INHERITANCE OF OIL PRODUCTION AND QUALITY FACTORS IN PEANUT

(*Arachis hypogaea* L.)

A Dissertation

by

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Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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August 2013

Major Subject: Plant Breeding

ABSTRACT

Peanut (*Arachis hypogaea* L.) has the potential to become a major source of biodiesel but for market viability, peanut oil yields must increase and specific quality requirements must be met. Oil yield in peanut is influenced by many components, including oil concentration, seed mass, and mean oil produced per seed. All of these traits can be improved through selection as long as there is sufficient genetic variation. Thus, elucidating the genetics of oil concentration, seed mass, and mean oil produced per seed in peanut is essential to advancing the development of genotypes with high oil yields. Additive genetic effects were predominant for oil concentration in two generation means analyses involving a proprietary high oil breeding line and additive genetic variance was highly significant in a complete four-parent diallel analysis. Genetic variance for weight of 50 sound mature kernels (50 SMK) and mean oil produced per SMK (OPS) was additive the diallel analysis. Narrow-sense heritability estimates were high for oil concentration in both the diallel and generation means analyses. Narrow-sense heritability was also high for 50 SMK, but was low for OPS. The low OPS heritability estimate was caused by the negative correlation between oil concentration and seed mass. Consequently, oil concentration and seed mass can be improved through early-generation selection, but large segregating populations from high oil crosses will be needed to identify progeny with elevated oil concentrations that maintain acceptable seed sizes.

Increasing the ratio of oleic to linoleic acid (O/L) in peanut oil and reducing the long chain saturated fatty acid concentration (which includes arachidic, behenic, and lignoceric acids) produces high quality, stable methyl esters for biodiesel. Therefore, elucidating the inheritance of these factors and their relationships in peanut populations segregating for high oil is critical. The results from generation means analysis confirm that the high-oleic trait is under simple genetic control and can be manipulated through selection. Oil concentration was negatively correlated with oleic acid concentration in the F_2 generations of both crosses and positively correlated with arachidic acid in most of the segregating generations that were evaluated. Therefore, developing a peanut genotype high in oil and oleic acid concentration that has reduced long chain saturates will require the evaluation of large numbers of segregating progeny.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank individuals who have kindly offered me their guidance through this long and challenging process. I would first like to thank Mr. Michael Baring for allowing me to work in his program for all these years. Mike has advocated for me through good times and some difficult times at Texas A&M. I always knew I could trust and count on Mike's guidance. I would also like to thank the members of my committee, Drs. Bill Rooney, Jim Starr, Mark Burow and Charles Simpson. Quite simply, no one could ask for a better, more professional group of mentors.

One of my primary priorities after arriving in College Station was to ask Dr. Rooney chair my committee. I strongly believe Dr. Rooney is one of the most erudite plant breeders in academia and an advocate for his students. Thanks to Drs. Starr and Burow for allowing me to work in their labs at various times. I have gained a tremendous amount of knowledge and experience in molecular genetics from my time in the lab. I would also like to thank Dr. Simpson for his support. None of this work would be possible without his brilliance in developing TxAG-6. Finally, I would like to thank my parents, Dr. Norman and Judy Wilson, for their unwavering support through this process. I have been in college since 2001, and they (and I) are ready for this experience to be over. My father inspired me to work in the agricultural industry as a plant breeder. He is the most professional and knowledgeable plant breeder and agronomist I have ever met.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Biodiesel has become an important source of alternative energy in the United States because of increased fuel prices and worldwide demand. Biodiesel is a renewable energy source that burns cleaner than petroleum based diesel and, unlike ethanol, requires no modification of current engines. Adding biodiesel to diesel engines may improve the currently mandated ultralow sulfur diesel by increasing lubricity.

The cultivated peanut (*Arachis hypogaea* L.) is an important annual oilseed crop planted as a food group throughout the world. In the U.S., one million acres of peanuts were planted in 2011 (USDA, National Agriculture Statistics Service). Major limitations to increased use of peanut oil as a fuel source are production of sufficient quantities of oil on a per acre basis to reduce the price and optimization fatty acid chemistry for fuel. By further increasing the oil concentration and reducing the cost of the oil, peanuts can be a significant source of biodiesel. Peanut oil currently produces an average of 1,060 liters oil per hectare, whereas the major oilseed legume, soy (*Gylcine max*), produces 446 liters per hectare (Pahl, 2008).

Reported variation in percentage oil concentration of cultivated genotypes ranges from 436 to 555 g kg^{-1} (Cherry, 1977), 358 to 542 g kg^{-1} (Salunkhe *et al*., 1992) and 37.0 to 53.0% (ICRISAT, 1986), with an overall mean around 450 g kg^{-1} (Dwivedi et al., 1990; Salunkhe et al., 1992). Cherry (1977) identified wild species in section *Erectoidis* and *Rhizomatosae* with oil contents above 600 g kg^{-1}. Attempts have been made to

exploit novel genes in wild species via interspecific hybridization. However, most crosses are incompatible or have a low success rate (Stalker and Simpson, 1995). Progeny from some *A. hypogaea* genotypes crossed to wild species *A. diogoi* exhibited symptoms that mimic Tomato Spotted Wilt (TSWV) virus and died prematurely (Stalker and Simpson, 1995). Other phenomena noted in interspecific crosses included embryo abortion at different stages after fertilization (Johansen and Smith, 1956; Halward and Stalker, 1987, Pattee and Stalker, 1992a,b; Tallury 1994), prolonged dormancy, sometimes lasting years (C.E. Simspson, personal communication), and healthy, yet infertile F_1 plants (Stalker and Simpson, 1995; C.E. Simpson, personal communication).

Dwivedi *et al*. (1993a) described the exploitation of genes for high oil concentration from wild species as a distant possibility. However, TxAG-6, an amphidiplopid created from a complex cross between wild species, contains approximately 620 g kg^{-1} oil (unpublished data) and is compatible with cultivated tetraploid (2n = 4x = 40) peanuts (Simpson *et al*., 1993). TxAG-6 was derived from crossing the diploid hybrid from *A. cardenasii* (PI 262141) x *A. chacoensis* (PI 276235) as male onto the female *A. batizocoi* (PI 298639). This hybrid was then chromosome doubled with colchicine.

Oil concentration in peanuts is influenced by genetic and environmental effects. Numerous studies have measured a significant interaction between environments (either seasonal or location) and genotypes (Holaday and Pearson, 1974; Tai and Young, 1975; Layrisse *et al*., 1980; Dwivedi *et al*., 1990; Dwivedi *et al*., 1993a; Upadhyaya and Nigam, 1999; Dwivedi *et al*., 2000; Isleib *et al*., 2008; Baring et al., 2013). Management

factors including fertility (Bhuiya and Chowdhury, 1974; Reddy and Murthy, 1989; Dwivedi *et al*., 1993a), fungicide applications (Dwivedi *et al*. 1993b), planting date (Gupta *et al*., 1983), harvest date (Court *et al*., 1984; Knauft *et al*., 1986), irrigation (Desai *et al*., 1992), and *Bradyrhizobium* inoculation (Singh and Ahuja, 1985) have been reported to affect oil concentration. Management factors, particularly planting and harvest date, directly affect maturity. Oil is the final major reserve to accumulate in seed (Pattee *et al*., 1974), therefore maturity directly affects oil concentration (Baring et al., 2013). Dwividi *et al*. (1990) reported a positive association between percentage oil content and 100-seed mass in graded samples but no association in non-graded samples, which indicated that uniform maturity within a genotype was required to maximize oil content.

Previous reports on the inheritance of oil concentration in peanut have been variable with respect to the importance of genetic effects and heritabilities, likely due to genetic differences in genotypes used in these experiments and environmental effects. Oil content was highly heritable in studies by Martin (1967) and Patil (1972), while Tai and Young (1975) were only able to measure heritability in 5 of 11 F_2 populations. Additive effects (general combining ability) were more important than non-additive effects (specific combining ability) for determining oil concentration on a dry weight basis in studies measuring F_1 populations (Sykes and Michaels, 1986; Isleib *et al*. 2004) and percentage oil content in an F_2 population (Layrisse *et al*., 1980). The performance of parental lines was generally a good predictor of oil content in hybrids (Layrisse *et al*., 1980; Isleib *et al*. 2004). Upadhyaya and Nigam (1999) found evidence of additive x

additive and dominance x dominance epitasis for oil content in one environment (dry season), but epitasis was not significant in a different environment (wet season). Cytoplasmic (maternal) effects were highly significant in an F_1 generation in a study by Isleib (2004), but were much less pronounced in a study using F_2's (Layrisse *et al*., 1980). F_2 populations resulting from crosses between different botanical types in a study by Tai and Young (1975) indicated transgressive segregation towards higher oil content (above parental mean) in a Spanish (subsp. *fastigiata* var. *vulgaris*) x Virginia cross and its reciprocal and lower oil content (less than the parental mean) in a runner (subsp. *hypogaea* var. *hypogaea*) x Valencia (subsp. *fastigiata* var. *fastigiata*) cross. Layrisse *et al*. (1980) found transgressive segregation toward lower oil content more common among diverse crosses.

Layrisse *et al*. (1980) determined that rank correlations of GCA (general combining ability) effects for oil content and seed yield were positive and significant, as were correlations among phenotypic means. The correlation between oil content and 100 pod weight was not significant. Dwivedi *et al*. (1990) determined that high oil content can be maintained when indirectly selecting for high yield through large seed size. Other studies have reported negative correlations between seed size and oil content (Holley and Hammons, 1968; Patil, 1972).

In biodiesel production, fatty acid chains are transesterified (process of exchanging the alkoxy group of an ester compound with another alcohol) with methanol to produce fatty acid methyl esters (FAME). FAME produced from saturated fatty acid chains (single carbon bonds) have higher resistance to oxidation compared to those

produced by less saturated fatty acids (double carbon bonds) and therefore increase the stability of biodiesel (Ramos et al., 2008).

Highly unsaturated fatty acids such as linoleic acid are associated with low cetane numbers (Bajpai and Tyagi 2006; Dermibas 2005; Knothe et al., 1998; Ramos et al., 2009). Cetane number is a diesel quality parameter that measures combustion quality during compression ignition and diesel fuel with a higher cetane number has improved ignition properties (Meher et al., 2006). Peanut oil contains relatively large amounts of long chain saturated fatty acids compared with soybean (*Glycine max*) and rapeseed (*Brassica napus*) oil which include arachidic, behenic, and lignoceric acids (Davis et al., 2009). These fatty acids affect the low temperature properties of biodiesel by promoting crystallization (Davis et al., 2009).

Approximately 80% of the total fatty acids in peanut oil are either oleic or linoleic acid (Lopez et al., 2000) and peanut breeders have been working to increase the oleic to linoleic acid ratios. The breeding line UF435 was the first genotype identified with 80% oleic and 2% linoleic acid (Norden et al., 1987). Inheritance of the high O/L trait in UF435 is under simple genetic control with two recessive mutations in *ahFAD2A* and *ahFAD2B* responsible for the trait (Moore and Knauft, 1989; Jung et al., 2000). One of the recessive alleles found in UF435 is common in runner and Virginia peanut genotypes, whereas the other allele is relatively rare (Knaft et al., 1993; Isleib et al., 1996).

Previous research indicates that additive effects are important in the inheritance of oleic acid (Mercer et al. 1990; Upadhyaya and Nigam 1999; Aruna and Nigam 2009)

as well as other fatty acids (Mecer et al., 1990; Aruna and Nigam, 2009). However, there are other reports of epistatic gene interactions governing the inheritance of the high O/L trait in peanut crosses (Lopez, et al., 2001, Muitia et al., 2006). Lopez *et al*. (2001) crossed six low O/L spanish lines with UF435. Segregation ratios in F_2 populations were consistent with digenic inheritance with allelic variation occurring in some lines. However, the O/L segregation ratios in some F_2 lines did fit the pattern of duplicative interaction, i.e. some plants that segregated at one only locus produced low O/L progeny, leading authors to postulate the presence of four to six genes controlling the high-oleic trait. Muitia *et al*. (2006) found that segregation ratios in the F_2 progeny resulting from a cross between NemaTAM and Tamrun OL01 fit a two gene epistatic model, while progeny from a cross between NemTAM and OLin were consistent with additive gene action.

