F_2 seed with normal and high oleic acid from crosses with F435 as a source of high oleic acid.

Parent	Seed with normal/high oleic acid	x^2 (3:1)	P
PI 262090	104/36	0.009	0.92
Marc I	82/21	1.080	0.30
F81206-1	60/16	0.495	0.48
F81206-2	74/15	3.653	0.06
F80202	56/15	0.428	0.51
F85410	70/18	0.856	0.35
F87113	53/20	0.108	0.74
F87113†	26/12	0.487	0.49
72x38	57/19	0.018	0.89
72x93	166/44	1.840	0.17
72x93‡	107/32	0.206	0.65
72x93§	56/15	0.428	0.51
OK FH-15	17/10	1.201	0.27
F79308-3	73/23	0.014	0.91
75x3A	19/9	0.368	0.54
		(15:1)	
91-1718	227/16	0.007	0.93

†(High oleic acid parent was F_2 from F87113 x F435) x 87113
‡(High oleic acid parent was F_2 from 72x93 x F435) x 80202
§(High oleic acid parent was F_2 from 72x93 x F435) x 81206

Table 2. Fatty acid composition of original F435 high oleic acid peanut and high oleic acid seed segregants from F_2 and six backcross generations. Crosses were made between F435 and F519-9, with F519-9 as the recurrent parent.

Fatty acid	F435	F_2	BC_1	BC_2	BC_3	BC_4	BC_5	BC_6
N	54	61	40	31	18	22	75	32
	- - - - - - - - - - - - - - - - - - % -
Palmitic (16:0)	8.8 ±0.2	6.5 ±0.2	6.4 ±0.1	6.5 ±0.1	6.4 ±0.1	6.6 ±0.2	7.6 ±0.1	5.6 ±0.2
Stearic (18:0)	2.9 ±0.1	2.8 ±0.2	2.7 ±0.2	2.8 ±0.2	2.6 ±0.2	2.5 ±0.3	3.0 ±0.2	3.0 ±0.2
Oleic (18:1)	79.2 ±0.3	79.1 ±0.5	79.1 ±0.3	79.1 ±0.3	78.6 ±0.4	79.5 ±0.6	79.9 ±0.3	81.4 ±0.4
Linoleic (18:2)	3.0 ±0.2	3.1 ±0.3	3.1 ±0.2	3.1 ±0.2	3.3 ±0.2	3.0 ±0.3	2.7 ±0.2	2.1 ±0.2
Arachidic (20:0)	1.2 ±0.1	1.3 ±0.2	1.4 ±0.1	1.3 ±0.1	1.3 ±0.1	2.0 ±0.2	1.4 ±0.1	1.5 ±0.1
Behenic (22:0)	2.4 ±0.1	3.0 ±0.1	3.1 ±0.1	3.0 ±0.1	3.3 ±0.2	3.9 ±0.2	2.3 ±0.1	2.7 ±0.1
Lignoceric (24:0)	0.6 ±0.1	1.8 ±0.1	1.9 ±0.1	1.8 ±0.1	2.0 ±0.1	0.7 ±0.1	1.3 ±0.1	1.8 ±0.1
Eicosenoic (20:1)	2.1 ±0.1	2.2 ±0.1	1.3 ±0.1	2.1 ±0.1	2.3 ±0.1	1.1 ±0.1	1.7 ±0.1	1.8 ±0.1

It is possible that families analyzed in this study contain a mixture of $01_101_1ol_2ol_2$ and $ol_1ol_101_201_2$ genotypes. However, if both genotypes were common in peanut germplasm, it would be expected that at least one cross made in breeding programs over the past 65 years would have produced a genetic combination uniting the two recessive genes. However, despite many evaluations of fatty acid composition in peanut, the high oleic acid trait was not reported until 1987 (Norden *et al.*, 1987).

Effects of the high oleic acid characteristic on other traits in peanut

There is little evidence that the high oleic acid trait affects other characteristics of peanut. Triangular taste tests between the high oleic acid line (F435) and the breeding line from which it was derived (F78-1339) revealed no detectible differences in color, aroma, flavor, or texture (Moore *et al.*, 1989). Pod yield and market grade of the two lines have been similar (unpublished data). Original undesirable attributes present in both F435 and F78-1339, such as the split pod characteristic and low yield, do not appear to be associated with the high oleic acid trait since high yielding, agronomically acceptable, lines have been developed from these backcross populations.

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