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Phenotypic and molecular dissection of ICRISAT mini core collection of peanut (*Arachis hypogaea* L.) for high oleic acid

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With 2 figures and 3 tables

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Abstract

High oleic acid (O) and low linoleic acid (L) make peanut oil ideal for longer storage and better human health. Among the ICRISAT mini core collection accessions, oleic acid ranged from 33.60% to 73.54%. Accessions belonging to ssp. *hypogaea* had higher oleic acid (54.16%) compared with those of ssp. *fastigiata* (45.70%). An O : L ratio up to 6.93 was found among ssp. *hypogaea*. Additional varieties, mutants, germplasm lines and breeding lines had oleic acid within the range of mini core accessions. Mutations in *ahFAD2A*, which along with its homologous gene, *ahFAD2B* code for delta-12-desaturase, resulted in higher oleic acid and O : L ratio. *ahFAD2A* mutant allele was found in 49.5% of the accessions, and its frequency was higher in ssp. *hypogaea* (84.52%) than in ssp. *fastigiata* (19.39%). *ahFAD2A* mutation had a maximum contribution of 18.82, 12.98 and 10.52 towards the phenotypic variance of O, L and O : L ratio, respectively. Genotypes with high oleic acid levels could not reveal 'A' insertion mutation in *ahFAD2B*. Accessions with high oleic acid could be employed for improving peanut oil quality.

Key words: Peanut, ICRISAT mini core collection — oleic acid — linoleic acid — mutation — *ahFAD2A*—*ahFAD2B*

Peanut (*Arachis hypogaea* L.) is an important oilseed crop with its kernels containing 44–56% oil. Peanut oil quality mainly depends on the composition of 12 fatty acids. Among them, oleic acid (O), a monounsaturated fatty acid (MUFA), and linoleic acid (L), a polyunsaturated fatty acid (PUFA), make up 80% of the oil composition. Linoleic acid (C18 : 2, Δ^9, Δ^{12}) contains one more double bond at the Δ^{12} position of the hydrocarbon chain than oleic acid (C18 : 1, Δ^9) (Töpfer et al. 1995, Schwartzbeck et al. 2001). Peanut oil quality is determined by the oleic acid and linoleic acid (O : L) ratio. High percentage of linoleic acid makes the oil prone for oxidation, leading to rancidity, off-flavours, and short shelf- life during seed storage. High level of oleic acid at the cost of linoleic acid, not only improves the shelf life, but also offers the health benefits such as reducing low-density lipoproteins, maintaining high-density lipoprotein, slowing down atherosclerosis, and reversing the inhibitory effect of insulin production (Vassiliou et al. 2009).

Delta-12-desaturase (oleoyl-PC desaturase) converts oleic acid into linoleic acid by introducing a double bond into the hydrocarbon chain of the former. It is coded by two homologous genes *ahFAD2A* and *ahFAD2B* (Jung et al. 2000a,b), which share 99 per cent nucleotide sequence homology in the coding region (Bruner et al. 2001). High oleic acid and O : L ratio results from a G to A transition at position 448 (448G > A) of the open reading frame (ORF) of *ahFAD2A* (Bruner et al. 2001, Yu et al. 2008) and an 'A' insertion mutation leading to a premature stop codon in *ahFAD2B* (Jung et al. 2000a, Lopez et al. 2002). In addition, insertion of a miniature-inverted-repeat-transposable element (MITE) at 665 and 997 bp of the coding region of *ahFAD2B* resulted in several premature stop codons (Patel et al. 2004) leading to high O : L ratio.

Cleaved amplified polymorphic sequence (CAPS) markers have been developed to detect 448G > A mutation in *ahFAD2A* (Chu et al. 2007) and 'A' insertion in *ahFAD2B* (Chu et al. 2009). A PCR to detect MITE insertion in *ahFAD2B* was also developed (Patel et al. 2004). Recently, a real-time polymerase chain reaction (PCR) marker has been developed from the *FAD2* genes (Barkley et al. 2010, 2011, Wang et al. 2011b). Further improvement has been the development of a simple PCR assay using allele-specific primers and alternating annealing temperatures to detect mutant and wild-type alleles of FAD2 on both the A and B genomes of peanut (Chen et al. 2010).

