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RESEARCH

Frequency of a Loss-of-Function Mutation in Oleoyl-PC Desaturase (*ahFAD2A*) in the Mini-Core of the U.S. Peanut Germplasm Collection

Y. Chu, L. Ramos, C. C. Holbrook, and P. Ozias-Akins*

ABSTRACT

High oleic acid to linoleic acid ratios (high O/L) in tetraploid peanut (*Arachis hypogaea* L.) are controlled by the activity of oleoyl-PC desaturase, which is encoded by two homeologous genes (*ahFAD2A* and *ahFAD2B*). In a naturally occurring high O/L peanut, a spontaneous mutation (G-to-A at position 448 resulting in a D150N amino acid substitution) has been found in *ahFAD2A*, which resulted in a dysfunctional desaturase. In normal × high O/L crosses, segregation ratios for high:normal O/L are either 1:3 or 1:15 suggesting that one gene in some normal O/L lines may be mutated. We designed a cleaved amplified polymorphic sequence (CAPS) marker to differentiate the mutant and wild-type *ahFAD2A* alleles at the critical point mutation. The mutant allele was present in 31.6% of the accessions from the mini-core collection of peanut germplasm and was confirmed by DNA sequence analysis. The mutant allele was frequent among subspecies *hypogaea* accessions but absent from subspecies *fastigiata* accessions and the putative diploid, A-genome progenitor of peanut, *Arachis duranensis*. These data will be useful to breeders who would like to transfer disease resistance traits from mini-core accessions to high oleic acid cultivars.

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Abbreviations: AFLP, amplified fragment polymorphism; CAPS, cleaved amplified polymorphic sequence; O/L, oleic acid to linoleic acid ratio; ORF, open reading frame; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat.

THE TRAIT FOR high oleic to linoleic acid ratio (high O/L) in peanut (*Arachis hypogaea* L.) is favored over low O/L because it confers health benefits and oil stability. High oleic acid in the diet can reduce blood cholesterol and aortic cholesterol accumulation thereby decreasing the risk of cardiovascular disease (O'Byrne et al., 1997; Wilson et al., 2006). Oleic acid also can be beneficial in preventing cancer, increasing insulin sensitivity, and ameliorating some inflammatory diseases (Chong et al., 2006; Colomer and Menendez, 2006; Mesa Garcia et al., 2006). Regarding oil stability, high O/L ratios in peanut extend shelf life by delaying the development of rancidity (O'Keefe et al., 1993). The high stability of oleic acid also makes the catalytic hydrogenation of vegetable oil unnecessary. Hydrogenated vegetable oil is the major source of trans fatty acids, compounds that exacerbate heart disease and diabetes (Chong et al., 2006). Replacing partially hydrogenated oil by high oleic acid peanut oil can minimize the consumption of trans fatty acids (Broun et al., 1999).

Published in Crop Sci. 47:2372–2378 (2007).
doi: 10.2135/cropsci2007.02.0117
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Normal oleate peanut genotypes have 36 to 70% oleic acid and 15 to 43% linoleic acid, whereas high oleate peanut genotypes have approximately 80% oleic acid and approximately 2% linoleic acid (Norden et al., 1987). The conversion from oleate to linoleate is catalyzed by oleoyl-PC desaturase which introduces the second double bond to oleic acid (Schwartzbeck et al., 2001). Loss-of-function of oleoyl-PC desaturase activity is solely responsible for the high O/L trait in peanut (Ray et al., 1993). Oleoyl-PC desaturase activity is governed by two genes, *ahFAD2A* and *ahFAD2B* (Jung et al., 2000a; Jung et al., 2000b). These genes share 99% nucleotide sequence homology in the coding region and both of them code for active desaturases (Bruner et al., 2001). The two genes are homeologous in that *ahFAD2A* belongs to the A subgenome of *A. hypogaea* and *ahFAD2B* to the B subgenome (Jung et al., 2000b). A spontaneous high O/L mutant F435 was discovered by Norden et al. (1987). This line has a nonfunctional ahFAD2A desaturase and the transcription of *ahFAD2B* is greatly suppressed (Jung et al., 2000a,b). In crosses between F435 and normal oleate peanut, F2 segregation ratios can be either 1:3 (genotype I) or 1:15 (genotype II) for high: normal O/L (Norden et al., 1987). Further molecular analysis showed that in some normal O/L peanut breeding lines, a natural point mutation was present in *ahFAD2A* which caused a nonsynonymous substitution of asparagine for aspartate at position 150 (D150N). This mutation results in a dysfunctional *ahFAD2A* desaturase, which explains the 3:1 segregation ratio in genotype I peanut breeding lines. As for genotype II, normal O/L peanut lines, *ahFAD2A* retains aspartate at position 150 and the *ahFAD2A* desaturase is functional along with *ahFAD2B*; therefore, the segregation ratio becomes 15:1 (Jung et al., 2000a). The functional importance of aspartate at position 150 was proved by site specific mutagenesis studies (Bruner et al., 2001). Consequently, the presence or absence of this D150N point mutation in ahFAD2A will control the segregation ratio in crosses with high-oleic acid genotypes.

