

Distribution of Allergen Composition in Peanut (*Arachis hypogaea* L.) and Wild Progenitor (*Arachis*) Species

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ABSTRACT

Ara h 1, Ara h 2, and Ara h 3 are the major allergens found in peanut (*Arachis hypogaea* L.) seed. We analyzed 60 accessions in the core of the U.S. peanut core collection, along with 88 Florida peanut breeding program lines, by quantification of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) patterns. The core of the core collection showed a wider range of allergen content than the Florida breeding lines; however, average levels of the allergens were the same for both germplasm sources. Geographic origin of the accession had no bearing on the levels of allergens measured. The accession PI 288210 from India had the lowest level of Ara h 1 (7.0%). Accession PI 372305 from Nigeria had the highest level of Ara h 1 (18.5%), but the lowest level of Ara h 2 (6.2%). Accession PI 494795 from Zambia had the highest level of Ara h 2 (13.2%), but the lowest level of Ara h 3 (21.8%). Null mutants for the major allergens were not detected; however, two accessions, 20 lines, and two peanut cultivars (Florunner and Georgia Red) contained no or little of a 36 kDa Ara h 3 isoform, Ara h 3-im. Comparing the seed protein profiles of the putative progenitors of the peanut A and B genomes, *A. duranensis* and *A. ipaensis*, respectively, to the commercial cultivar Georgia Green, allowed the subgenome origin of each allergen polypeptide to be determined. Collectively, these results indicate that eliminating all of the major allergens to create a hypoallergenic peanut is limited with the peanut germplasm currently available and would benefit from reverse genetic approaches.

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Abbreviations: SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

ANNUAL CULTIVATED PEANUT (*Arachis hypogaea* L.) is an allo-tetraploid ($2n = 4x = 40$; $C = 2813$ Mbp) composed of A and B genomes in the Leguminosae family (Husted, 1933; Strebins, 1957). It is one of the most popular foods in the world due to its low cost and high nutrition; however, peanut allergy is the most common cause of food-related, fatal anaphylaxis (Bock et al., 2001). The prevalence of peanut allergy in Western society has been estimated at 1:10 000 to 1:200, and it appears to be on the rise (Grundy et al., 2002; Burks, 2003; Sicherer et al., 2003).

Peanut allergy is due to the ingestion of seed proteins or seed protein products. Three peanut proteins, Ara h 1, Ara h 2, and Ara h 3, are considered major allergens because they are recognized by >50% of peanut-allergic patients in the USA (Burks et al., 1991, 1992). Interestingly, Ara h 1 may be only a minor allergen for some European patients (de Jong et al., 1998). Genes for these allergens have been cloned and their corresponding proteins identified as a vicilin (conarachin, 7S globulin Ara h 1; Burks et al., 1991; Viquez et al., 2003) a conglutinin (Ara h 2; Stanley et al.,

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1997; Chatel et al., 2003; Viquez et al., 2001; Ramos et al., 2006), and a glycinin (arachin, 11S Ara h 3; Burks et al., 1998; Rabjohn et al., 1999; Kleber-Janke et al., 1999). On reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), Ara h 1 migrates as a 63-kDa band (Burks et al., 1991; Buschmann et al., 1996), Ara h 2 consists of a doublet of 20 and 17 kDa (Burks et al., 1992; de Jong et al., 1998), and Ara h 3 is composed of a series of polypeptides from 45 to 14 kDa (Koppelman et al., 2003). Most recently, an isoform of Ara h 3 designated Ara h 3-im appears to be less allergenic than Ara h 3. It was cloned as a partial cDNA (GenBank Accession; AY618460; Kang and Gallo, 2007), and it migrates as a 36-kDa band.

Seed protein patterns obtained by SDS–PAGE in the genus *Arachis* have been used to study the general characteristics of peanut proteins, identify cultivars, examine seed development, determine the relationship of proteins to other genetic traits, detect seed protein polymorphisms, and compare species (Bianchi-Hall et al., 1993, 1994). Although the total number of protein bands observed among peanut accessions can fluctuate between 11 and 18 (Bianchi-Hall et al., 1993), most of the peanut seed proteins consist of Ara h 1, Ara h 2, and Ara h 3 (Burks et al., 1991; Rabjohn et al., 1999; Koppelman et al., 2001).

Quantitative studies using SDS–PAGE showed no significant differences in the amounts of Ara h 1 (12–16%) and Ara h 2 (5.9–9.3%) in seeds based on market type (Valencia, Virginia, Spanish, or runner) or the location where the peanut was grown (Koppelman et al., 2001). This study analyzed only 13 peanut genotypes from different parts of the world. Therefore, it is necessary to extend this study to a larger number of genotypes that are more representative of the peanut gene pool.

The U.S. peanut germplasm collection contains 10004 accessions representing a large amount of genetic diversity. A peanut core collection of 831 accessions was created from the U.S. germplasm collection that minimizes repetitiveness and maintains the genetic diversity of peanut (Holbrook et al., 1993). The peanut core collection has been evaluated for 16 morphological traits and resistance to four diseases. These data were used to produce a “core of the core collection” containing 112 accessions (Holbrook and Dong, 2005). This subset of the core collection still contains the genetic diversity of the entire U.S. peanut germplasm collection, but its reduced size allows researchers to more efficiently locate valuable genes and traits.