Oleic acid concentration has been significantly correlated with other fatty acids in different studies (Isleib et al., 1996; Anderson and Gorbet, 2002; Isleib et al., 2006; Barkley et al., 2011). These correlations indicate that the O/L genotype influences the levels of other fatty acids and those genetic modifiers may be involved in fatty acid inheritance. Specifically, oleic acid and linoleic acid are negatively correlated because their proportions are controlled by the Δ12 fatty acid desaturase that catalyzes the first step in the biosynthesis of polyunsaturated fatty acids (Groff et al., 1995). The significant negative association between oleic acid and palmitic acid observed in multiple studies (Isleib et al., 1996; Anderson and Gorbet, 2002; Barkley et al., 2011) is

likely because of palmitic acid elongation to steraric acid, which is rapidly desaturated to oleic acid through Δ9 desaturase (Groff et al., 1995).

Correlations between oil concentration and fatty acid chemistry have varied. Mercer *et al*. (1990) determined that selection for fatty acid composition should not affect oil content because no fatty acids were correlated with percentage oil content in an F_2 population. He also concluded that additive gene effects are important in the inheritance of fatty acid content. Sykes and Michaels (1986) found no significant correlations between oil content and fatty acids in F_1 progeny. Dwivedi *et al*. (1993a) found significant positive phenotypic correlations between oleic and eicosenoic acids and oil content and significant negative correlation between oil content and all other fatty acids tested except lignoceric acid.

The heritability of wild-species derived genes for oil concentration is not known, nor is the impact of high oil concentration on fatty acid composition and seed size. These questions must be answered before we can successfully exploit potentially novel genes contained in TxAG-6. The objectives of these studies are 1) determine genetic variance components, narrow sense heritabilities, and combining ability estimates for oil concentration and seed size; 2) investigate the types of gene action governing the inheritance of oil concentration and fatty acid composition 3) determine the relationship between fatty acids, oil concentration and seed size, and oil concentration and fatty acid composition in segregating progeny. These objectives were accomplished through analyses of a full diallel mating design and a generation means mating design containing

segregating progeny derived from a crossing a high oil concentration breeding line containing oil genes from TxAG-6 with adapted high O/L cultivars.

CHAPTER II

GENERATION MEANS ANALYSIS OF OIL CONCENTRATION IN PEANUT*

Synopsis

The current interest in biodiesel production has resulted in a concurrent interest in increasing the oil concentration in high yielding cultivars, which could make peanuts (*Arachis hypogaea* L.) more desirable as a biofuel source. Currently, peanut seed is approximately 450 to 500 g kg^{-1} oil on a dry weight basis, depending upon location grown, and there is relatively little genetic variation for oil concentration among adapted high yielding cultivated peanut genotypes. Thus, identifying sources of variation and elucidating the genetics of oil concentration in peanut is essential to advancing the development of high oil genotypes. The objective of this study was to determine the types of gene action governing the inheritance of oil concentration in peanut by generation means analysis. The F_1, F_2, and backcross generations of two different runner peanut crosses segregating for oil concentration were evaluated in College Station, TX in 2010. Significant differences in oil concentration among the generations were detected and generation means analysis revealed significant additive, dominance, and epistatic effects for oil concentration in both crosses. The broad-sense heritability estimates were 0.85 and 0.78 and narrow-sense heritability estimates were 0.55 and 0.53 for each of the crosses. Our data indicate that transgressive segregants for high oil were observed

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and there is sufficient additive variation present to improve the oil concentration of current runner cultivars.

Introduction

Biofuels are becoming important as worldwide demand for fuels is increasing and prices are rising. Biodiesel has become an important source of alternative energy in the USA. In 2007, over 450 million gallons of biodiesel were produced in the United States (Pahl, 2008). Biodiesel is a renewable energy source that burns cleaner than petroleum-based diesel and, unlike ethanol, does not require modification of current engines. Use of biodiesel may improve the current mandated ultralow sulfur diesel by increasing lubricity (Pahl, 2008).

The major limitations to increased use of vegetable oil for biodiesel are production of sufficient quantities of oil and value of the oil for food purposes. Currently, rapeseed (*Brassica napus*) and soybean (*Glycine max*) oil comprise approximately 85% of total global biodiesel raw-material sources (Pahl, 2008). Peanut (*Arachis hypogaea* L.) produces an average of 1,060 liters oil per hectare, while soy oil produces only around 446 liters per hectare (Pahl, 2008). There is the potential to increase the oil concentration in peanut, which would reduce the cost of the oil and make peanuts a significant source of oil as a raw material for biodiesel production. Peanuts are such an excellent source of biodiesel that Rudolf Diesel's original engine ran on peanut oil around the turn of the 19th century (Pahl, 2008).

Genetic variation for total oil concentration is known to exist in cultivated peanut genotypes with oil concentrations ranging from 436 to 555 g kg^{-1} (Cherry, 1977) and 358

to 542 g kg^{-1} (Knauft and Ozias-Akins, 1995) with an overall mean around 450 g kg^{-1} (Dwivedi et al., 1990; Salunkhe et al., 1992). Even more variation is available in wild species relatives of cultivated peanut. Specifically, oil concentration above 600 g kg^{-1} has been identified in the wild species in the Section *Erectoidis* and *Rhizomatosae* (Cherry, 1977) Attempts have been made to exploit novel genes in wild species via interspecific hybridization, but success has been limited as hybridization of these species is difficult at best (Stalker and Simpson, 1995).

In addition to variation attributable to genotype, the oil concentration of peanuts is also influenced by the environment and the interaction of genotype and environment (Holaday and Pearson, 1974; Dwivedi et al., 1990; Dwivedi et al., 1993a; Upadhyaya and Nigam, 1999; Dwivedi et al., 2000; Isleib et al., 2008). Variation in oil concentration and quality attributable to environment are commonly associated with management factors such as fertility (Bhuiya and Chowdhuty, 1974; Reddy and Murthy, 1989; Dwivedi et al., 1993a), fungicide applications (Dwevidi et al., 1993b), planting date (Gupta et al., 1983), harvest date (Court et al., 1984; Knauft et al., 1986), irrigation (Desai et al., 1992), and *Bradyrhizobium* inoculation (Singh and Ahuja, 1985). Management factors, particularly planting and harvest date, also affect maturity and therefore likely affect oil concentration because oil is the final major reserve to accumulate in seed (Pattee et al., 1974).

Previous reports on the inheritance of oil concentration in peanut have been variable in reporting the importance of genetic effects and heritabilities, likely because of differences in the genotypes and environments used in these experiments. Patil (1972)

reported that oil content was highly heritable whereas Tai and Young (1975) reported measurable heritability (ie, non-zero) in only 5 of 11 F_2 populations. Additive effects (general combining ability) were more important than non-additive effects (specific combining ability) for determining oil concentration on a dry weight basis in diallel studies measuring F_1 populations (Sykes and Michaels, 1986; Isleib et al., 2004) and percentage oil content in an F_2 diallel population (Layrisse et al., 1980). However, Singkham et al. (2011) observed that the magnitude of specific combining ability was greater than that of general combining ability in an F_2 population. Without regard to gene action, the performance of parental lines has generally been a good predictor of their performance in crosses for oil concentration (Layrisse et al., 1980; Isleib et al., 2004).

In addition to main genetic effects, there is some evidence of epistatic interactions and trangressive segregation for oil concentration. Upadhyaya and Nigam (1999) reported the presence of additive x additive and dominance x dominance epitastic effects for oil concentration in the dry season, but not in the wet season. Cytoplasmic effects were highly significant in an F_1 generation (Isleib et al., 2004), but were much less pronounced in a study using F_2 progeny (Layrisse et al., 1980). F_2 populations from crosses between different botanical types indicated transgressive segregation towards higher oil content (above parental mean) in a Spanish (subsp. *fastigiata* var. *vulgaris*) x Virginia (subsp. *hypogaea* var. *hypogaea*) cross and its reciprocal and lower oil content (less than the parental mean) in a runner (subsp. *hypogaea* var. *hypogaea*) x Valencia (subsp. *fastigiata* var. *fastigiata*) cross (Tai and Young, 1975). Layrisse et al. (1980)

found transgressive segregation toward lower percentage oil content more common among diverse crosses.

Most of the research on peanut oil concentration has focused on cultivars with oil concentrations that are typically at or below the concentration in peanuts cultivated in the USA. While there are specific sources of high oil concentration in wild peanut species, there has not been a study of the inheritance of these sources (Dwivedi et al., 1993a). A primary limitation for the use of these sources was cross-incompatibility between genotypes (Stalker and Simpson, 1995). To alleviate this issue, TxAG-6, an amphidiplopid created from a complex cross between wild species was developed. TxAG-6 contains approximately 620 g kg^{-1} oil (unpublished data) and is compatible with cultivated tetraploid (2n = 4x = 40) peanuts (Simspson et al., 1993). TxAG-6 was derived by crossing the diploid hybrid *A. cardenasii* (Krapov. and W.C. Gregory) x *A. diogoi* (Hoehne) as male onto an *A. batizocoi* (Krapov. and W.C. Gregory) female. The chromosomes of this hybrid were doubled with colchicine, creating in a plant that was fertile when self-pollinated and when crossed with *A. hypogaea*.

A working knowledge of the inheritance of oil concentration is necessary if peanut cultivars are to be developed with elevated oil concentration for biofuel use. Using this high oil germplasm, the objectives of this study were to (i) determine the inheritance of elevated oil concentration in peanut and (ii) estimate heritability and the number of genes controlling oil concentration.

Materials and Methods

Germplasm Development and Experimental Design

Parental lines included in this study were high-oil runner breeding line 31-08-05-02 and two adapted, high-oleic runner genotypes Tamrun OL01 (Simpson et al., 2003) and Tamrun OL07 (Baring et al., 2006). The breeding line 31-08-05-02 had the pedigree Florunner^2 // (TxAG-6 / Florunner BC_3). Line 31-08-05-02 was crossed as the male parent to both Tamrun OL01 and Tamrun OL07. Crosses and backcrosses (BC) were completed in College Station, TX between 2008 and 2010. F_2 seed were produced by self-pollinating F_1 plants in the greenhouse at College Station and BC_1 seed were produced by manually crossing the F_1 back to each parent (Table 2.1).

For each cross, the numbers of individual plants evaluated varied by generation based on the expectation of genetic segregation; therefore more individuals were evaluated in the F_2, and BC generation than in the parents and F_1 (Table 2.1) (Mather and Jinks, 1977). Peanuts were planted 0.6 m apart at a 0.9 m row spacing with five plants per row. The non-segregating P_1, P_2, and F_1 entries had single-row plots, whereas the segregating F_2, BC_1F_1A and BC_1F_1B entries had three-row plots. In order to minimize spatial effects due to variation in the field, this design was replicated three times for each cross and the entries were randomized within each replication. All plots were planted at the TAMU research farm in Brazos Co, TX in 2010 and standard agronomic and pest-control practices were employed throughout the growing season. Supplemental irrigation was provided on an as-needed basis.

Individual plants were harvested at maturity and allowed to dry before threshing. After threshing and dehulling, an equal weight (approximately 20 g) sample of sound mature kernels (seeds that ride a 6 x 19 mm screen) from each plant was taken and tested for oil content. Oil content was measured using nuclear magnetic resonance spectroscopy (NMR), which gives percentage total oil content of seed on a dry-weight basis. Percentage oil content readings were converted to oil concentrations in g kg^{-1}. Peanut samples were dried to less than 5% moisture and stored indoors prior to the NMR assay. The mean oil concentration from each generation was subjected to analysis of variance using PROC GLM of SAS® 9.2 (SAS Institute Inc.). Fischer's protected LSD test was used to determine whether differences existed among generation means.

Generation Means Analysis

Generation means obtained from the analysis of variance were used to estimate mean, additive, and dominance effects by a joint scaling test described by Singh and Chaudhary (1985). The joint scaling test derived genetic estimates using the procedure of weighted least squares, where weights were the reciprocals of the variances of each generation. A three-parameter model that estimated mid-parent (*m*), additive (*d*), and dominance (*h*) effects was applied to the data using the joint-scaling test and tested for goodness of fit via a chi-square test with three degrees of freedom. Failure of the three parameter model was an indicator of epitasis and a full model (six genetic parameters) was then applied. The epistatic genetic parameters are as follows:

i = additive x additive gene interaction

j = additive x dominance gene interaction

l = dominance x dominance interaction

Calculations were performed using JNTSCALE software (Ng, 1990).

Broad-sense heritability, standard error for broad-sense heritability, narrow-sense heritability, standard error for narrow-sense heritability, and minimum number of genes controlling the trait were calculated for oil concentration. Formulas described for generation means analysis by Rodriguez-Herrera et al (2000) were used for this purpose.