Previously, fatty acid genotypes of five Virginia-type peanuts were screened by measurement of oleic and linoleic acid content in segregating populations and all five were found to be "genotype I" (Isleib et al., 1996). Up to now there has been no systematic study on the frequency of the D150N point mutation in ahFAD2A among peanut germplasm. One method for sampling a germplasm collection is to use a core collection developed for peanut (Holbrook et al., 1993). The peanut core collection of 831 accessions, representing approximately 10% of the total U.S. germplasm collection was selected based on country of origin and available morphological data to maximize genetic diversity. The core collection has been extensively studied for a variety of disease resistances such as nematode, tomato spotted wilt, and leaf spot, among others (Anderson et al., 1996; Franke et al., 1999; Holbrook and Anderson, 1995; Holbrook and Dong, 2005; Holbrook et al., 2000b). Fatty acid composition in this core collection also was examined (Hammond et al., 1997). More recently, a mini-core collection which preserves the majority of genetic variation in the core collection was further developed (Holbrook and Dong, 2005). It is highly possible that the natural D150N mutation in ahFAD2A would be present in the U.S. peanut germplasm collection. Therefore, we designed a cleaved amplified polymorphic sequence (CAPS) marker using the available sequence data for *ahFAD2A* to screen the mini-core collection as well as 27 accessions of *Arachis duranensis*, the putative A subgenome progenitor of peanut. The mutant allele frequency between these populations allows one to infer when such a mutation may have occurred, that is, before or after polyploidization. Furthermore, this research will provide important genetic information for peanut breeders engaged in the development of peanut cultivars with the trait for high O/L ratio.

MATERIALS AND METHODS

Mini-Core Growth Condition

One hundred twelve accessions for the mini-core collection were planted in field plots in Tift County Georgia on 5 June 2006. Plots consisted of two rows, 2 m long and 0.9 m apart. Standard cultural practices for peanut production were followed.

DNA Extraction

For samples from the mini-core collection, a maximum of 15 leaflets were sampled from each accession with one leaflet per individual plant. A 6 mm leaf disk from each leaflet was excised with a hole-punch. All leaf disks from the same accession were pooled for DNA extraction. The leaf disks were frozen in liquid N and ground into powder by vortexing with three to four 3 mm stainless steel ball-bearings. Subsequently, DNA was extracted according to a CTAB extraction method (Singsit et al., 1997). For samples from *Arachis duranensis*, individual young leaves were frozen in liquid N and ground with two stainless steel ball-bearings. Genomic DNA was prepared from Qiagen DNeasy 96 plant kit (Qiagen, Valencia, CA). DNA quantification was performed by fluorometry and the concentration was adjusted to 25 ng/μL^{-1} with TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA, pH 8.0).

Polymerase Chain Reaction Amplification and Restriction Digestion

The polymerase chain reaction (PCR) performed was with sense primer, 5′-GATTACTGATTATTGACTT-3′, and anti-sense primer, 5′-CCAACCCAAACCTTTCAGAG-3′. The sense primer sequence was identical to primer aF19 (Patel et al., 2004), which includes the 19-bp insertion specific to the 5′UTR of the *ahFAD2A* gene. The anti-sense primer anneals within the coding sequence of *ahFAD2A*. The expected size of the specific *ahFAD2A* PCR product is 826 bp. The PCR