In the present study, we assessed the levels of allergenic proteins using the peanut core of the core collection to find accessions that have reduced levels of allergens. Because peanut is a regional crop with relatively few individuals involved in breeding and genetic research in the USA, we also examined 88 breeding lines developed by the University of Florida peanut breeding program to investigate the abundance of the allergens within this germplasm. Additionally, since

cytological and molecular investigations strongly suggest that two wild diploid species, *A. duranensis* and *A. ipaensis*, are the most likely progenitors of *A. hypogaea*, representing the A and B genomes, respectively (Jung et al., 2003; Kochert et al., 1996), we compared their seed protein profiles to those of several cultivars to determine their seed protein contribution to commercially cultivated peanut.

MATERIALS AND METHODS

Peanut Genotypes and Protein Extraction

Genotypes sampled included 99 of the 112 accessions in the peanut core of the core collection (provided by Dr. C.C. Holbrook, USDA-ARS, Tifton, GA), 100 breeding lines from the Florida breeding program (provided by Dr. D.W. Gorbet, University of Florida, Marianna), cultivars Florunner, Georgia Green, Georgia Red, Pronto, Spangcross, and Tifrunner (provided by Dr. P. Ozias-Akins, University of Georgia, Tifton), A genome donor *A. duranensis* (PI 262133 and PI 468202), and B genome donor *A. ipaensis* (PI 468322) (provided by Dr. R. Pitman, USDA-ARS, Griffin, GA). Proteins were extracted from 20 seeds of each of the 208 genotypes by the modified method of Koppelman et al. (2001). Protein extracts were made by mixing 100 mg of ground seed with 1 mL of 20 mM Tris-HCl (pH 8.2). After 2 h of stirring at room temperature (RT), the aqueous fraction was collected by centrifugation ($3000 \times g$) for 5 min at RT. The aqueous phase was subsequently centrifuged ($10000 \times g$) for 15 min at RT to remove residual traces of oil and insoluble particles, and then extracts were stored at -20°C until use. Soluble protein content was determined using the Dc Protein Assay kit with BSA as a standard (BioRad, Hercules, CA). Following protein extraction, 60 of the 99 core accessions and 88 of the 100 breeding lines were used in this study because protein yield of the other accessions and lines was low.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

The SDS–PAGE was performed essentially according to Laemmli (1970) with a Mini Protein II System (BioRad, Hercules, CA). Protein extract (10 μg) was mixed with an equal volume of $2\times$ SDS–PAGE sample buffer (0.09 M Tris-HCl at pH 6.8, 20% glycerol, 2% SDS, 0.02% bromophenol blue, and 0.1 M dithiothreitol [DTT]). The mixture was boiled 5 min for denaturation and then centrifuged for approximately 5 to 10 s. Electrophoresis was performed on 12% acrylamide gels (15 by 10 cm) in gel running buffer (glycine 14.4 g/L, Tris-base 3.03 g/L, 20% SDS 5 mL/L) for 1 h and 10 min at 150 V. Gels were stained with 0.1% Coomassie brilliant blue R-250 and then destained. Perfect Protein Markers (Novagen, Madison, WI) with molecular weights of 10, 15, 25, 35, 50, 75, 100, 150, and 225 kDa were used as references.

Analysis of Quantitative Data

The SDS–PAGE gels were destained, and scanned with a Gel Doc 1000/2000 gel documentation system (BioRad, Hercules, CA). Data were transformed into protein banding patterns using Quantity One software (Version 4.1, BioRad, Hercules, CA). Peak areas were calculated after background subtraction

to estimate the percentage of proteins per band in each sample. The approximate molecular weight (MW) of the bands was calculated based on the positions of the molecular markers using standard MW curves. Allergen protein levels were determined and expressed as the percentage of total detectable protein. Two independent experiments were performed, which validated the analytic precision of this method with standard deviations of <2%.

RESULTS

Allergen Content

The range of protein content for the three major allergens was wider in the core of the core collection than in the Florida breeding lines (Table 1). Of the six polypeptides measured, five had greater variability in the core accessions. For example, the amount of the 63-kDa Ara h 1 polypeptide ranged from approximately 7.0 to 18.5% for the collection, compared with 9.2 to 16.2% for the breeding lines. The average amount of total Ara h 1, Ara h 2, and Ara h 3, however, was not significantly different between the core of the core accessions (11.6 ± 2.0 , 10.9 ± 1.0 , and 31.3 ± 4.1 , respectively) and the breeding lines (12.1 ± 1.3 , 11.3 ± 1.1 , and 30.0 ± 1.6 , respectively).

Percentages of the allergen polypeptides are provided for each accession (Table 2) and breeding line examined (Table 3) with those samples having the highest and lowest levels indicated. The lowest levels of each allergen could be found within accessions from various countries of origin, and average levels of each polypeptide grouped according to country of origin were not significantly different ($P = 0.05$, data not shown). Among the core of the core collection, PI 288210 from India had the lowest level of Ara h 1 (7.0%), an average amount of Ara h 2, and slightly above average amounts of Ara h 3. The accession PI 372305 from Nigeria had the highest level of Ara h 1 (18.5%), but the lowest level of Ara h 2 (6.2%) and a relatively low level of Ara h 3. Accession PI 494795 from Zambia had the highest level of Ara h 2 (13.2%), but the

