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Analysis of Crude Protein and Allergen Abundance in Peanuts (*Arachis hypogaea* cv. Walter) from Three Growing Regions in Australia

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Supporting Information

ABSTRACT: The effects of plant growth conditions on concentrations of proteins, including allergens, in peanut (*Arachis hypogaea* L.) kernels are largely unknown. Peanuts (cv. Walter) were grown at five sites (Taabinga, Redvale, Childers, Bundaberg, and Kairi) covering three commercial growing regions in Queensland, Australia. Differences in temperature, rainfall, and solar radiation during the growing season were evaluated. Kernel yield varied from 2.3 t/ha (Kairi) to 3.9 t/ha (Childers), probably due to differences in solar radiation. Crude protein appeared to vary only between Kairi and Childers, whereas Ara h 1 and 2 concentrations were similar in all locations. 2D-DIGE revealed significant differences in spot volumes for only two minor protein spots from peanuts grown in the five locations. Western blotting using peanut-allergic serum revealed no qualitative differences in recognition of antigens. It was concluded that peanuts grown in different growing regions in Queensland, Australia, had similar protein compositions and therefore were unlikely to show differences in allergenicity.

KEYWORDS: *peanut, Arachis hypogaea, allergen, allergenicity, Ara h 1, Ara h 2, 2D-DIGE, growth conditions*

INTRODUCTION

Allergy to kernels of peanut (*Arachis hypogaea* L.) is a growing health problem worldwide. Although comparisons of allergen composition between peanut varieties have been made in several studies,[1−4] little attention has been paid to the allergenic characterization of peanuts derived from a single variety grown in distinct growing regions with different environmental conditions.

Because climatic factors, such as temperature and solar radiation, influence the development of peanut plants,[5−7] peanuts from different growing regions might have different abundances of allergens. Environmental conditions can significantly influence the seed protein content in some legumes.[8−11] Variations in weather, including extreme events such as very high temperature that induce a stress response, may affect plant performance by influencing the expression pattern of certain proteins[5] and might result in altered concentrations of allergens. Environmental effects that are associated with nitrogen nutrition, such as drought, soil density, root diseases, and pests, may also influence seed protein content.[8,10,12−14] The pollen of ragweed (*Ambrosia artemisiifolia*), assayed using ELISA, contained 1.8-fold more of the major ragweed allergen, Amb a 1, when plants were grown at current compared to preindustrial atmospheric CO_2 concentrations and 1.6-fold more Amb a 1 at future atmospheric CO_2 concentrations (700 ppm CO_2).[15] Protein bands corresponding to the major birch pollen allergen, Bet v I, were more intense in samples collected from a site where average temperatures were 1.0−2.5 °C higher than controls, and the plants grown in warmer conditions had a 2 week shorter growing season.[16] Because environmental variables such as solar radiation have an impact on the rate of photosynthesis, allergen abundance might be influenced by the growing conditions in peanut.

In the only study comparing peanuts grown in several locations,[1] no differences were found in crude protein and Ara h 1 and 2 contents in runner peanuts derived from the United States and Argentina. However, the allergen content was not correlated with the environmental conditions to which the peanut plants were exposed, and the methods used have since been outdated in this otherwise valuable study; for example, densitometry measurements of protein bands separated using 1D-gel electrophoresis (1D-GE) were performed to quantify Ara h 1 and 2, and none of the protein bands was identified rigorously, but rather classified by running a purified standard in a neighboring lane of the gel. Given that each band in 1D-GE of crude protein extracts is likely to contain a mixture of proteins, this identification method is not optimal and the

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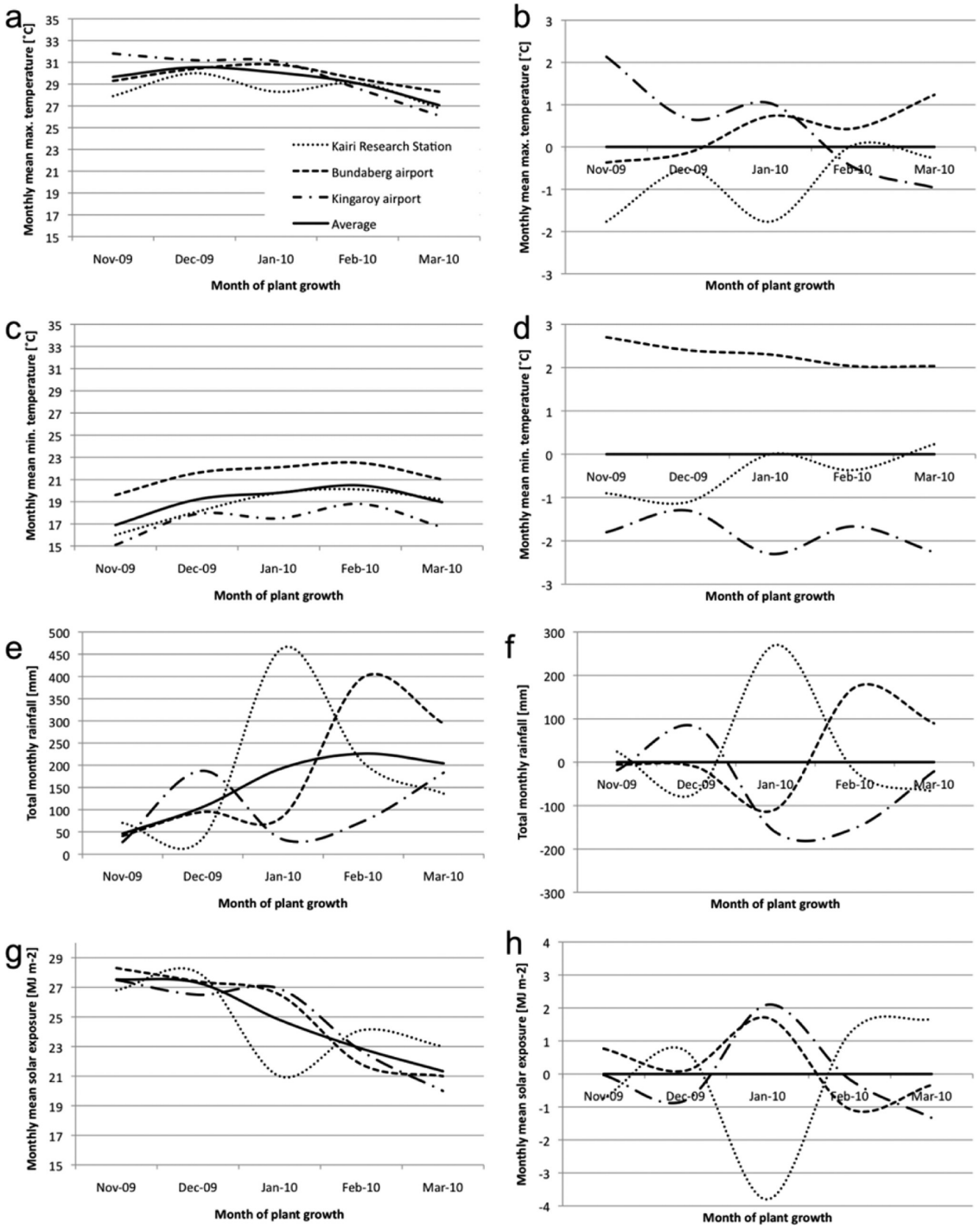

Figure 1. Climatic conditions during the growth period (November 2009 to March 2010) at weather stations closest to peanut-growing regions in Kingaroy, Bundaberg, and Kairi. (Left panels) Absolute values and their averages for each of the climate variables. (Right panels) anomalies illustrating relative differences in climatic variables over the average.

quantification method imprecise. Furthermore, the IgE binding studies performed did not distinguish between Ara h 1 and 2 and other allergens.[1] The authors could give only a broad overall estimation of allergenicity, without being able to identify which allergens were responsible.[1] A recent study that combined 2D-difference gel electrophoresis (2D-DIGE) with Western blotting analysis of two different peanut varieties from Indonesia and Germany demonstrated that more than 100 differences between the protein components could be resolved.[4] The extent to which these differences were a function not of the variety grown but of the differences in growth conditions is not known.

Most peanut allergens are seed storage proteins that make up a substantial proportion of the total protein in the kernel; for example, Ara h 1 is 12−16% and Ara h 2 is 5.9−9.3% of the total protein.[1,17] Here we used a combination of more recently developed and relatively sophisticated methods to provide a precise analysis of the effect of plant growth conditions on peanut allergen abundance. We aimed to determine whether the concentration of crude protein, as well as its proteome (i.e.,

the complement of proteins that make up the crude protein, including the allergens), varies when peanuts of a single variety (Walter) were grown in different regions in Queensland, Australia.

MATERIALS AND METHODS

Peanut-Growing Regions. Peanut plants of a Spanish runner variety, Walter, were grown as part of a regional variety evaluation trial conducted by the Peanut Company of Australia (PCA) from November 2009 to March 2010 in three peanut-growing regions in Queensland, Australia. Two regions were in southern Queensland, one close to Kingaroy (latitude, 26.5333 S; longitude, 151.8330 E) and the other at Bundaberg (latitude, 24.8500 S; longitude, 152.3500 E) around 250 km northeast of Kingaroy, whereas the third region was in northern Queensland, close to Kairi (latitude, 17.2167 S; longitude, 145.5500 E), around 1500 km north of Bundaberg (Supporting Information, Supplementary Figure 1). In Kingaroy, two sites were set up at the Queensland Department of Agriculture, Fisheries and Forestry Taabinga and Redvale Research Stations, which are located within 5 km of each other. In Bundaberg, one site was located at the Bundaberg Research Station and the other site on a peanut grower's farm (Russo Farms) close to Childers, around 50 km southwest of Bundaberg. In Kairi, one site was set up at the Queensland Department of Agriculture, Fisheries and Forestry Kairi Research Station (Supporting Information, Supplementary Figure 1).

The soil type at the Kingaroy, Bundaberg, and Kairi Research Station sites was an acidic Red Ferrosol Kraznozem, whereas the Bundaberg on-farm site at Russo Farms was an acidic Earthy Sand.[18] Peanut plants at all sites were subject to differing conditions of foliar disease pressure (mainly *Cercosporidium personatum* (Berk. & Curt) Deighton, *Cercospora arachidicola* Hori, and *Puccinia arachidis* Speg.), which were controlled by regular fungicide application. The foliar disease pressure was greatest at Kairi, intermediate at Bundaberg, and lowest at Kingaroy.

Growing Conditions, Experimental Design, and Kernel Samples. Regional variety trials consisting of 20 genotypes were established at each of the five sites. The trial was in a randomized complete block design with three replicate blocks. Each trial was sown using a cone planter using 90 cm row spacing, with weed control achieved by chemical methods. Nutrients were applied to ensure nonlimiting levels based on a preplanting soil test were present. *Rhizobium*, as a peat-based inoculant, was applied with the seed during the planting operation. No fertilizer nitrogen was applied to any of the trials because N-fixation by *Rhizobium* has been shown to provide all of the crop N requirements.[19] Planting and harvesting dates varied for each site, as did rainfall and irrigation applied to the crop, as well as planting density (Supporting Information, Supplementary Table 1).

Peanut crops at all sites received sufficient rainfall and irrigation such that nonlimiting water conditions prevailed throughout the crop growth cycle. At harvest, the entire 2 × 5 m plot was dug and plants inverted into a windrow, where field-drying occurred for the next 3−5 days. The windrow was then threshed using a stationary threshing machine, and the harvested pods were artificially dried in a fan-forced dehydrator at 30 °C to obtain 9% kernel moisture. The pods were processed in a grading room, where they were shelled and total pod yield, kernel percent, and kernel yield determined (Supporting Information, Supplementary Table 2). Peanut kernels from each replicate plot from the five sites were prepared and pooled, and a subsample was allocated for biochemical analysis.

The (total) cumulative radiation received for each crop was collated and plotted as a regression against pod yield (Supporting Information, Supplementary Figure 2).

Climate Data for the Three Peanut-Growing Regions. The closest weather station to the peanut field sites—one in each of the three regions (Kingaroy, Bundaberg and Kairi)—was identified, and the monthly maximum and minimum temperatures, total monthly rainfall, and monthly average solar radiation were obtained from the Bureau of Meteorology, Australia (www.bom.gov.au) and plotted using Excel (Microsoft Office, 2008). The monthly averages of the conditions measured in each station as well as the overall averages were plotted against time; anomalies were also plotted so that the individual averages could be compared with the overall average of the conditions (Figure 1).

Measurement of Crude Protein Content. Using an optimized protocol, samples of 10 raw peanuts per replicate (3 replicates per location) were pooled and seed coats removed before homogenization. The homogenate was defatted with *n*-hexane, and crude protein was extracted by adding 20 mM Tris (pH 8.5) to 45 mg of peanut flour, vortexing vigorously and incubating for 30 min at 21 °C on a shaker. The aqueous fraction was collected after centrifugation at 12600*g* at room temperature. Levels of crude protein and Ara h 1 and 2 were measured with a 2D Quant kit (GE Healthcare) and commercial Ara h 1 and 2 ELISA kits (Indoor Biotechnologies, Charlottesville, VA, USA), respectively, and expressed as milligrams per gram flour. Box plots and means with 95% confidence intervals were plotted, and one-way ANOVA was applied.

1D- and 2D-Gel Electrophoresis, 2D-DIGE, Mass Spectrometry, and Western Blotting. 1D-GE was performed under reducing conditions by loading approximately 25 μg of protein (in NuPAGE LDS 4× sample buffer containing 80 mM DTT) per lane onto 4−12% NuPAGE Novex Bis-Tris Mini gels. The gels were run at constant 100 V for 1 h in an XCell SureLock Mini Cell (Invitrogen Life Technologies). Gel electrophoresis was performed with all three biological replicates per location.