Results and Discussion

Oil concentration of 31-08-05-02 was significantly higher than that of either of the Tamrun parents and all other generations tested in both crosses (Table 2.2). Analysis of variance revealed significant differences among generations for oil concentration in both crosses ($P < 0.001$). The F_1 and F_2 means fell between the two parents for both crosses, suggesting additive gene action. Distribution of F_2 progeny for cross I was unimodal and asymmetrical with a negative skew (Figure 2.1), while the F_2 progeny distribution in cross II was bimodal with a slightly positive skew (Figure 2.2). This indicated that Tamrun OL07 was a more favorable parent than Tamrun OL01 when combined with 31-08-05-02 in crosses designed to increase oil concentration. One transgressive segregant for increased oil concentration was observed in cross II (Figure 2.2). Transgressive segregation for high oil concentration in F_2 peanut progeny has been observed (Si-long et al., 2009). A larger F_2 population may be necessary to obtain additional genotypes with oil concentrations greater than the high parent.

For crosses I and II, the three-parameter model (additive and dominance) was not sufficient to explain the genetic variation for oil concentration based on significant χ^2

values (Table 2.3). The lack of fit the three-parameter model (additive-dominance model) indicated the genes controlling oil concentration were not independent and/or the genes and alleles are epistatically interacting to produce the observed phenotypes. Therefore, the six parameter model was used to determine the type and magnitude of gene action involved in the inheritance of oil concentration.

In the six-parameter model, significant additive (*d*) and dominance (*h*) effects were obtained in both crosses (Table 2.3). However, these estimates were biased in the presence of epistasis and their relative importance to epistatic effects cannot be directly assessed (Bernardo, 2002). Analysis indicated significant dominance x dominance epistasis (*l*) for cross I. For cross II, both dominance x dominance (*l*) and additive x dominance epistases (*j*) were significant. Dominance effects were negative while dominance x dominance effects were positive in both crosses. Gene interactions are considered to be duplicative when dominance and dominance x dominance estimates have different signs, confirming the importance of dominance effects (Grewal, 1988).

Broad-sense heritability (H^2) estimates were 0.78 for cross I and 0.85 for cross II (Table 2.4). These broad-sense heritability values are much higher than those reported by Tai and Young (1975). Isleib et al. (2008) observed that most of the variation in oil content of peanut grown in the uniform peanut performance test was overwhelmingly environmental rather than genetic. Narrow-sense heritability (h^2) estimates were 0.53 and 0.55 for crosses I and II, respectively (Table 2.4), indicating that additive effects and variation account for the majority of genetic control for oil concentration in these populations. A recent report indicated that narrow-sense heritabilities for oil content

were very low in runner crosses (Singkham et al., 2010). Differences in heritability estimates between studies may be a function of environment, experimental or mating design, and perhaps most importantly genetic background, because parents with elevated oil concentrations were not utilized in these studies.

The estimated minimum number of genes controlling oil concentration was 27.53 and 27.58 for crosses I and II, respectively (Table 2.4). Tai and Young (1975) indicated that oil content in peanut is a quantitative trait, but they did not estimate the number of genes involved. The current study is one of the first to estimate the number of genes controlling oil concentration in peanut therefore it is not possible to make direct comparisons. Because numerous different physiological processes and factors could potentially and ultimately influence oil concentration (Dwivedi et al., 1993a), the number of genes present in any cross could be high as is observed herein. Furthermore, given the number of potential factors, genetic linkage may also result in the underestimation of genes controlling a trait (Rao and Rana, 1989; Rodriguez-Herrera et al., 2000).

The large number of genes controlling oil concentration increases the likelihood for environmental interactions (Gamble, 1962; Upadhyaya and Nigam, 1999). Our genetic estimates could vary under different environmental conditions. Specifically, epistatic interactions would be more likely to interact with environmental effects than main effects (additive and dominance) because additive x additive effects for oil concentration were strongly influenced by environment in a previous study (Upadhyaya and Nigam, 1999).

Effective selection for quantitative traits is primarily determined by the genetic effects controlling their inheritance. Our research clearly demonstrates that inheritance of oil concentration is quantitative and controlled by a large number of genes that have additive, dominance and epistatic effects. Furthermore, when many genes are involved in the inheritance of a trait, epistasis and linkage are likely to be also involved. It should be noted that the dominance x dominance epistasis found in crosses I and II and dominance x additive epistasis found in cross II cannot be fixed in inbred peanut lines. Because both the genetic effects model and the narrow–sense heritability estimates indicate that additive variance is important in the inheritance of oil concentration in both crosses, conventional pedigree and early generation selection methods should be effective in improving oil concentration in peanut.

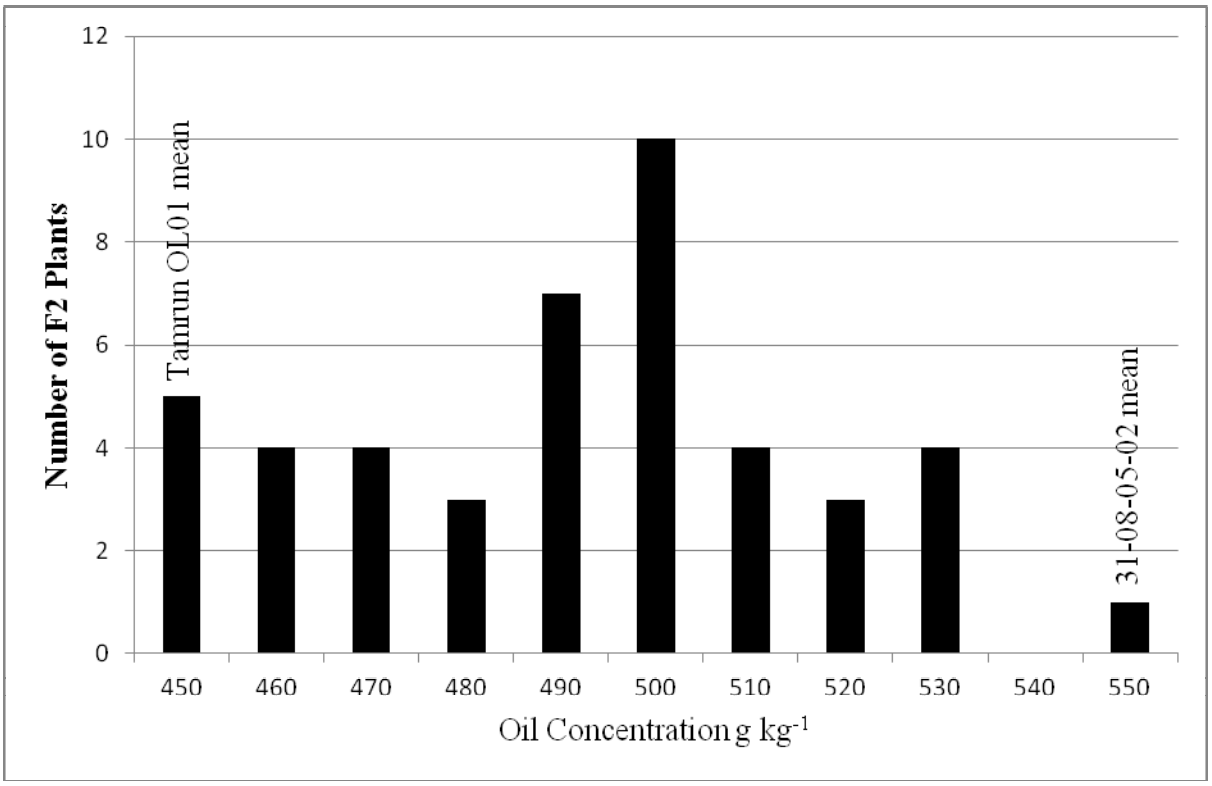

FIGURE 2.1 Distribution of oil concentration on a dry weight basis measured by nuclear magnetic resonance spectroscopy (NMR) among F_2 progeny from a cross between Tamrun OL01 and 31-08-05-02 (Cross I) evaluated in College Station, TX in 2010.

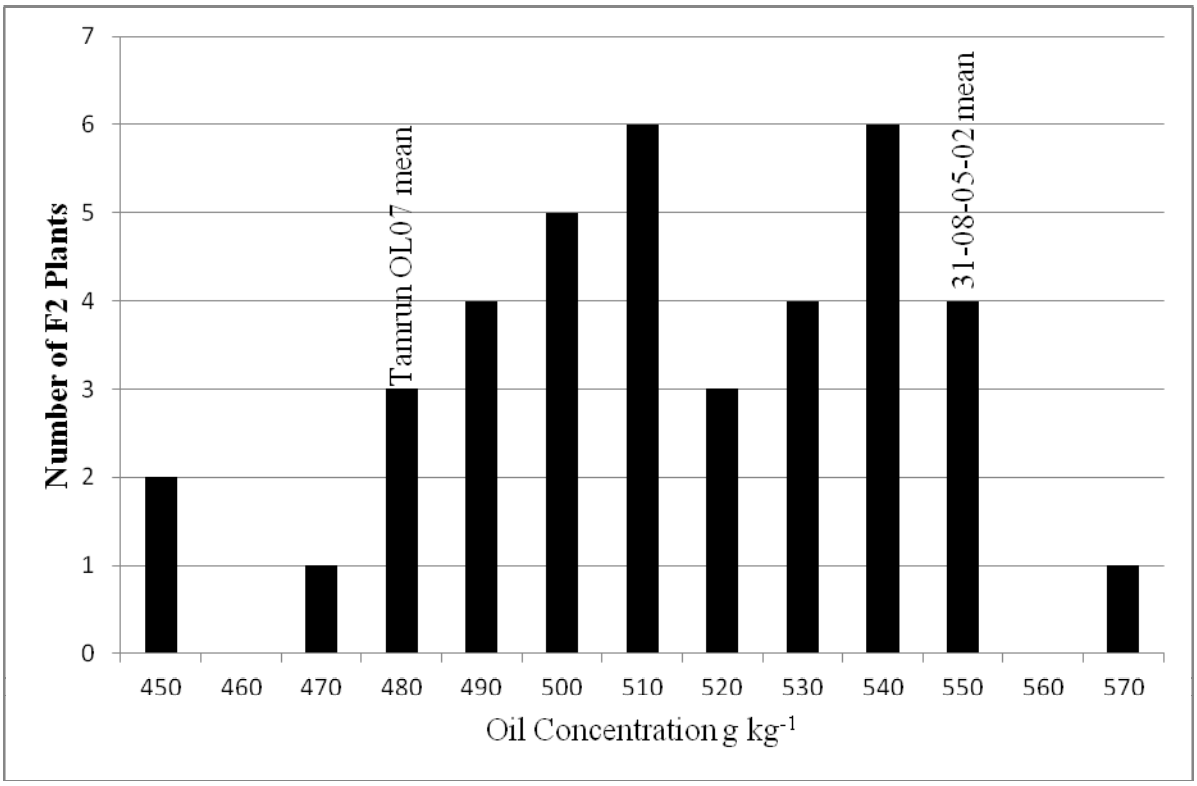

FIGURE 2.2 Distribution of oil concentration on a dry weight basis measured by nuclear magnetic resonance spectroscopy (NMR) among F_2 progeny from a cross between Tamrun OL07 and 31-08-05-02 (Cross II) evaluated in College Station, TX in 2010.

TABLE 2.1 Generations and number of plants evaluated per generation (n) in two generation means experiments planted in College Station, TX in 2010

Generation	Cross I	n Cross I[a]	Cross II	n Cross II
P_1	Tamrun OL01	15	Tamrun OL07	13
P_2	31-08-05-02	15	31-08-05-02	14
F_1	P_1 x P_2	13	P_1 x P_2	13
F_2	F_1 self	45	F_1 self	39
BC_1A	F_1 x Tamrun OL01	25	F_1 x Tamrun OL07	38
BC_2B	F_1 x 31-08-05-02	39	F_1 x 31-08-05-02	37

[a]n Cross I and n Cross II refer to the number of individuals in that cross for the generation shown

TABLE 2.2 Mean and standard error of oil concentration on a dry-weight basis measured by nuclear magnetic resonance spectroscopy (NMR) in parents (P_1 and P_2), and their F_1, F_2, BC_1A (F_1 x P_1) and BC_1B (F_1 x P_2) generations in two crosses grown in College Station, TX in 2010.

	Cross I	**Cross II**
Generation	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
	g kg^{-1}	
P_1	454.32 ± 3.42 d[a]	484.55 ± 2.46d
P_2	555.13 ± 2.34 a	554.71 ± 2.83 a
F_1	497.43 ± 4.06 b	499.89 ± 3.48 bc
F_2	498.21 ± 3.91 b	512.37 ± 4.94 b
BC_1A	470.15 ± 4.25 c	492.31 ± 3.90 cd
BC_2B	510.07 ± 3.79 b	513.33 ± 3.85 b

[a]The same letters in the same column indicate no significant differences at the 5% level based on Fischer's protected LSD

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TABLE 2.3 Estimates of additive, dominance, and epistatic effects (and their standard errors) from the joint scale test for oil concentration on a dry weight basis measured by nuclear magnetic resonance spectroscopy (NMR) in parents (P_1 and P_2) and their F_1, F_2, BC_1A (F_1 x P_1) and BC_1B (F_1 x P_2) in two crosses grown in College Station, Texas in 2010.