Identification of genetic sources with mutations in *ahFAD2A* and *ahFAD2B* leading to high O : L ratio is important for peanut breeding for quality traits. Because direct testing of the seed oil content requires expensive instrumentation, availability of genetic markers to detect these mutations will be useful. The mini core collection (10% of core collection, 1% of entire collection) of the US peanut germplasm collection (Holbrook and Dong 2005) was screened with CAPS marker for 448G > A mutation in *ahFAD2A*. Mutant allele was found in 30 of total 95 accessions tested (Chu et al. 2007). Accessions belonging to ssp. *hypogaea* had the mutant allele of *ahFAD2A*, whereas those of ssp. *fastigiata* were devoid of this mutant allele. Twenty-seven accessions of *Arachis duranensis*, the putative diploid A genome donor of cultivated peanut, contained wild-type allele of *ahFAD2A*.

Upadhyaya et al. (2002) have reported the construction of a mini core collection consisting of 184 accessions from a core collection (Upadhyaya et al. 2003) consisting of about 10% of

world germplasm accessions, but representing the genetic variability of the entire germplasm collection. Hence, the mini core collection preserving the entire variation available in the core collection would enhance the exploitation of peanut genetic resources (Upadhyaya et al. 2002). Therefore, as prerequisite to improve oil quality in peanut, an attempt has been made to identify accessions with high O : L ratio by screening the mini core collection through *ahFAD2A*- and *ahFAD2B*–specific markers and direct fatty acid profiling.

Materials and Methods

Plant materials and profiling of oleic and linoleic acid

The mini core collection was obtained from ICRISAT Patancheru, Hyderabad. It is a subset of core collection bearing the genetic variability of entire world germplasm collection (Upadhyaya et al. 2002). This mini core subset consisted of 184 accessions of peanut germplasm representing botanical varieties, *hypogaea* (85) and *hirsuta* (1), *fastigiata* (37), *vulgaris* (58), *aequetorina* (1) and *peruviana* (2). ICG 5016 and ICG 5051, both belonging to botanical var. *hypogaea*, could not be studied. In addition, 19 genotypes consisting of released cultivars ('M 13', 'GPBD 4', 'M 28-2', 'TAG 24', 'JL 24', 'TPG 41', 'TG 26' and 'TMV 2'), their mutants (GM 4-3 and GM 6-1, both derived from GPBD 4 by gamma mutagenesis), ICRISAT germplasm lines (ICG 13942, ICG 2738, ICG 10565, ICG 6236, ICG 13941 and ICG 4716) and other breeding lines (R 9227, MN 1-28 and MN 1-35) were included in the study. Per cent oleic acid and linoleic acid was measured from five randomly selected mature kernels of same size and maturity from each genotype using Near Infra Red Spectroscopy (Panford and Deman 1990, Misra et al. 2000). Average of five consistent scans from each replication was taken for analysis. These observations were made for the seeds obtained by growing the mini core accessions during the 2008 rainy and 2009 summer seasons. The average of two seasons was used for analysis.

Detection of mutant allele in *ahFAD2A* and *ahFAD2B*

Young leaves were collected from 2-week-old seedlings grown in field and were used for DNA isolation by following CTAB method (Saghai-Maroof et al. 1984). Transition mutation G > A at 448 bp in *ahFAD2A* was detected in 182 accessions and other set of 19 genotypes by a CAPS marker by polymerase chain reaction (PCR) performed with the forward (5′ GATTACTGATTATTGACTT 3′) (Patel et al. 2004) and reverse (5′ CCAACCCAAACCCTTTCAGAG 3′) (Chu et al. 2007) primers. A PCR product of 826 bp was expected. The PCR was performed with 1 unit of *Taq* DNA polymerase (New England Biolabs, Ipswich, MA, USA), 10× reaction buffer supplied by the manufacturer, 0.1 mM dNTPs, 10 pmol/μl each primer and 50 ng DNA template in a total reaction volume of 20 μl. The PCR conditions included 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 48.5°C for 30 s, 72°C for 1 min with a final extension step at 72°C for 7 min. All the PCR amplifications were performed with mastercycler (Eppendorf, Hamburg, Germany). Five microlitres of PCR product was run on agarose gel (1%) to confirm the success of amplification. Remnant PCR product (15 μl) was digested with 1 U of *Hpy*99I restriction enzyme (New England Biolabs) at 37°C for 1 h. The digested products were separated on a 2% agarose gel. Two restricted products of 598 bp and 228 bp were expected from wild-type allele of *ahFAD2A*. Linear regression analysis was performed using trait and marker data to know total phenotypic variance contributed by the marker.