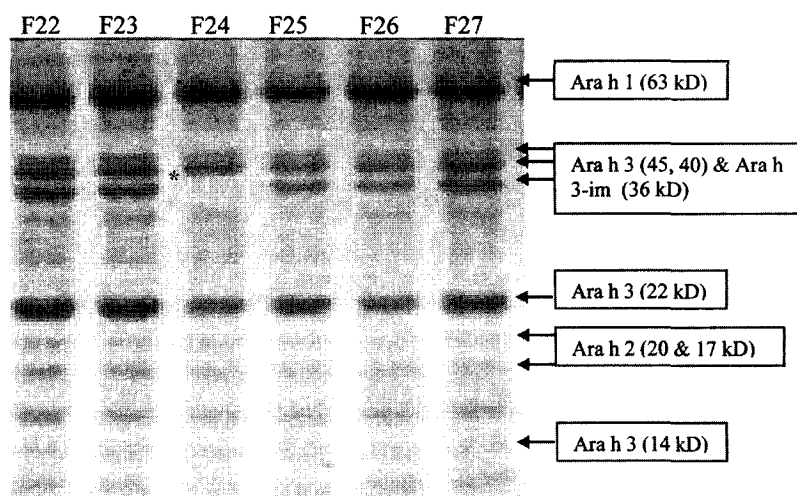


Figure 1. Seed protein profiles of screened peanut (*Arachis hypogaea* L.) germplasm. Examples of the Florida breeding lines tested (F22–F27, six lines). The asterisk indicates the missing 36 kDa band (F24). Allergen polypeptides are labeled.

lowest level of Ara h 3 (21.8%) and a moderate amount of Ara h 1. Among the breeding lines, F32 had the lowest level of Ara h 1 (9.2%), and average amounts of Ara h 2 and Ara h 3. Line F25 had the lowest amount of Ara h 2 (9.2%), but much higher than average levels of Ara h 1 and average amounts of Ara h 3. Line F62 had the lowest level of Ara h 3 (26.2%), but an above average level of Ara h 1 and an average amount of Ara h 2.

Seed Protein Profiles

For a majority of the germplasm tested, seed protein profiles were consistent. Band patterns showed 13 polypeptides; however, the recently identified 36-kDa novel allergen polypeptide that is an isoform of Ara h 3 (Kang and Gallo, 2007), was very low or nonexistent in 20 of the 88 Florida breeding lines and in two of the 60 core of the core collection accessions. Figure 1 illustrates a typical SDS-PAGE gel showing the protein profile of several Florida breeding lines with an example of one line, F24, that is missing the 36-kDa band. Analysis of several cultivars also showed that the 36-kDa band was not consistently present; Tifrunner, Georgia Green, Pronto, and Spancross showed the band, while Georgia Red and Florunner were missing this polypeptide (Fig. 2).

Protein profiles from *A. duranensis* and *A. ipaensis* revealed the contribution of the allergen polypeptides from these putative diploid progenitors to the protein profiles seen in most peanut cultivars (Fig. 3). The 63-kDa (Ara h 1), 36-kDa (Ara h 3-im), 22-kDa (Ara h 3), and 14-kDa (Ara h 3) polypeptides are found in both diploids. The 45-kDa (Ara h 3) and 20-kDa (Ara h 2) polypeptides are seen in the B genome donor *A. ipaensis*, and the 40-kDa (Ara h 3) and 17-kDa (Ara h 2) polypeptides are found in the A genome donor *A. duranensis*.

Table 1. Seed allergen content within different sources of peanut (*Arachis hypogaea* L.) germplasm.[†]

Polypeptide	Breeding lines [‡]	Core of the core
	%	
Ara h 1 (63 kD)	9.2–16.2	7.0–18.5
Ara h 2 (20 kD)	3.9–7.9	3.0–6.5
Ara h 2 (17 kD)	4.7–7.8	3.2–6.9
Ara h 3 (45 kD)	6.6–12.1	5.7–11.5
Ara h 3 (40 kD)	7.7–12.3	6.5–14.6
Ara h 3 (22 kD)	8.7–12.2	8.9–19.0

[†]Two sources of peanut germplasm: Florida peanut breeding lines (88 samples), and the core of the U.S. peanut germplasm core collection (60 samples).

[‡]Percentage of each polypeptide out of a total of 13 major peanut seed polypeptides on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Table 2. Percentage of allergens in 60 accessions from the peanut (*Arachis hypogaea* L.) core of the core collection.