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was performed with 0.8 U of Jumpstart *Taq* DNA polymerase (Sigma, St. Louis, MO), 1x reaction buffer supplied by the manufacturer, 0.1 mM dNTPs, 0.2 μM each primer, and 40 ng DNA template in a total reaction volume of 40 μL. The PCR reaction conditions were 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 48.5°C for 30 s, and 72°C for 1 min with a final extension step at 72°C for 7 min. All PCR amplifications were performed with the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Agarose gels (1%) were run with 5 μL of the PCR reaction to confirm the success of amplification. Each PCR reaction was purified with Qiaquick PCR purification kit (Qiagen, Valencia, CA), and PCR products were eluted with 30 μL of water. Purified PCR products (8 μL) were digested with 0.4 U of *Hpy*99I restriction enzyme (New England Biolabs, Ipswich, MA) at 37°C for 1 h. The digested products were separated on a 2% agarose gel and stained with SYBR Green I (Invitrogen, Carlsbad, CA) for 30 min. Gel images were recorded with a Molecular Imager Gel Doc XR System (Bio-Rad, Hercules, CA). The PCR products from ahFAD2A mutant lines were sequenced directly with the anti-sense primer reported above. Sequencing was performed with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). The Vector NTI software program (Invitrogen, Carlsbad, CA) was used for sequence analysis.

A Chi-square test was performed to determine if the number observed was significantly different from the expected number. The expected number was calculated based on the allele frequency in the entire mini-core.

RESULTS AND DISCUSSION

The point mutation resulting in the D150N amino acid substitution in ahFAD2A was a natural allelic variant in *Arachis hypogaea* and this mutation was encoded by a G to A transition at position 448 of the open reading frame (ORF). To search for a restriction enzyme recognition site that would span this nucleotide position, we submitted the *ahFAD2A* sequence from *Arachis duranensis* (AF272951) to the New England Biolabs cutter website (http://tools.neb.com/NEBcutter2/index.php). A unique restriction site for *Hpy*99I (CGWCG W = A/T) was found in the ORF, recognizing the wild-type sequence beginning at position 477 (C**<u>G</u>**ACG). The G in bold and underlined is the key nucleotide position replaced by A in high O/L mutant peanut lines (Bruner et al., 2001; Patel et al., 2004). Therefore, PCR products amplified from a functional *ahFAD2A* gene should be recognized by *Hpy*99I and cut into two fragments, 598 and 228 bp in size. In the *ahFAD2A* mutant lines, the G-to-A transition at position 478 would make the sequence unrecognizable for *Hpy*99I and no digestion products should be observed. DNA was obtained from 95 out of 112 accessions in the mini-core collection. We could not obtain DNA from the remaining accessions because either the seeds did not germinate in the field or the plants had died. After PCR amplification and digestion with *Hpy*99I, 30 samples (31.6%) remained undigested, and therefore carried the mutant allele, whereas 65 (68.4%) yielded the two fragments expected for the wild-type allele. Data for each accession along with its country of origin are summarized in Table 1. A representative gel image of digested and undigested samples is shown in Fig. 1. The 826-bp PCR-amplified fragment is readily observed in all the samples that were digested by *Hpy*99I, although the 228-bp fragment could not be easily visualized after staining with ethidium bromide. Use of SYBR Green I, which has 50 to 100 times higher sensitivity than ethidium bromide, allowed the smaller fragment to be observed. A small amount of undigested PCR product routinely was detected by this staining method even when the amount of *Hpy*99I enzyme per reaction was increased from 0.4 to 2 U. All of the *A. duranensis* DNAs were extracted from single plants and the undigested PCR products still were detectable; therefore, a mixture of PCR products resulting from a mixture of templates could not account for the incomplete digestion. Incomplete digestion of a PCR product is not unusual. Jung et al. (2000b) also observed incomplete digestion for their CAPS marker that could distinguish between *ahFAD2A* and *ahFAD2B*. Incomplete digestion could be caused in part by the low fidelity of the *Taq* polymerase used for PCR amplification.

Germplasm lines that did not show digestion products were confirmed as G448A mutants by DNA sequencing. Twenty-seven out of 30 PCR products from ahFAD2A mutant lines gave quality sequence with the anti-sense primer. All of the sequences contained the nucleotide substitution that would result in an A at position 448. Therefore, the mutation of *ahFAD2A* detected by restriction digestion in these lines is the G448A point mutation that leads to loss-of-function of the ahFAD2A desaturase.