2D-GE was performed with three biological replicates of crude protein extracts obtained from peanut kernels from Redvale, Childers, and Kairi. For the 2D-GE, 25 μg of protein per sample was added to immobilized pH gradient (IPG) buffer (5 M urea, 2 M thiourea, 2% CHAPS, 2% (w/v) sulfobetaine 3−10, 1% (v/v) Carrier Ampholytes, 65 mM DTT (0.05% (w/v), Bromophenol blue, 40 mM Tris (pH 8.8)). The protein samples were absorbed into IPG strips (pH 3−10 nonlinear) and focused up to 100,000 kVh with a maximum of 5000 V. After reduction (375 mM Tris (pH 8.8), 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS, 2.5% (v/v) acrylamide solution, 0.5% DTT) and alkylation (same buffer but 2.5% iodacetamide instead of DTT) of the proteins on the strips, the second dimension was run on 12% Bis-Tris gels (12% Criterion XT Bis-Tris precast gels, Bio-Rad). The 1D- and 2D-gels were used for Western blotting or stained with either Sypro Ruby (Invitrogen Life Technologies) or Coomassie Brilliant Blue G-250 (Sigma-Aldrich).

For the 2D-DIGE, samples were concentrated using a Speedy Vac (Eppendorf AG) and resuspended in DIGE buffer (10 mM Tris (pH 8.8), 7 M urea, 2 M thiourea, 4% CHAPS, 2% DTT) to a concentration of 5 mg/mL. After labeling with CyDye (GE Healthcare Life Sciences), the samples were suspended in sample buffer (10 mM Tris (pH 8.8), 7 M urea, 2 M thiourea, 4% CHAPS, 1% (v/v) carrier ampholytes (SB 3−10), 2% DTT) before loading a total of 25 μg of protein following the standard procedure for 2D-GE. Three biological replicates from each of the five locations were used. This was in contrast to the conventional 2D-GE, where three replicates from each of three locations (one from each growing region) were used. It was taken into account that one Cy-Dye might have a higher affinity to some protein spots than the other Cy-Dye. To avoid misleading results, each gel was therefore run with alternate Cy-Dye combinations. The 2D-GE and 2D-DIGE gels were analyzed using Progenesis Same Spot software (Nonlinear Dynamics).

For Western blotting, the gel and nitrocellulose membrane (0.45 μm, Bio-Rad Laboratories Inc.) were equilibrated in transfer buffer (10 mM Tris, 100 mM glycine, 10% (v/v) methanol) for 15 min and placed into a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories Inc.). The transfer was performed in a coolroom (4 °C) at 100 V for 2 h and 10 V overnight. Ponceau S staining was used to check that transfer was successful. After blocking for 2.5−7 h in 0.2% (w/v) bovine serum albumin (BSA), the membranes were incubated under constant agitation at 4 °C overnight in 1.5−3 mL serum of a peanut-allergic individual (diluted 1:10 in antibody buffer: 20 mM Tris base, 0.1% (v/v) Tween 20, 500 mM NaCl, 0.2% (w/v) BSA, pH 7.5). The secondary antibody (anti-human IgE (ε-chain specific)−peroxidase antibody produced in goat (Sigma-Aldrich Co); diluted

1:20000 with antibody buffer) was incubated for a maximum of 2 h at 21 °C. The detection of allergens on the membrane was conducted with the BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit; Roche Diagnostics). Luminescence was captured after 1 min of exposure in a G:Box using GeneSnap software (Synoptics Ltd.). Proteins representing IgE binding spots were identified by comparing the position of the protein spots on the membranes stained with the reversible Ponceau S stain to the protein spots on 2D-GE.

Ethics Approval and Serum Testing. Ethics approval for the collection of human blood was obtained from the Human Ethics Research Committee at Macquarie University, Sydney (HE30OCT2009-D00168). Peanut-allergic human sera from participants with a history of peanut allergy were kindly collected by Dr. Karl Baumgart either at his private allergy practice or at Douglass Hanly Moir Pathology. The sera were subjected to a Phadia ImmunoCap 1000 test (Thermo Fisher Scientific), which showed that the participant whose serum was used had a very high titer of IgE to recombinant Ara h 1 and 2, moderate levels of IgE to Ara h 3, and no response to Ara h 8 and 9. All sera collected were tested on a 1D-Western blot prepared with peanut crude protein. One additional participant was recruited who was negative to the major peanut allergens Ara h 1, 2, and 3 by RAST (Schottdorf GmbH, Germany).

Mass Spectrometry. The preparation of protein plugs for mass spectrometry was adopted from a previous study.[4] PerfectPure C18 tips (Eppendorf AG) were used for preparation before running the sample in a nanoLC electrospray ionization MS-MS in a Q-Star Elite mass spectrometer (Applied Biosystems). The LC component consisted of a 150 mm separation column (Zorbax column 300SB C18) driven by an Agilent Technologies 1100 series nano/capillary liquid chromatography system. Peptides were separated over 1 h (5−40% acetonitrile) and eluted directly into the mass spectrometer, which was run in positive ion mode with MS scans run over a range of m/z 400−1500 and at 4 spectra s^{-1}. Precursor ions were selected for auto MS/MS at an absolute threshold of 500 and a relative threshold of 0.01, with a maximum of three precursors per cycle. Precursor charge-state selection and preference was set to 2+ and then 3+, and precursors were selected by charge then abundance. For the Mascot search, an in-house allergen database, which contained 52 nonredundant allergen sequences found on the allergome database (www.allergome.org) and NCBI (http://www.ncbi.nlm.nih.gov/protein/) in December 2011, was created. A similar approach was used previously with a smaller subset of sequences.[20] Furthermore, the NCBInr database, which has been used regularly to search for peanut proteins and allergens,[2,4] was used to identify peanut proteins that were not recognized as allergens.

A database containing nonredundant allergen sequences for Ara h 1−Ara h 11 acquired in FASTA format from the allergome webpage (www.allergome.org, December 2011) was created and used for protein identification. The allergome database has links to uniprot (www.uniprot.org December 2011) and NCBI Entrez (http://www.ncbi.nlm.nih.gov/, December 2011). BLAST was used to find similar sequences in the NCBInr database that might be considered isoforms of the allergen. Before the database was made available through ProteinPilot (Applied Biosystems, version 2.01), the sequences were aligned with ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/, February 2012) to ensure there were no redundant sequences.

The peptide masses obtained were identified using Mascot (Matrix Science). Only peptides that could not be matched to the peanut allergen database were then compared to the NCBInr (NCBI nonredundant) database to identify peanut proteins that were not allergens. The parameters for the Mascot search allowed up to one missed cleavage and identified peptides with variable modifications such as carbamidomethylation (C) and methionine oxidation (M). The peptide charge was set to 2+ and 3+ (monoisotopic) and the MS/MS tolerance to 0.2 Da. When using the NCBInr database, the taxonomy was set to Viridiplantae ("green plants"). The protein matches were regarded as positive if the Mascot search marked them as significant and if at least two different peptides could be matched. The percentage of the full-length protein for which there were peptide matches (percent coverage) was calculated. For each protein spot only the first, most significant, match with the highest MOWSE score was listed (Supporting Information, Supplementary Tables 4 and 5) and the number of isoforms present was given.

RESULTS

Climate Data for the Three Growing Regions. *Temperature.* For the growing period (November 2009−March 2010), the average daily maximum temperature in Kingaroy decreased from 32 °C in November, when it was 2 °C above average, to 26 °C in March, when it was 1 °C below average (Figure 1a,b). The temperature in Bundaberg during the study was relatively constant with only a slight increase from 29 to 31 °C between November and January, after which the temperature decreased to 28 °C in March. The temperature in Bundaberg increased steadily when compared with the overall averages between all regions, being below the long-term average at the beginning of the growing season and above average at the end. The temperature at Kairi fluctuated between 27 and 30 °C throughout the growth season and was up to 2 °C below the long-term average until January. Overall, the average daily maximum temperature was up to 4 °C different between the three regions.

In Kingaroy the daily minimum temperature was 15−19 °C, which was 1.0−2.3 °C below average (Figure 1c,d). Kingaroy recorded the greatest difference between daily maximum and minimum temperatures. In Bundaberg the daily minimum temperature was 19.5−23 °C, which was 2−3 °C above average, and this site had the lowest difference between daily maximum and minimum temperatures. In Kairi, the daily minimum temperatures were on average 16−20 °C, which is close to the long-term average of all three regions. There was a slight increase in daily minimum temperature in all regions from November to March.

Rainfall. Of the various weather variables, rainfall differed most between the peanut-growing regions (Figure 1e,f). In November the total monthly rainfall was low in all three regions. In December total rainfall in Kingaroy was 190 mm, which was ∼90 mm above average, but rainfall then declined and remained below average over the next 3 months. In Bundaberg the rainfall was between 50 and 100 mm from November until March but then increased to 400 mm in February (∼180 mm above average) and 300 mm (∼90 mm above average) in March. Kairi was the driest region in December but the wettest in January, with 465 mm of rain, which was 270 mm above average. In February and March the rainfall in Kairi was 200 and 150 mm, respectively, which are values close to the average rainfall between the regions.

Solar Radiation. Solar radiation in Kingaroy and Bundaberg remained between 26 and 28 $MJ \cdot m^2$ from November until January and then decreased gradually to around 20−21 $MJ \cdot m^2$ in March (Figure 1g,h). Solar radiation in Kairi was similar in November and December but 4 $MJ \cdot m^2$ lower on average in January, whereas it was around 2 $MJ \cdot m^2$ above average in Kingaroy and Bundaberg.

Summary of Climate. Kingaroy had the largest differences between daily maximum and minimum temperature, less rainfall in January and February, and a decrease in solar radiation from January to March compared to the other regions. Bundaberg had the smallest differences between daily maximum and minimum temperature, the highest rainfall in February and March, and a very similar solar radiation compared to Kingaroy. Kairi had the lowest maximum temperatures between November and January, but the

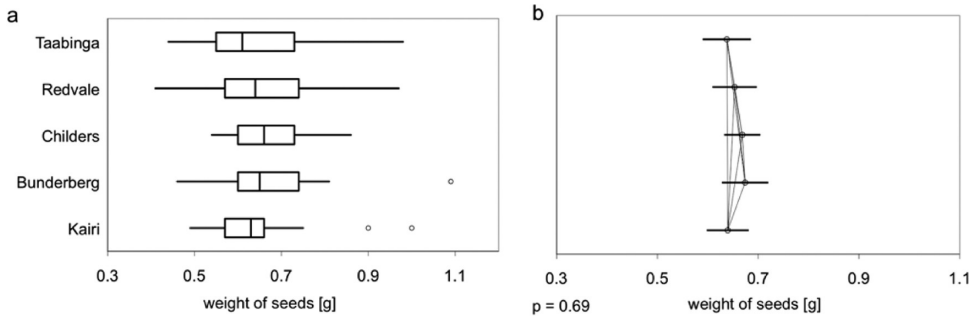

Figure 2. Fresh weights of peanut seeds from five locations in Queensland, Australia. Box plots (a) and means with 95% confidence intervals (b) are given. A line between two means represents a significant similarity, whereas the absence of a line represents a significant difference between these means. The *p* values are given for comparison of all data groups with one-way ANOVA. In all panels n = 10 plants per replicate and 3 replicates per treatment.

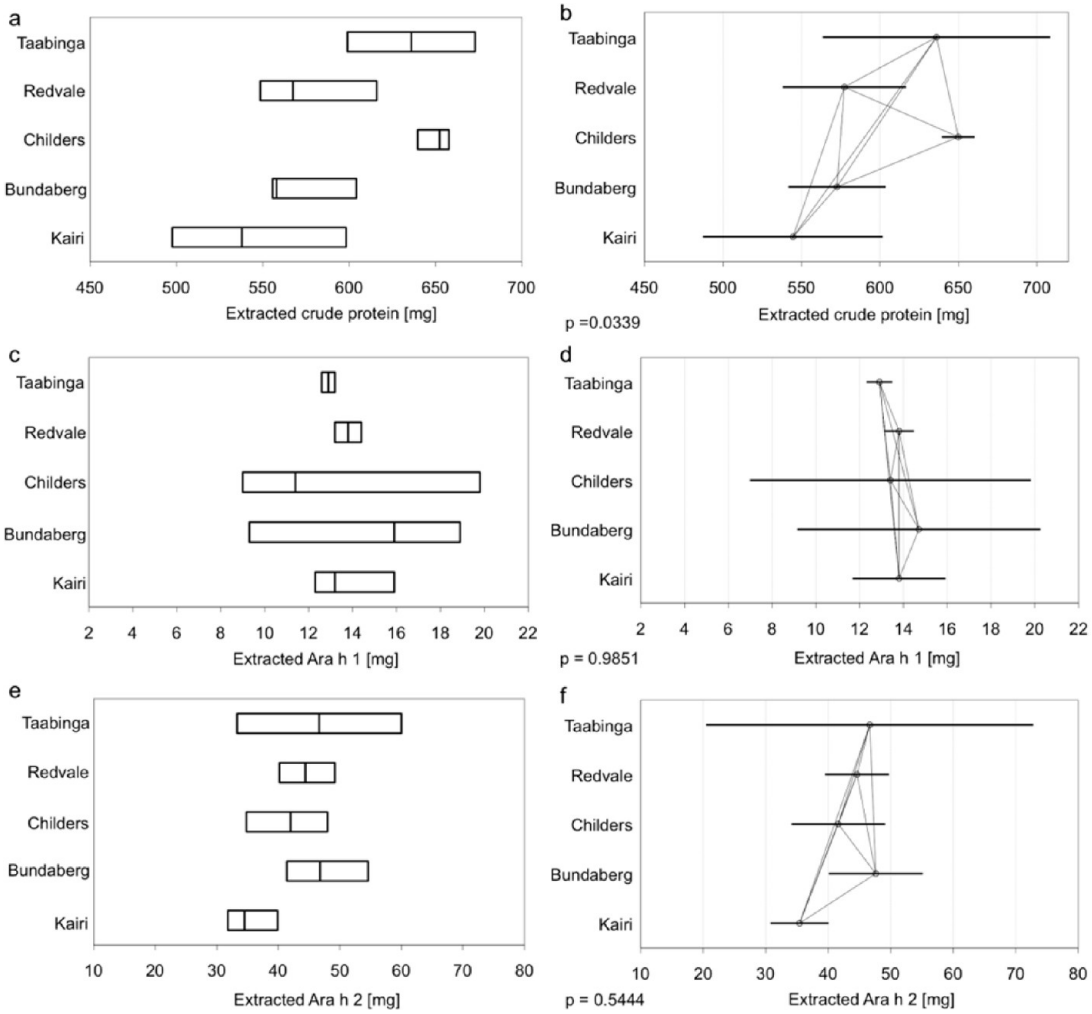

Figure 3. Relative abundance of crude protein and Ara h 1 and 2 of peanuts grown in five locations in Queensland, Australia. Box plots and means with 95% confidence intervals are given: (a, b) crude protein; (c, d) Ara h 1; (e, f) Ara h 2. Each extraction was derived from a pool of 10 seeds per sample, and 3 biological replicates were used for each location. All measurements are based on milligrams per gram of peanut flour.

minimum temperatures were close to average. In January it rained more in Kairi compared to any other region throughout the period of plant growth. At the same time, the solar radiation in Kairi was well below the long-term average for that region.