PI no.	Allergen content							Total Ara h 3	Origin
	Ara h 1 (63 kD)	Ara h 2 (20 kD)	Ara h 2 (17 kD)	Total Ara h 2	Ara h 3 (45 kD)	Ara h 3 (40 kD)	Ara h 3 (22 kD)		
				%					
493329	10.5	6.0	6.9 [†]	12.9	7.4	10.7	10.1	28.2	Argentina
493356	10.7	6.2	6.8	13.0	9.4	11.8	10.2	31.4	Argentina
493547	10.1	6.3	6.7	13.0	8.8	11.8	11.3	31.9	Argentina
493581	10.9	6.5 [†]	6.6	13.1	9.3	11.3	11.1	31.7	Argentina
493631	11.2	6.0	6.3	12.3	8.0	11.2	11.4	30.6	Argentina
493717	11.5	5.3	6.0	11.3	8.2	11.0	11.0	30.2	Argentina
493729	13.0	6.0	6.4	12.4	8.1	9.8	10.0	27.9	Argentina
493880	11.3	6.0	6.7	12.7	8.3	10.2	10.5	29.0	Argentina
494034	10.1	6.0	6.5	12.5	8.2	10.7	10.9	29.8	Argentina
331314	11.0	5.9	6.4	12.3	7.3	10.3	11.3	28.9	Argentina
331297	12.3	5.8	6.1	11.9	7.6	9.8	11.2	28.6	Argentina
403813	10.1	5.6	5.8	11.4	9.0	12.9	13.5	35.4	Argentina
475863	10.5	4.9	6.7	11.6	8.2	11.2	11.2	30.6	Bolivia
497318	12.6	5.9	6.6	12.5	5.7 [‡]	8.1	9.3	23.1	Bolivia
497395	10.2	6.0	6.5	12.5	8.2	10.0	10.7	28.9	Bolivia
274193	13.6	5.6	5.8	11.4	6.4	7.8	11.3	25.5	Bolivia
475918	11.7	6.0	5.6	11.6	8.6	12.5	13.0	34.1	Bolivia
497517	10.5	6.0	6.9 [†]	12.9	7.4	10.7	10.1	28.2	Brazil
262038	11.1	5.4	5.7	11.1	9.4	11.8	12.1	33.3	Brazil
496448	14.0	6.2	6.6	12.8	6.3	7.6	8.9 [‡]	22.8	Burkina Faso
157542	11.8	4.4	5.3	9.7	7.5	10.4	14.7	32.6	China
158854	10.0	5.6	5.7	11.3	9.3	12.4	13.4	35.1	China
504614	11.5	5.5	5.9	11.4	6.5	8.5	10.1	25.1	Colombia
259658	14.5	5.6	5.3	10.9	6.6	9.2	13.2	29.0	Cuba
497639	12.6	5.4	5.8	11.2	6.9	8.4	10.2	25.5	Ecuador
290560	10.4	5.8	6.7	12.5	8.4	11.4	11.0	30.8	India
290620	8.8	5.7	6.3	12.0	8.6	10.9	11.8	31.3	India
290566	10.9	5.5	5.5	11.0	8.4	10.5	11.5	30.4	India
290594	11.9	5.6	5.8	11.4	7.3	9.8	11.3	28.4	India
290536	13.2	5.2	5.5	10.7	6.6	9.6	10.8	27.0	India
288146	10.2	4.9	5.4	10.3	9.2	12.4	13.4	35.0	India
288210	7.0 [‡]	4.9	6.1	11.0	8.7	11.9	13.5	34.1	India
478819	11.4	4.5	4.6	9.1	9.3	14.6 [†]	14.4	38.3	India
343398	10.4	5.8	6.2	12.0	7.6	9.8	10.7	28.1	Israel
343384	11.0	6.2	6.6	12.8	9.4	11.7	11.1	32.2	Israel
371521	10.9	5.9	6.4	12.3	7.4	10.5	11.5	29.4	Israel
319768	9.8	5.0	5.6	10.6	9.7	12.4	13.4	35.5	Israel
295250	11.9	4.0	4.2	8.2	8.3	12.0	13.7	34.0	Israel
295309	11.4	4.4	4.9	9.3	7.4	9.4	14.0	30.8	Israel
370331	8.5	4.5	4.5	9.0	9.0	12.6	14.6	36.2	Israel
319770	17.4	3.4	4.1	7.5	7.7	8.9	15.8	32.4	Israel
196622	16.8	3.3	4.0	7.3	7.7	11.7	15.5	34.9	Ivory Coast
200441	9.8	5.7	6.3	12.0	9.4	10.8	12.4	32.6	Japan
196635	9.3	5.8	6.2	12.0	8.9	11.4	12.1	32.4	Madagascar
259851	11.3	5.7	6.4	12.1	7.3	10.2	11.9	29.4	Malawi
259836	13.6	3.3	3.2 [‡]	6.5	9.7	13.4	15.0	38.1	Malawi
355268	12.6	3.8	4.4	8.2	9.0	13.6	14.2	36.8	Mexico
337399	10.1	5.0	5.4	10.4	10.7	14.0	13.8	38.5 [†]	Morocco
481795	14.9	3.6	3.9	7.5	8.3	10.6	19.0 [†]	37.9	Mozambique
372271	11.3	5.5	5.6	11.1	8.9	11.2	11.5	31.6	Nigeria
476596	12.3	3.4	3.6	7.0	7.5	11.9	17.1	36.5	Nigeria
372305	18.5 [†]	3.0 [‡]	3.2 [‡]	6.2 [‡]	5.8	6.6	14.9	27.3	Nigeria
337406	11.5	5.0	5.4	10.4	11.5 [†]	13.0	12.7	37.2	Paraguay
502040	14.1	5.4	6.6	12.0	6.5	7.6	10.2	24.3	Peru
502111	12.7	5.6	5.9	11.5	6.7	8.0	11.6	26.3	Peru
240560	12.8	3.8	3.7	7.5	7.7	8.5	14.7	30.9	South Africa
478850	10.0	4.8	4.4	9.2	9.5	12.3	13.5	35.3	Uganda
494795	13.2	6.3	6.9 [†]	13.2 [†]	5.8	6.5 [‡]	9.5	21.8 [‡]	Zambia
471954	10.3	5.2	5.1	10.3	8.8	13.4	14.4	36.6	Zimbabwe
429420	9.5	5.9	6.7	12.6	7.8	13.5	14.1	35.4	Zimbabwe

[†]Indicates the highest level for the allergen polypeptide specified.

[‡]Indicates the lowest level for the allergen polypeptide specified.

Table 3. Percentage of allergens among Florida peanut breeding lines.

