

## 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 we also plan to clone Ara h 8, purify and produce antibodies against this allergen. The number of interested collaborators is increasing every year. Accomplishments and objectives will also be discussed below.

### Experimental goals proposed by the PI in 2007:

1. peanut F2 and F3 progeny samples (repeat) from the continuous NC crosses (lost due to Katrina) have been re-crossed by Dr. Isleib after initial re-screening of parental samples (by our lab). We will be screening the new progeny this year as soon as they are sent to us. Progeny of any new crosses, which are continuously being produced by Dr. Isleib will be screened.
2. other varieties and germplasms. Additionally, we utilize the anti-Ara h 3 and Ara h 6 antibodies to complete screening the core of the core collection levels of Ara h 3 and Ara h 6.
3. peanuts from TILLING and silencing projects by Dr. Ozias-Akins, parental strains have been and are being screened as a continuous collaborative project. Other samples from 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), USDA Univ of Raleigh, NC (Dr. T. Sanders), etc are continuously being screened and results reported to collaborators on a regular basis. Several publications will arise from these studies.
4. Ara h 8 has been cloned into an expression vector and the protein will be expressed towards producing anti-Ara h 8 antibodies. As before, all antibodies produced are readily available to all interested collaborators towards advancing the overall research with maximum efficiency.

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 & recombinant Ara h 3, for use as standards in all experiments. (GPC /USDA contribution)
- Produce, purify and develop assays for antibodies against Ara h 3 & Ara h 6 and screen variety of peanut samples from different collaborators (GPC funded)

### Overall accomplishments to date:

Two rabbit **anti-Ara h 1 antibodies** and a **chicken anti-Ara h 2 antibody** were obtained used to screen for the levels of Ara h 1, 2 levels in approximately 500 peanut varieties. 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 all 380 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 380 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 Ara h 1 or Ara h 2, but **several were recently identified that were missing an isoform of Ara h 3**. All 380 samples from the NC peanuts, including progeny from crosses, and most of the core collection have been screened for levels of Ara h 3. Most encouraging of all, was finding a peanut variety from Tom Isleib's NC samples that completely lacks one of the Ara h 2 isoforms, a few missing Ara h 3 isoforms and two had significantly reduced levels of Ara h 1. We were able to cross breed and found progeny that lacked both one of the Ara h 3 isoforms and the Ara 2 isoform. These samples and some additional varieties with reduced Ara h 1 have been re-bred (due to Katrina) by Dr. Isleib and must be screened. Of the parental strains from Dr. Simpson, we have found very interesting type A and B genome species that lack at least one of the allergens or isoforms thereof.

### What is currently in the works:

- Continued Screening of silencing and TILLING peanut varieties for Dr. Ozias-Akins and processing treated samples for reduced allergens as well as others mentioned in the past year's accomplishments.
- A method for the large-scale purification of natural and recombinant Ara h 6 from peanuts is still in progress.
- A method for developing a better purification method for recombinant and native Ara h 3 is being pursued.
- We have cloned recombinant Ara h 8 and plan to express this protein and produce antibodies and immunoassays.
- Helping several national and international companies developing treatments and diagnostic tools for peanut allergy by providing purified proteins and antibodies to them.