	Cross I	**Cross II**
Model[a]	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
Three parameter		
m	503.46 ± 1.95**	518.6 ± 1.78**
d	-49.56 ± 1.93**	-33.5 ± 1.18**
h	-12.96 ± 4.12**	-21.51 ± 3.69**
χ^2	13.59**	11.18*
Six parameter		
m	537.10 ± 19.45**	557.83 ± 21.3**
d	-50.41 ± 2.07**	-35.01 ± 1.88**
h	-115.91 ± 46.89*	-123.9 ± 49.45*
i	-32.38 ± 19.34	-38.20 ± 21.22
j	20.95 ± 12.11	28.12 ± 11.58*
l	76.23 ± 29.08**	65.96 ± 29.54*

*, ** indicate terms are significant at $P < 0.05$ and $P < 0.01$, respectively.

[a]*m* = mid-parent effect, *d* = additive effect, *h* = dominance effect, *i* = additive x additive effect, *j* = additive x dominance effect, and *l* = dominance x dominance effect.

TABLE 2.4 Broad (H^2) and narrow (h^2) sense heritability estimates, and estimates for the minimum number of genes controlling oil concentration in peanuts. The estimates were made using generation means analysis of generations from the cross of Tamrun OL01 and 31-08-05-02 (Cross I) and Tamrun OL07 and 31-08-05-02 (Cross II) that were evaluated in College Station, Texas in 2010.

Cross	H^2	h^2	Minimum no. of genes
I	0.78 ± 0.21	0.53 ± 0.17	27.53
II	0.85 ± 0.23	0.55 ± 0.23	27.58

CHAPTER III

GENERATION MEANS ANALSIS OF FATTY ACID COMPOSITION IN PEANUT*

Synopsis

Optimizing the chemical composition of peanut (*Arachis hypogaea* L.) oil is essential for the production of biodiesel. Specifically, increasing the ratio of oleic to linoleic acid (O/L) in peanut oil and reducing the long chain saturated fatty acid concentration (which includes arachidic, behenic, and lignoceric acids) produces high quality, stable methyl esters for biodiesel. Therefore, elucidating the inheritance of these factors and their relationships in peanut populations segregating for high oil is critical. The F_1, F_2, and backcross generations derived from two crosses, both involving a high oil concentration, low O/L runner breeding line (31-08-05-02) and two high O/L, normal oil concentration, adapted runner genotypes (Tamrun OL01 and Tamrun OL07), were evaluated in College Station, TX in 2010. The results from generation means analysis confirm that the high-oleic trait is under simple genetic control and can be manipulated through breeding and selection. Most fatty acids were controlled primarily by additive gene action which is highly selectable. Dominance effects also played an important part in the inheritance of most fatty acids. Additive x dominance interaction was significant in the inheritance of stearic and arachidic acids in the cross involving Tamrun OL07.

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Oil concentration was also negatively correlated with oleic acid concentration in the F_2 generations of both crosses and positively correlated with arachidic acid in most of the segregating generations that were evaluated. Therefore, developing a peanut genotype high in oil and oleic acid concentration that has reduced long chain saturates will require the evaluation of large numbers of segregating progeny.

Introduction

Concomitant rises in fuel demand and prices led to an increased interest in biodiesel. Peanut (*Arachis hypogaea* L.) has the potential to become a significant source of biodiesel worldwide; assuming a sufficient quantity of high quality oil is produced in the seed. The physical properties of peanut oil are determined by its fatty acid composition. Recently, several studies have examined the chemical properties of fatty acid methyl esters most suitable for biodiesel. Highly unsaturated fatty acids such as linoleic acid are associated with low cetane numbers (Bajpai and Tyagi 2006; Dermibas 2005; Knothe et al., 1998; Ramos et al., 2009). Cetane number is a diesel quality parameter that measures combustion quality during compression ignition and diesel fuel with a higher cetane number has better ignition properties (Meher et al., 2006). Polyunsaturated methyl esters also decrease the oxidative stability of biodiesel (Ramos et al., 2008). Peanut oil contains relatively large amounts of long chain saturated fatty acids compared with soybean (*Glycine max*) and rapeseed (*Brassica napus*) oil which includes arachidic, behenic, and lignoceric acids (Davis et al., 2009). These fatty acids affect the low temperature properties of biodiesel by promoting crystallization (Davis et al., 2009). Thus, one objective of our peanut breeding program for biodiesel production

is to recover high O/L ratios in progeny as they have reduced long chain saturated fatty acid concentration.

Approximately 80% of the total fatty acids in peanut oil are either oleic or linoleic acid (Lopez et al., 2000) and peanut breeders have been working to increase the oleic to linoleic acid ratios. The breeding line UF435 was the first genotype identified with 80% oleic and 2% linoleic acid (Norden et al., 1987). Inheritance of the high O/L trait in UF435 is under simple genetic control with two recessive mutations in *ahFAD2A* and *ahFAD2B* responsible for the trait (Moore and Knauft, 1989; Jung et al., 2000). One of the recessive alleles found in UF435 is common in runner and Virginia peanut genotypes, whereas the other allele is relatively rare (Knaft et al., 1993; Isleib et al., 1996). Previous research indicates that additive effects are important in the inheritance of oleic acid (Mercer et al. 1990; Upadhyaya and Nigam 1999; Aruna and Nigam 2009) as well as other fatty acids (Mecer et al., 1990; Aruna and Nigam, 2009). However, there are other reports of epistatic gene interactions governing the inheritance of the high O/L trait in peanut crosses (Lopez, et al., 2001, Muitia et al., 2006).

Significant genotype-by-environment interactions for fatty acid concentration are also well documented. Dwivedi et al. (1993) positively correlated oil concentration and O/L ratio with soil pH and Fe concentration. Worthington et al. (1972) reported seasonal differences in oil stability, some of which were not explained by differences in fatty acid levels. Low growing-season temperatures tend to increase the activity of oleic desaturase, thereby decreasing the O/L ratio (Golombek et al., 1995). Brown et al. (1975) found increases in lineoleic acid at more northerly latitudes both in Texas and

across other sites. Anderson and Gorbet (2002) found significant yearly differences in fatty acid chemistry and a significant relationship between planting date and oil chemistry in two of the three years tested. However, genotype effects on fatty acid composition were much more important than planting date. The O/L ratio tended to increase with seed maturity in most studies (Worthington et al., 1972; Sanders et al., 1982; Mozingo et al., 1988).

Oleic acid concentration has been significantly correlated with other fatty acids in different studies (Isleib et al., 1996; Anderson and Gorbet, 2002; Isleib et al., 2006; Barkley et al., 2011). These correlations indicate that the O/L genotype influences the levels of other fatty acids and those genetic modifiers may be involved in fatty acid inheritance. Specifically, oleic acid and linoleic acid are negatively correlated because their proportions are controlled by the Δ12 fatty acid desaturase that catalyzes the first step in the biosynthesis of polyunsaturated fatty acids (Groff et al., 1995). The significant negative association between oleic acid and palmitic acid observed in multiple studies (Isleib et al., 1996; Anderson and Gorbet, 2002; Barkley et al., 2011) is likely because of palmitic acid elongation to steraric acid, which is rapidly desaturated to oleic acid through Δ9 desaturase (Groff et al., 1995).

The purpose of this study was to elucidate the inheritance of fatty acid concentration in crosses between adapted, high O/L runner genotypes and a normal oleic runner genotype that is high in oil concentration via a generation means mating design. In addition, we determined the correlations between different fatty acids and between fatty acid composition and oil concentration in our populations.

Materials and Methods

Germplasm Development and Generation Means Analysis

Parental lines included in this study were a high-oil (550 to 560 g kg^{-1}, Wilson et al., 2013), normal O/L (1.5:1) runner breeding line 31-08-05-02 and two adapted, normal oil (45 to 48%), high O/L (18:1) runner genotypes Tamrun OL01 (Simpson et al., 2003) and Tamrun OL07 (Baring et al., 2006). Development of these populations through forward crossing and backcrossing, planting and field layout, and harvest techniques were described in a previous study (Wilson et al., 2013). Six generations were developed and analyzed per cross, including the parents, F_1, F_2, and backcrosses to both parents. The experiments were grown in the field at College Station, TX in 2010. Oil concentration was measured on an individual plant basis using nuclear magnetic resonance spectroscopy (NMR) as described previously on the same populations (Wilson et al., 2013). Eleven seeds from each plant were crushed and analyzed for fatty acid composition via gas chromatography (GC).

The mean fatty acid concentration from each generation was subjected to analysis of variance using PROC GLM of SAS® 9.2 (SAS Institute Inc.) Fisher's protected LSD test was used to determine whether differences existed among generation means at the 5% level of significance. A joint-scaling test was applied to the generation means to estimate mean (*m*), additive (*d*), and dominance (*h*) effects using JNTSCALE software (Ng, 1990). A chi-squared test was utilized to test the adequacy of the three parameter model. A significant chi-square value indicated a lack of fit and a six

parameter epistatic model was then fitted to the data. The epistatic genetic parameters are as follows:

i = additive x additive interaction

j = additive x dominance interaction

l = dominance x dominance interaction

Pearson's correlation coefficients for fatty acids and oil concentration were derived in the F_2 and backcross generations using PROC CORR of SAS® 9.2 (SAS Institute Inc.).

To examine the inheritance of the high O/L trait, seed samples were initially classified in the F_2 as high or low (normal) oleic. An O/L ratio of 9:1 was considered to be high O/L (Knauft et al., 1999). For further F_2 testing and for backcross analysis, samples with O/L ratios of less than 9:1 were classified as either low oleic or mid-oleic as described by Muitia et al. (2006). The chi-square statistic was applied to the O/L ratios to test conformity to the expected segregation ratios for monogenic inheritance.

Fatty Acid Methyl Ester Preparation and Analysis

Fatty acid methyl ester (FAME) analysis was performed similar to Jungman (2000). For each sample, 11 seeds were crushed using a Carver hydraulic press and oil was separated from the meal using cheesecloth. The methylation solution consisted of 29.1 ml of 14% borontrifloride in methanol, 20 ml toluene, and 50.9 ml methanol. One drop of oil was added to 1.5 ml methylation solution in a 5 ml reaction vial (Chromatography Research Supplies, Louisville, KY). The sample was placed in a heating block at 95°C for 30 min, cooled to room temperature, and 1.5 ml distilled water was then added to the solution. The solution was decanted into a test tube and 1.5 ml of

hexane was then added. After vortexing, the organic phase was transferred to a new test tube and the solvent was evaporated under a stream of nitrogen gas. The residue was dissolved in 1 ml hexane and decanted into a 2 ml gas chromatograph vial (Agilent Technologies, Santa Clara, CA) for analysis.

Prepared vials were placed in a HP 7673B automatic liquid sampler, from which 5 µl were injected into a HP 5890 Series II gas chromatograph with a Supelco Omegawax capillary column, 30 m long x 0.53 mm inner diameter x 0.50 µm film thickness. Temperatures at the injection port and flame ionization detector (FID) were 300°C and 320°C, respectively. The initial oven temperature was 180°C which increased to 230°C at a rate of 5°C/min. Total run time was 25 minutes. Signal output from the FID was recorded by a HP 3396 Series II integrator. The FAME analytical standards RM-3 and RM-5 (Sigma-Aldrich, St. Louis, MO) were analyzed for identification of fatty acid peaks.

Results and Discussion

Previous research clearly indicates that the high O/L acid trait is simply inherited in many peanut populations (Moore and Knauft, 1989; Barkley et al., 2011). Therefore, segregation ratios of 1:3 or 1:15 (high O/L : low O/L) were expected depending on the genetics of the low oleic parent. For both crosses, data were highly consistent with a 1:3 segregation ratio under monogenic inheritance (Table 3.1). This indicates that 31-08-05-02 is homozygous recessive for one of the *FAD2* genes. The chi-square test for the three-class model (low, 1.76:1 and below; mid, 1.77:1 to 8.9:1; and high O/L ratio, 9:1 and above) was consistent with monogenic inheritance and partial dominance in the cross

between Tamrun OL01 and 31-08-05-02. Results from the cross between Tamrun OL07 and 31-08-05-02 did not conform to the 1:2:1 segregation ratio because of the low number of low O/L (1.85:1 O/L ratio and below) F_2 progeny produced. All backcross populations conformed to a 1:1 segregation ratio expected under monogenic inheritance (Table 3.2). Epistatic inheritance patterns were not observed in this study.