Breeding line	Allergen content								Breeding line	Allergen content							
	Ara h 1 (63 kD)	Ara h 2 (20 kD)	Ara h 2 (17 kD)	Total Ara h 2	Ara h 3 (45 kD)	Ara h 3 (40 kD)	Ara h 3 (22 kD)	Total Ara h 3		Ara h 1 (63 kD)	Ara h 2 (20 kD)	Ara h 2 (17 kD)	Total Ara h 2	Ara h 3 (45 kD)	Ara h 3 (40 kD)	Ara h 3 (22 kD)	Total Ara h 3
	%									%							
F2	10.8	7.9 [†]	7.8 [†]	15.7 [†]	10.2	11.7	10.0	31.9	F50	12.3	5.7	5.8	11.5	9.8	11.1	10.4	31.3
F3	12.5	5.8	6.0	11.8	7.9	10.2	10.0	28.2	F52	11.5	5.4	6.1	11.5	7.7	10.8	11.9	30.4
F4	12.8	5.0	5.2	10.1	8.7	10.4	10.8	29.9	F53	15.0	5.4	6.6	12.0	7.6	8.7	10.1	26.3
F5	13.2	6.0	6.3	12.3	9.7	11.5	9.9	31.1	F54	11.1	6.2	6.5	12.7	8.7	11.1	11.5	31.3
F6	12.8	5.2	6.2	11.4	8.3	10.0	9.9	28.2	F55	11.6	5.6	5.8	11.5	9.5	11.3	11.5	32.3
F7	10.6	5.2	5.8	11.0	9.0	10.6	10.5	30.0	F56	12.0	6.0	7.1	13.1	9.1	11.6	10.9	31.5
F8	11.4	5.1	5.9	11.0	8.8	10.3	10.1	29.1	F57	14.0	5.5	5.9	11.4	7.2	9.2	10.9	27.3
F9	11.9	4.8	5.7	10.6	8.8	11.0	10.5	30.3	F58	12.2	5.9	7.2	13.0	8.4	11.8	10.9	31.0
F10	12.0	5.6	5.6	11.2	8.9	10.1	9.9	29.0	F61	12.5	6.0	6.7	12.7	8.6	10.8	10.2	29.5
F12	11.0	6.1	5.9	12.0	7.9	10.4	10.5	28.8	F62	13.4	4.9	5.6	10.5	7.0	8.8	10.4	26.2 [‡]
F13	10.1	5.5	6.1	11.5	8.5	10.1	10.9	29.5	F63	13.7	5.3	5.9	11.2	7.1	10.3	9.5	26.9
F14	11.3	4.8	5.3	10.2	7.9	10.1	11.7	29.7	F64	11.9	5.7	5.9	11.5	8.4	10.8	11.0	30.1
F15	11.4	6.6	7.2	13.8	8.8	10.6	10.3	29.7	F65	12.0	4.4	5.1	9.5	7.4	10.2	12.1	29.8
F16	11.7	5.0	5.5	10.5	7.7	10.5	10.2	28.4	F66	11.4	5.6	6.0	11.5	8.6	11.3	10.8	30.7
F17	11.6	5.3	6.1	11.4	7.5	9.8	10.5	27.7	F67	11.7	4.9	5.3	10.2	7.0	11.2	12.0	30.2
F18	12.0	5.4	6.3	11.7	9.2	11.5	11.0	31.6	F68	12.3	5.9	6.3	12.2	8.4	11.0	10.8	30.1
F19	11.7	5.0	5.7	10.7	7.8	10.2	10.9	29.0	F69	12.1	5.7	5.6	11.3	8.9	10.3	10.8	29.9
F20	11.9	5.2	5.5	10.7	7.1	8.9	10.4	26.5	F70	14.0	5.7	5.7	11.4	7.6	9.6	10.2	27.4
F21	15.0	5.1	5.1	10.3	7.7	9.5	9.8	27.0	F72	11.4	5.6	6.5	12.1	8.5	10.8	10.4	29.7
F22	12.4	5.1	5.6	10.7	7.6	9.9	11.0	28.5	F73	11.3	5.6	5.6	11.2	8.4	10.9	11.2	30.5
F23	13.8	4.9	5.4	10.3	7.9	10.1	10.9	28.9	F74	11.1	5.6	5.6	11.2	8.9	11.0	10.7	30.5
F24	16.2 [†]	5.1	5.6	10.7	8.1	10.5	9.7	28.4	F75	10.6	5.5	5.9	11.4	8.5	10.6	11.1	30.1
F25	15.2	4.2	5.0	9.2 [‡]	8.0	9.9	11.0	28.9	F76	12.5	5.3	5.6	10.9	7.7	10.3	10.6	28.5
F26	15.0	4.4	5.3	9.7	7.8	9.6	9.2	26.5	F77	11.6	5.2	5.7	10.8	8.1	10.6	11.3	29.9
F27	13.9	3.9 [‡]	4.7 [‡]	8.6	7.3	10.4	10.4	28.1	F78	12.9	5.5	5.8	11.3	8.2	10.5	10.3	29.0
F28	15.4	4.8	5.1	9.9	9.3	11.5	8.7 [‡]	29.5	F79	11.1	5.6	5.9	11.5	7.9	11.3	11.5	30.6
F29	11.6	4.8	5.2	10.0	8.9	10.8	10.7	30.4	F80	12.6	5.9	6.5	12.4	9.2	11.7	10.0	30.8
F30	11.3	4.8	5.2	10.0	9.0	10.7	10.4	30.0	F81	10.9	5.2	5.5	10.7	8.3	10.4	12.0	30.7
F31	9.5	5.8	6.4	12.2	6.6 [†]	10.4	12.0	29.0	F82	10.2	6.0	5.9	11.9	7.5	11.3	11.4	30.2
F32	9.2 [‡]	5.7	6.2	11.9	6.9	10.6	11.9	29.4	F83	12.1	4.8	5.7	10.5	8.6	11.0	11.9	31.6
F33	11.8	5.2	5.8	10.9	8.1	11.2	11.6	30.9	F84	12.6	6.5	7.1	13.7	9.6	10.9	11.6	32.1
F34	11.5	5.5	6.0	11.5	7.3	10.8	11.2	29.3	F85	12.8	5.8	6.6	12.4	10.2	12.3 [†]	11.6	34.1 [†]
F35	13.8	5.2	5.7	10.9	9.0	11.7	10.1	30.8	F86	10.5	5.9	6.1	12.0	9.6	11.7	11.1	32.3
F36	12.0	4.5	5.6	10.1	8.3	10.8	10.5	29.7	F87	11.1	6.0	6.3	12.2	8.2	10.7	11.7	30.5
F37	11.1	4.6	5.1	9.6	8.3	11.1	11.1	30.5	F88	10.3	5.7	5.7	11.4	8.6	11.5	11.6	31.7
F38	12.9	5.1	5.9	11.0	9.6	12.3 [†]	10.8	32.8	F89	10.8	5.5	6.1	11.6	9.1	11.0	10.9	30.9
F39	12.9	5.1	6.0	11.2	9.0	12.3 [†]	9.7	31.1	F90	11.7	5.5	5.9	11.4	8.1	11.9	11.9	31.9
F42	11.6	6.4	6.4	12.8	9.0	11.6	10.8	31.3	F93	12.3	6.6	6.9	13.6	11.9	9.2	11.1	32.2
F43	11.9	4.8	5.6	10.4	8.3	11.1	10.9	30.3	F94	11.8	4.8	5.2	10.0	11.8	9.2	11.9	32.9
F44	10.4	5.8	6.2	11.9	9.2	10.3	9.8	29.4	F95	11.6	5.3	5.6	10.9	11.3	8.9	11.0	31.3
F45	11.3	5.0	5.7	10.7	9.4	10.8	10.4	30.6	F96	12.1	5.8	6.4	12.2	12.1 [†]	8.4	11.1	31.6
F46	12.2	5.8	6.7	12.4	9.4	11.0	9.6	30.0	F97	12.8	5.7	6.3	11.9	10.7	7.7 [‡]	10.3	28.7
F47	12.0	6.2	6.7	13.0	10.3	11.2	10.0	31.5	F98	11.2	5.1	5.7	10.8	11.2	9.0	12.2 [†]	32.5
F48	11.5	4.8	5.3	10.0	8.5	10.6	10.7	29.8									
F49	11.7	5.3	6.4	11.7	9.6	11.7	10.3	31.7									