The selected seven genotypes with varying levels of oleic acid were investigated for 'A' insertion mutation at 441_442 bp of *ahFAD2B* by CAPS involving PCR amplification with the forward (5′ GGAGCTTTAACAACACAA 3′) and reverse (5′ ATA-TGGGAGCATAAGGGT 3′) primer (Chu et al. 2009) and restriction digestion of the PCR product with *Hpy*188I. The PCR was performed with 1 unit of *Taq* DNA polymerase, 10× reaction buffer supplied by the manufacturer, 0.1 mM dNTPs, 10 pmol/μl each primer and 50 ng DNA template in a total reaction volume of 25 μl. The PCR amplification conditions were initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 30 s with a final extension at 72°C for 7 min. Amplification was confirmed by loading 17 μl of PCR product on agarose gel (1%). Eight microlitres of the PCR product was digested by adding 4.8 μl of water and 0.2 μl or 2 U of *Hpy*188I (New England Biolabs). The digestion was performed at 37°C overnight. The digested product was separated on a 2% (w/v) agarose gel. An expected PCR product of 658 bp and restricted products of 521 and 137 bp were expected from *ahFAD2B* mutant allele.

Results

ICRISAT mini core accessions differed significantly for the per cent oleic acid, linoleic acid and O : L. Although the difference because of seasons was significant, genotype X season interaction was non-significant for these traits. Oleic acid content ranged from 33.60% to 73.54% among the mini core accessions (Data S1). Both oleic acid and linoleic acid contents varied between the subspecies and between the botanical varieties within a subspecies. In general, oleic acid content was higher in accessions belonging to ssp. *hypogaea* (54.16%) compared with those of ssp. *fastigiata* (45.70%) (Table 1). Within ssp. *fastigiata*, botanical var. *aequatoriana* had highest oleic acid (67.18%), followed by botanical var. *peruviana* (59.19%), botanical var. *fastigiata* (46.96%) and botanical var. *vulgaris* (44.05%). Mean linoleic acid content was more in ssp. *fastigiata* (32.97%) compared with ssp. *hypogaea* (26.42%). The botanical var. *vulgaris* had relatively high mean linoleic acid (33.84%) followed by botanical var. *fastigiata* (32.43%), botanical var. *peruviana* (24.34%) and botanical var. *aequatoriana* (19.20%). Botanical var. *hirsuta*, *aequatoriana* and

Table 1: Oleic acid and linoleic acid content along with the type of *ahFAD2A* allele among the ICRISAT mini core accessions of peanut

Botanical variety	No. of accessions	Oleic acid (%)		Linoleic acid (%)		O : L ratio		Type of *ahFAD2A* allele	
		Mean	Range	Mean	Range	Mean	Range	Wild	Mutant
hypogaea	83	54.02	39.23–73.54	26.50	10.59–39.03	2.25	1.01–6.93	13 (15.66)	70 (84.34)
hirsuta	1	65.46	–	19.82	–	3.30	–	0 (0.00)	1 (100.00)
fastigiata	37	46.96	38.17–66.56	32.43	15.81–39.76	1.62	0.96–4.21	27 (72.97)	10 (27.03)
vulgaris	58	44.05	33.60–66.41	33.84	17.49–43.74	1.37	0.77–3.80	52 (89.66)	6 (10.34)
aequatoriana	1	67.18	–	19.20	–	3.50	–	0 (0.00)	1 (100.00)
peruviana	2	59.19	55.20–63.18	24.34	21.02–27.66	2.50	2.00–3.01	0 (0.00)	2 (100.00)
LSD @5%		4.26		3.47		0.45			
CV (%)		4.35		5.87		12.38			

Values in the parenthesis indicate percentage.