Plant Performance. *Yield.* Pod and kernel yield performance for Walter, along with kernel percentage, is shown in Supplementary Table 1 of the Supporting Information. Pod yield varied widely between regions, being lowest at Kairi Research Station (3.5 t/ha) and highest at Bundaberg Research Station (5.6 t/ha). Variation in yield was at least in part due to variation in radiation during the crop growth cycle (less at Kairi, northern Queensland), as well as higher foliar disease pressure in northern Queensland, which, despite regular fungicide control, is known to reduce yield potentials in this region.[21] This is further substantiated by the strong positive relationship

between total radiation received and pod yield (Supporting Information, Supplementary Figure 2).

Seed Weight. Fresh weights of 10 individual peanut kernels from each of the samples (triplicates per site) were measured. One-way ANOVA was applied, and the results are shown as box plots and means with 95% confidence intervals (Figure 2). The mean weight of the individual peanut kernels was between 0.6 and 0.7 g. Weights of the peanut kernels from the five sites did not show any significant differences ($p > 0.5$). The peanut kernels from all locations had similar weights and did not show any unusual characteristics, indicating that the Walter peanut crops were grown under near-optimal conditions.

Crude Protein Content. Peanuts grown in Taabinga, Redvale, Bundaberg, Childers, and Kairi varied slightly in crude protein content. Peanuts from Taabinga contained on average 621 mg (±45 mg), Redvale, 577 mg (±34 mg), and Bundaberg, 573 mg (±27 mg), crude protein/g peanut flour. Peanuts grown in Childers contained on average the highest crude protein at 650 mg (±9 mg), whereas Kairi peanuts contained the lowest protein content at 545 mg (±51 mg) (Figure 3a). Given that peanuts contain around 50% lipid (www.pca.com.au) and the peanut flour used was defatted, and assuming the lipid content was the same in peanuts from each location, the total protein content in the peanut kernels measured was estimated to be on average 31% for Taabinga, 29% for Redvale, 29% for Bunderberg, 32% for Childers, and 27% for Kairi. According to Koppelman et al., runner peanut kernels (such as Walter) contain 24−28% protein.[1] The extraction yield of crude protein from ground peanuts is therefore around 100%. The yield value obtained might exceed 100% due to variation in the measurement or the protein content of the variety Walter. When the means and 95% confidence intervals were compared (Figure 3b), protein extracts from Taabinga, Redvale, and Bundaberg were statistically similar to each other and to protein extracts from peanuts grown in Kairi and Childers. Crude protein in peanuts from Kairi and Childers differed significantly from each other, but the respective data were associated with different levels of variation, meaning this result cannot be verified.

Ara h 1 and 2 Content. The average Ara h 1 content in the peanuts from all five locations was 12.9−13.8 mg/g peanut flour and statistically similar between locations ($p > 0.9$; Figure 3c,d). The content of Ara h 2 was 35.4−46.6 mg/g peanut flour and also similar between locations ($p > 0.5$) (Figure 3e,f).

Comparison of 1D- and 2D-GE and 2D-DIGE. The qualitative and quantitative differences in proteins other than Ara h 1 and 2 in the peanuts grown at the five different locations were examined using 1D- and 2D-GE. Due to the very high similarity between the biological replicates on 1D-GE, only one gel is shown (Figure 4). The banding patterns for protein extracts subjected to 1D-GE from all locations were similar.

Mass spectrometry revealed that many bands on the 1D-GE included Ara h 1. These bands ranged in molecular mass from 32 to 140 kDa (including the monomeric band of ∼64 kDa) indicating post-translational processing and possibly aggregation of Ara h 1. Ara h 3 was present in protein bands between 22 and 60 kDa, also suggesting it was post-translationally processed (see Discussion). Furthermore, two protein bands at around 28 and 14 kDa contained Ara h 10, and a band at around 12 kDa contained Ara h 11. Details of the mass spectrometry results are listed in the Supporting Information and Table 3.

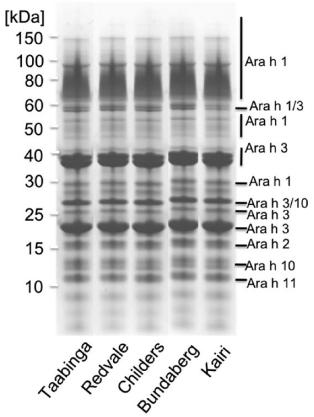

Figure 4. 1D-GE of crude protein extracts of peanuts grown in five locations in Queensland, Australia. Equal volumes of extract were run on each lane of the gel. The protein identities are derived from mass spectrometry analysis.

2D-GE was performed to obtain a high-resolution separation of proteins in the protein extracts and to allow identification of protein spots via mass spectrometry. Of the 455 protein spots obtained, 154 spots were identified with the in-house peanut allergens database or, if no match was found, with the NCBI nonredundant database (http://www.ncbi.nlm.nih.gov/; Figure 5 has a summary of results; details can be found in Supplementary Figure 4 and Supplementary Tables 4 and 5 in the Supporting Information). When three biological replicates of samples from Redvale, Childers, and Kairi were compared, 45 protein spots differed significantly in volume (Figure 6; significantly different spots are marked). These spots ranged in molecular mass from 10 to 100 kDa with p*I* values of 4−9. Only 13 of these protein spots could be identified using mass spectrometry (Table 1; a detailed list of all protein spots with significantly different spot volumes, detailed images and graphics showing the normalized volumes is in Supplementary Table 2 in the Supporting Information). Seven of the 13 protein spots identified were Ara h 3 and 3 were Ara h 1 (2 protein spots were a mixture of both allergens). All proteins visualized were present in peanuts from each location; the spots differed only in volume. None of the significant protein spots that were not matched to proteins in the in-house allergen database could be identified using the NCBInr database (http://www.ncbi.nlm.nih.gov/).

It was not clear whether the differences in spot volumes of the protein spots were due to different protein concentrations in the protein extracts or gel-to-gel variation. To overcome gel-to-gel variation that might have occurred and further substantiate the results, 2D-DIGE was performed, where two protein samples and one internal standard were labeled with different fluorescent dyes (Cy2, Cy3, and Cy5) before they were run in equal concentrations on the same 2D-gel.

The results of the 2D-DIGE experiments were analyzed using Progenesis Same Spot software (Nonlinear Dynamics). Surprisingly, there were only two protein spots (with p*I* values of 5.8 and 6.9 and molecular weights of 81 and 29 kDa, respectively) with significantly different spot volumes between peanut protein extracts from the different locations (Figure 7; Table 2). These spots were minor and could not be identified with mass spectrometry. The two protein spots (Figure 7) were not significantly different after conventional 2D-GE (Figure 6). Because gel-to-gel variation is reduced in 2D-DIGE experi-

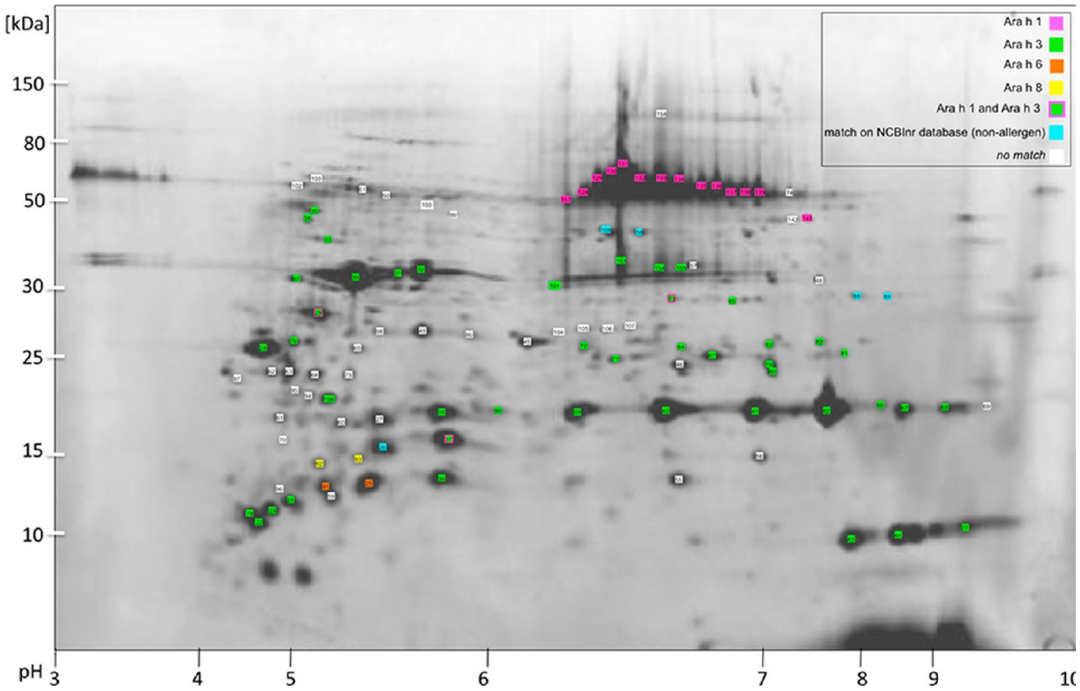

Figure 5. Summary of 2D-GE protein identities derived from mass spectrometry and MASCOT searches using an in-house peanut allergen database. Protein spots that could not be identified with this database were matched using the NCBInr database. Most of the protein spots represent fragments of allergens. A detailed list of protein identities, including NCBI accession numbers, numbers of matched isoforms, protein name, molecular mass, MOWSE score, sequence coverage, p*I*, queries matched, and matched peptides is given in Supplementary Figure 4 and Supplementary Tables 4 and 5 in the Supporting Information.

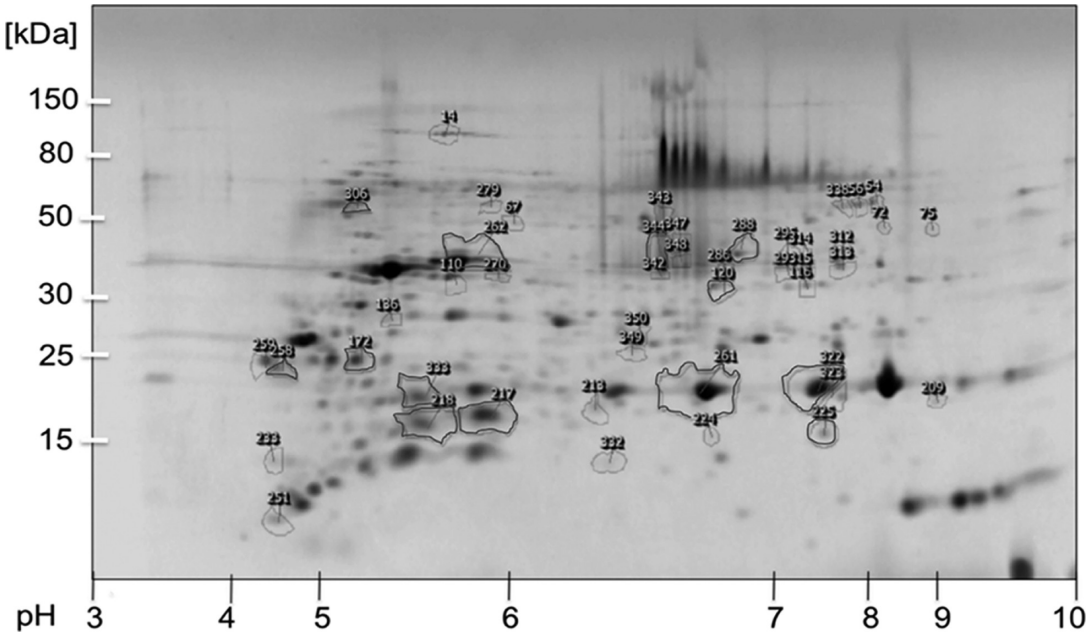

Figure 6. 2D-GE of crude protein extracts from peanuts grown in Redvale, Childers and Kairi. Protein spots with significantly different spot volumes ($p < 0.05$) between crude proteins samples were obtained using Progenesis Same Spot software (Nonlinear Dynamics Ltd.) and are highlighted. Spots marked in bold were used for protein identification with mass spectrometry (Table 1 and Supplementary Table 2 in the Supporting Information).

ments, it is likely that identification of the significantly different protein spots in the 2D-DIGE was more reliable than those identified with the conventional 2D-GE. The different spot volumes that were detected using 2D-GE were therefore assumed to be due to gel-to-gel variation. Only the volumes of the protein spots detected on 2D-DIGE were different (as opposed to the lack of specific protein spots in peanuts from one or more locations).