[†]Indicates the highest level for the allergen polypeptide specified.

[‡]Indicates the lowest level for the allergen polypeptide specified.

DISCUSSION

Numerous treatment strategies have been suggested and used for peanut allergies. Although the best strategy is to avoid the allergens by eliminating them from the diet, this can be difficult. Therefore, it is worthwhile to discover peanut plants that are null for the allergens, contain lower levels of these allergens, or have modified proteins that have reduced or no allergenicity, and then crossbreed to develop hypoallergenic peanut plants. There are peanut plants with variable allergenic properties depending on the human

populations (Burks et al., 1991, 1992; Clarke et al., 1998; de Jong et al., 1998; Kleber-Janke et al., 1999). It has been suggested that these variations result from genetic variation, different cooking methods, and different dietary regimes among human populations. Also, it was hypothesized that variation in peanut allergenicity may be a result of the geographic origin of the peanut. To answer these questions, Koppelman et al. (2001) analyzed four different peanut market types and concluded that peanut market type is not an important factor for allergen content. Also, allergen content

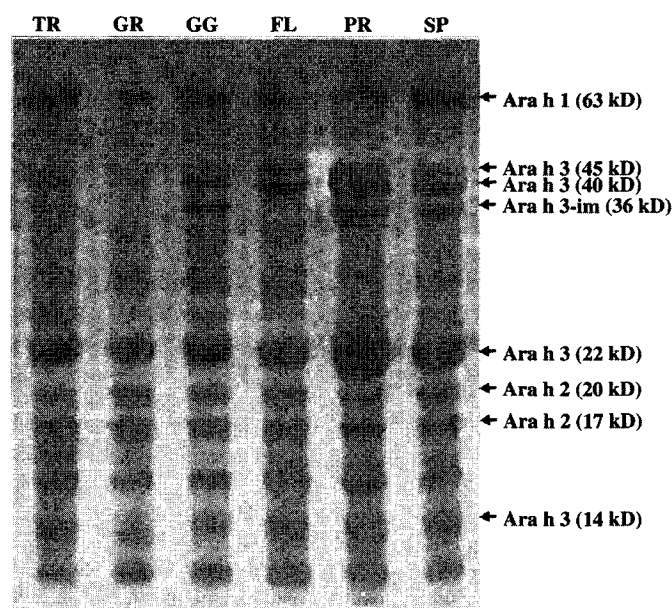


Figure 2. Seed protein profiles of peanut (*Arachis hypogaea* L.) cultivars: Tifrunner (TI); Georgia Red (GR); Georgia Green (GG); Florunner (FL); Pronto (PR); and Spancross (SP). Allergen polypeptides are labeled.