peruviana were not well represented as they had 1, 1 and 2 accessions, respectively in the ICRISAT mini core collection. Hence, the results may be different when more accessions are processed. By the virtue of high oleic acid and low linoleic acid, ssp. *hypogaea* had a high mean O : L ratio of 2.23 with a maximum 6.93, whereas ssp. *fastigiata* recorded a low mean of O : L ratio (1.51), and the highest O : L ratio observed was only 3.80. Among the 19 additional genotypes tested, all were within the range of mini core collection for O, L and O : L ratio. High oleic acid was observed in two mutants, GM 4-3 (71.00%) and GM 6-1 (70.85%). However, GM 6-1 had a higher O : L ratio (6.62) than GM 4-3 (4.39).

ahFAD2A and *ahFAD2B* were specifically checked for two reported mutant alleles; 448 G > A substitution and 'A' insertion at 441_442 bp, respectively. Alleles without these mutations were considered wild types. The CAPS marker for 448 G > A substitution mutation at *ahFAD2A* showed 826-bp PCR product, but did not yield 598-bp and 228-bp restricted products with *Hpy*99I enzyme. In contrast, those with wild-type allele showed the two restricted products (Fig. 1). Of the 182 accessions, 90 carried 448 G > A substitution mutation at *ahFAD2A*. The majority (84.52%) of the accessions belonging to ssp. *hypogaea* types carried mutant allele with G > A substitution, whereas only 19.39% of ssp. *fastigiata* types carried this mutant allele (Table 1). Within ssp. *fastigiata*, mutant allele was more common in the botanical var. *fastigiata* (27.03%) compared with the botanical var. *vulgaris* (10.34%). Among the accessions carrying the mutant allele at *ahFAD2A*, the oleic acid ranged from 41.38% to 73.54% with a mean of 56.13%. But those without G > A substitution had oleic acid range of 33.60–58.23% with a mean of 43.22%. Also, the accessions with mutant *ahFAD2A* had a range of 1.11–6.93 with an average of 2.43 O : L ratio. In contrast, the mean O : L ratio was 1.26 with a range of 0.77–2.55 among the accessions not carrying G > A substitution. Of the 19 additional genotypes, all the three belonging to ssp. *hypogaea* carried mutant allele. Eight of the 17 ssp. *fastigiata* types had mutant allele of *ahFAD2A*. Again, these genotypes were characterized by high oleic acid and O : L ratio as compared to remaining nine genotypes with wild-type *ahFAD2A* (Table 2).

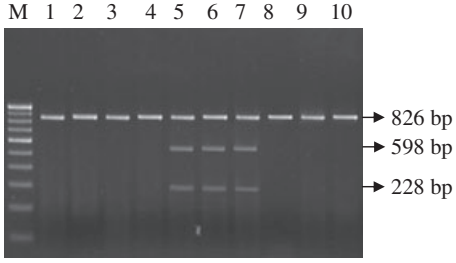

Fig. 1: CAPS marker analysis for *ahFAD2A* among ICRISAT mini core accessions of peanut (M: 100-bp DNA ladder, 1: ICG 10185, 2: ICG 2925, 3: ICG 12625, 4: ICG 7243, 5: ICG 4543, 6: ICG 4684, 7: ICG 4955, 8: ICG 5286, 9: ICG 6913 and 10: ICG 2381)

Because many of the mini core accessions and other genotypes showed 448G > A mutation at *ahFAD2A*, and also they had high oleic acid and O : L ratio, the contribution of this mutation towards the total phenotypic variance of oleic acid, linoleic acid and O : L ratio, was investigated. Significant linear regression was observed between mutant allele of *ahFAD2A* and high oleic acid, low linoleic acid and high O : L ratio (Table 3). This mutation had a maximum contribution towards the phenotypic variance of oleic acid (18.82), followed by linoleic acid (12.98) and O : L ratio (10.52).

The selected six genotypes along with a mini core accession, ICG 2381 (Table 2) with high oleic acid, were screened for 'A' insertion mutation in *ahFAD2B*. PCR product of 658 bp when digested with *Hpy*188I, restricted products of 521 and 137 bp, was not observed, indicating the absence of 'A' insertion

Table 2: Oleic acid and linoleic acid content along with the type of allele at *ahFAD2A* and *ahFAD2B* among the botanical varieties, their mutants and some breeding lines of peanut