In summary, peanuts grown in three peanut-growing regions in Queensland, Australia, had the same relative concentrations of individual proteins, including major allergens Ara h 1 and Ara h 2. These findings were substantiated by the Ara h 1 and 2

Table 1. Protein Spots with Significantly Different Spot Volumes from 2D-GE of Crude Protein Extracts of Peanuts from Redvale, Childers, and Kairi (Highlighted in Figure 6)

	identification MS/MS									average normalized volumes		
spot	NCBI accession no.	isoallergen or other protein	fragment (full length, kDa)	MS spot[a]	Western blot spot[b]	ANOVA (p)	fold[c]	MW (kDa)	pI	Redvale	Childers	Kairi
262	gil57669861	Ara h 3	yes [60]	32	14	0.001	2.1	38	5.6	8616	1.8e+004	1.2e+004
333	no match			27	21	0.003	1.3	19	5.5	2730	3577	2684
261	gil37789212	Ara h 3	yes [61]	40		0.003	1.4	21	6.7	1.6e+004	2.0e+004	1.4e+004
258	gil9864777	Ara h 3	yes [60]	97		0.009	2.2	23	4.3	305.6	234.4	511.1
218	gil115187464	thioredoxin fold[d]	no	26	23	0.010	1.3	17	5.5	3084	4137	3484
344	gil21314465	Ara h 3	yes [62]	153		0.012	4.8	42	6.5	8110	4277	1686
288	no match			57		0.019	1.9	42	6.9	1604	943.8	859.6
120	gil1168391	Ara h 1	yes [71]	73		0.021	1.4	34	6.8	277.3	352.2	390.3
217	gil37789212	Ara h 3	yes [61]	37	24	0.030	1.3	18	5.8	6532	7034	5521
	gil1168391	Ara h 1	yes [71]									
306	gil9864777	Ara h 3	yes [60]	35		0.031	1.6	53	5.1	514.7	526.7	329.3
	gil1168390	Ara h 1	yes [70]									
322	gil9864777	Ara h 3	yes [60]	41		0.034	1.5	21	7.4	9274	1.3e+004	8584
225	no match			56	29	0.035	1.5	17	7.5	616.4	473.5	398.3
172	gil1708792	galactose-binding lectin[d]	yes [29]	64		0.045	1.4	25	5.2	1429.6	1115.9	1507.1

[a]MS spot number in the mass spectrometry table (Supporting Information). [b]Western blot number in Figure 8. [c]Fold difference in mean abundance of protein spots. [d]Results from NCBInr database; protein spot 172 (64) was not matched to a peanut proteins database but to a *Viridiplantae* database.

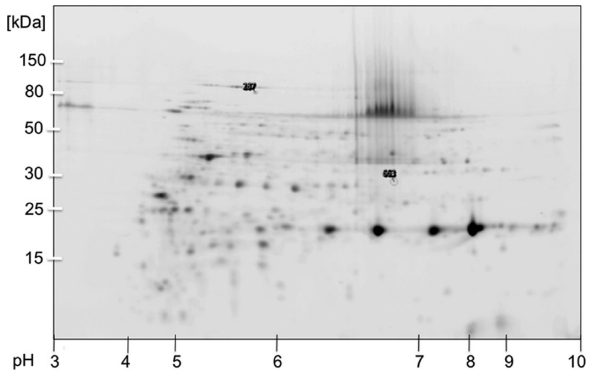

Figure 7. 2D-DIGE of crude protein extracts from peanuts grown in Taabinga, Redvale, Childers, Bundaberg, and Kairi. Protein spots were analyzed using the Progenesis Same Spot software (Nonlinear Dynamics Ltd.), and significantly different spot volumes ($p < 0.05$) between crude proteins samples are highlighted (Table 2).

results obtained with ELISA, which showed no difference between Ara h 1 and 2 contents in peanut kernels obtained from the five locations.

Western Blots. The recognition of antigens in protein extracts from peanuts grown in the different locations by antibodies in the serum of a peanut-allergic individual was tested by performing Western blotting after 2D-GE (Figure 8). Limited available serum allowed two biological replicates to be run for three locations: Redvale, Childers, and Kairi. Only one representative Western blot per treatment is shown. The same patterns of IgE binding intense spots were obtained for protein extracts from plants grown at the three sites. Almost all IgE binding spots were Ara h 1 and 3. Furthermore, faint protein spots were detected on the Western blots, which were identified as Ara h 3/4−9, but it could not be clarified whether the majority of the faint protein spots were real signals on the blots or merely the result of increased background, given that the serum did not contain antibodies against Ara h 8 and 9 (see Material and Methods: Ethics Approval and Serum Testing).

Overall, there appeared to be no qualitative difference in recognition of antigens in Western blots among protein extracts from peanuts grown in the three locations.

DISCUSSION

Commercial peanuts of the Spanish variety, Walter, grown at five sites across three peanut-growing regions in Queensland, Australia, had different pod yields, probably as a result of different levels of total radiation. Strictly, total "intercepted" radiation (i.e., accounting for early canopy development before full crop cover) should be used, but no canopy data were available to enable this calculation to be made. However, the total radiation received by the crop is a reasonable approximation of total intercepted radiation, and the results

Table 2. Protein Spots with Significantly Different Spot Volumes from 2D-DIGE of Crude Protein Extracts of Peanuts from Five Locations in Queensland, Australia (Highlighted in Figure 7)

					average normalized volumes				
spot	ANOVA (p)	fold[a]	MW (kDa)	pI	Taabinga	Redvale	Childers	Bundaberg	Kairi
287	0.016	1.5	81	5.8	0.658	0.972	0.748	0.990	0.910
663	0.041	1.4	29	6.9	1.135	0.962	0.831	1.099	1.113

[a]Fold difference in mean abundance of protein spots.

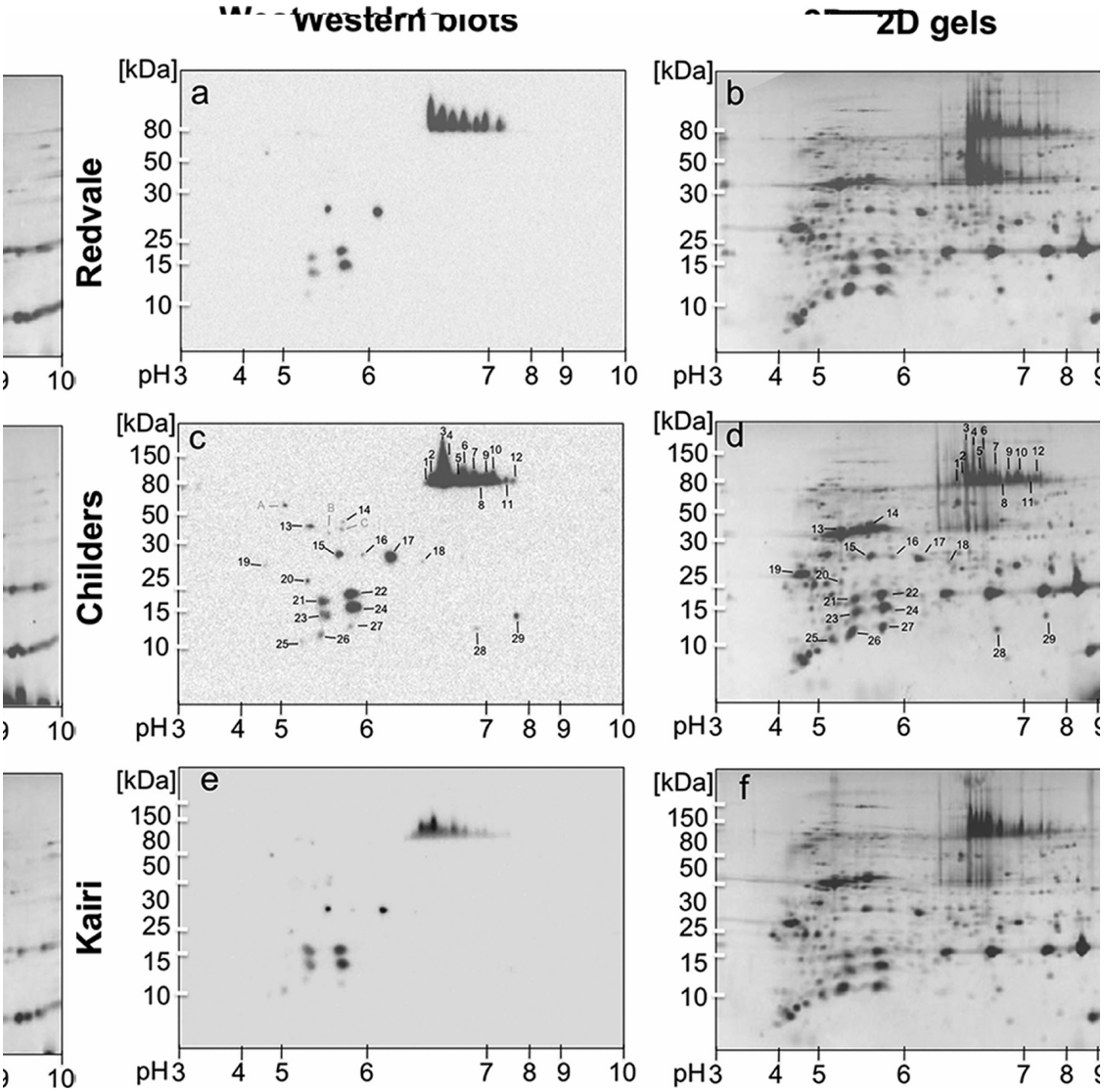

Figure 8. 2D-GE and Western blots using serum of a peanut-allergic patient for extracts of peanuts grown in Redvale (a, b), Childers (c, d), and Kairi (e, f) in Queensland, Australia. The 2D gels in the right panel facilitate the identification of the location of the protein spots. The identities of most protein spots on the Western blots are listed in Table 3.

correspond to the theory that total radiation received drives biomass and hence pod yield production under nonlimiting water conditions (applicable to all peanut plants grown in this experiment).[22]

The peanuts grown at the five sites had the same relative concentrations of individual proteins in crude extracts, except for extracts from Kairi and Childers, which differed significantly from each other. This is probably due to the small sample size tested and, given the high similarity in kernel weights and in 1D- and 2D-GE patterns, as well as the similar amounts of Ara h 1 and 2 in all extracts tested, it is very likely that the protein content would be similar in those extracts if more samples were tested. Walter is known to be a "high-oleic" variety of peanuts (www.pca.com.au). The high content of oleic fatty acid does not affect peanut allergenicity, and high-oleic peanuts confer the same risk of allergy as normal peanuts.[23]

Ara h 1 was identified mainly on 1D- and 2D-GE in a group of protein spots at ∼64 kDa corresponding to the monomeric form of this molecule.[4,20,24−26] Ara h 1 was also identified in a range of bands between 32 and 140 kDa, mainly on 1D-GE, meaning that both fragments and aggregates of this protein were present. The 140 kDa band might represent the trimeric

Table 3. Identities and Intensities of Peanut Protein Spots Detected in Western Blots (Figure 8)

	identification MS/MS			intensity of protein spot[a]		
spot	isoallergen or protein	fragment	MS spot[b]	Redvale	Childers	Kairi
1−12	Ara h 1	yes	128−139	+++	+++	+++
13	Ara h 3	yes	30		+	+
14	Ara h 3	yes	32		+	+
15	no match		49	+++	+++	+++
16	no match		86		+	+
17	Ara h 3	yes	45	+++	+++	+++
18	Ara h 3	yes	72		+	
19	Ara h 3	yes	28		+	
20	no match		75		+	+
21	no match		27	+++	+++	+++
22	Ara h 3	yes	38	+++	+++	+++
23	thioedoxin fold[c]	no	26	+++	+++	+++
24	Ara h 1 and 3	yes	37	+++	+++	+++
25	Ara h 6	yes	91			+
26	Ara h 6 and 8	yes	25	+	+	+
27	Ara h 5[d]	no	36		+	+
28	Ara h 9[d]	yes	55		+	
29	Ara h 7 precursor[d]	no	56		+	
A[e]				+	+	+
B[e]					+	+
C[e]					+	+

[a]+++, high intensity; +, low intensity. [b]MS spot number in the mass spectrometry table (Supporting Information). [c]Result from NCBInr database; no match in in-house peanut allergen database. [d]Not significant, no other match. [e]Not used for mass spectrometry.

form of Ara h 1.[27] The precursor of Ara h 3 is known to have a molecular weight of 60 kDa and could be identified in the 1D-GE, whereas the other Ara h 3 bands are likely to be post-translationally processed into various subunits as shown previously.[25] Other protein bands found in this study, such as Ara h 10 and 11 in 1D-GE or Ara h 8 in 2D-GE (Supporting Information, Supplementary Tables 4 and 5), have not, however, been identified previously by mass spectrometry. Only one protein band on the 1D-GE was identified as Ara h 2, although it is usually present as two bands of around 17 and 18 kDa, corresponding to the two isoforms.[26] The detection of Ara h 2 by mass spectrometry in 2D-GE might be impeded due to its function as a trypsin inhibitor,[28] which would inhibit production of peptides from the protein in samples run on the mass spectrometer. The presence of Ara h 2 in four protein spots at 17 and 19 kDa and between p*I* 5 and 6 was confirmed in a study in which pancreatin (rather than trypsin) was used[20] and is substantiated by the recognition of these proteins on 2D-Western blots by patients' sera containing Ara h 2 IgE, indicating a partially or wholly native structure with intact epitopes.

Analysis using 2D-DIGE showed that there was no difference in individual protein expression between the growing regions, including a range of allergens that were identified via mass spectrometry. Western blotting with peanut-allergic human serum highlighted the same major protein spots. Taken together, these data suggest that peanuts of the Walter variety from the three peanut-growing regions in Queensland, Australia, are most likely to have the same allergenic properties.