In the cross between Tamrun OL01 and 31-08-05-02, differences were present among progeny generations for all fatty acids measured and the O/L ratio (Table 3.3). In the cross between Tamrun OL07 and 31-08-05-02, differences were present among generations for O/L ratio and all fatty acids except stearic, arachidic, and behenic acids. For both crosses, all F_1 generation fatty acid concentrations were between the means of the parents. F_2 means for all fatty acids were between both parents except for behenic acid in the Tamrun OL07 cross. Elevated behenic acid concentration in the F_2 indicates transgressive segregation that is undesirable in oil used for biodiesel.

In this study, standard errors of the means for most fatty acids in both crosses were as high as or higher in the non-segregating generations than segregating generations. This phenomenon was present for certain fatty acids in a similar study with peanut, but was not discussed (Aruna and Nigam, 2009). Elevated standard errors in non-segregating generations make calculating variance components such as heritabilities and number of genes impractical because they fail to satisfy basic assumptions of genetic model. These observations may be indicative of larger environmental variance in non-segregating generations. Relatively few plants were sampled in non-segregating generations, because of biological constraints of peanut crossing to produce F_1s.

Therefore, a few plants with relatively large environmental differences have a larger relative effect on environmental variation in non-segregating generations compared with segregating generations.

The preponderance of genetic effects were additive for all fatty acids in both crosses (Table 3.4). In the Tamrun OL07 cross, only additive effects were significant for palmitic, oleic, linoleic, arachidic, gadoleic, and lignocenic acids. In the Tamrun OL01 cross, both additive and dominance effects were significant for palmitic, oleic, linoleic, and behenic acids and the O/L ratio, whereas only additive effects were important in the inheritance of lignocenic acid.

The three-parameter model was sufficient to explain the inheritance of most fatty acids in both crosses but additive x dominance epistatic interactions were detected for stearic and gadoleic acids in the Tamrun OL07 cross. Observed differences in the importance of dominance and epistatic effects for some fatty acids between the two crosses may partly be caused by genetic factors and the inherent difficulty in reproducing minor genetic effects. While additive x dominance epistasis and dominance effects are present in this study, they are of little value because they are not selectable in a peanut cultivar. Additive effects are easily selected and expressed in homozygous peanut cultivars developed through pedigree or other appropriate selection methods.

Our data indicate that when sufficient variation is present, fatty acid concentration can be manipulated in peanut oil through selection. In the development of new peanut cultivars for biodiesel production, increasing oleic acid concentration while reducing long chain saturated fatty acid concentrations is critical for optimizing oil

chemistry (Davis et al., 2009). The relationships between these fatty acids in peanut oil have varied. Barkley et al. (2011) noted a weak positive correlation between oleic acid and lignoceric acid, while another report noted weak negative correlations between oleic acid and behenic and lignoceric acids (Anderson and Gorbet, 2002). Isleib (1996) found weak correlations between oleic acid and lignoceric, behenic, and arachidic fatty acids that varied in direction with different crosses.

In this study, correlations between oleic and arachidic and lignocenic acids were generally weak and negative (Table 3.5). Therefore, selection against either of these long chain saturated fatty acids while simultaneously selecting for high O/L could be accomplished in both crosses assuming sufficient genetic variation exists. Correlations between oleic and behenic acids were negative in both crosses with the exception of the BC_1B generation in the Tamrun OL07 cross, which indicates that selection for the high O/L trait and low behenic acid can be done simultaneously.

The negative correlations between oleic and linoleic, oleic and palmitic, and linoleic and gadoleic acids were relatively consistent across crosses and generations. The positive correlations between gadoleic and oleic, arachidic and behenic, and lignocenic and behenic acids were also stable across genetic backgrounds. These pairs of fatty acids are tightly linked in pathways controlling fatty acid synthesis in oilseed crops (Barker et al., 2007). Therefore, reducing behenic acid concentration should simultaneously reduce lignocenic and arachidic acid concentrations because of their observed correlations, which are based on strong biosynthetic associations (Barker et al., 2007). The same or similar genes likely control many of these correlated fatty acids, indicating pleiotropic

effects. Other correlations between fatty acids were highly variable between generations within a cross and/or between the two crosses. Particular attention should be paid to genetic background when selecting for or against these fatty acids pairs.

Reducing the cost of peanut oil by increasing oil concentration in seed is essential to make peanut biodiesel economically viable. Our research indicates that improving oil concentration in these populations in this environment is possible (Wilson et al., 2013). However, the associations between oil and fatty acid composition in this study differed significantly depending on the genetic background of the segregating generation measured. There was no consistent correlation between oil concentration and any fatty acid that across all segregating generations in either cross. There may have been pleotropic effects between oil concentration and fatty acid chemistry, but the strength of these effects was dependent on genetic background. A moderate negative correlation between oil concentration and oleic acid concentration was present in the F_2 generations of both crosses. There were weak to moderate positive correlations between oil concentration and arachidic acid in five of six genetic combinations measured. Developing a peanut genotype containing elevated oil and oleic acid concentrations that possesses reduced quantities of long chain saturates is possible, but will require the evaluation of substantial numbers of segregating progeny.

TABLE 3.1 Segregation for oleic to linoleic acid ratio (O/L) of F_2 peanut progeny and chi-square values for the crosses of either Tamrun OL01 or Tamrun OL07 with 31-08-05-02 grown in College Station, TX in 2010.

Recessive Gene Action Two-Class Model			
	Number of Seeds		χ^2
Cross	Low O/L[a]	High O/L	3:1
Tamrun OL01 x 31-08-05-02	32	13	0.37 ns[b]
Tamrun OL07 x 31-08-05-02	25	14	2.47 ns
Recessive Gene Action Three-Class Model			
	Number of Seeds		χ^2
Cross	Low O/L	Mid O/L	High O/L	1:2:1
Tamrun OL01 x 31-08-05-02	10	22	13	0.31 ns
Tamrun OL07 x 31-08-05-02	2	23	14	8.64*

[a]Low-oleic genotypes had O/L ratios of 1.76:1 or below for the Tamrun OL01 cross and 1.85:1 and below for the Tamrun OL07 cross. Mid O/L genotypes were above the low-oleic threshold to a ratio of 8.9:1, and high O/L types had O/L ratios of 9:1 and above.

[b]ns, does not significantly depart from expected genetic ratio at 5% level of significance; * departs significantly from the expected ratio at the 5% level of significance.

TABLE 3.2 Segregation for oleic to linoleic acid ratio (O/L) of BC_1F_1 peanut progeny and chi-square (χ^2) values for the crosses of either Tamrun OL01 or Tamrun OL07 with 31-08-05-02 grown in College Station, TX in 2010.

Recessive Gene Action Two-Class Model			
	Number of Seeds		χ^2
Cross	Low O/L[a]	Mid O/L	1:1
(Tamrun OL01 x 31-08-05-02) x 31-08-05-02	16	23	1.26 ns[b]
(Tamrun OL07 x 31-08-05-02) x 31-08-05-02	12	22	2.94 ns
Recessive Gene Action Two-Class Model			
	Number of Seeds		χ^2
Cross	Mid O/L	High O/L	1:1
(Tamrun OL01 x 31-08-05-02) x Tamrun OL01	13	12	0.08 ns
(Tamrun OL07 x 31-08-05-02) x Tamrun OL07	20	18	0.11 ns

[a]Low-oleic genotypes had O/L ratios of 1.76:1 or below for the Tamrun OL01 cross and 1.85:1 and below for the Tamrun OL07 cross. Mid O/L genotypes were above the low-oleic threshold to a ratio of 8.9:1, and high O/L types had O/L ratios of 9:1 and above.

[b]ns, does not significantly depart from expected genetic ratio at 5% level of significance

TABLE 3.3 Mean and standard error (SE) of fatty acid concentration in parents, F_1, F_2, BC_1A (F_1 x P_1) and BC_1B (F_1 x P_2) generations of peanut grown in College Station, TX in 2010.

	Mean ± SE	**Mean ± SE**
Trait	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
P_1	5.62 ± 0.09e[a]	4.69 ± 0.29c
P_2	9.31 ± 0.09a	7.67 ± 0.37a
F_1	7.89 ± 0.16bc	6.38 ± 0.25b
F_2	7.56 ± 0.22cd	6.67 ± 0.32ab
BC_1A	7.00 ± 0.28d	6.13 ± 0.37b
BC_1B	8.57 ± 0.18ab	6.83 ± 0.37ab
Stearic acid (18:0)		
P_1	2.09 ± 0.10c	1.64 ± 0.10ns
P_2	2.72 ± 0.05a	1.93 ± 0.16
F_1	2.46 ± 0.09b	1.67 ± 0.08
F_2	2.36 ± 0.06b	1.83 ± 0.09
BC_1A	2.54 ± 0.10ab	1.55 ± 0.10
BC_1B	2.36 ± 0.06b	1.69 ± 0.12
Oleic acid (18:1)		
P_1	79.43 ± 0.31a	82.31 ± 0.68a
P_2	49.99 ± 0.58e	52.40 ± 0.75e
F_1	61.48 ± 1.25cd	64.48 ± 1.40cd
F_2	64.41 ± 1.84c	68.78 ± 1.76bc
BC_1A	70.27 ± 2.19b	72.90 ± 1.74b
BC_1B	57.12 ± 1.32d	61.63 ± 1.55d

TABLE 3.3 Continued

Trait	Mean ± SE Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Mean ± SE Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
Linoleic acid (18:2)		
P_1	4.74 ± 0.20e	3.61 ± 0.17d
P_2	30.30 ± 0.60a	31.57 ± 0.86a
F_1	20.63 ± 1.10bc	20.59 ± 1.29b
F_2	18.18 ± 1.64c	15.06 ± 1.56c
BC_1A	12.66 ± 1.88d	12.01 ± 1.39c
BC_1B	24.81 ± 1.13b	23.12 ± 1.61b
Arachidic acid (20:0)		
P_1	1.11 ± .03c	1.09 ± 0.04ns
P_2	1.43 ± 0.02a	1.34 ± 0.06
F_1	1.29 ± 0.03b	1.16 ± 0.04
F_2	1.28 ± 0.02	1.22 ± 0.04
BC_1A	1.28 ± 0.03b	1.15 ± 0.06
BC_1B	1.28 ± 0.03b	1.21 ± 0.05
Gadoleic acid (20:1)		
P_1	2.40 ± 0.06a	2.29 ± 0.08a
P_2	1.22 ± 0.03d	1.20 ± 0.05e
F_1	1.66 ± 0.04bc	1.77 ± 0.07c
F_2	1.69 ± 0.05b	1.87 ± 0.06bc
BC_1A	1.82 ± 0.05b	2.02 ± 0.06b
BC_1B	1.50 ± 0.04c	1.53 ± 0.05d
Behenic acid (22:0)		
P_1	2.32 ± 0.06c	2.47 ± 0.11ns
P_2	2.71 ± 0.05a	2.72 ± 0.16
F_1	2.68 ± 0.07a	2.65 ± 0.11
F_2	2.65 ± 0.05a	2.89 ± 0.10

TABLE 3.3 Continued

Trait	Mean ± SE Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Mean ± SE Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
BC_1A	2.47 ± 0.05bc	2.58 ± 0.13
BC_1B	2.64 ± 0.05ab	2.62 ± 0.09
Lignocenic acid (24:0)		
P_1	1.55 ± 0.05a	1.51 ± 0.06a
P_2	1.18 ± 0.03c	1.05 ± 0.07d
F_1	1.36 ± 0.04b	1.12 ± 0.11cd
F_2	1.35 ± 0.03b	1.35 ± 0.05ab
BC_1A	1.36 ± 0.03b	1.26 ± 0.07bc
BC_1B	1.30 ± 0.03b	1.15 ± 0.05cd
O/L ratio		
P_1	17.20 ± 0.78a	23.49 ± 1.21a
P_2	1.66 ± 0.05c	1.69 ± 0.08c
F_1	3.12 ± 0.23c	3.38 ± 0.34c
F_2	8.60 ± 1.42b	9.56 ± 1.42b
BC_1A	12.42 ± 2.05b	11.85 ± 1.50b
BC_1B	2.645 ± 0.21c	4.15 ± 0.85c

[a]Different letters denote means between generations were significantly different at 5% level of probability by LSD test.

TABLE 3.4 Estimates of additive, dominance, and epistatic effects (and their standard errors) from the joint scaling test for concentration of fatty acids in parents (P_1 and P_2) and their F_1, F_2, BC_1A (F_1 x P_1) and BC_1B (F_1 x P_2) in two peanut crosses grown in College Station, Texas in 2010.