SN	Name	Botanical variety	Oleic acid (%)	Linoleic acid (%)	O : L ratio	*ahFAD2A* (CAPS)	*ahFAD2B* (CAPS)
1	ICG 13942	*hypogaea*	60.35	21.60	2.79	–	
2	M 13	*hypogaea*	56.91	23.85	2.39	–	
3	ICG 2381[1]	*hypogaea*	73.54	11.15	6.60	–	+
4	ICG 2738	*fastigiata*	42.60	36.09	1.18	+	
5	MN 1-28	*fastigiata*	49.56	32.23	1.54	–	
6	MN 1-35	*fastigiata*	53.64	28.25	1.90	–	
7	ICG 10565	*vulgaris*	41.60	37.28	1.12	+	
8	ICG 6236	*vulgaris*	43.88	33.57	1.31	+	
9	ICG 13941	*vulgaris*	46.91	33.62	1.40	+	
10	ICG 4716	*vulgaris*	39.14	38.58	1.01	+	
11	GPBD 4	*vulgaris*	50.39	28.77	1.75	–	+
12	M 28-2	*vulgaris*	48.21	32.34	1.49	–	
13	TAG 24	*vulgaris*	40.69	38.34	1.06	+	+
14	JL 24	*vulgaris*	43.83	35.24	1.24	+	+
15	R 9227	*vulgaris*	56.25	25.62	2.20	–	
16	GM 4-3	*vulgaris*	71.00	16.18	4.39	–	+
17	TPG 41	*vulgaris*	60.13	26.11	2.30	–	+
18	GM 6-1	*vulgaris*	70.85	10.70	6.62	–	+
19	TG 26	*vulgaris*	40.15	38.03	1.06	+	
20	TMV 2	*vulgaris*	41.50	39.18	1.06	+	
	LSD @5%		3.62	2.88	0.71		
	CV (%)		3.36	4.68	15.38		

+ indicates wild-type allele and – indicates mutant allele.
[1]Included from the mini core collection.

Table 3: Linear regression between *ahFAD2A* mutation and oil traits among the ICRISAT mini core accessions of peanut

Trait	Pooled phenotypic variance	R^2
Oleic acid	39.25	18.82[1]
Linoleic acid	25.34	12.98[1]
O : L ratio	0.61	10.52[1]

[1]Significant at 5% probability.

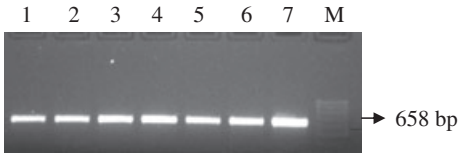

Fig. 2: CAPS marker analysis for *ahFAD2B* (1: JL 24, 2: TAG 24, 3: GPBD 4, 4: ICG 2381, 5: GM 4-3, 6: GM 6-1, 7: TPG 41 and M: 100-bp DNA ladder)

mutation in *ahFAD2B* in all these genotypes (Fig. 2). It was observed that the accession, ICG 2381 with the highest oleic acid of 73.54% and O : L ratio of 6.62, carried mutant allele of *ahFAD2A* and no 'A' insertion mutation at *ahFAD2B*.

Discussion

This study reports the profile of oleic acid and linoleic acid, which are the key determinants of peanut oil quality, among the ICRISAT mini core accessions. This mini core consisting of 184 accessions was developed (Upadhyaya et al. 2002) using 1704 accessions of ICRISAT peanut core collection, representing 14 310 global (92 countries) peanut germplasm collection in ICRISAT genebank (Upadhyaya et al. 2003). Mini core collection has been evaluated for drought tolerance (Upadhyaya 2005), high yield potential, high shelling percentage and 100-seed weight (H. D. Upadhyaya, unpublished data 2009), multiple disease resistance (Kusuma et al. 2007) and salinity tolerance (Srivastava 2010). Good success has been recorded in identifying diverse sources for these traits for use in improvement programmes.

A wide range for oleic acid content (33.60–73.54%) and O : L ratio (0.77–6.93) was recorded among 182 accessions studied. Accessions belonging to ssp. *hypogaea* had higher oleic acid content (54.16%) and high O : L ratio (2.23 with a maximum 6.93) than those of ssp. *fastigiata*. Earlier study using 83 accessions of the US germplasm collection of over 10 000 accessions also reported higher proportion of oleic acid in ssp. *hypogaea* than in ssp. *fastigiata* (Wang et al. 2009). Mini core accessions were also screened for mutations in *ahFAD2A* and *ahFAD2B* using the CAPS markers (Chu et al. 2007, 2009) developed for G to A transition at 448 bp of *ahFAD2A* (Bruner et al. 2001, Yu et al. 2008) and an 'A' insertion at 441_442 bp of *ahFAD2B* (Jung et al. 2000a, Lopez et al. 2002) for identifying mutant alleles to select for high oleic acid content.