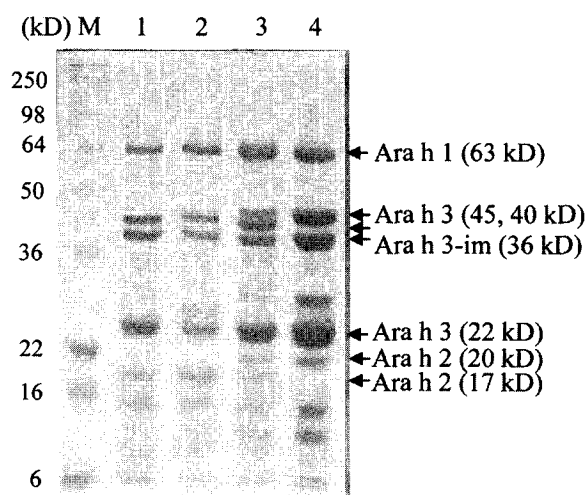


Figure 3. Seed protein profiles of diploid wild *Arachis* species and peanut (*Arachis hypogaea* L.) cultivar Georgia Green (M: size marker SeeBlue Plus2 Pre-Stained Standard, Invitrogen; 1: *A. duranensis* [PI 262133, A genome donor]; 2: *A. duranensis* [PI 468202, A genome donor]; 3: Georgia Green; 4: *A. ipaensis* [PI 468322, B genome donor]).

was not related to the origin of these plants. Previous studies indicated that Ara h 1 content was between 12 and 16%, and Ara h 2 content was between 5.9 and 9.3% (Koppelman et al., 2001). In the present study, more genetically diverse peanut samples were analyzed, the core of the core collection of peanut accessions, and also numerous Florida breeding lines. The Ara h 1 and Ara h 2 content in this germplasm varied between 7.0 to 18.5% and 6.2 to 15.7%, respectively. Our results show that the germplasm used in this study is more diverse than those studied in the past, and that lower allergen levels were detected.

In soybean [*Glycine max* (L.) Merr.], it was demonstrated that high protein lines that contained more glycinin than normal plants did not contain more allergens (Yaklich et al., 1999). We found a peanut accession with low Ara h 1 (7.0%) that contained higher Ara h 3 content (34.1%), whereas an accession with the highest Ara h 1 content (18.5%) showed the lowest level of Ara h 2 (6.2%) and lower Ara h 3 (27.3%) than the average (Table 2). Therefore, these major allergens, which constitute most of the seed storage proteins in peanut, may compensate for each other to balance the total amino acid composition in peanut seed.

Florida breeding lines have been developed from relatively few superior genotypes, and Florunner, which is missing the 36-kDa band corresponding to Ara h 3-im (Fig. 2), has been used extensively. Consequently, many more of the Florida lines do not contain this polypeptide or have it at low levels compared with the core of the core accessions (Table 2 and 3, Fig. 1). So, nulls of Ara h 3-im were discovered. Interestingly, before the identity of Ara h 3-im was known, the 36-kDa polypeptide was first reported to be polymorphic among peanut cultivars as a marker that was associated with poor blanchability (Shokraii et al., 1985). In that study, 22 Virginia-type peanuts were examined and seven of them lacked the polypeptide. In the present study, one runner type (Florunner) lacked the polypeptide while two others (Georgia Green and Tifrunner) contained it. The single Valencia market type tested (Georgia Red) did not contain this polypeptide, but both Spanish types (Pronto and Spancross) did possess it. Taken collectively, these results indicate that the presence or absence of Ara h 3-im is not influenced by market type. Unlike Ara h 3-im, nulls of Ara h 1, Ara h 2, and Ara h 3 were not found in this germplasm screen. Similarly, nulls for the major allergen in soybean, P34, were not found following an extensive screening of the soybean core collection (Yaklich et al., 1999). The major peanut allergen proteins are conserved in most *A. hypogaea* that are being used for cultivar development.

Results with the wild diploid species further strengthen the conclusion that *A. duranensis*, particularly PI 262133, and *A. ipaensis* are the most likely current representatives of the cultivated peanut progenitors. By comparing the protein profiles between the cultivar Georgia Green and the diploids, we were able to identify the allergen proteins that derived from either the A or B subgenomes (Fig. 3).

Using SDS-PAGE as a primary screen, our results indicate that examining the peanut germplasm for plants with low allergen levels is possible for the identification of parents useful in a breeding program. For further testing, it will be necessary to use more sensitive quantitative methods such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting. With so many major allergens, however, it appears unlikely that their elimination will be possible solely through selective peanut breeding using the current available germplasm. Reverse genetic approaches such as

targeting induced local lesions in genomes (TILLING) of mutagenized peanut populations followed by breeding and the generation of transgenics engineered to suppress the expression of these proteins are more viable alternatives for the development of a hypoallergenic peanut.