Environmental conditions can significantly influence seed protein content in some legumes.[8−11] However, in grain/seed crops generally, nitrogen supply is considered to be the most important factor affecting protein content and composition.[29] Therefore, environmental and other conditions that interact with nitrogen nutrition, such as drought, *Rhizobium* infection, soil density, root diseases, and pests might also influence seed protein content.[8,10,12−14]

The quantity and quality of the protein content and Ara h 1 and 2 fractions, as well as the protein pattern on Western blots, were dependent on both the extraction yield and the influence of the extraction buffer on the proteins in the peanuts. Allergens that were not sufficiently extracted were not investigated and might have different abundance in peanuts from the different production regions. However, because runner peanut kernels (such as Walter) contain 24−28% protein,[1] the extraction yield of 27.2−32.5% crude protein from ground kernels using the chosen extraction protocol was thought to be around 100%.

The significant difference in crude protein content from peanuts derived from Childers and Kairi should be interpreted with caution, as the standard deviation for the crude protein content in peanuts from Childers was very low compared to the standard deviations for the other samples. Because the crude protein content of peanuts from Bundaberg, which is located very near Childers, was statistically similar to the protein content from Kairi, and the 2D-DIGE experiments did not reveal major differences, it is most likely that the crude protein contents of peanuts from Kairi and Childers were similar. Because Western blots were performed with a single patient's serum (plus a non-peanut-allergic serum as a negative control), only subsets of the epitopes present would have been recognized. On Western blots the proteins were denatured and therefore some conformational epitopes may have been lost. However, denaturation may increase the accessibility of linear epitopes within the allergen molecules, such as the 24 known linear IgE binding epitopes on the Ara h 1 molecule,[30−32] although three of these epitopes are considered to be irrelevant because they are located in the N-terminal region of native Ara h 1,[33] regarded as the signal peptide, which is cleaved from the mature protein.[27,34]

Krause et al. identified Ara h 1-deficient peanuts from Southeast Asia by 2D-GE, demonstrated the different compositions of the tested extracts, and revealed a number of variations of the allergen patterns in peanuts from different varieties.[3] However, mediator-release experiments of these peanut extracts demonstrated similar allergenicity when compared with standard peanut extract. These results indicate that the allergenicity of peanuts with reduced Ara h 1 content might be compensated for by the other allergens and, thus, not necessarily cause a reduction of allergenicity.[3] For peanuts, however, even trace amounts of residual allergens can elicit clinical responses in a very atopic individual,[35−39] and thus differences in allergen abundance might affect some people. For individuals allergic only to Ara h 1, Ara h 1-deficient peanuts would be of significant benefit.

For future research it would be interesting to test the influence of nitrogen supply on the expression of the major peanut allergens. This might involve treatment of plants with not only different amounts of nitrogen fertilizer but also differences between external nitrogen supply and the endogenous supply of nitrogen supplied via nitrogen fixation by symbiotic rhizobia bacteria.

In conclusion, proteins from peanut plants grown in three regions in Queensland, Australia, were similar and provide the same set of allergens and, therefore, a similar threat to peanut-allergic individuals.

■ ASSOCIATED CONTENT

Supporting Information

Map showing the peanut-growing sites from which the peanuts were obtained for analysis; a plot with the relationship between cumulative solar radiation and pod yield, as well as the growth period and yield for the analyzed peanuts; individual images of significantly different protein spots on 2D-GE between different growth locations and graphics showing normalized averages of spot volumes; finally, tables and accompanying gel images containing details regarding the mass spectrometry results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

1D-GE, one-dimensional gel electrophoresis; 2D-GE, two-dimensional gel electrophoresis; 2D-DIGE, two-dimensional difference gel electrophoresis; Ara h 1, 2, *Arachis hypogaea* allergens 1 and 2; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; IPG, immobilized pH gradient; MS, mass spectrometry

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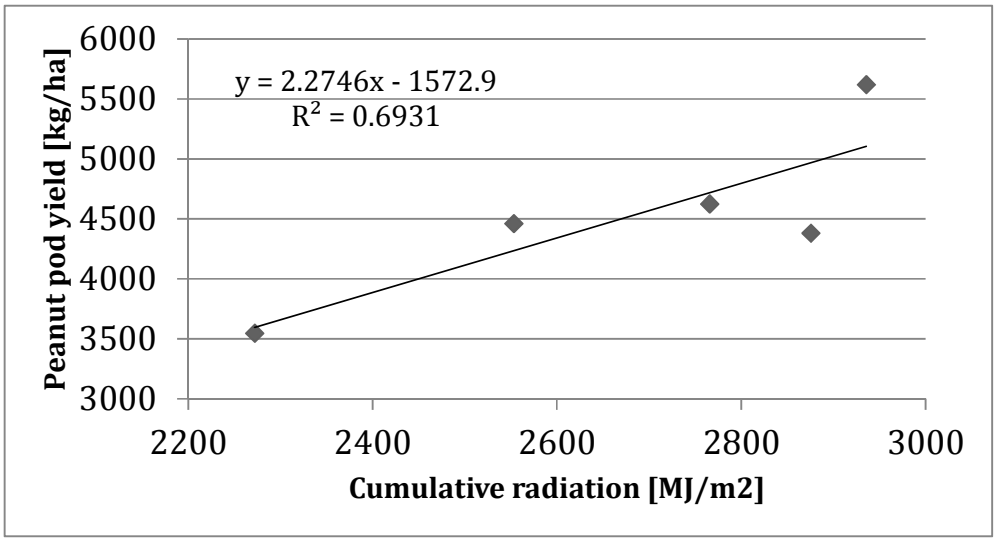
Supplementary Figure 1. Commercial peanut-growing regions and sites from which the peanut kernels were obtained for analysis.



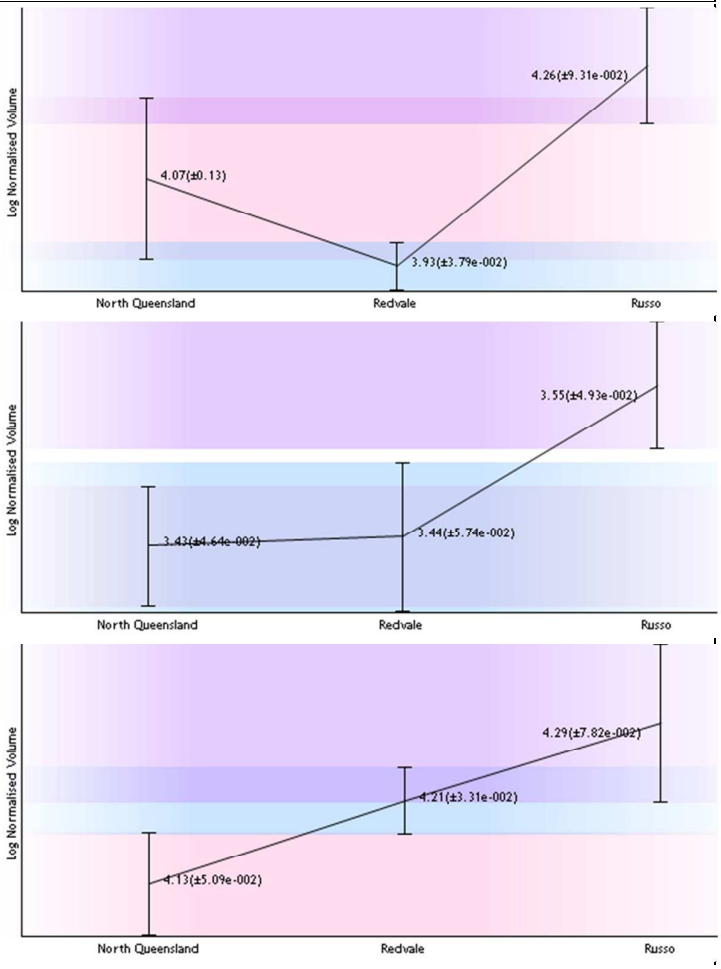
Supplementary Figure 2. Correlation between cumulative solar radiation and pod yield.

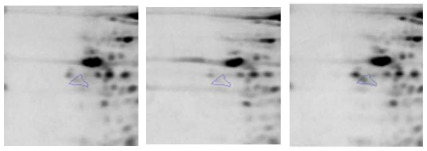
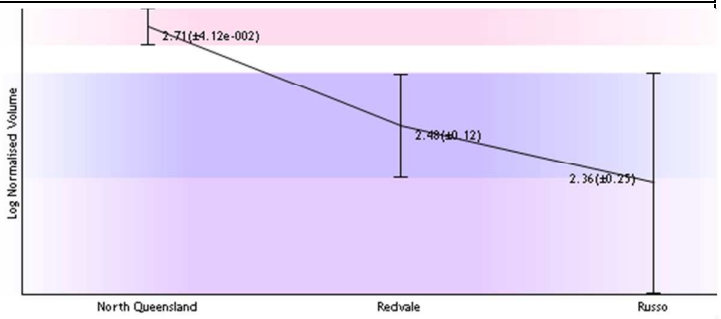
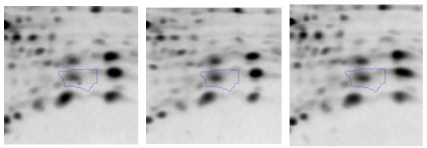
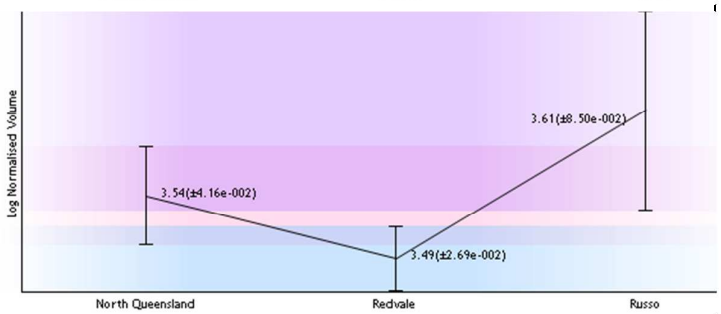
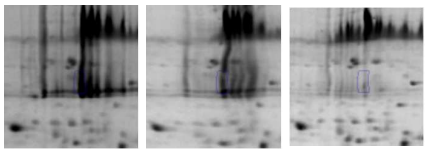
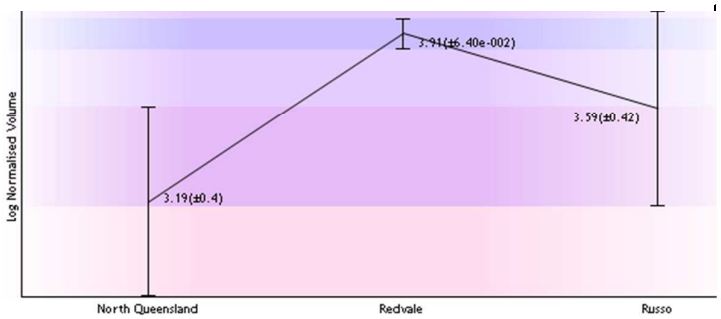
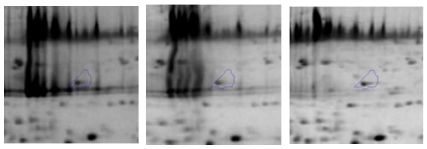
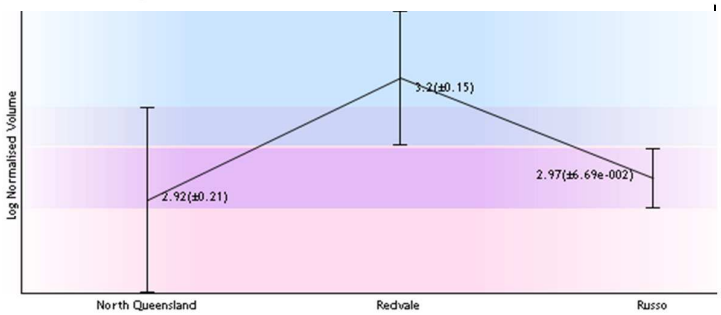
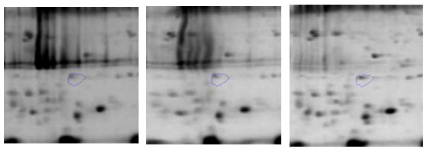
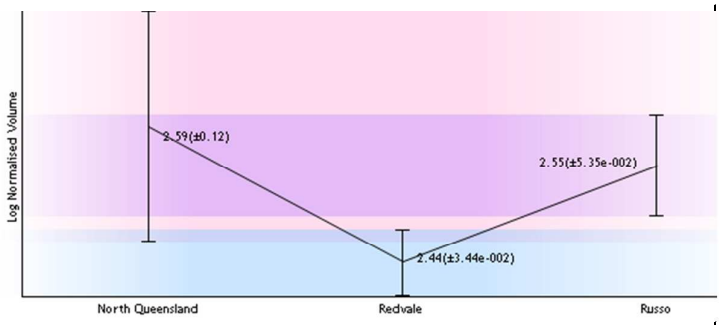
Supplementary Table 1. Yield and Grade Performance of Walter Peanuts in Ultra Early Regional Variety Trials among Five Growing Locations in Queensland, Australia Conducted during 2009/10.