G to A transition mutation in *ahFAD2A* was found in 49.45% of the accessions, which was higher than that found (31.6%) among the mini core of the US peanut germplasm collection (Chu et al. 2007). Mini core collection of ICRISAT and US peanut germplasm collection consisting of 112 accessions (Holbrook and Dong 2005) differed for their origin as the latter was developed using 836 US peanut core collection (Holbrook et al. 1993) representing 7432 accessions.

Again, the mutant allele was more common (84.52%) among ssp. *hypogaea* than among ssp. *fastigiata* (19.39%). This molecular analysis for *ahFAD2A* alleles clearly supports the earlier view of ssp. *fastigiata* being more primitive, from which ssp. *hypogaea* originated as a result of mutation event (Singh 1988). However, the other hypothesis proposes the independent origin of ssp. *hypogaea* (Singh 1988). Accessions carrying mutant allele differed from those containing wild allele in having higher oleic acid and O : L ratio. Similar distribution pattern of G to A transition mutation and oleic acid and O/L ratio was also observed among 19 additional genotypes. Linear regression analysis showed a significant contribution of mutation in *ahFAD2A* towards the phenotypic variance of oleic acid (18.82), linoleic acid (12.98) and O : L ratio (10.52).

Seven selected genotypes with varying levels of oleic acid were devoid of 'A' insertion mutation in *ahFAD2B*. ICG 2381 with the highest oleic acid of 73.54% and O : L ratio of 6.62 carried mutant allele of *ahFAD2A*, but *ahFAD2B* allele was devoid of 'A' insertion mutation. In addition to 'A' insertion mutation at *ahFAD2B*, insertion of a 225-bp MITE at 665 and 997 bp of its coding region is known to result in high oleic acid mutants (Patel et al. 2004). A PCR using bF19 and R1/FAD primers has been developed to test length polymorphism at *ahFAD2B* resulting from the insertion of MITE. But the genotypes used in this study need to be tested for MITE insertion. However, Chu et al. (2009) reported that 'A' insertion mutation was more frequent (58%) than MITE insertion mutation among the progenies of a high oleic acid mutant, C458. Recently, a C to T transition in 281 position of the coding region of *ahFAD2B* which caused an I94T (isoleucine to threonine) mutation in the amino acid sequences of the delta-12-desaturase in high oleic acid (68.69%) mutant has been reported (Wang et al. 2011a). Hence, high oleic acid types identified in this study are being screened for novel mutations by gene cloning and sequencing in our laboratory.

For oleic acid, a significant genotypic effect and non-significant genotype x environment interaction effect has been found among high oleic acid types (Singkham et al. 2010). Also, Sarvamangala et al. (2011) reported three QTLs governing oleic acid level in peanut. Hence, high oleic acid being regulated by only two genes, *ahFAD2A* and *ahFAD2B*, an effective selection for high oleic acid trait in peanut, might be possible. The high oleic acid genotypes identified in this study like ICG 2381, GM 6-1 and GM 4-3 may be utilized in future breeding programmes.

References

Barkley, N. A., K. D. C. Chamberlin, M. L. Wang, and R. N. Pittman, 2010: Development of a real-time PCR genotyping assay to identify high oleic acid peanuts (*Arachis hypogaea* L.). Mol. Breed **25,** 541—548.

Barkley, N. A., M. L. Wang, and R. N. Pittman, 2011: A real-time PCR genotyping assay to detect FAD2A SNPs in peanuts (*Arachis hypogaea* L.). Electron. J. Biotechnol. **14,** 12.

Bruner, A. C. J., S. Abbott, A. G. Powell, and L. Gary, 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.

Chen, Z., M. Wang, N. Barkley, and R. Pittman, 2010: A simple allele-specific PCR assay for detecting *FAD2* alleles in both A and B genomes of the cultivated peanut for high-oleate trait selection. Plant Mol. Biol. Rep. **28,** 542—548.