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References

- Bianchi-Hall, C.M., R.D. Keys, and H.T. Stalker. 1994. Use of seed protein profiles to characterize peanut cultivars. *Peanut Sci.* 21:152-159.
- Bianchi-Hall, C.M., R.D. Keys, H.T. Stalker, and J.P. Murphy. 1993. Diversity of seed storage protein patterns in wild peanut (*Arachis*, Fabaceae) species. *Plant Syst. Evol.* 186:1-15.
- Bock, S.A., A. Muñoz-Furlong, and H.A. Sampson. 2001. Fatalities due to anaphylactic reactions to foods. *J. Allergy Clin. Immunol.* 107:191-193.
- Burks, A.W. 2003. Peanut allergy: A growing phenomenon. *J. Clin. Invest.* 111:950-952.
- Burks, A.W., H.A. Sampson, and G.A. Bannon. 1998. Review article series II: Peanut allergens. *Allergy* 53:725-730.
- Burks, A.W., L.W. Williams, C. Connaughton, G. Cockrell, T.J. O'Brien, and R.M. Helm. 1992. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J. Allergy Clin. Immunol.* 90:962-969.
- Burks, A.W., L.W. Williams, R.M. Helm, C. Connaughton, G. Cockrell, and T.J. O'Brien. 1991. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J. Allergy Clin. Immunol.* 88:172-179.
- Buschmann, L., A. Petersen, M. Schlaak, and W.M. Becker. 1996. Reinvestigation of the major peanut allergen Ara h 1 on molecular level. *Monogr. Allergy* 32:92-98.
- Chatel, J.M., H. Bernard, and F.M. Orson. 2003. Isolation and characterization of two complete Ara h 2 isoforms cDNA. *Int. Arch. Allergy Immunol.* 131:14-18.
- Clarke, M.C., S.A. Kilburn, J.O. Hourihane, K.R. Dean, J.O. Warner, and T.P. Dean. 1998. Serological characteristics of peanut allergy. *Clin. Exp. Allergy* 28:1251-1257.
- de Jong, E.C., M. van Zijverden, S. Spanhaak, S.J. Koppelman, H. Pellegroni, and A.H. Penninks. 1998. Identification and partial characterization of multiple major allergens in peanut proteins. *Clin. Exp. Allergy* 28:743-751.
- Grundy, J., S. Matthews, B. Bateman, T. Dean, and S.H. Arshad. 2002. Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts. *J. Allergy Clin. Immunol.* 110:784-789.
- Holbrook, C.C., W.F. Anderson, and R.N. Pittman. 1993. Selection of a core collection from the U.S. germplasm collection of peanuts. *Crop Sci.* 33:859-861.
- 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.
- Husted, L. 1933. Cytological studies of the peanut *Arachis*: I. Chromosome number and morphology. *Cytologia* 5:109-117.
- 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.
- Kang, I.-H., and M. Gallo. 2007. Cloning and characterization of a novel peanut allergen Ara h 3 isoform displaying potentially decreased allergenicity. *Plant Sci.* 172:345-353.
- Kleber-Janke, T., R. Cramer, U. Appenzeller, M. Schlaak, and W.M. Becker. 1999. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int. Arch. Allergy Immunol.* 119:265-274.
- Kochert, G., H.T. Stalker, M. Gimenes, L. Galgaro, C.R. Lopes, and K. Moore. 1996. RFLP and cytological evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *Am. J. Bot.* 83:1282-1291.
- Koppelman, S.J., E.F. Knol, R.A.A. Vlooswijk, M. Wensing, A.C. Knulst, S.L. Heffle, H. Gruppen, and S. Piersma. 2003. Peanut allergen Ara h 3: Isolation from peanuts and biochemical characterization. *Allergy* 58:1144-1151.
- Koppelman, S.J., R.A. Vlooswijk, L.M. Knippels, M. Hessing, E.F. Knol, F.C. van Reijssen, and C.A. Bruijnzeel-Koomen. 2001. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. *Allergy* 56:132-137.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
- Rabjohn, P., E.M. Helm, J.S. Stanley, C.M. West, H.A. Sampson, A.W. Burks, and G.A. Bannon. 1999. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J. Clin. Invest.* 103:535-542.
- Ramos, M., 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.
- Shokraii, E.H., A. Esen, and R.W. Mozingo. 1985. Relation of a 36,000-dalton arachin subunit to blanchability in peanuts (*Arachis hypogaea* L.). *J. Agric. Food Chem.* 33:1114-1116.
- Sicherer, S.H., A. Muñoz-Furlong, and H.A. Sampson. 2003. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: A 5-year follow-up study. *J. Allergy Clin. Immunol.* 112:1203-1207.
- Stanley, J.S., N. King, A.W. Burks, S.K. Huang, H. Sampson, G. Cockrell, R.M. Helm, C.M. West, and G.A. Bannon. 1997. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch. Biochem. Biophys.* 342:244-253.
- Stebbins, G.L. 1957. Genetics, evolution, and plant breeding. *Indian J. Genet. Plant Breed.* 17:129-141.
- Viquez, O.M., K.N. Konan, and H.W. Dodo. 2003. Structure and organization of the genomic clone of a major peanut allergen gene, Ara h 1. *Mol. Immunol.* 40:565-571.
- Viquez, O.M., C.G. Summer, and H.W. Dodo. 2001. Isolation and molecular characterization of the first genomic clone of a major peanut allergen, Ara h 2. *J. Allergy Clin. Immunol.* 107:713-717.
- Yaklich, R.W., R.M. Helm, G. Cockrell, and E.M. Herman. 1999. Analysis of the distribution of the major soybean seed allergens in a core collection of *Glycine max* accessions. *Crop Sci.* 39:1444-1447.