		Cross	
Trait	**Model[a]**	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
Palmitic acid (16:0)	Three parameter		
	m	7.47 ± 0.06**	6.22 ± 0.20**
	d	-1.84 ± 0.06**	-1.33 ± 0.20**
	h	0.42 ± 0.15**	0.25 ± 0.34
	χ^2	1.00	3.82
Stearic acid (18:0)	Three parameter		
	m	2.42 ± 0.05**	1.78 ± 0.08**
	d	-0.23 ± 0.05**	-0.15 ± 0.08
	h	-0.06 ± 0.10	-0.12 ± 0.12
	χ^2	17.885**	3.38
	Six parameter		
	m	2.02 ± 0.33**	
	d	-0.32 ± 0.06**	
	h	0.92 ± 0.84	
	i	0.38 ± 0.32	
	j	0.98 ± 0.25**	
	k	-0.48 ± 0.55	
Oleic acid (18:1)	Three parameter		
	m	64.78 ± 0.32**	67.54 ± 0.50**
	d	14.66 ± 0.32**	20.59 ± 0.50**
	h	-2.68 ± 1.14*	-1.79 ± 1.31
	χ^2	1.24	5.29

TABLE 3.4 Continued

Trait	Model[a]	Cross	
		Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
Linoleic acid (18:2)	Three parameter		
	m	17.48 ± 0.31**	17.33 ± 0.42**
	d	-12.74 ± 0.31**	-13.72 ± 0.42**
	h	2.81 ± 1.01**	1.82 ± 1.17
	χ^2	0.46	7.48
Arachidic acid (20:0)	Three parameter		
	m	1.28 ± 0.02**	1.21 ± 0.03**
	d	-0.13 ± 0.02**	-0.11 ± 0.03**
	h	0.00 ± 0.03	-0.05± 0.05
	χ^2	13.20**	1.39
	Six parameter		
	m	1.27 ± 0.12**	
	d	-0.16 ± 0.02**	
	h	0.02 ± 0.31	
	i	0.00 ± 0.12	
	j	0.32 ± 0.09**	
	k	0.00 ± 0.20	
Gadoleic acid (20:1)	Three parameter		
	m	1.76 ± 0.03**	1.76 ± 0.04**
	d	0.54 ± 0.03**	0.54 ± 0.04**
	h	-0.12 ± 0.05*	0.05 ± 0.08
	χ^2	15.07**	2.91
	Six parameter		
	m	1.93 ± 0.25**	
	d	0.59 ± 0.03**	

TABLE 3.4 Continued

Trait	Model[a]	Cross	
		Tamrun OL01 (P_1) x 31-08-05-02 (P_2)	Tamrun OL07 (P_1) x 31-08-05-02 (P_2)
	h	-0.70 ± 0.58	
	i	-0.12 ± 0.24	
	j	-0.54 ± 0.14**	
	k	0.44 ± 0.34	
Behenic acid (22:0)	Three parameter		
	m	2.51 ± 0.04**	2.62 ± 0.08**
	d	-0.19 ± 0.04**	-0.10 ± 0.08
	h	0.16 ± 0.08*	0.08 ± 0.14
	χ^2	3.16	6.78
Lignocenic acid (24:0)	Three parameter		
	m	1.34 ± 0.02**	1.30 ± 0.04**
	d	0.15 ± 0.02**	0.21 ± 0.04**
	h	0.00 ± 0.05	-0.09 ± 0.10
	χ^2	5.90	6.45
O/L ratio	Three parameter		
	m	9.59 ± 0.38**	12.48 ± 0.56**
	d	7.92 ± 0.38**	10.79 ± 0.55**
	h	-6.32 ± 0.43**	-8.98 ± 0.67**
	χ^2	4.67	5.75

*, ** indicate terms are significant at 5% and 1% levels of probability, respectively.

[a]*m* = mid-parent effect, *d* = additive effect, *h* = dominance effect, *i* = additive x additive effect, *j* = additive x dominance effect, and *l* = dominance x dominance effect.

TABLE 3.5 Pearson's correlation coefficients between paired comparisons of eight fatty acids, O/L ratio, and oil concentration in three segregating peanut populations (F_2, BC_1A, and BC_1B) resulting from crosses between either Tamrun OL01 or Tamrun OL07 (P_1) and 31-08-05-02 (P_2).

Correlation between	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)			Tamrun OL07 (P_1) x 31-08-05-02 (P_2)		
	Generation			Generation		
	F_2	BC_1A[a]	BC_1B	F_2	BC_1A	BC_1B
1-2[b]	0.31*	0.39*	0.26	0.39*	0.26	- 0.22
1-3	0.25	0.38*	0.29	0.38*	0.29	- 0.06
1-4	- 0.32*	- 0.31*	- 0.16	- 0.31*	- 0.16	0.24
1-5	0.32*	0.23	0.08	0.23	0.08	- 0.17
1-6	0.37*	0.49**	0.41*	0.49**	0.41*	0.14
1-7	- 0.42**	- 0.47**	- 0.16	- 0.47**	- 0.16	- 0.35*
1-8	0.13	0.40**	0.17	0.40**	0.17	- 0.10
1-9	- 0.24	- 0.08	- 0.09	- 0.08	- 0.09	- 0.18
1-10	- 0.27	- 0.23	- 0.14	- 0.23	- 0.14	0.10
2-3	- 0.09	0.16	0.74**	0.16	0.74**	0.59**
2-4	- 0.97**	- 0.78**	- 0.81**	- 0.78**	- 0.81**	- 0.23
2-5	0.97**	0.61**	0.56**	0.61**	0.56**	- 0.14
2-6	0.04	0.37*	0.74**	0.37*	0.74**	0.63**
2-7	- 0.72**	- 0.38*	- 0.02	- 0.38*	- 0.02	0.31
2-8	0.45**	0.72**	0.61**	0.72**	0.61**	0.73**

TABLE 3.5 Continued

Correlation between	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)			Tamrun OL07 (P_1) x 31-08-05-02 (P_2)		
	Generation			**Generation**		
	F_2	BC_1A	BC_1B	F_2	BC_1A	BC_1B
2-9	0.06	0.20	- 0.15	0.45**	0.22	0.60**
2-10	- 0.88**	- 0.89**	- 0.88**	- 0.68**	- 0.64**	- 0.18
3-4	0.02	- 0.27	- 0.18	- 0.06	- 0.44**	- 0.03
3-5	- 0.03	0.24	0.14	- 0.08	0.13	- 0.28
3-6	0.92**	0.93**	0.88**	0.89**	0.88**	0.87**
3-7	- 0.45**	- 0.60**	- 0.71**	- 0.11	0.17	0.43**
3-8	0.03	0.38	0.13	0.56**	0.68**	0.72**
3-9	- 0.49**	- 0.24	- 0.50**	0.33*	0.28	0.50**
3-10	0.03	- 0.26	- 0.16	0.10	- 0.23	- 0.14
4-5	- 1.00**	- 1.00**	- 1.00**	- 0.96**	- 0.93**	- 0.92**
4-6	- 0.14	- 0.30	- 0.23	- 0.17	- 0.38*	0.10
4-7	0.68**	0.70**	0.55**	0.54**	0.21	0.40*
4-8	- 0.59**	- 0.63**	- 0.43**	- 0.39*	- 0.36*	- 0.08
4-9	- 0.15	- 0.26	- 0.09	- 0.07	- 0.24	- 0.03
4-10	0.89**	0.94**	0.96**	0.91**	0.91**	0.78**
5-6	0.13	0.26	0.20	- 0.01	0.08	- 0.39*
5-7	- 0.70**	- 0.70**	- 0.54**	- 0.59**	- 0.36*	- 0.58**
5-8	0.56**	0.60**	0.39*	0.15	0.10	- 0.27

TABLE 3.5 Continued

Correlation between	Tamrun OL01 (P_1) x 31-08-05-02 (P_2)			Tamrun OL07 (P_1) x 31-08-05-02 (P_2)		
	Generation			**Generation**		
	F_2	BC_1A	BC_1B	F_2	BC_1A	BC_1B
5-9	0.12	0.23	0.07	- 0.15	0.10	- 0.27
5-10	- 0.89**	- 0.94**	- 0.96**	- 0.89**	- 0.91**	- 0.70**
6-7	- 0.46**	- 0.53**	- 0.62**	- 0.26	0.25	0.34*
6-8	0.31*	0.55**	0.48**	0.75**	0.48**	0.77**
6-9	- 0.34*	0.15	- 0.26	0.32*	- 0.01	0.44**
6-10	- 0.09	- 0.25	- 0.22	- 0.06	- 0.22	0.00
7-8	- 0.09	- 0.32	- 0.12	0.00	- 0.06	0.56**
7-9	0.53**	0.25	0.52**	0.50**	0.19	0.60**
7-10	0.62**	0.64**	0.49**	0.51**	0.20	0.17
8-9	0.61**	0.71**	0.56**	0.73**	0.62**	0.79**
8-10	- 0.50**	- 0.62**	- 0.40*	- 0.30	- 0.22	- 0.13
9-10	- 0.11	- 0.22	- 0.11	- 0.01	- 0.22	- 0.13

[a]BC_1A refers to the backcross of the F_1 (P_1 x P_2) to P_1. BC_1A refers to the backcross of the F_1 (P_1 x P_2) to P_2.

[b]1 = oil concentration; 2 = palmitic acid; 3 = stearic acid; 4 = oleic acid; 5 = linoleic acid; 6 = arachidic acid; 7 = gadoleic acid; 8 = behenic acid; 9 = lignocenic acid; 10 = O/L ratio,

*, ** indicate terms are significant at the 5% and 1% levels of probability, respectively.

CHAPTER IV

DIALLEL ANALYSIS OF OIL PRODUCTION COMPONENTS IN PEANUT

Synopsis

Peanut (*Arachis hypogaea* L.) has the potential to become a major source of biodiesel, but the current peanut oil price is prohibitive. Oil yield in peanuts is influenced by many different components, including oil concentration, seed mass, and mean oil produced per seed. All of these traits can potentially be improved through selection as long as there is sufficient genetic variation. A diallel mating design was conducted to measure additive and dominance variation associated with these traits. Our data indicate the importance of additive effects for oil concentration, weight of 50 sound mature kernels (50 SMK), and mean milligrams oil produced per SMK (OPS). Dominance variation was also a significant factor in the inheritance of oil concentration. However, dominance variance cannot be fixed in inbred peanut lines. Reciprocal effects were detected for weight of 50 SMK and OPS. Heritability was very high for oil concentration and weight of 50 SMK, and low for OPS. The low OPS heritability estimate was caused by the negative correlation between oil concentration and seed size. Consequently, oil concentration and seed mass alone can be improved through early-generation selection but large segregating populations from high oil crosses will be needed to identify progeny with elevated oil concentrations that maintain acceptable seed sizes.

Introduction

The cultivated peanut (*Arachis hypogaea* L.) is an important annual oilseed crop planted widely in North America, South America, Africa, Asia, and Australia. Over one million acres of peanuts were planted in the United States in 2011 (USDA, National Agriculture Statistics Service). Peanut has potential as a source of biofuel, but current price of peanut oil is high due to demands from the edible markets. Increasing the oil production of peanut on a per acre basis is essential, if peanuts are to become a large scale source of biodiesel.

Our previous research on oil concentration in peanut has focused on estimating genetic effects as determined from analyses of progeny means (Wilson et al., 2013). Underlying gene action, as calculated using a generation means mating design, may not reflect genetic variance available for a trait (Bernardo, 2002). While improving the mean is our ultimate goal, sufficient genetic variation is required for selection. Previous diallel studies conducted with peanut indicate that selectable genetic variation exists for oil content. Additive effects (general combining ability, GCA) were more important than non-additive effects (specific combining ability, SCA) for determining oil content in studies measuring F_1 populations (Sykes and Michaels, 1986; Isleib et al. 2004) and an F_2 population (Layrisse et al., 1980). The performance of parental lines was generally a good predictor of hybrid oil content (Layrisse et al., 1980; Isleib et al. 2004). Cytoplasmic (maternal) effects were highly significant in an F_1 generation in a study by Isleib (2004), but were much less pronounced in a study using F_2's (Layrisse et al., 1980).

Layrisse et al. (1980) determined that rank correlations of GCA (general combining ability) effects for oil content and seed yield were positive and significant, as were correlations among phenotypic means. No correlations were significant between oil content and 20-pod length and 20-pod weight. Dwivedi et al. (1990) determined that high oil content can be maintained when indirectly selecting for large seed size. Other studies have reported negative correlations between seed size and oil content (Holley and Hammons, 1968; Patil, 1972).