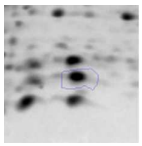
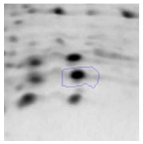
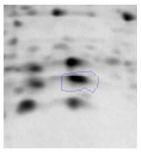
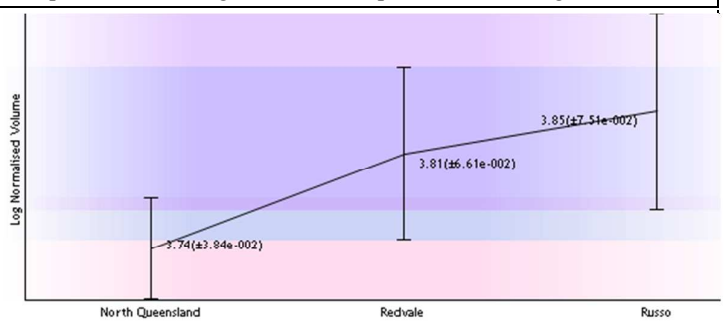
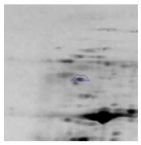
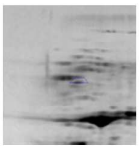
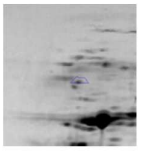
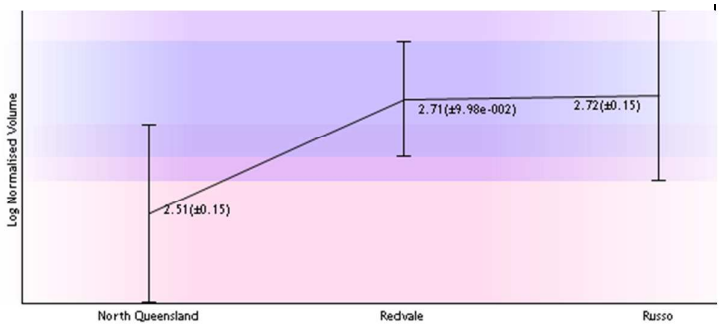
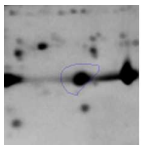
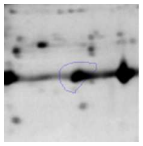
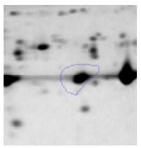
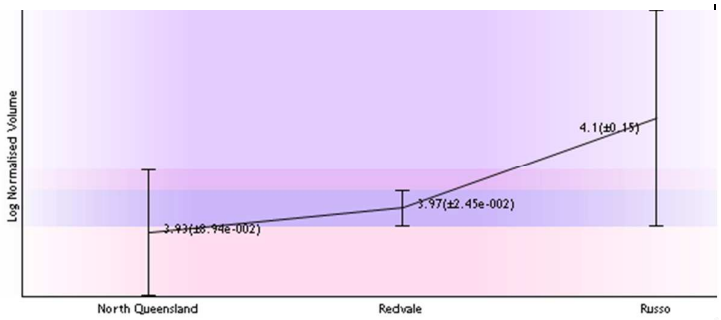
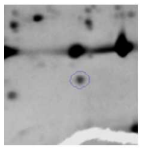
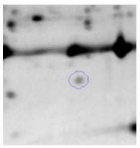
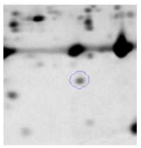
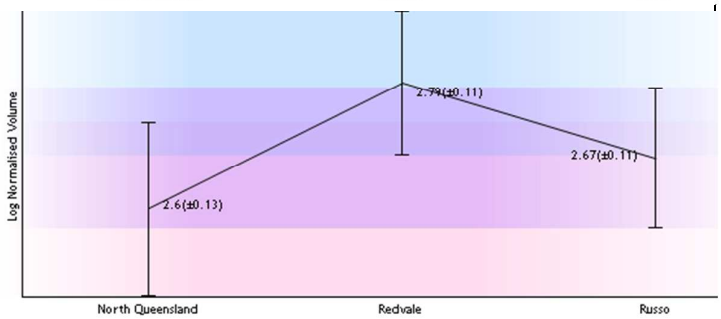
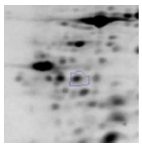
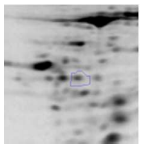
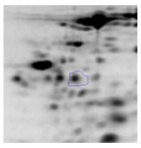
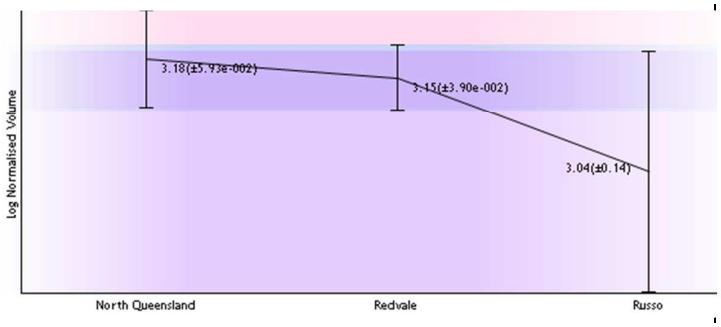
Site	Planting Date (dd/mm/yy)	Harvest Date	Pod Yield (kg/ha)	Kernel Yield (kg/ha)	Kernel %
Taabinga	16/11/09	15/03/10	4379	2770	63.2
Redvale	15/12/09	19/04/10	4458	2976	66.7
Childers	12/11/09	11/03/10	5618	3896	69.4
Bundaberg	03/12/09	29/03/10	4621	2989	64.7
Kairi	16/12/09	09/04/10	3544	2260	63.5

Supplementary Table 2. Individual Images of Averaged Individual Spot Volumes and Graphics showing Normalised Averages of Protein Spots that have been Detected as Significantly Different on 2D-Gels of Protein Extracts from Peanuts Grown in Different Locations. (North Queensland = Kairi; Russo = Childers)

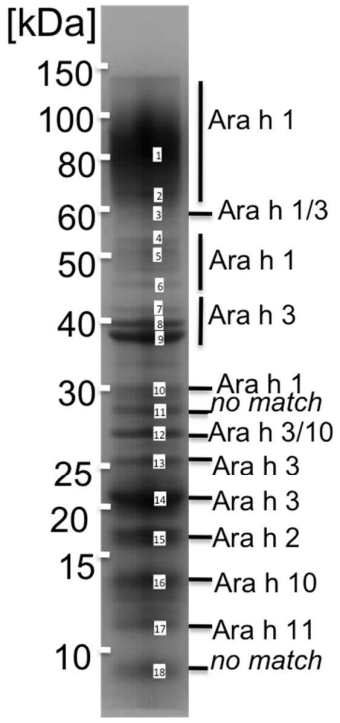
Spot no.	Redvale	Childers	Kairi	Comparison of averaged individual spot volumes in regions
262				
333				
261				



Spot no.	Redvale	Childers	Kairi	Comparison of averaged individual spot volumes in regions
258				
218				
344				
288				
120				

Spot no.	Redvale	Childers	Kairi	Comparison of averaged individual spot volumes in regions
217				
306				
322				
225				
172				

Mass spectrometry



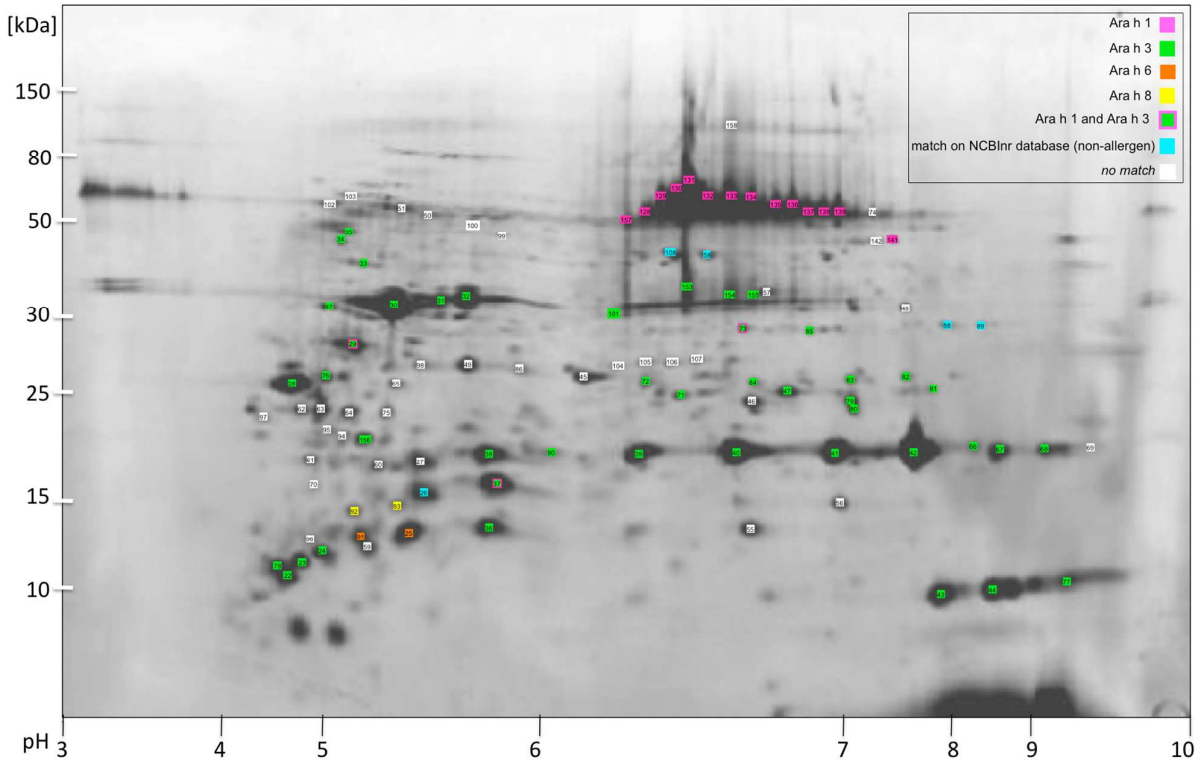
Supplementary Figure 3. Peanut Protein Spots Selected for Mass Spectrometry from a 1D-gel. To identify the proteins on 2D-gels the protein spots were picked and digested used for mass spectrometry. The mass spectrometry results are listed in Supplementary Table 3.

Supplementary Table 3. Identification of Peanut Proteins after 1D-gel Electrophoresis. An in-house peanut allergen database was used for the identification of protein spots in Mascot (Matrix Science Inc.). Proteins that did not match in the in-house database were identified using the NCBInr database. The first significant hit of each allergen in the protein spotsand the number of fragments as isoforms are listed in the table. The protein matches were regarded as positive if the Mascot search marked them as significant and if at least two different peptides could be matched. The spot numbers correspond to those in Supplementary Figure 3.

Spot no.	NCBI acc no	Allergen	Isoforms present	Protein name	Molecular mass (M_r)[a)]	Score	Sequence coverage [%]	pI[b)]	Queries matched	Matched peptides
1	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	589	16%	6.62	11	K.AMVIVVVNK.G K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H K.QAKDLAFPGSGEQVEK.L R.VLLEENAGGEQEERGQR.R
2	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	377	13%	6.62	7	K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.SSENNEGVIVKVSK.E R.IVQIEAKPNTLVLPK.H
3	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	333	11%	6.62	6	K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.IVQIEAKPNTLVLPK.H

4	gi|9864777	Ara h 3	1	Gly1	60412	241			4	K.TANDLNLLILR.W K.FFVPPSQQSLR.A R.SPDIYNPQAGSLK.T R.NRSPDIYNPQAGSLK.T
5	gi|1168390	Ara h 1	3	Full=Allergen Ara h 1, clone P17	70240	253	7%	6.43	4	K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.VLLEENAGGEQEER.G
6	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	212	6%	6.43	4	K.AMVIVVVNK.G + Oxidation (M) R.RPSHQQPR.K R.DQSSYLQGFSR.N R.VLLEENAGGEQEER.G
7	gi|21314465	Ara h 3	4	allergen Arah3/Arah4	61700	182	4%	5.52	2	R.QILQNLR.G R.GENESDEQGAIVTVR.G
8	gi|21314465	Ara h 3	4	allergen Arah3/Arah4	61700	173	4%	5.52	2	R.QILQNLR.G R.GENESDEQGAIVTVR.G
9	gi|9864777	Ara h 3	1	Gly1	60412	233	8%	5.48	3	R.GENESEEEGAIVTVK.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G
10	gi|1168391	Ara h 1	1	Full=Allergen Ara h 1, clone P41B			11%	6.62		K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.IVQIEAKPNTLVLPK.H
11	*no match*									
12	gi|118776570	Ara h 3	1	arachin 6	60339	368	11%	5.40	5	R.SPDIYNPQAGSLK.T R.GENESEEQGAIVTVK.G R.NRSPDIYNPQAGSLK.T R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G
13	gi|118776570	Ara h 3	6	arachin 6	60339	408	10%	5.40	5	K.TANELNLLILR.W R.SPDIYNPQAGSLK.T K.SQSENFEYVAFK.T R.GENESEEQGAIVTVK.G R.NRSPDIYNPQAGSLK.T
14	gi|9864777	Ara h 3	6	Gly1	60412	964	17%	5.48	17	K.TANDLNLLILR.W K.FFVPPSQQSLR.A R.SPDIYNPQAGSLK.T K.SQSDNFEYVAFK.T R.NRSPDIYNPQAGSLK.T R.NALFVPHYNTNAHSIIYALR.G R.VYDEELQEGHVLVVPQNFAVAGK.S
15	*no match*									
16	gi|52001239	Ara h 10	2	oleosin 2	15517	147	16%	9.36	2	R.MADVAGYVGQK.T + Oxidation (M) R.QTHGSVPEQLEMAK.H + Oxidation (M)
17	gi|118776570	Ara h 3	6	arachin 6	60339	310			4	R.SPDIYNPQAGSLK.T R.QQPEENACQFQR.L R.GENESEEQGAIVTVK.G R.RPFYSNAPQEIFIQQGR.G
18	*no match*									

Ara h 1 was present in various bands ranging from 80 to 32 kDa in the Tris extracts, indicating a aggregation and fragmentation of Ara h 1. Ara h 3 was present in protein bands between 60 and 22 kDa, also indiction fragmentation. Furthermore, two protein bands at around 28 and 14 kDa contained Ara h 10 and a protein band at around 12 kDa contained Ara h 11. Three bands did not give a match using the in-house allergen database.



Supplementary Figure 4. Mass Spectrometry of 2D-gel. To identify the proteins on 2D gels the protein spots were picked and digested used for mass spectrometry. The mass spectrometry results are listed in Supplementary Table 4 and 5.

