

Use of Antibodies Against The Major Peanut Allergens to Screen Peanut Cultivars

Soheila J. Maleki, Research Scientist, USDA-ARS-SRRC, 1100 Robert E. Lee Blvd. New Orleans, LA 70124

We proposed to develop polyclonal antibodies and immunoassays specific to the major peanut allergens. These antibodies are to be used in a variety of screening projects with multiple collaborators. Our overall goal of this project is to find naturally existing, engineered or processed peanuts with reduced allergenic proteins. Funding was received to produce antibodies against the peanut allergens. We are happy to report that we have successfully produced four antibodies and have been screening a variety of samples with these antibodies this year. This year we also plan to clone Ara h 8, purify and produce antibodies against this allergen. Previously, we found errors in the sequence of Ara h 8 clone and therefore repeated cloning and sequencing multiple times. We were finally able to get the correct Ara h 8 sequence following mutagenesis and recloning and have produced the antibody this year and plan to purify it. Meanwhile, we have continued to screen peanut samples from all over the world using existing antibodies. The number of interested collaborators is increasing every year. Accomplishments and objectives will also be discussed below.

Accomplishments:

1. Progeny of any new crosses (performed in the last years), which are continuously being produced by Dr. Isleib, have been screened by SDS-PAGE see additional Table on next page for the newest crosses that were screened this year).
2. **We are very excited to find peanut varieties that have significantly reduced levels of Ara h 1 and 3 (possibly missing both) AND contain the missing Ara h 2 isoform previously incorporated. We still have to screen these antibodies with the antibodies to determine if they are missing Ara h 1 and 3 or just have very low levels of these allergens.**
3. Many peanut samples either have been or are continuously being screened and results reported to collaborators for example peanuts from: TILLING and silencing projects by Dr. Ozias-Akins; differential processing, UC Davis (Dr. Teuber), North Carolina A&T State University (Dr. M. Ahmenda), LSU (Dr. S. Bahna), Univ. Nebraska (Dr. S. Taylor), Cal. State Univ. LA (Dr. Singh), and USDA Univ of Raleigh, NC (Dr. T. Sanders), peanut samples in the environment and on surfaces with King's College, UK (Dr. Gideon lack), development of peanut vaccine n mouse model, McMaster's University (Dr. Manel Jordana) etc. Several publications have and will continue to arise from these studies.
4. Ara h 8 was cloned into an expression vector and the protein was expressed towards producing anti-Ara h 8 antibodies this year. As before, all antibodies produced are readily available to all interested collaborators towards advancing the overall research with maximum efficiency.
5. We are in the process of purifying and developing assays for Ara h 8 antibody.

We continue to:

- improve current assays and implement new and more rapid immunoassays to accelerate the screening process
- Continuous purification of Ara h 1, Ara h 2, Ara h 3, Ara h 6 for use as standards in all experiments. (GPC /USDA contribution)
- Produce, purify and continue to develop and optimize assays for antibodies against Ara h 3 & Ara h 6 and screen variety of peanut samples from different collaborators.

Overall accomplishments to date:

- ◆ We have thus far purified Ara h 1, 2, and 6 from peanuts and recombinant Ara h 3 (acidic subunit) and used them to make 6 antibodies. We have also made four more antibodies against raw and roasted peanut in both rabbit and chicken for a total of 10 very useful antibodies. Antibodies against specific Ara h 3 subunits (basic) are in the works.
- ◆ Antibodies were purified and various immunoassays such as Western blot, Slot Blot, immunoprecipitation and ELISA have been developed, optimized and used to screen literally 1000s of samples for all of these 10 antibodies.
- ◆ In addition to proteins purified from peanut, recombinant Ara h 3 and Ara h 6 protein were cloned, expressed, and a method for purification of the recombinant and native Ara h 3 was developed for antibody production.
- ◆ Ara h 8 cDNA was recently cloned into an expression vector and we have made antibodies to this allergen.
- ◆ The antibodies produced have been used for screening literally over a 1000 samples and have been shared with many as discussed in proposal and above. The production of these antibodies has been much more impactful than originally imagined at the beginning of this project. The impact is apparent from the number of research projects in which these antibodies have been used and the resultant high quality publications.

What is currently in the works or continuing:

-We hope to continue to share the antibodies or continue to screen existing peanut varieties, peanuts subject to silencing and TILLING or other manipulations and many processing treated samples for reduced allergens as well as others mentioned in the past year's accomplishments.

-Helping several national and international companies developing treatments and diagnostic tools for peanut allergy by

providing purified proteins and antibodies to them as controls.

Table of crosses performed last year and seeds available for screening using GPC funded antibodies.