Seed mass is not a critical factor for processors when peanuts are processed for oil (Dwivedi et al., 1990). However, there is a positive correlation between seed mass and yield (Layrisse et al., 1980; Singh et al., 1984). Therefore, maximizing seed size and oil mass per seed in early generations of germplasm evaluation for biodiesel production are important considerations. The objectives of this research were to determine genetic variance components for oil content, seed mass of sound mature kernels (SMK), and mean milligrams oil produced per SMK (OPS) through a diallel mating design. The relationship between oil concentration and seed mass in segregating progeny was also elucidated.

Materials and Methods

Germplasm Development and Experimental Design

A four-parent diallel cross, including reciprocals, was made in a greenhouse in College Station in 2009 and 2010. Individual F_1 plants were grown to maturity in the greenhouse and field site. Seed collected from individual F_1 plants was pooled to provide enough F_2 seed for the experiment. Sixteen F_2 seed were planted by hand in 2.4 m long

twin row plots arranged in a randomized complete block design with four replications in 2010. Standard agronomic and pest control practices were employed throughout the growing season and plots were irrigated. The following parents were included:

1. Tamrun OL01 (Simpson et al., 2003): Large seeded, adapted runner variety with oil concentration between 430 and 460 g kg^{-1}
2. Tamrun OL07 (Baring et al., 2006): Adapted runner variety with medium sized seeds and oil concentration between 470 and 490 g kg^{-1}
3. Lub 268: Advanced early-maturing runner breeding line, medium to large seed size with oil concentration between 500 and 530 g kg^{-1}
4. 31-08-05-02: runner breeding line with pedigree Florunner^2 // TxAG-6 (Simpson et al., 1993) / Florunner BC_3; small seeded with oil concentration above 550 g kg^{-1}

At maturity, plants were harvested individually and seed was dried to 5% moisture content. A sample of 50 sound mature kernels (50 SMK) was randomly selected from seed that would not pass through a 6 x 19 mm mesh screen. This SMK sample was weighed and 20 g of seed were used to estimate oil content using nuclear magnetic resonance (NMR), which measures total oil content on a percentage dry-weight basis. These readings were converted to oil concentrations in g kg^{-1}. Oil yield per SMK (OPS) in milligrams was calculated by multiplying percent oil content by 50 SMK weight in grams, divided by 50 and multiplied by 100.

Statistical Analysis Using the Jinks-Hayman Model

The Hayman analysis of variance (Hayman, 1954) was performed using GenStat® 14 (VSN International). This approach partitions sums of squares into the following components: additive (*a*), non-additive (*b*, which is subdivided into b_1, which if significant indicates unidirectional dominance; b_2, which indicates asymmetry of gene distribution; and b_3, which indicates dominance deviations not attributable to b_1 or b_2), *c* (maternal), and *d* (reciprocal differences other than *c*).

To test for genotype differences, an analysis of variance for each measured parameter was performed via PROC GLM of SAS® 9.2 (SAS Institute Inc.). Fisher's protected LSD test was used to determine whether differences existed among plot means at the 5% level of significance When genotype differences were confirmed, variances and covariances were calculated from the diallel table via GenStat® 14 (VSN International). Genetic components estimated based on variances and covariances outlined by Hayman (1954) included the following: D, additive variance, H_1, dominance variance, H_2, proportion of dominance due to positive and negative effect of genes, and F, relative frequency of dominant and recessive alleles, h^2, dominance effect (sum over all loci in heterozygous phase in all crosses) and E_2, environmental effects. These estimates were calculated for F_2 populations as described by Singh and Chaudhary 1985) where:

$$D = V_{0l0} - E_2$$

$$H_1 = 16V_{1L2} - 16W_{0l02} + 4V_{0l0} - (4(5n - 4) / n)E_2$$

$$H_2 = 16V_{1L2} - 16W_{0l02} - (16(n - 1) / n)E_2$$

$$F = 4V_{0l0} - 8W_{0l02} - (4(n - 2) / n)E_2$$

$$h^2 = (4M_{L2} - 4\ M_{LO})^2 - (16(n\text{-}1) /n)\ E_2$$

$$E_2 = [(\text{Error SS} + \text{Rep SS}) / \text{d.f.}] / \text{number of replications}$$

F_2 generation data analysis differs from F_1 analysis due to inbreeding, therefore coefficients of H_1 and H_2 are reduced by 1/4 and the coefficients of F are reduced by 1/2 (Singh and Chaudhary, 1985). Genetic components were used to estimate narrow-sense heritability as described by Singh and Chaudhary (1985), where:

$$h^2 = (1/4D) / (1/4D + 1/16H_1 - 1/8F + E)$$

To test the validity of assumptions in the Jinks-Hayman model, the uniformity of *Wr*, *Vr*, was calculated using the following formula described by Singh and Chaudhary (1985):

$$t^2 = (n - 2) / 4[(Var\ V_r - Var\ W_r)^2 / (Var\ V_r \text{ x } Var\ W_r)^2 - \text{Cov}^2(V_r, W_r)]$$

Another general test for epsistasis utilized in this study was the regression of covariance on the variance, which was calculated using the following formula described by Singh and Chaudhary (1985):

$$b = C_{ov}(Wr, Vr) / Var(Vr)$$

This significance of the regression coefficient (*b*) from zero and one was then tested. The regression coefficient is expected to be significantly different from zero but not from 1.0 if all assumptions are met.

Statistical Analysis Using the Griffing Model

The diallel data for each parameter was subjected to a fixed effect analysis using model I, method 1 of Griffing (1956). Using mean sums of squares estimates, GCA

effects for each parent, SCA effects for each cross, and reciprocal effects were calculated using DIALLEL software (Burow and Coors, 1994). The phenotypic correlation between oil concentration and weight of 50 SMK across all populations was computed using PROC CORR of SAS® 9.2 (SAS Institute, Inc).

Results

Analyses of variance indicated significant genotype differences for oil concentration ($P < 0.0001$), 50 SMK ($P < 0.0001$) and OPS ($P = 0.011$). Plot means for the three traits are presented in Table 4.1. Compared to oil concentration and 50 SMK, variation for OPS was limited. Across all F_2 progeny, oil concentration tended to decrease as 50 SMK increased with a correlation of $r = - 0.45$ ($P < 0.0001$).

In this study, the t^2 value was not significant for the three traits (Table 4.2), indicating no general violation of underlying assumptions. Regression coefficients for oil concentration and weight of 50 SMKs were significantly different than zero and not significantly different than unity at the 5% level, also indicating no general violation of assumptions. However, the regression coefficient for OPS was not significantly different than zero, indicating a partial violation of assumptions.

Results from Griffing and Hayman's detailed analyses of variance were similar for GCA and SCA effects (Tables 4.3, 4.4, and 4.5). Data indicate that both the *a* term in Hayman's analysis and GCA were important in the inheritance of all three traits (Tables 4.3, 4.4, and 4.5). Previous research indicates the importance of GCA in the inheritance of seed weight in peanut (Dwivedi et al., 1989; Layrisse et al., 1980). Both Hayman's *a* term and GCA are analogous to additive effects. Dominance effects, tested by *b* and

SCA, were also significant in the inheritance of oil concentration (Table 4.3). However, the ratio of *a* to *b* and GCA to SCA indicated that additive effects were more important than dominance effects, particularly for oil concentration and weight of 50 SMK (Tables 4.3 and 4.4).

There were differences in the partitioning of sums of squares in the two analyses for reciprocal effects. Hayman's *c* term was significant for 50 SMK weight, while reciprocal effects in Griffing's analysis were not significant (Table 4.4). Mather and Poysa (1983) noted differences in reciprocal effects between the two analyses in a diallel of triticale (× *Triticosecale* Wittmack). Reciprocal effects were detected in both analyses for OPS, which is a violation of assumptions in the genetic model (Table 4.5).

As expected, the high oil parent 31-08-05-02 gave the highest GCA estimated for oil concentration (Table 4.6). However, the GCA for weight of 50 SMK was highly negative for this breeding line and was significant and positive for the low oil genotype Tamrun OL01. The highest SCA effects observed in this study tended to vary widely for each parent and trait depending on the cross (Table 4.7). None of the progeny populations had positive SCA values for all three traits. Tamrun OL07 had negative SCA values for all traits in crosses with Lub 268 and 31-08-05-02.

Hayman's genetic variance components confirm the importance of additive variation for oil concentration and weight of 50 SMK along with the error associated with OPS measurements (Table 4.8). Narrow-sense heritiabilities (h^2) based on Hayman's genetic components were extremely high for oil concentration and 50 SMK (0.97 and 0.83, respectively) and were low for OPS (0.13)

Discussion

The diallel cross is a powerful tool to study the various variance components of the genetic systems controlling a quantitative trait. The diallel analysis, as outlined by Griffing (1956), partitions phenotypic variation into genotypic and error variation and further divides genotypic variation into additive and dominance components. These values can then be used to calculate heritability estimates, draw inferences about the genetic system, and determine the most efficient breeding procedures.

Diallel analyses, along with other mating designs, are based on several assumptions with regard to the genetic system. The failure of one or more of these assumptions may influence and could to some extent invalidate inferences derived from the analysis. Our data indicate a partial failure of assumptions in the general test for epistasis for OPS. Estimates of additive and dominance genetic variance cannot be accurately obtained from a diallel analysis in the presence of epistasis (Baker, 1978), which skews the relative contribution of the genotypic values associated with the parents. Previous research indicates that inheritance of oil concentration is a more complex genetic system than a simple additive-dominance model (Upadhyaya and Nigam, 1999, Wilson et al., 2013). Despite these constraints, a diallel design can be used to estimate genetic variance components (Hayman, 1954; Wilson et al., 1978) and combining abilities (Baker, 1978), although less reliably than if all assumptions in the genetic model were satisfied.

Because our study is based on a limited number of selected parents, the inferences are applicable to these populations alone. Authors have suggested that genetic

variance estimates and therefore heritability estimates are unreliable in a fixed model (Baker, 1978; Bernardo, 2002; Sughroue and Hallauer, 1997). However, the preponderance of evidence from this study and other published papers clearly demonstrate the importance of additive effects in the inheritance of peanut oil concentration (Layrisse et al., 1980; Sykes and Michaels, 1986; Isleib et al. 2004; Wilson et al; 2013) and seed mass (Layrisse et al., 1980; Dwivedi et al., 1989).

The importance of additive effects and lack of error associated with oil concentration measurements are reflected in the high narrow-sense heritability estimate. Wilson et al. (2013) also reported a high h^2 for oil concentration and the trait exhibited continuous variation in a normal distribution in F_2 generations (Wilson et al, 2013). The high heritability estimate indicates that most of that genetic variation is additive and responsive to selection.

The inverse relationship between oil concentration and seed weight was also observed in previous studies, which implies that the use of metabolic resources to produce elevated oil content in peanut seeds causes a concurrent decrease in endosperm weight (Holley and Hammons, 1968; Patil, 1972). Although a negative correlation existed between seed size and oil concentration, there were outliers within F_2 progeny derived from 31-08-05-02 that had high OPS compared to the plot average.

Progress can be made toward developing seed with improved oil concentration since the vast majority of variation for this trait is genetic. Because the relationship between oil concentration and seed weight is negative in our populations, large segregating populations will need to be evaluated to improve both traits. The low

narrow-sense heritability of OPS is a product of the negative correlation between oil concentration and seed weight in our populations and error associated with these measurements. Based on the presented data, early-generation selection based on OPS would not be effective but selection for either oil concentration and/or seed size would be. Given that higher yields result in higher total oil yields, a selection index that maximizes one trait while maintaining performance of the second may be an appropriate approach to improving oil yield in peanut.

TABLE 4.1 Mean of oil concentration, weight of 50 sound mature kernels (SMKs) in grams, and mean milligrams oil produced per SMK (OPS) of F_2 progeny and parents in a four-parent diallel of peanut.

	Trait		
Pedigree	**Oil Concentration**	**50 SMK**	**OPS**
	g kg^{-1}	g	mg
31-08-05-02	553a[a]	27.4i	303bc
31-08-05-05 x Lub268	521b	28.5hi	297bc
Lub268 x 31-08-05-05	520b	29.5gh	307abc
Lub 268	507c	30.4fgh	308abc
Tamrun OL07 x 31-08-05-05	504c	29.6gh	298bc
Tamrun OL01 x 31-08-05-05	503c	29.8gh	300bc
31-08-05-05 x Tamrun OL07	496cd	30gh	296bc
31-08-05-05 x Tamrun OL01	495cd	31.4efg	311ab
Lub 268 x Tamrun OL07	483de	32def	309ab
Tamrun OL07 x Lub 268	479e	30.4fgh	291c
Tamrun OL07	477e	33.7bcd	322a
Tamrun OL07 x Tamrun OL01	471ef	34.3abc	323a
Tamrun OL01 x Tamrun OL07	465fg	33.2cde	308abc
Tamrun OL01 x Lub 268	458g	33.1cde	304bc
Lub 268 x Tamrun OL01	455gh	35.4ab	322a
Tamrun OL01	446h	35.8a	311ab
Coefficient of Variation	1.8	4.3	4.0

[a]The same letters in the same column indicate no significant differences at the 5% level based on Fisher's protected LSD.

