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Project Title: Creating drought tolerant peanut breeding lines for West Texas

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Summary: Our early works indicated that water-deficit induced expression of an isopentenyltransferase gene, *IPT*, could dramatically increase drought tolerance in peanut. However, these works were conducted in small scale experiments and not with homozygous *IPT*-transgenic lines. To determine accurately how much improvement that *IPT*-expression can make in peanut, we must conduct more field-trial experiments with homozygous *IPT*-transgenic plants. The major goal of our research in 2012 was to identify homozygous lines of *IPT*-transgenic peanut plants, so that future field-trial experiments will be conducted by using homozygous lines. Due to the severe weather of 2011, only limited seeds were obtained for each *IPT*-transgenic lines. Consequently, we had to grow the *IPT*-transgenic peanut plants for one more generation under well watered conditions. We grew about 60 plants for each of the four independent lines selected for detailed study. During the summer, we isolated genomic DNAs from half of them and conducted PCR experiments to confirm if they still contain the *IPT* transgene. All PCR positive lines were harvested individually at the end of the 2012 growth season. Now plants from each lines are being analyzed using two methods: physiological response to drought condition and PCR analysis. Only lines with all progenies showing drought resistance phenotype and containing the *IPT* transgene will be considered homozygous lines and these lines will be identified in 2013.

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Objectives: Drought is the No. 1 limiting factor for agricultural production in America's Southwest. Drought-caused agricultural loss in Texas usually averages over \$1 billion per year. To maintain a vibrant agriculture industry and to diversify crops in West Texas, we must increase crop's tolerance to drought. The proposed research is to increase peanut yield and to make peanut production profitable in West Texas. Rotating growing cotton with peanut in West Texas is a beneficial agronomic practice, because peanut is a legume crop that can fix nitrogen, which is good for soil in West Texas. Our overall goal is to create peanut that can use water more efficiently and increase peanut production in the arid/semi-arid land of West Texas.

Procedure:

a. Field analysis of IPT-expressing peanut plants

Because of the record hot summer temperatures and worst drought conditions in 2011, we did not harvest enough seeds to conduct the low irrigation experiments in the field in 2012. Instead, we planted all seeds in the irrigated plot and conducted PCR analysis with leaf tissues of each plant to identify transgenic plants. We analyzed about 30 plants for each of the four transgenic lines and majority of them are *IPT*-transgenic plants. All PCR positive lines were harvested at the end of the growth season.

b. Obtaining homozygous IPT-expressing peanut plants

Because there is no easy and fast way to test if a peanut plant is transgenic plant in laboratory, we have to grow each individual T₃ or later generation plant and isolate DNA to test if it contains the *IPT* transgene. The seeds from 2011 were not enough for us to conduct screening experiments for homozygous lines, we had to grow the *IPT*-transgenic plants for one more generation to get enough seeds. In 2012, we obtained enough seeds that are being used for drought treatment in greenhouse. If no drought sensitive progenies will be found in a particular line, and all progenies in this line contain

the *IPT* transgene based PCR analysis, then this line is homozygous. We believe that we can identify homozygous *IPT*-expressing peanut plants for four transgenic lines, i.e. 3, 7, 11, and 13, in 2013.

Results and Discussion: Although we were not able to conduct a low irrigation experiment in 2012, we were able to generate more than 100 seeds for 30 to 40 segregated progenies of the four independent *IPT*-transgenic lines. In theory, about a quarter of these 30 to 40 segregated progenies should be homozygous already. It will be just a matter of time before we identify them. We are testing the seeds of these 30 to 40 segregated progenies for their resistance to drought and the presence of the *IPT* transgene in their genome. If a homozygous line is found, all of its progenies should be drought tolerant and contain the *IPT* transgene. Early on we observed that *IPT*-transgenic peanut plants produced larger and longer seed pods than wild-type and segregated non-transgenic peanut plants (Fig. 1 below). We observed similar result from last year's experiment, although this time plants were grown in the irrigated plot. We need to confirm this result in future experiments, as this phenotype is a great agronomic trait.

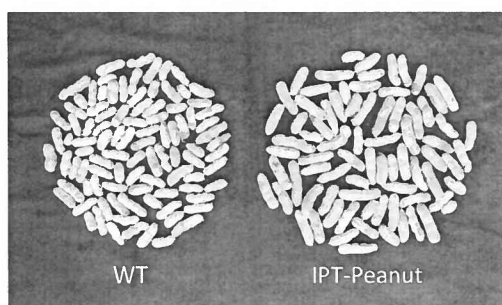


Fig. 1. The phenotype of seeds from wild-type and *IPT*-transgenic peanut plants grown under reduced irrigation conditions. This phenotype was found among the irrigated peanut plants as well.