TILLING for a Reduced Allergen Peanut

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Peanut (Arachis hypogaea L.) is a crop species with little genetic diversity at the DNA level and limited resources for disease resistance and agronomic traits within the species. Wild relatives contain much more genetic diversity, although they are diploid while cultivated peanut is tetraploid. Since peanut is an allotetraploid, it contains four copies of any one gene. Two of these copies belong to one of the peanut's progenitor genomes (probably A. *duranensis* - A-genome) and two belong to the other progenitor genome (probably A. *ipaensis* - B-genome). The two pairs of copies (A and B) do not normally exchange with each other because of the lack of chromosome pairing between the two. We have used funding from the Georgia Peanut
Commission to characterize the DNA sequence of the multiple copies of two allergen genes, *ara h* 2 and *ara h* 6, and to develop a small TILLING population. TILLING will be described below. We have designed primers based on the DNA sequence generated to allow specific amplification of each of the two *ara h* 2 genes in separate PCR reactions. This approach will simplify TILLING in polyploid peanut.

We are exploring the potential for inducing mutations in DNA sequence (knockout) specifically to alter the allergen composition and content of peanut seeds. In order to achieve gene knockouts, we have employed a technique known as TILLING, Targeting Induced Local Lesions IN Genomes (McCallum et al. 2000. Plant Physiol. 123:439). This method has proved to be valuable for recovering mutations in known genes of *Arabidopsis thaliana*, a model for plant genomics, and several programs are underway to TILL for genes in crop plants such as rice, com, soybean, wheat, and barley. It is almost guaranteed that this method can eventually achieve the intended goal of mutations in allergen genes. We have developed a population of mutagenized peanut that 1) can be used as a genetic/genomics tool for determining the function of a gene and 2) promises to yield valuable germplasm due to the knockout of allergen genes, *ara h* 1 and/or *ara h* 2, which encode for the most allergenic proteins. Knockout of an allergen by mutation would be a safer and more acceptable method for modifying the allergen content of peanut than a transgenic approach to “knockdown” expression. Knockout is the preferred approach at this time if such a peanut will have the potential for commercialization in the near future. While the mutation approach may take several years to succeed, it is not expected to be subject to the same regulatory concerns as transgenic peanut.

The TILLING population resulted from ethyl methane sulfonate (EMS) mutagenesis of Tifrunner (a recent release from Corley Holbrook). EMS has been used because it induces single nucleotide changes in the DNA. DNA contains four different nucleotides, A, T, G, and C, which provide the basis of the genetic code. Mutagenesis with EMS typically causes C/G to T/A transitions, and thereby can alter the amino acid sequence of a protein or truncate it. In fall 2004, we harvested M2 seeds from 1700 EMS-mutagenized M1 plants. Approximately 1450 M2 plants survived in the field in 2005 to produce M3 seeds. Leaves for DNA extraction were collected from each plant in summer/fall 2005. DNA was extracted from ~400 plants and leaves from the remaining M2 plants were frozen at -80C. We have begun to TILL for mutations on 4-fold DNA pools of the first 384 plants. TILLING on each new species requires optimization of DNA extraction procedures and concentration, primer design, amplification conditions, gel runs, gel visualization and scoring. Seed funds from the Georgia Peanut Commission have been instrumental in our receipt of a commitment from the Consortium for Plant Biotechnology to fund a more intensive TILLING effort.

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