Supplementary Table 4. Identification of Peanut Proteins after 2D-gel Electrophoresis. An in-house peanut allergen database was used for the identification of protein spots in Mascot (Matrix Science Inc.). Proteins that did not match in the in-house database were identified using the NCBInr database. The first significant hit of each allergen in the protein spots and the number of fragments as isoforms are listed in the table. The protein matches were regarded as positive if the Mascot search marked them as significant and if at least two different peptides could be matched. The Spot numbers correspond to Supplementary Figure 4.

Spot no.	NCBI acc no	Isoallergen	Isoforms present	Protein name	Molecular mass (M_r[a])	MOWSE Score	Sequence coverage [%]	pI	Queries matched	Matched peptides
22	gi| 118776570	Ara h 3	4	arachin 6	60339	421	8	5.40	7	R.QIVQNLR.G R.GENESEEQGAIVTVK.G K.SPDEEEEYDEDEYAEEER.Q R.QIVQNLRGENESEEQGAIVTVK.G K.SPDEEEEYDEDEYAEEERQQDR.R
23	gi|21314465	Ara h 3	6	allergen Arah3/Arah4	61700	204	6	5.52	7	R.QILQNLR.G R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y
24	gi|21314465	Ara h 3	2	allergen Arah3/Arah4	61700	178	4	5.52	3	R.QILQNLR.G R.GENESDEQGAIVTVR.G
25	gi|17225991	Ara h 6	7	conglutin	16779	143	15	5.74	3	R.QMVQQFKR.E R.IMGEQEQYDSYDIR.S + Oxidation (M)
25[a)]	gi|17225991	Ara h 6	3	conglutin	16779	164	27%	5.74	4	R.ELMNLPQQCNFR.A + Carbamidomethyl (C); Oxidation (M) R.VNLKPCEQHIMQR.I + Carbamidomethyl (C); Oxidation (M) R.IMGEQEQYDSYDIR.S + Oxidation (M)
26	*no match*									

27	*no match*									
28	gi| 118776570	Ara h 3	8	arachin 6	60339	2113	13	5.40	50	R.QIVQNLR.G R.FNLAGNHEQEFLR.Y R.GENESEEQGAIVTVK.G R.RFNLAGNHEQEFLR.Y R.SLPLSPYSPQPGQEDR.E K.SPDEEEEYDEDEYAEEER.Q
29	gi| 1168391	Ara h 1	1	Full=Allergen Ara h 1, clone P41B	71302	281	14%	6.62	11	K.AMVIVVVNK.G + Oxidation K.GTGNLELVAVR.K R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation K.DLAFPGSGEQVEK.L K.GSEEEGDITNPINLR.E K.KGSEEEGDITNPINLR.E R.IFLAGDKDNVIDQIEK.Q
29[b)]	gi|9864777	Ara h 3	3	Gly1	60412	3			4	R.FNLAGNHEQEFLR.Y 11R.GENESEEEGAIVTVK.G R.QIVQNLRGENESEEEGAIV R.QIVQNLRGENESEEEGAIV
30	gi|9864777	Ara h 3	8	Gly1	60412	4514	16	5.48	83	R.QIVQNLR.G R.FNLAGNHEQEFLR.Y R.GENESEEEGAIVTVK.G R.SLPYSPYSPQSQPR.Q R.RFNLAGNHEQEFLR.Y R.PFYSNAPQEIFIQQGR.G R.RPFYSNAPQEIFIQQGR.G R.QIVQNLRGENESEEEGAIVTVK.G R.GADEEEEYDEDEYEYDEEDRR.R
31	gi|37789212	Ara h 3	6	storage protein	61461	786	13	5.52	22	R.QILQNLR.G R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.A R.QILQNLRGENESDEQGAIVTVR.G R.AYFGLIFLGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
31[a)]	gi|21314465	Ara h 3	6	allergen Arah3/Arah4	61700	404	12%	5.52	11	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QILQNLRGENESDEQGAIVTVR.G
31[a)]	gi|9864777	Ara h 3	8	Gly1	60412	141	10%	5.48	8	R.FNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
32	gi|57669861	Ara h 3	3	arachin Ahy-4	60699	300	9	5.36	9	R.FNLAGNHEQEFLR.Y R.GENESEEEGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QIVQNLRGENESEEEGAIVTVR.G
32[a)]	gi|21314465	Ara h 3	6	allergen Arah3/Arah4	61700	319	12%	5.52	9	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QILQNLRGENESDEQGAIVTVR.G
33	gi|9864777	Ara h 3	1	Gly1	60412	254	10	5.48	7	R.FNLAGNHEQEFLR.Y R.GENESEEEGAIVTVK.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QIVQNLRGENESEEEGAIVTVK.G
34	*no match*									
35	gi|9864777	Ara h 3	9	Gly1	60412	30	5	5.48	2	R.SPDIYNPQAGSLK.T R.RFNLAGNHEQEFLR.Y
35[a)]	*no match*									
36	*no match*									
37	gi|37789212	Ara h 3	9	storage protein	61461	1125	29	5.52	27	R.QILQNLR.G K.TANELNLLILR.W K.FFVPPSEQSLR.A R.SPDIYNPQAGSLK.T K.SQSENFEYVAFK.T R.WLGLSAEYGNLYR.N R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.NRSPDIYNPQAGSLK.T R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.A R.NALFVPHYNTNAHSIIYALR.G R.VFDEELQEGHVLVVPQNFAVAGK.S
37[b)]	gi|1168391	Ara h 1	3	full=Allergen Ara h 1, clone P41B	71302	972	29	6.62	24	K.PNTLVLPK.H

										K.SQSENFEYVAFK.T
38[a)]	gi|3703107	Ara h 3	4	glycinin	58315	106	4%	5.68	2	K.TANDLNLLILR.W
										R.SPDIYNPQAGSLK.T
39	gi|37789212	Ara h 3	7	storage protein	61461	4114	19	5.52	86	K.TANELNLLILR.W
										K.FFVPPSEQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSENFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NALFVPHYNTNAHSIIYALR.G
										R.VFDEELQEGHVLVVPQNFAVAGK.S
39[a)]	gi|37789212	Ara h 3	2	storage protein	61461	478	9%	5.52	10	K.TANELNLLILR.W
										K.FFVPPSEQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSENFEYVAFK.T
										R.NRSPDIYNPQAGSLK.T
40	gi|37789212	Ara h 3	7	storage protein	61461	4735	27	5.52	95	K.TANELNLLILR.W
										R.AHVQVVDSNGDR.V
										K.FFVPPSEQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSENFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NALFVPHYNTNAHSIIYALR.G
										R.VFDEELQEGHVLVVPQNFAVAGK.S
										K.TDSRPSIANLAGENSFIDNLPEEVVANSYGLPR.E
40[a)]	gi|37789212	Ara h 3	8	storage protein	61461	1985	9%	5.52	54	K.TANELNLLILR.W
										K.FFVPPSEQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSENFEYVAFK.T
										R.NRSPDIYNPQAGSLK.T
41	gi|9864777	Ara h 3	9	Gly1	60412	3872	22	5.48	68	K.TANDLNLLILR.W
										R.AHVQVVDSNGNR.V
										K.FFVPPSQQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NRSPDIYNPQAGSLK.T
										R.NALFVPHYNTNAHSIIYALR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
41[a)]	gi|9864777	Ara h 3	8	Gly1	60412	1783	13%	5.48	52	K.TANDLNLLILR.W
										K.FFVPPSQQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.NRSPDIYNPQAGSLK.T
										R.VYDEELQEGHVLVVPQNFAVAGK.S
42	gi|9864777	Ara h 3	9	Gly1	60412	4264	21	5.48	68	K.TANDLNLLILR.W
										R.AHVQVVDSNGNR.V
										K.FFVPPSQQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NALFVPHYNTNAHSIIYALR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
42[a)]	gi|9864777	Ara h 3	9	Gly1	60412	7186	24%	5.48	146	K.TANDLNLLILR.W
										R.AHVQVVDSNGNR.V
										K.FFVPPSQQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NRSPDIYNPQAGSLK.T
										K.SQSDNFEYVAFKTDSR.P
										K.NNNPFKFFVPPSQQSLR.A
										R.NALFVPHYNTNAHSIIYALR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
43	gi|9864777	Ara h 3	6	Gly1	60412	684	16	5.48	12	K.TANDLNLLILR.W
										K.TANDLNLLILR.W
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.RPFYSNAPQEIFIQQGR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
44	gi|3703107	Ara h 3	5	glycinin	58315	454	8	5.68	8	K.TANDLNLLILR.W
										R.SPDIYNPQAGSLK.T
										R.RPFYSNAPQEIFIQQGR.G
45	*no match*									
46	*no match*									
47	gi|9864777	Ara h 3	8	Gly1	60412	890	23%	5.48	23	K.TANDLNLLILR.W
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.FNLAGNHEQEFLR.Y
										R.RFNLAGNHEQEFLR.Y
										R.PFYSNAPQEIFIQQGR.G
										R.RPFYSNAPQEIFIQQGR.G
										R.NALFVPHYNTNAHSIIYALR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
48	gi|9864777	Ara h 3	8	Gly1	60412	1035	25%	5.48	26	K.TANDLNLLILR.W
										K.FFVPPSQQSLR.A
										R.SPDIYNPQAGSLK.T
										K.SQSDNFEYVAFK.T
										R.WLGLSAEYGNLYR.N
										R.NRSPDIYNPQAGSLK.T
										R.RFNLAGNHEQEFLR.Y
										R.RPFYSNAPQEIFIQQGR.G
										R.NALFVPHYNTNAHSIIYALR.G
										R.VYDEELQEGHVLVVPQNFAVAGK.S
49	*no match*									
50	*no match*									
51	*no match*									
55	*no match*									

56	*no match*									
57	*no match*									
58	*no match*									
59	*no match*									
60	*no match*									
61	*no match*									
62	*no match*									
63	*no match*									
64	*no match*									
65	*no match*									
66	gi|9864777	Ara h 3	6	Gly1	60412	330	15%	5.48	11	K.TANDLNLLILR.W K.FFVPPSQQSLR.A R.SPDIYNPQAGSLK.T K.SQSDNFEYVAFK.T R.NRSPDIYNPQAGSLK.T R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G
67	gi|9864777	Ara h 3	6	Gly1	60412	817	16%	5.48	22	K.TANDLNLLILR.W K.FFVPPSQQSLR.A R.SPDIYNPQAGSLK.T K.SQSDNFEYVAFK.T R.WLGLSAEYGNLYR.N R.NRSPDIYNPQAGSLK.T R.VYDEELQEGHVLVVPQNFAVAGK.S
68	gi|9864777	Ara h 3	6	Gly1	60412	466	15%	5.48	13	K.TANDLNLLILR.W K.FFVPPSQQSLR.A R.SPDIYNPQAGSLK.T R.WLGLSAEYGNLYR.N R.VYDEELQEGHVLVVPQNFAVAGK.S
69	*no match*									
70	*no match*									
71	gi|9864777	Ara h 3	8	Gly1	60412	643	17%	5.48	14	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C) R.LESEGGYIETWNPNNQEFECAGVALSR.L + Carbamidomethyl (C)
71[a)]	*no match*									
72	gi|9864777	Ara h 3	9	Gly1	60412	50	5%	5.48	3	R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G
72[a)]	*no match*									
73	gi|1168391	Ara h 1	1	Full=Allergen Ara h 1, clone P41B	71302	89	9%	6.62	5	K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R K.SFNLDEGHALR.I K.DLAFPGSGEQVEK.L R.IVQIEAKPNTLVLPK.H
73[a)]	gi|21314465	Ara h 3	6	allergen Arah3/Arah4	61700	682	16%	5.52	18	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QILQNLRGENESDEQGAIVTVR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
74	*no match*									
75	*no match*									
75[a)]	*no match*									
76	gi|21314465	Ara h 3	4	allergen Arah3/Arah4	61700	100	4%	5.52	2	R.QILQNLR.G R.GENESDEQGAIVTVR.G
77	gi|9864777	Ara h 3	8	Gly1	60412	378	9%	5.48	10	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.PFYSNAPQEIFIQQGR.G R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
78	gi|9864777	Ara h 3	7	Gly1	60412	288	6%	5.48	6	R.QIVQNLR.G R.GENESEEEGAIVTVK.G R.RFNLAGNHEQEFLR.Y R.QIVQNLRGENESEEEGAIVTVK.G
79	gi|9864777	Ara h 3	8	Gly1	60412	394	12%	5.48	10	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
80	gi|9864777	Ara h 3	8	Gly1	60412	321	12%	5.48	10	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
81	gi|37789212	Ara h 3	5	storage protein	61461	101	4%	5.52	2	K.TANELNLLILR.W R.SPDIYNPQAGSLK.T
82	gi|9864777	Ara h 3	7	Gly1	60412	339	19%	5.48	10	K.TANDLNLLILR.W R.SPDIYNPQAGSLK.T K.SQSDNFEYVAFK.T R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.VYDEELQEGHVLVVPQNFAVAGK.S
83	gi|9864777	Ara h 3	8	Gly1	60412	342	12%	5.48	9	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
84	gi|9864777	Ara h 3	8	Gly1	60412	373	12%	5.48	11	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
85	gi|21314465	Ara h 3	4	allergen	61700	464	12%	5.52	10	R.QILQNLR.G

