

TILLING for a Reduced Allergen Peanut

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Georgia Agricultural Commodity Commission Reports, Feb. 9, 2005

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, and their use for transferring this diversity into cultivated peanut requires years (or decades in some cases) of breeding and selection. 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 them. However, each copy of most genes is likely to be functional unless there has been a mutation since the relatively recent origin of tetraploid peanut. Such natural mutations must be relatively rare since DNA polymorphisms are few. Mutation breeding of peanut where an altered trait is sought probably has not been very successful because of this allotetraploid constitution. We presently are using a mutagenesis approach 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, but it requires that the DNA sequence of genes be known.

We have begun to develop a population of mutagenized peanut that 1) can be used as a genetic/genomic tool for determining the function of a gene and 2) promises to yield valuable germplasm due to the knockout of allergen genes, particularly *ara h 2*, which encodes for one of the most allergenic proteins. Very little presently is known about the allergenicity of Ara h VI, a protein related to Ara h II. Because of their similarities, it has been necessary for us to characterize the genomic sequence of *ara h 6* as well as *ara h 2* prior to the initiation of TILLING. We have determined that it will be more difficult to knock out all of the *ara h 6* genes because there are 3 genes instead of the 2 for *ara h 2* found in cultivated peanut. It also would be difficult to independently knock out one *ara h 2* and one *ara h 6*, then combine the knockouts by recombination, because they are closely linked (within 8000 nucleotides of one another). A knockout of only *ara h 2*, however, could reduce the allergenicity of peanut because these genes are expressed at a higher level than the *ara h 6* genes. In the 2004-05 funding cycle, we have produced a second, and larger TILLING population. This population resulted from ethyl methane sulfonate (EMS) mutagenesis of Tifrunner (a recent release from Corley Holbrook). In fall 2004, we harvested M2 seeds from 1700 EMS-mutagenized M1 plants. We also have completed DNA sequence upstream from the *ara h 2* coding region. This additional sequence has been necessary in order for us to design gene-specific primers for TILLING. In the past year, we have screened a few individuals from the 2003 TILLING population to determine if the mutagenesis treatment had the expected effect. Since we did not have enough DNA sequence information at the time to screen for *ara h 2* mutants, we carried out AFLP (amplified fragment length polymorphism) analysis to search for mutations. Four M3 progeny from each of three putative mutants recovered that showed loss of a band (and a control) will be tested in the next couple of months to verify the absence of the mutated band. Our goal will be to induce at least 1 mutation in every 100,000 base pairs in each plant.