Krapovickas and Rigoni (1960) proposed the subdivision of *A. hypogaea* L. into two subspecies: *A. hypogaea* L. spp. *hypogaea* and *A. hypogaea* L. spp. *fastigiata*. One of the primary distinctions between the two subspecies is the presence or absence of flowering on the central axis (main stem). Subspecies *fastigiata* has flowering on the main stem, *hypogaea* does not. In the United States, peanuts are classified into four market types. Runner and Virginia market types are spp. *hypogaea*, whereas Spanish and Valencia are spp. *fastigiata* (Stalker and Simpson, 1995).

Inheritance studies with different market-type cultivars have indicated that the *ahFAD2A* mutation is frequent in cultivars with no flowering on the main stem but absent in cultivars with flowering on the main stem. Knauft et al. (1993) examined the inheritance of this trait in crosses with 13 cultivars and breeding lines of the runner and Virginia market types (no flowering on the main stem). The trait exhibited monogenic inheritance in 12 of the 13 cross combinations. Isleib et al. (1996) examined five different cultivars of the Virginia-type cultivars and found that four exhibited monogenic inheritance. Lopez

Table 1. Presence or absence of the *ahFAD2A* point mutation as detected by *Hpy*99I restriction digestion at the site of G448A. "Individuals pooled" column indicates the actual number of leaf disks from individual plants that were pooled for each DNA sample. m, mutant allele; wt, wild-type allele.

Country of origin	PI no.	Individuals pooled	*ahFAD2A* allele
Argentina	331314	15	m
Argentina	339960	7	wt
Argentina	356004	15	wt
Argentina	403813	10	wt
Argentina	493356	11	wt
Argentina	493547	9	wt
Argentina	493581	10	wt
Argentina	493631	10	wt
Argentina	493693	12	m
Argentina	493717	10	wt
Argentina	493729	10	wt
Argentina	493880	8	wt
Argentina	493938	6	wt
Argentina	494018	14	wt
Argentina	494034	15	wt
Bolivia	274193	8	m
Bolivia	475863	6	wt
Bolivia	475918	10	wt
Bolivia	475931	9	m
Bolivia	497318	8	m
Bolivia	497395	8	m
Brazil	152146	13	wt
Brazil	262038	10	wt
Brazil	337293	9	wt
Brazil	408743	12	wt
Brazil	497517	14	wt
Burkina Faso	496401	7	m
Burkina Faso	496448	15	m
Burma	295730	12	m
China	158854	4	wt
China PR	461427	8	wt
Colombia	504614	15	wt
Cuba	259617	9	wt
Ecuador	497639	2	wt
Ecuador	497668	7	wt
India	288146	11	wt
India	290536	8	m
India	290560	7	wt
India	290566	13	m
India	290594	15	m
India	290620	8	m
India	478819	9	wt
Israel	295250	10	m
Israel	295309	10	m
Israel	296550	15	wt
Israel	296558	15	m
Israel	319768	14	wt
Israel	343384	9	wt
Israel	343398	15	m
Israel	370331	8	m
Israel	371521	15	wt
Ivory Coast	196622	14	m
Japan	200441	13	wt
Madagascar	196635	15	wt
Malawi	259836	13	wt
Malawi	259851	15	m
Morocco	337399	10	wt
Mozambique	481795	8	wt
Nigeria	372271	15	m
Nigeria	372305	12	m
Nigeria	399581	14	wt
Nigeria	476432	9	wt
Nigeria	476596	15	m
Nigeria	476636	11	wt
Pakistan	323268	14	m
Paraguay	337406	15	wt
Peru	502040	10	wt
Peru	502120	7	wt
Senegal	159786	13	m
South Africa	240560	7	wt
South Africa	268696	14	wt
South Africa	292950	7	m
South Africa	298854	14	wt
Sudan	162857	8	m
Sudan	268868	15	m
Taiwan	313129	14	m
Uganda	478850	7	wt
Uruguay	155107	6	wt
Uruguay	162655	15	wt
Venezuela	325943	9	wt
Venezuela	338338	6	wt
Zambia	268586	12	wt
Zambia	268755	15	wt
Zambia	268806	15	wt
Zambia	270786	9	wt
Zambia	270905	11	wt
Zambia	270907	9	wt
Zambia	270998	10	wt
Zambia	271019	14	wt
Zambia	494795	10	wt
Zimbabwe	429420	2	wt
Zimbabwe	442768	11	m
Zimbabwe	471952	3	wt
Zimbabwe	471954	3	wt
Zimbabwe	482189	8	wt