NC Cross No.	Identity or Parentage	Pedigree	Seed Source	Number of F ₂ seeds	FAG	Total seeds for cross
X06342	NC Ac 00013 (Rusty) / NC Ac 02953 (Seed coat color pink)	F1-01: F02	07 L F1N 190-1	115	++	
X06342	NC Ac 00013 (Rusty) / NC Ac 02953 (Seed coat color pink)	F1-02: F02	07 L F1N 190-2	67	++	182
X06343	NC Ac 02262 (Robusto) / NC Ac 00013 (Rusty)	F1-01: F02	07 L F1N 191-1	90	++	
X06343	NC Ac 02262 (Robusto) / NC Ac 00013 (Rusty)	F1-02: F02	07 L F1N 191-2	68	++	
X06343	NC Ac 02262 (Robusto) / NC Ac 00013 (Rusty)	F1-03: F02	07 L F1N 191-3	139	++	297
X06344	NC Ac 02953 (Seed coat color pink) / NC Ac 00013 (Rusty)	F1-01: F02	07 L F1N 192-1	66	++	
X06344	NC Ac 02953 (Seed coat color pink) / NC Ac 00013 (Rusty)	F1-02: F02	07 L F1N 192-2	59	++	
X06344	NC Ac 02953 (Seed coat color pink) / NC Ac 00013 (Rusty)	F1-03: F02	07 L F1N 192-3	43	++	
X06344	NC Ac 02953 (Seed coat color pink) / NC Ac 00013 (Rusty)	F1-04: F02	07 L F1N 192-4	104	++	272

These are the F2 seeds available for analysis with the antibodies. We will be analyzing a sample of F2 seeds from each F1 to look for segregation of the allergens before analyzing all F2 seeds from any single F1 plant, especially for crosses X06343 and X06344. If we want to try associating the reduced allergen phenotypes with molecular markers for ease of selection, it will be important to preserve the identities of the specific F1 and F2 seeds for future reference.

All crosses and the table above were produced by Dr. Thomas G. Isleib
 Department of Crop Science, Box 7629
 North Carolina State University
 Raleigh, NC 27695-7629
 Tel.: (919) 515-3281
 Fax: (919) 515-5657

Most recent publications using GPC funded antibodies (and more are in the works).

1. Guo, B.Z., Liang, X., Chung, S.Y., Holbrook, C.C., Maleki, S.J. (2008) Proteomic analysis of peanut seed storage proteins and genetic variation in a potential peanut allergen, *Protein Peptide Letters*, 15: 567-577.
2. Guo, B.Z.; Liang, X.; Chung, S-Y; Maleki, S.J. (2008) Proteomic screening points to the potential importance of the basic subunit of Ara h 3 in allergenicity of peanut. *Inflammation & Allergy – Drug Targets*, Volume 7, Number 3, pp. 163-166(4)
3. Du Toit, G, Katz, Y., Sasieni, P., Mesher, D., Maleki, S.J, Fisher H., Fox, A.T., Amir, T, Gali, Nitzan G., Kaluski, D., Livne, I., Lack, G. (2008) Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J. Allergy Clin Immunol.* 122(5): 984-91.
4. Clare, D.A., Ghra, G., Maleki, S. J., Sanders, T.H. (2008) Effects of Transglutaminase Catalysis on the Functional and Immunoglobulin Binding Properties of Peanut Flour Dispersions Containing Casein, *J. Agric. Food Chem.* 56 (22), 10913-10921.
5. Chu, Y., Faustinelli, P., Ramos, M.L., Hajduch, M., Thelen J.J., Maleki S.J., Ozias-Akins, P., (2008) Reduction of IgE Binding and Nonpromotion of *Aspergillus flavus* Fungal Growth by Simultaneously Silencing Ara h 2 and Ara h 6 in Peanut. *J. Agric. Food Chem.* 56 (23), 11225-11233.
6. Ozias-Akins, P., Ramos, M.L., Faustinelli, P., Chu Y., Maleki, S.J., Thelen, J.J., Huntley, J., Arias, K., Jordana, M. (2008) Spontaneous and induced variability of allergens in commodity crops: Ara h 2 in peanut as a case study. *Reg. Toxicology and Pharmacology*, 54: 37–40.
7. Chassaing, H., Trégoat, V, Nørgaard. J.V., Maleki, S.J., van Hengel, A.J. (2009). Resolution and identification of major peanut allergens using a combination of fluorescence two-dimensional differential gel electrophoresis, Western blotting and Q-TOF mass spectrometry. *J Proteomics.*72 (3), 511-26. Ramos, M.L., Huntley, J. J., Maleki S.J., Ozias-Akins, P. (2009) Identification and characterization of a hypoallergenic ortholog of Ara h 2.01. *Plant Mol Biol.* 69:325–335.
8. Schmitt, D.A, Nesbit, J.B., Hurlburt, B.K., Cheng, H., Maleki, S.J. (2010) Processing Can Alter the Properties of Peanut Extract Preparations. *J. Agric. Food Chem.* 58 (2), 1138–1143.