TABLE 4.2 Scaling tests (t^2, regression coefficient) of the additive-dominance model for oil concentration, weight of 50 sound mature kernels (50 SMK) in grams and mean milligrams oil produced per SMK (OPS) in a four-parent F_2 diallel of peanut.

Trait	**t^2**	**Regression Coefficient**
Oil Concentration	1.29	1.07 ± 0.08
Weight of 50 SMKS (g)	-2.89	0.82 ± 0.15
Oil Per Seed (mg)	0.76	0.71 ± 0.27

TABLE 4.3 Griffing and Hayman's analyses of variance of oil concentration in a four-parent F_2 diallel of peanut.

Griffing			Hayman		
Source	*df*	Mean Square	Source	*df*	Mean Square
Blocks	3	168.1	Blocks	3	168.1
Genotypes	15	3236.1**	Genotypes	15	3236.1**
GCA	3	14779.6**	*a*	3	14779.6**
SCA	6	628.3**	*b*	6	628.3**
			b_1	1	850.1*
			b_2	3	262.1
			b_3	2	1066.7**
Reciprocal	6	72.1	*c*	3	66.6
			d	3	77.7
Error	45	78.1	Error	45	78.1

*, ** indicates terms are significant at the 5 and 1% levels of probability, respectively

TABLE 4.4 Griffing and Hayman's analyses of variance of weight of 50 sound mature kernels (50 SMK) in grams in a four-parent F_2 diallel of peanut.

Griffing			Hayman		
Source	*df*	Mean Square	Source	*df*	Mean Square
Blocks	3	4.87	Blocks	3	3.65
Genotypes	15	24.71**	Genotypes	15	24.72**
GCA	3	107.98**	*a*	3	107.98**
SCA	6	3.65	*b*	6	3.65
			b_1	1	1.71
			b_2	3	3.53
			b_3	2	4.80
Reciprocal	6	4.14	*c*	3	8.24*
			d	3	0.04
Error	45	1.87	Error	45	1.87

*, ** indicates terms are significant at the 5 and 1% levels of probability, respectively

TABLE 4.5 Griffing and Hayman's analyses of variance of mean milligrams oil produced per sound mature kernel (OPS) in a four-parent F_2 diallel of peanut.

Griffing			Hayman		
Source	*df*	Mean Square	Source	*df*	Mean Square
Blocks	3	442.8*	Blocks	3	442.8*
Genotypes	15	359.8*	Genotypes	15	359.8*
GCA	3	508.5*	*a*	3	508.5*
SCA	6	286.4	*b*	6	286.4
			b_1	1	344.0
			b_2	3	358.0
			b_3	2	150.3
Reciprocal	6	358.9*	*c*	3	664.4*
			d	3	53.4
Error	45	149.8	Error	45	149.8

* indicates terms are significant at the 5% levels of probability

TABLE 4.6 Estimates of GCA effects and standard errors for oil concentration, weight of 50 sound mature kernels (50 SMK) in grams, and mean milligrams oil produced per SMK (OPS) in a four-parent F_2 diallel of peanut.

Parent	**Oil Concentration**	**50 SMK**	**OPS**
	g kg^{-1}	g	mg
Tamrun OL01	-22.3**	2.06**	4.30*
Tamrun OL07	-8.0**	0.58**	1.86
Lub268	1.7	-0.31	-1.23
31-08-05-02	28.6**	-2.33**	-4.92**
SE (g_i)	1.4	0.21	1.87

*, ** indicates terms are significant at the 5 and 1% levels of probability, respectively

TABLE 4.7 Estimates of SCA effects and standard errors for oil concentration, weight of 50 sound mature kernels (50 SMK) in grams, and mean milligrams oil produced per SMK (OPS) in a four-parent F_2 diallel of peanut.

	Parent			
Parent	Tamrun OL01	Tamrun OL07	Lub 268	31-08-05-02
	0.75[a]	8.65**	-12.31**	2.91
Tamrun OL01	0.11	-0.44	0.97*	-0.64
	-4.64	2.55	2.77	-0.67
		3.81	-2.41	-10.06**
Tamrun OL07		1.04*	-0.58	-0.01
		11.48*	-7.55*	-6.48
			14.13**	0.59
Lub 268			-0.54	0.14
			3.42	-1.36
				6.56*
31-08-05-02				0.52
				5.80

	Oil Concentration	50 SMKs (g)	OPS (mg)
$SE(s_{ii})$	3.3	0.51	4.89
$SE(s_{ij})$	2.5	0.38	3.42

[a]Top number oil concentration; middle 50 SMK; bottom OPS

*, ** indicates terms are significant at the 5 and 1% levels of probability, respectively

TABLE 4.8 Estimates and standard errors of genetic and environmental components of the Jinks-Hayman diallel model for oil concentration, weight of 50 sound mature kernels (50 SMK) in grams and mean milligrams oil produced per SMK (OPS) of peanut.

	Trait		
Component	**Oil Concentration**	**50 SMK**	**OPS**
D	2079.10** ± 26.29	13.16** ± 0.31	24.49 ± 23.69
H_1	1052.61 ± 305.66	2.95 ± 3.62	79.32 ± 275.46
H_2	1005.26 ± 282.15	1.18 ± 3.34	68.46 ± 254.27
F	528.71 ± 133.20	0.23 ± 1.58	54.89 ± 120.04
h^2	882.23* ± 186.21	-3.84 ± 2.27	-45.68 ± 172.47
E_2	20.94 ± 11.43	0.51 ± 0.14	42.03* ± 10.59
h^2	0.97	0.83	0.13

*, ** indicates terms are significant at the 5 and 1% levels of probability, respectively

CHAPTER V

CONCLUSIONS

Developing new peanut genotypes for biodiesel production will require simultaneous improvement of multiple traits. Because peanut oil currently commands a high value within the edible market, oil yield must be increased for peanut oil to become a viable source of biofuel. My research is primarily concerned with the improvement of oil yield and quality by identifying traits that can be manipulated in early generations.

This study demonstrates that improvement of oil concentration of peanut seeds in early generations, via selection of wild-species derived genes, is possible. A majority of the phenotypic variation and gene action observed for oil concentration was due to additive effects, which can be fixed in new inbred genotypes. Seed mass was also highly heritable in this study. Seed mass is critical because larger seeds produce more oil on a per seed basis and perhaps more importantly, seed yield is positively correlated with seed mass in peanut. An inverse correlation between oil concentration and seed mass in these populations suggest that improving both traits simultaneously will be difficult. Large populations will need to be screened to improve oil concentration while maintaining an acceptable seed size. Improvement of total oil yield will require development of genotypes with elevated oil concentrations and suitable seed size capable of yielding as much as conventional varieties on a per hectare basis. Extensive yield testing comparing breeding lines developed from these populations and the best available adapted varieties is essential.

Fatty acid composition of peanut oil used in biodiesel is also important. Recent research has proven that the high-oleic acid trait improves the oxidative stability of plant based biodiesel. This study demonstrates that the high-oleic acid trait is under simple genetic control in these populations and can be manipulated through early-generation selection. The inverse correlation between oil concentration and oleic acid in the two F_2 generations evaluated in the generation means experiment indicates that screening large segregating populations will be necessary for simultaneous improvement. Reduction in long chain saturated fatty acids to improve biodiesel quality is also a valid consideration, however the traits discussed previously should be addressed first. Later-generation screening may be appropriate for selection against these fatty acids.

Based on these observations, crossing high-oil breeding lines derived from TxAG-6 with adapted, high-yielding, high-oleic, and disease resistant runner varieties and breeding lines should result in F_2 progeny segregating for desired characteristics. A large number of plants derived from these crosses could be screened in the F_2 generation for the high-oleic trait (based on individual seed) and elimination of individuals with low oil concentrations and small seed size. Single seed selection of high-oleic seeds from high oil F_2s with acceptable oil concentration and seed size would fix the high-oleic trait in subsequent generations and reduce the number of undesirable progeny. Populations could then be screened for oil concentration and seed size in the $F_{2:3}$ and $F_{2:4}$ generations and seed of desirable breeding lines increased. Oil concentrations of $F_{2:4}$ high-oil breeding lines derived from 31-08-05-02 are given in table A1. Oil yield testing of selected breeding lines should begin no later than the $F_{2:5}$ generation.

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APPENDIX

TABLE A1 Oil concentrations of $F_{2:4}$ breeding lines derived from crossing high-oleic runner genotypes with 31-08-05-02 grown in a replicated experiment in Yoakum, TX in 2012.

Genotype	Pedigree	Oil Concentration
		g kg-1
7573	Lub 268/31-08-05-02 F2:3	591a[a]
7518	Tamrun OL07/31-08-05-02 F2:3	585ab
7522	Tamrun OL07/31-08-05-02 F2:3	583a-c
31-08-05-02	TxAG-6 / Florunner BC_3	583a-c
7565	Tamrun OL01/31-08-05-02 F2:3	582a-d
7514	Tamrun OL07/31-08-05-02 F2:3	578a-e
7544	Tamrun OL01/31-08-05-02 F2:3	578a-f
7556	Tamrun OL07/31-08-05-02 F2:3	575a-g
7563	Lub 268/31-08-05-02 F2:3	574a-g
7527	Tamrun OL01/31-08-05-02 F2:3	574a-h
7557	31-08-05-02/Tamrun OL07 F2:3	570a-i
7524	Tamrun OL01/31-08-05-02 F2:3	569a-j
7516	Tamrun OL07/31-08-05-02 F2:3	569a-j
7531	31-08-05-02/Tamrun OL07 F2:3	568a-j
7515	Tamrun OL07/31-08-05-02 F2:3	568b-k
7554	Tamrun OL07/31-08-05-02 F2:3	567b-l
7548	31-08-05-02/Lub 268 F2:3	566b-m
7569	31-08-05-02/Lub 268 F2:3	566b-n
7562	Lub 268/31-08-05-02 F2:3	565b-n

TABLE A1 Continued

Genotype	Pedigree	Oil Concentration
7520	Tamrun OL07/31-08-05-02 F2:3	564b-n
7542	Lub 268/31-08-05-02 F2:3	562b-n
7567	31-08-05-02/Lub 268 F2:3	561c-o
7535	31-08-05-02/Lub 268 F2:3	561c-p
7541	Lub 268/31-08-05-02 F2:3	560c-p
7519	Tamrun OL07/31-08-05-02 F2:3	559d-p
7538	Lub 268/31-08-05-02 F2:3	559d-p
7512	Tamrun OL07/31-08-05-02 F2:3	556e-q
7513	Tamrun OL07/31-08-05-02 F2:3	556e-q
7521	Tamrun OL07/31-08-05-02 F2:3	555e-q
7546	Tamrun OL01/31-08-05-02 F2:3	555f-q
7555	Tamrun OL07/31-08-05-02 F2:3	552g-q
7526	Tamrun OL01/31-08-05-02 F2:3	552g-q
7553	Tamrun OL07/31-08-05-02 F2:3	551g-q
7572	Lub 268/31-08-05-02 F2:3	551h-q
7532	Lub 268/31-08-05-02 F2:3	549i-r
7525	Tamrun OL01/31-08-05-02 F2:3	547i-r
7571	Lub 268/31-08-05-02 F2:3	546l-r
7564	Lub 268/31-08-05-02 F2:3	544k-r
7534	31-08-05-02/Lub 268 F2:3	544l-r
7559	31-08-05-02/Lub 268 F2:3	543m-r
7547	31-08-05-02/Lub 268 F2:3	542o-r
7561	Lub 268/31-08-05-02 F2:3	539o-s
7536	31-08-05-02/Lub 268 F2:3	538p-s
7539	Lub 268/31-08-05-02 F2:3	537p-s
7568	31-08-05-02/Lub 268 F2:3	535q-s

TABLE A1 Continued

Genotype	Pedigree	Oil Concentration
7560	31-08-05-02/Tamrun OL01 F2:3	534q-s
7540	Lub 268/31-08-05-02 F2:3	534q-s
Lub 268	TAMU breeding line	526rs
Tamrun OL07	TAMU variety	516st
Tamrun OL01	TAMU variety	502t
Coefficient of Variation		2.59

[a]The same letters in the same column indicate no significant differences at the 5% level based on Fisher's protected LSD.