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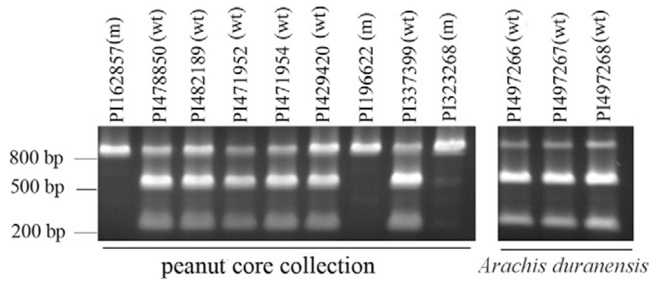

Figure 1. *Hpy*99I-digested *ahFAD2A* fragments from peanut mini-core collection and *Arachis duranensis* accessions.

Table 2. Comparison of the frequency of mutant *ahFAD2A* alleles in mini-core accessions with and without flowering on the main stem, a primary distinction between subspecies. No = spp. *hypogaea*. Yes = spp. *fastigiata*.

Flowering on the main stem	Mutant	Wild type	Total	X^2
No	30	10	40	58.2**
Yes	0	52	52	
Total	30	62	92	

**Significant at $P \leq 0.01$.

Table 3. Number and frequency of mini-core accessions carrying mutations of *ahFAD2A* and exhibiting flowering on the main stem for the representatives from the 26 clusters of the core collection.

Cluster	Total mini-core accessions	*ahFAD2A* mutant number (%)	Flowers on main stem no. (%)
1	5	1 (20)	3 (60)
2	3	3 (100)	0 (0)
3	5	0 (0)	5 (100)
4	7	0 (0)	7 (100)
5	5	0 (0)	5 (100)
6	7	1 (14)	6 (86)
7	4	4 (100)	0 (0)
8	4	2 (50)	0 (0)
9	3	0 (0)	3 (100)
10	3	2 (67)	0 (0)
11	4	0 (0)	4 (100)
12	6	2 (33)	1 (17)
13	6	1 (17)	0 (0)
14	5	3 (60)	2 (40)
15	4	1 (25)	3 (75)
16	5	0 (0)	5 (100)
17	8	0 (0)	8 (100)
18	2	2 (100)	0 (0)
19	2	0 (0)	1 (50)
20	4	2 (50)	0 (0)
21	2	0 (0)	1 (50)
22	2	0 (0)	2 (100)
23	2	0 (0)	2 (100)
24	3	1 (33)	1 (33)
25	6	3 (50)	0 (0)
26	5	2 (40)	2 (40)

et al. (2001) examined the inheritance of the high oleic acid in six Spanish market-type peanut cultivars (flowering on the main stem). When these were crossed with a high oleic parent, all the resulting populations segregated for two major genes. Our molecular data are consistent with these previously observed frequencies. Our investigation of the accessions in the mini-core collection indicated that the *ahFAD2A* mutation is frequently present in accessions with no flowering on the main stem but absent in accessions with flowering on the main stem (Table 2).

The mini-core was developed by randomly selecting 10% of the core accessions after they had been grouped into 26 clusters based on multivariate analysis of data for 16 morphological characteristics. Previous studies have demonstrated that this clustering method can be used to improve the efficiency of identifying desirable traits in the core collection and in the entire collection (Holbrook and Anderson, 1995; Holbrook and Dong, 2005). In 17 clusters with both flowering habits, a reasonable chance to identify the distribution of ahFAD2A mutation among the accessions exists (Table 3). All the mini-core accessions in clusters 2, 7, and 18 contain the mutation and do not have flowering on the main stem. At least half of the mini-core accessions in clusters 8, 10, 14, 20, and 25 contain the mutation, and most of the mini-core accessions in these clusters do not have flowering on the main stem. These should be promising clusters for breeders who are looking for spp. *hypogaea* germplasm that could be crossed with high oleic cultivars to produce progeny that would segregate 3:1 for normal to high oleic. If a particular cluster also contains a disease resistance trait of interest, the disease resistant accessions now can be screened for the dysfunctional desaturase before their introduction into a breeding program.