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

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

Holbrook, C. C., and W. Dong, 2005: Development and evaluation of a mini core collection for the US peanut germplasm collection. Crop Sci. **45,** 1540—1544.

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

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. Teulé, 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.

Kusuma, V. P., G. Yugandhar, B. C. Ajay, M. V. C. Gowda, and H. D. Upadhyaya, 2007: Identification of Sources of Multiple Disease Resistance in Groundnut (*Arachis hypogaea* L.) Mini Core ISOR National Seminar, 31—32, Directorate of Oilseeds Research, Rajendranagar, Hyderabad, India.

Lopez, Y., H. L. Nadaf, O. D. Smith, C. E. Simpson, and A. K. Fritz, 2002: Expressed variants of 12-fatty acid desaturase for the high oleate trait in Spanish market-type peanut lines. Mol. Breed **9,** 183—192.

Misra, J. B., R. S. Mathur, and D. M. Bhatt, 2000: Near infrared transmittance spectroscopy: a potential tool for non destructive determination of oil content in groundnuts. J. Sci. Food Agric. **80,** 237—240.

Panford, J. A., and J. M. Deman, 1990: Determination of oil content of seeds by NIR: influence of fatty acid composition on wavelength selection. J. Am. Oil Chem. Soc. **67,** 473—482.

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.

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen, and R. W. Allard, 1984: Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U.S.A. **81,** 8014—8018.

Sarvamangala, C., M. V. C. Gowda, and R. K. Varshney, 2011: Identification of quantitative trait loci for protein content, oil content and oil quality for groundnut (*Arachis hypogaea* L.). Field Crops Res. **122,** 49—59.

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.

Singh, A. K., 1988: Putative genome donors of *Arachis hypogaea* (Fabaceae), evidence from crosses with synthetic amphidiploids. Plant Syst. Evol. **160,** 143—151.

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

Srivastava, N., 2010: Identification of Contrasting Parental Material for Salinity Tolerance Based on Morphological, Physiological, and Biochemical Study and Molecular Diversity by SSR Molecular Markers in Groundnut (*Arachis hypogaea*). Jawaharlal Nehru Technological University of Hyderabad, Kukatpally, 500 085, A. P., India.

Töpfer, R., N. Martini, and J. Schell, 1995: Modification of plant lipid synthesis. Science **268,** 681—686.

Upadhyaya, H. D., 2005: Variability for drought resistance related traits in the mini core collection of peanut. Crop Sci. **45,** 1432—1440.

Upadhyaya, H. D., P. J. Bramel, R. Ortiz, and S. Singh, 2002: Developing a mini core of peanut for utilization of genetic resources. Crop Sci. **42,** 2150—2156.

Upadhyaya, H. D., R. Ortiz, P. J. Bramel, and S. Singh, 2003: Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. Genet. Resour. Crop Evol. **50,** 139—148.

Vassiliou, E. K., A. Gonzalez, C. Garcia, J. H. Tadros, G. Chakraborty, and J. H. Toney, 2009: Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-alpha both *in vitro* and *in vivo* systems. Lipids Health Dis. **8,** 25.

Wang, M. L., C. Y. Chen, J. Davis, B. Guo, H. T. Stalker, and R. N. Pittman, 2009: Assessment of oil content and fatty acid composition variability in different peanut subspecies and botanical varieties. Plant Genet. Resour. **1,** 71—73.

Wang, C. T., Y. Y. Tang, X. Z. Wang, S. W. Zhang, G. J. Li, J. C. Zhang, and S. L. Yu, 2011a: Sodium azide mutagenesis resulted in a peanut plant with elevated oleate content. Electron. J. Biotechnol. **14,** 10.

Wang, M. L., N. A. Barkley, Z. Chen, and R. N. Pittman, 2011b: FAD2 gene mutations significantly alter fatty acid profiles in cultivated peanuts (*Arachis hypogaea*). Biochem. Genet., **49,** 748—759.

Yu, S., L. Pan, Q. Yang, P. Min, Z. Ren, and H. Zhang, 2008: Comparison of the [Delta] 12 fatty acid desaturase gene between high-oleic and normal-oleic peanut genotypes. J. Genet. Genomics **35,** 679—685.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Oleic acid and linoleic acid content along with the type of *ahFAD2A* allele among the mini core accessions of peanut used in this study can be obtained from the author.

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