

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

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Peanut allergy is a serious problem that needs to be addressed at multiple levels. We proposed to develop polyclonal antibodies and immunoassays specific to three of the major peanut allergens. These antibodies are to be used in a variety of screening projects with multiple collaborators. The overall goal of this project is to find naturally existing peanut cultivars with reduced levels of the allergenic proteins. Funding was received to reproduce antibodies against the Ara h 1, Ara h 2 and Ara h 3 allergens. We are happy to report that we have successfully produced all three antibodies and have been screening a variety of samples with these antibodies. We have also produced antibody against Ara h 6 and will be screening our peanut varieties with this antibody. Other accomplishments will also be discussed below.

Experimental goals proposed by the PI in 2004:

- Protein Purification (Continuous purification of Ara h 1, Ara h 2, Ara h 3, Ara h 6 & recombinant Ara h 3, for use as standards in all experiments. (USDA contribution)
- Improve current assays and implement new and more rapid immunoassays for large scale screening to confirm current screens that have been performed, to accelerate the screening process and purify and develop new immunoassays for the antibodies being made (USDA/GPC funded).
- Production of antibodies against two more of the peanut allergens; Ara h 6 & Ara h 8 (GPC funded)
- Continue to screen various cultivars provided by our collaborators (USDA/GPC funded).

What has been accomplished to date:

Two rabbit **anti-Ara h 1 antibodies** and used to screen for the levels of Ara h 1 in approximately 400 peanut varieties.

A **chicken anti-Ara h 2 antibody** was obtained on the fourth attempt and utilized to screen approximately 400 peanut samples.

Recombinant Ara h 3 protein was cloned, expressed, purified and utilized to produce both **Chicken and Rabbit anti-Ara h 3 antibodies**, which were then purified. Western blot and ELISA immunoassays were developed for these antibodies and utilized to screen 70 of the NC samples and 50 of the 99 samples of the core of the core.

The level of Ara h 1, 2 and Ara h 3 in the core of the core peanut germplasm collection and approximately 300 NC peanut samples was determined. As determined for Ara h 1, some variation was seen in the levels of Ara h 2 in the various cultivars tested, and even though some varieties were found to have reduced levels of Ara h 2 and Ara h 3, none of the 99 samples from the core of the core completely lacked these allergens. Most encouraging of all, is finding a peanut variety from Tom Isleib's NC samples that completely lacks one of the Ara h 2 isoforms and two had significantly reduced levels of Ara h 1. All 300 samples from the NC peanuts and 50 of the core collection have been screened for levels of Ara h 3. Some varieties have been found to lack one or more of the Ara h 3 isoforms. The most promising samples were chosen and sent to Dr. Isleib for breeding this winter. The progeny of these bred peanuts were obtained from Dr. Isleib and screened this summer. **We are very excited to report that we were able obtain cross bred progeny that lacked both one of the Ara h 3 isoforms and the Ara 2 isoform**. The inheritance pattern followed the classic Mendelian (1:15) genetics

Some of the accomplishments not funded by GPC (unless indicated), but related to overall goals:

- A core of the core peanut collection has been formed containing 99 peanut samples (USDA in GA).
- All 99 samples and 270 more from NC have been ground and defatted into peanut meals (Univ. of FL, USDA, LA).
- The percentage of protein in each of 369 peanut samples has been determined from the peanut meals (USDA, LA)
- The amount of soluble peanut protein has been determined for each of these 369 samples (USDA, LA)
- Samples from the NC collection (270) have been screened for the levels of Ara h 1 and Ara h 2 and 3 protein. One cultivar was found to lack an Ara h 2 isoform, one had significantly lower Ara h 1 levels. (USDA, NC, Partially GPC funded).
- Antibodies against Ara h 2, 3 and Ara h 6 have been successfully produced, purified, and optimized for immunoassay (GPC).

What is currently in the works:

- A method for the full purification of natural (or native) Ara h 3 from peanuts is still in progress. (accomplished this year)
- A method for developing a better purification method for recombinant Ara h 3 is being pursued.
- More efficient and rapid immunoassay screening procedures will continue to be analyzed for use in this laboratory.
- We have identified and purified Ara h 6 from peanuts and sequenced it with HPLC it to confirm that it is Ara h 6 and produced antibodies against it for screening studies.

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