All the mini-core accessions in clusters 3, 4, 5, 9, 11, 16, 17, 22, and 23 were spp. *fastigiata* and did not contain the mutation. Breeders using other accessions from these clusters are likely to produce progeny that segregate 15:1 for normal to high oleic. Based on the associations presented in Tables 2 and 3, it appears unlikely that there are naturally occurring spp. *fastigiata* accessions that contain the ahFAD2A mutation. Plant breeders working to develop high oleic Spanish-type cultivars should use one of the recently released high oleic Spanish cultivars (Baring et al., 2006; Simpson et al., 2003b) as one of the parents in their crosses.

Since *ahFAD2A* is from the A genome and diploid *Arachis duranensis* is the putative A-genome donor (Jung et al., 2003; Ramos et al., 2006; Seijo et al., 2004), we further tested available *Arachis duranensis* accessions (Table 4) for the same CAPS marker. All 27 accessions could be digested with *Hpy*99I indicating that all contain the functional wild-type allele. This result implies that the spontaneous G448A point mutation in *ahFAD2A* occurred after

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the polyploidization event that gave rise to allotetraploid peanut. The mutant allele appears to have arisen before the global distribution of peanut from its center of origin (South America) since both mutant and wild-type alleles were found in most geographic regions (Table 1).

The U.S. peanut germplasm collection contains a great deal of genetic diversity. It is a valuable resource for peanut breeding programs. In-depth studies on the genetic variation for this collection are warranted. However, due to the time and expense that would be incurred to evaluate the entire collection, a more economical approach is to develop core and minicore collections that are representative of the diversity found in the entire collection (Holbrook and Dong, 2005; Holbrook et al., 1993). Our survey of the mini-core collection for the loss-of-function *ahFAD2A* allele now allows a breeder to select not only a disease resistant genotype but also one that could facilitate the combination of disease resistance and the high O/L trait.

During the past 10 yr, numerous peanut cultivars with the high O/L trait have been released. Among them, Sunoleic 95R, Sunoleic 97R, Tamrun OL1, Tamrun OL2, Olin, and Georgia 04S are cultivars whose pedigree included F435 as the high O/L trait donor (Branch, 2005; Gorbet and Knauft, 1997, 2000; Simpson et al., 2003a,b, 2006) and would be expected to carry the mutant *ahFAD2A* allele. Georgia HiO/L peanut was produced using T2636M as the high O/L parent (Branch, 2000). T2636M is a mutant line induced by gamma radiation. The other parents for the high O/L cultivars confer disease resistance, high yield, or other desirable agronomic traits. Continued breeding efforts to produce elite high O/L peanut cultivars are needed to counteract a broad range of disease and abiotic challenges in the United States. Germplasm collections are a rich resource of resistance genes. It has been shown that the germplasm in the U.S. core collection has a varied level of resistance to nematodes, tomato spotted wilt virus, late leaf spot, and other fungi (Anderson et al., 1996; Franke et al., 1999; Holbrook and Anderson, 1995; Holbrook et al., 2000a,b), and some of the disease resistance patterns are preserved in the mini-core collection (Holbrook and Dong, 2005). These genotypes can serve as parents to improve the resistance gene diversity in peanut cultivars. None of the germplasm in the core collection, however, is high O/L (Hammond et al., 1997). PI288178 demonstrated the highest oleic acid content (60.3% oleate, 19.9% linoleate), only a 3:1 ratio. Therefore, it is unlikely that any B-genome alleles are nonfunctional in the core although there is a 30% chance that the A-genome loss-of-function allele is present based on our survey of the mini-core collection.

Previously, peanut genetic polymorphism has been explored using other PCR-based molecular markers including random amplified polymorphic DNA (RAPD) markers, amplified fragment polymorphism (AFLP) markers, and microsatellite DNA (simple sequence repeat, SSR) markers (Halward et al., 1992; He et al., 2003; Krishna et al., 2004; Lanham et al., 1992; Milla et al., 2005; Moretzsohn Mde et al., 2004). The present study is the first report on the genetic polymorphism of a specific gene in the mini-core collection using a CAPS marker. Our study provides information for a breeder to predict the F2 segregation ratio of either 3:1 or 15:1 for high O/L if they were to use a mini-core collection genotype in crosses with a high O/L parent.