				Arah3/Arah4						R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G
86	*no match*									
87	gi|9864777	Ara h 3	6	Gly1	60412	669	16%	5.48	15	R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESEEEGAIVTVK.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QIVQNLRGENESEEEGAIVTVK.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
88	*no match*									
89	*no match*									
90	gi|37789212	Ara h 3	9	storage protein	61461	464	13%	5.52	13	K.TANELNLLILR.W K.FFVPPSEQSLR.A R.SPDIYNPQAGSLK.T K.SQSENFEYVAFK.T R.NRSPDIYNPQAGSLK.T R.VFDEELQEGHVLVVPQNFAVAGK.S
91	gi|17225991	Ara h 6	3	conglutin	16779	400	32%	5.74	11	R.QMVQQFKR.E R.ELMNLPQQCNFR.A + Carbamidomethyl (C); Oxidation (M) R.VNLKPCEQHIMQR.I + Carbamidomethyl (C); Oxidation (M) R.IMGEQEQYDSYDIR.S + Oxidation (M)
92	gi|145904610	Ara h 8	1	Ara h 8 allergen isoform	16402	1185	64%	5.07	44	K.VSVTFHTK.G K.AIEGYVLSN.- K.IKVSVTFHTK.G K.ATVVDGDELTPK.L K.GDAPLPDEVHQDVK.Q K.VTAVEDGKTSYVLHK.I M.GVHTFEEESTSPVPPAK.L K.LIPAIQSIEIVEGNGGPGTVK.K K.LIPAIQSIEIVEGNGGPGTVKK.V K.VSVTFHTKGDAPLPDEVHQDVK.Q
93	gi|145904610	Ara h 8	1	Ara h 8 allergen isoform	16402	319	54%	5.07	18	K.VSVTFHTK.G K.AIEGYVLSN.- K.IKVSVTFHTK.G K.ATVVDGDELTPK.L K.GDAPLPDEVHQDVK.Q M.GVHTFEEESTSPVPPAK.L K.LIPAIQSIEIVEGNGGPGTVK.K K.LIPAIQSIEIVEGNGGPGTVKK.V
94	*no match*									
95	*no match*									
96	*no match*									
97	*no match*									
98	*no match*									
99	*no match*									
100	*no match*									
101	gi|9864777	Ara h 3	8	Gly1	60412	59	9%	5.48	3	R.FNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.GYFGLIFPGCPSTYEEPAQQGR.R + Carbamidomethyl (C)
102	*no match*									
103	*no match*									
104	*no match*									
105	*no match*									
106	*no match*									
107	*no match*									
108	*no match*									
108[a)]	*no match*									
109	*no match*									
110	*no match*									
111	*no match*									
112	*no match*									
113	*no match*									
114	*no match*									
116	*no match*									
117	*no match*									
118	*no match*									
119	*no match*									
120	*no match*									
121	*no match*									
122	*no match*									
123	*no match*									
124	*no match*									
125	*no match*									
126	*no match*									
127	*no match*									
128	gi|1168390	Ara h 1	7	Full=Allergen Ara h 1, clone P17	70240	64	3%	6.43	2	K.GTGNLELVAVR.K K.DLAFPGSGEQVEK.L
129	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	1234	32%	6.43	38	R.PNTLVLPK.H R.LFEVKPDK.K K.EHVQELTK.H K.AMVIVVVNK.G + Oxidation (M) K.DNVIDQIEK.Q K.GTGNLELVAVR.K R.NNPFYFPSR.R K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H

										R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.RVLLEENAGGEQEER.G R.IFLAGDKDNVIDQIEK.Q K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
130	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	2611	41%	6.43	73	R.WGPAEPR.E R.PNTLVLPK.H K.AMVIVVVNK.G R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R K.GTGNLELVAVRK.E K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I R.EGEQEWGTPGSEVR.E R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.RVLLEENAGGEQEER.G R.IFLAGDKDNVIDQIEK.Q R.NTLEAAFNAEFNEIRR.V K.NPQLQDLDMMLTCVEIK.E + Carbamidomethyl (C); Oxidation (M) R.EGEQEWGTPGSEVREETSR.N K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
131	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	3759	32%	6.62	102	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.DNVIDQIEK.Q K.GTGNLELVAVR.K R.NNPFYFPSR.R R.SSENNEGVIVK.V K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I R.VLLEENAGGEQEER.G K.GSEEEGDITNPINLR.E R.IVQIEAKPNTLVLPK.H R.RVLLEENAGGEQEER.G K.KGSEEEGDITNPINLR.E R.IFLAGDKDNVIDQIEK.Q K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
131[a)]	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	2515	35%	6.62	66	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.DNVIDQIEK.Q K.GTGNLELVAVR.K R.NNPFYFPSR.R K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I R.VLLEENAGGEQEER.G K.GSEEEGDITNPINLR.E R.IVQIEAKPNTLVLPK.H R.RVLLEENAGGEQEER.G R.NTLEAAFNAEFNEIR.R K.KGSEEEGDITNPINLR.E R.IFLAGDKDNVIDQIEK.Q R.NTLEAAFNAEFNEIRR.V K.NPQLQDLDMMLTCVEIK.E + Carbamidomethyl (C); 2 Oxidation (M) K.KNPQLQDLDMMLTCVEIK.E + Carbamidomethyl (C); 2 Oxidation (M) K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
131[a)]	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	833	32%	6.43	25	R.WGPAEPR.E R.PNTLVLPK.H R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.EGEQEWGTPGSEVR.E R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.IFLAGDKDNVIDQIEK.Q K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
131[a)]	gi|1168390	Ara h 1	3	Full=Allergen	70240	1310	34%	6.43	40	R.WGPAEPR.E

				Ara h 1, clone P17						R.PNTLVLPK.H K.AMVIVVVNK.G R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.DNVIDQIEK.Q K.GTGNLELVAVR.K R.NNPFYFPSR.R K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I R.EGEQEWGTPGSEVR.E R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.RVLLEENAGGEQEER.G R.IFLAGDKDNVIDQIEK.Q K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
132	gi|1168390	Ara h 1	6	Full=Allergen Ara h 1, clone P17	70240	156	5%	6.43	3	K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.VLLEENAGGEQEER.G
132[a)]	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	368	13%	6.62	9	K.PNTLVLPK.H K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G
132[a)]	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	637	20%	6.62	19	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H
132[a)]	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	657	23%	6.62	20	K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G K.GSEEEGDITNPINLR.E R.IVQIEAKPNTLVLPK.H R.IFLAGDKDNVIDQIEK.Q
133	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	1038	29%	6.62	37	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R K.GTGNLELVAVRK.E K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.NNPFYFPSRR.F R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.KSFNLDEGHALR.I R.SSENNEGVIVKVSK.E R.VLLEENAGGEQEER.G K.GSEEEGDITNPINLR.E R.IVQIEAKPNTLVLPK.H R.RVLLEENAGGEQEER.G R.EETSRNNPFYFPSR.R K.KGSEEEGDITNPINLR.E R.IFLAGDKDNVIDQIEK.Q R.VLLEENAGGEQEERGQR.R
133[a)]	gi|1168390	Ara h 1	3	Full=Allergen Ara h 1, clone P17	70240	819	34%	6.43	26	R.PNTLVLPK.H R.LFEVKPDK.K K.EHVQELTK.H K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.IFLAGDKDNVIDQIEK.Q R.NTLEAAFNAEFNEIRR.V K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
133[a)]	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone	70240	232	11%	6.43	11	K.AMVIVVVNK.G + Oxidation (M) R.NNPFYFPSR.R

				P17						R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G
134	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	545	16%	6.62	15	K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H
134[a)]	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	470	18%	6.62	17	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H R.IFLAGDKDNVIDQIEK.Q
135	gi|1168391	Ara h 1	3	Full=Allergen Ara h 1, clone P41B	71302	316	16%	6.62	10	K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H
135[a)]	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17; AltName	70240	321	16%	6.43	11	R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G R.IVQIEARPNTLVLPK.H
136	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	385	16%	6.43	11	R.PNTLVLPK.H K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G
136[a)]	gi|46560472	Ara h 1	5	conarachin	34112	121	10%	5.07	3	R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) R.VLLEENAGGEQEER.G
137	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	417	17%	6.43	12	R.PNTLVLPK.H R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G
138	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	267	12%	6.43	7	R.PNTLVLPK.H K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.VLLEENAGGEQEER.G
139	gi|1168391	Ara h 1	1	Full=Allergen Ara h 1, clone P41B	71302	260	15%	6.62	8	K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.DLAFPGSGEQVEK.L R.VLLEENAGGEQEER.G R.IVQIEAKPNTLVLPK.H
141	gi|1168390	Ara h 1	4	Full=Allergen Ara h 1, clone P17	70240	88	3%	6.43	2	R.NNPFYFPSR.R R.DQSSYLQGFSR.N
141[a)]	*no match*									
142	*no match*									
142[a)]	*no match*									
143	*no match*									
144	*no match*									
145	*no match*									
146	gi|1168391	Ara h 1	2	Full=Allergen Ara h 1, clone P41B	71302	629	23%	6.62	13	K.PNTLVLPK.H K.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.GTGNLELVAVR.K R.NNPFYFPSR.R R.DQSSYLQGFSR.N R.EGEPDLSNNFGK.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I

										R.VLLEENAGGEQEER.G K.GSEEEGDITNPINLR.E
149	*no match*									
153	gi|21314465	Ara h 3	4	allergen Arah3/Arah4	61700	159	9%	5.52	4	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.GENESDEQGAIVTVR.G R.RPFYSNAPQEIFIQQGR.G
154	gi|21314465	Ara h 3	5	allergen Arah3/Arah4	61700	322	9%	5.52	7	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.GENESDEQGAIVTVR.G R.RPFYSNAPQEIFIQQGR.G
155	gi|21314465	Ara h 3	6	allergen Arah3/Arah4	61700	345	12%	5.52	10	R.QILQNLR.G R.QQPEENACQFQR.L + Carbamidomethyl (C) R.FNLAGNHEQEFLR.Y R.GENESDEQGAIVTVR.G R.RFNLAGNHEQEFLR.Y R.RPFYSNAPQEIFIQQGR.G R.QILQNLRGENESDEQGAIVTVR.G
156	gi|37789212	Ara h 3	8	storage protein	61461	187	9%	5.52	8	K.TANELNLLILR.W K.FFVPPSEQSLR.A R.SPDIYNPQAGSLK.T K.SQSENFEYVAFK.T R.NRSPDIYNPQAGSLK.T
157	gi|1168390	Ara h 1	2	Full=Allergen Ara h 1, clone P17	70240	1385	34%	6.43	41	R.WGPAEPR.E R.PNTLVLPK.H K.AMVIVVVNK.G R.LFEVKPDK.K K.AMVIVVVNK.G + Oxidation (M) K.DNVIDQIEK.Q K.GTGNLELVAVR.K R.NNPFYFPSR.R K.SFNLDEGHALR.I R.DQSSYLQGFSR.N R.DGEPDLSNNFGR.L K.EGALMLPHFNSK.A + Oxidation (M) K.DLAFPGSGEQVEK.L R.IPSGFISYILNR.H R.KSFNLDEGHALR.I R.EGEQEWGTPGSEVR.E R.VLLEENAGGEQEER.G K.GSEEEDITNPINLR.D R.IVQIEARPNTLVLPK.H K.KGSEEEDITNPINLR.D R.RVLLEENAGGEQEER.G R.IFLAGDKDNVIDQIEK.Q K.ISMPVNTPGQFEDFFPASSR.D + Oxidation (M)
158	*no match*									

[a)] Replicate: this protein spot was picked from more than one gel for mass spectrometry

[b)] More than one allergen was detected in this protein spot

Most protein spots on the 2D gel were identified as allergens, predominantly as fragments of Ara h 3 and Ara h 1. In most protein spots more than one isoform of the respective allergens was dectected. Protein spots 92 and 93 were identified as Ara h 8, which was not reported previously.

Supplementary Table 5. Identification of Peanut Proteins after 2D-Gel Electrophoresis Using the Publically Available NCBInr (non-redundant) Database in Mascot (Matrix Science Inc.). The protein matches were regarded as positive if the Mascot search marked them as significant and if at least two different peptides could be matched. Only the protein spots had a match are listed. The spot numbers correspond to Supplementary Figure 4.

Spot no.	NCBI acc no	protein name	MOWSE score	Queries matched	Molecular mass (M_r)[a)]	Sequence coverage [%]	pI[b)]	Matched peptides
26	gi|115187464	thioredoxin fold	62	2	17411	12%	5.57	R.FALLVEDLK.V K.HVTFLADGSAK.Y
58	gi|2342884	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	100	3	51493	5%	6.19	R.IPISYIK.T K.DTDILAAFR.V K.LTYYTPEYETK.D
75	gi|951114	galactose-binding lectin precursor	74	2	29116	7%	5.39	R.VLYAMPVR.I + Oxidation (M) K.FGFSASGSLGGR.Q

88	gi|315229327	annexin-like protein	959	34	36131	47%	8.30	K.FLLTLLGK.Q K.TIIAILGHR.N R.DAVLANVAIR.N K.TQLVATFNR.Y R.DIKELYYK.R K.FLLTLLGKQD.- R.NSVHLEDAVAK.E R.ELLVGLVSSFR.Y R.LESEISGDFER.A K.LLDNQASDDFYK.A R.LAQSEANILHETVK.E K.KLLDNQASDDFYK.A R.SLEEDVAANTTGHLR.E R.KAYEELHQEDLIK.R K.AYEELHQEDLIKR.L R.LAQSEANILHETVKEK.K
89	gi|315229327	annexin-like protein	683	21	36131	46%	8.30	K.FLLTLLGK.Q K.TIIAILGHR.N R.DAVLANVAIR.N K.TQLVATFNR.Y R.DIKELYYK.R K.FLLTLLGKQD.- R.NSVHLEDAVAK.E R.ELLVGLVSSFR.Y R.LESEISGDFER.A R.YRDEHAISISK.K K.LLDNQASDDFYK.A R.LAQSEANILHETVK.E K.KLLDNQASDDFYK.A R.SLEEDVAANTTGHLR.E R.KAYEELHQEDLIK.R R.LAQSEANILHETVKEK.K
108	gi|2342884	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	255	5	51493	12%	6.19	R.IPISYIK.T K.DTDILAAFR.V K.LTYYTPEYETK.D K.TFQGPPHGIQVER.D R.GGLDFTKDDENVNSQPFMR.W + Oxidation (M)

Only six protein spots were identified in peanut using the NCBInr database (http://www.ncbi.nlm.nih.gov/). These proteins were not previously classified as allergens.