Table 4. ***Arachis duranensis*** **accessions that were screened for the point mutation in** ***ahFAD2A*****. All of them possess the wild-type allele because polymerase chain reaction products were digested by** ***Hpy*****99I.**

PI no.		
Grif 15035	PI 468320	PI 475885
Grif 15036	PI 468372	PI 475886
Grif 15037	PI 475844	PI 497265
Grif 15038	PI 475845	PI 497266
PI 262133	PI 475846	PI 497267
PI 468197	PI 475847	PI 497268
PI 468201	PI 475882	PI 497270
PI 468203	PI 475883	PI 497483
PI 468319	PI 475884	PI 497484

Acknowledgments

We thank Evelyn P. Morgan for technical assistance.

References

Anderson, W.F., C.C. Holbrook, and A.K. Culbreath. 1996. Screening the peanut core collection for resistance to tomato spotted wilt virus. Peanut Sci. 23:57–61.

Baring, M.R., Y. Lopez, C.E. Simpson, J.M. Cason, J. Ayers, and M. Burow. 2006. Registration of 'Tamnut OL06' peanut. Crop Sci. 46:2720–2721.

Branch, W.D. 2000. Registration of 'Georgia Hi-O/L' peanut. Crop Sci. 40:1823–1824.

Branch, W.D. 2005. Registration of 'Georgia-04S' peanut. Crop Sci. 45:1653–1654.

Broun, P., S. Gettner, and C. Somerville. 1999. Genetic engineering of plant lipids. Annu. Rev. Nutr. 19:197–216.

Bruner, A.C., S. Jung, A. Abbott, and G. Powell. 2001. The naturally occurring high oleate oil character in some peanut varieties results from reduced oleoyl-PC desaturase activity from mutation of aspartate 150 to asparagine. Crop Sci. 41:522–526.

Chong, E.W., A.J. Sinclair, and R.H. Guymer. 2006. Facts on fats. Clin. Exp. Ophthalmol. 34:464–471.

Colomer, R., and J.A. Menendez. 2006. Mediterranean diet, olive oil, and cancer. Clin. Transl. Oncol. 8:15–21.

Franke, M.D., T.B. Brenneman, and C.C. Holbrook. 1999. Identification of resistance to rhizoctonia limb rot in a core collection of peanut germplasm. Plant Dis. 83:944–948.

Gorbet, D.W., and D.A. Knauft. 1997. Registration of 'SunOleic 95R' peanut. Crop Sci. 37:1392.

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

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Halward, T., T. Stalker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. 18:315–325.

Hammond, E.G., D. Duvick, T. Wang, H. Dodo, and R.N. Pittman. 1997. Survey of the fatty acid composition of peanut (*Arachis hypogaea*) germplasm and characterization of their epoxy and eicosenoic acids. J. Am. Oil Chem. Soc. 74:1235–1239.

He, G., R. Meng, M. Newman, G. Gao, R.N. Pittman, and C.S. Prakash. 2003. Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol. 3:3.

Holbrook, C.C., and W.F. Anderson. 1995. Evaluation of a core collection to identify resistance to late leaf spot in peanut. Crop Sci. 35:1700–1702.

Holbrook, C.C., and W. Dong. 2005. Development and evaluation of a mini-core collection for the U.S. peanut germplasm collection. Crop Sci. 45:1540–1544.

Holbrook, C.C., A.W. Johnson, and M.G. Stephenson. 2000a. Level and geographical distribution of resistance to *Meloidogyne arenaria* in the U.S. peanut germplasm collection. Crop Sci. 40:1168–1171.

Holbrook, C.C., R.N. Pittman, and W.F. Anderson. 1993. Selection of a core collection from the U.S. germplasm collection of peanut. Crop Sci. 33:859–861.

Holbrook, C.C., P. Timper, and H.Q. Xue. 2000b. Evaluation of the core collection approach for identifying resistance to *Meloidogyne arenaria* in peanut. Crop Sci. 40:1172–1175.

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

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

Jung, S., D. Swift, E. Sengoku, M. Patel, F. Teule, G. Powell, K. Moore, and A. Abbott. 2000b. The high oleate trait in the cultivated peanut [*Arachis hypogaea* L.]: I. Isolation and characterization of two genes encoding microsomal oleoyl-PC desaturases. Mol. Gen. Genet. 263:796–805.

Jung, S., P.L. Tate, R. Horn, G. Kochert, K. Moore, and A.G. Abbott. 2003. The phylogenetic relationship of possible progenitors of the cultivated peanut. J. Hered. 94:334–340.

Knauft, D.A., K. Moore, and D.W. Gorbet. 1993. Further studies on the inheritance of fatty acid composition in peanut. Peanut Sci. 20:74–76.

Krapovickas, A., and V.A. Rigoni. 1960. La nomenclatura de las subspecies y variedades de *Arachis hypogaea* L. Rev. Invest. Agric. 14:197–228.

Krishna, G.K., J. Zhang, M. Burow, R.N. Pittman, S.G. Delikostadinov, Y. Lu, and N. Puppala. 2004. Genetic diversity analysis in Valencia peanut (*Arachis hypogaea* L.) using microsatellite markers. Cell. Mol. Biol. Lett. 9:685–697.

Lanham, P.G., S. Fennell, J.P. Moss, and W. Powell. 1992. Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. Genome 35:885–889.

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

Mesa Garcia, M.D., C.M. Aguilera Garcia, and A. Gil Hernandez. 2006. Importance of lipids in the nutritional treatment of inflammatory diseases. Nutr. Hosp. 21:28–41.

Milla, S.R., T.G. Isleib, and H.T. Stalker. 2005. Taxonomic relationships among *Arachis* sect. *Arachis* species as revealed by AFLP markers. Genome 48:1–11.

Moretzsohn Mde, C., M.S. Hopkins, S.E. Mitchell, S. Kresovich, J.F. Valls, and M.E. Ferreira. 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol. 4:11.

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

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

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

Patel, M., S. Jung, K. Moore, G. Powell, C. Ainsworth, and A. Abbott. 2004. High-oleate peanut mutants result from a MITE insertion into the FAD2 gene. Theor. Appl. Genet. 108:1492–1502.

Ramos, M.L., G. Fleming, Y. Chu, Y. Akiyama, M. Gallo, and P. Ozias-Akins. 2006. Chromosomal and phylogenetic context for conglutin genes in *Arachis* based on genomic sequence. Mol. Genet. Genomics 275:578–592.

Ray, T.K., S.P. Holly, D.A. Knauft, A.G. Abbott, and G.L. Powell. 1993. The primary defect in developing seed from the high oleate variety of peanut (*Arachis hypogaea* L.) is the absence of Δ12–desaturase activity. Plant Sci. 91:15–21.

Schwartzbeck, J.L., S. Jung, A.G. Abbott, E. Mosley, S. Lewis, G.L. Pries, and G.L. Powell. 2001. Endoplasmic oleoyl-PC desaturase references the second double bond. Phytochemistry 57:643–652.

Seijo, J.G., D. Ducasse, E.A. Moscone, A. Krapovickas, G.I. Lavia, and A. Fernandez. 2004. Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaensis* are the wild diploid progenitors of *A. hypogaea* (*Leguminosae*). Am. J. Bot. 91:1294–1303.

Simpson, C.E., M.R. Baring, A.M. Schubert, M.C. Black, H.A. Melouk, and Y. Lopez. 2006. Registration of 'Tamrun OL 02' peanut. Crop Sci. 46:1813–1814.

Simpson, C.E., M.R. Baring, A.M. Schubert, H.A. Melouk, M.C. Black, Y. Lopez, and K.A. Keim. 2003a. Registration of 'Tamrun OL01' peanut. Crop Sci. 43:2298.

Simpson, C.E., M.R. Baring, A.M. Schubert, H.A. Melouk, Y. Lopez, and J.S. Kirby. 2003b. Registration of 'OLin' peanut. Crop Sci. 43:1880–1881.

Singsit, C., M.J. Adang, R.E. Lynch, W.F. Anderson, A. Wang, G. Cardineau, and P. Ozias-Akins. 1997. Expression of a *Bacillus thuringiensis* cryIA(c) gene in transgenic peanut plants and its efficacy against lesser cornstalk borer. Transgenic Res. 6:169–176.

Stalker, H.T., and C.E. Simpson. 1995. Genetic resources in *Arachis, In* H. E. Pattee and H. T. Stalker (ed.) Advances in peanut science. Am. Peanut Res. Educ. Soc., Stillwater, OK.

Wilson, T.A., D. Kritchevsky, T. Kotyla, and R.J. Nicolosi. 2006. Structured triglycerides containing caprylic (8:0) and oleic (18:1) fatty acids reduce blood cholesterol concentrations and aortic cholesterol accumulation in hamsters. Biochim. Biophys. Acta 1761:345–349.