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FINAL REPORT FOR 2017 NPB/SPRI PROJECT

PROJECT TITLE: Development of artificial tetraploid species for accelerating introgression of disease resistance genes into cultivated peanut

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I. Layman's Summary

One of the most serious problems confronting peanut breeders is the limited genetic diversity within cultivated peanut that results in them being vulnerable to disease and insect epidemics. Wild peanut species contain many traits that are absent in cultivated peanut including disease and pest resistance and drought tolerance. However, direct utilization of these unique genes by conventional hybridization between wild and cultivated peanuts is challenging as the vast majority of wild species are diploid and cannot be crossed directly with tetraploid cultivated peanut.

The goal of this project is to create artificial, wild-derived tetraploid peanut species and to improve the efficiency of interspecific hybridization. During the project period, we treated over 1,000 steam cuttings from the crosses between different wild peanuts. We generated three synthetic tetraploid wild peanut accessions that can be directly used to make crosses with cultivated peanuts. We also conducted backcross by using an elite peanut cultivar, TifNV-High O/L, as the recurrent parent, and the real hybrids were identified by a combination of phenotypic traits and molecular markers. Our efforts provide valuable resource for introgression of disease and pest resistance genes from wild species into cultivated peanuts and will benefit to the peanut community.

II. Project accomplishments:

1. Cutting treatment

In 2017, we treated a total of 1,718 cuttings from 12 different crosses, 435 pods and 93 seedlings which were derived from germinated seeds were obtained from three crosses made at UGA in 2016. However, no pod was harvested from other genotypes (Table 1).

To produce more seeds from the potential tetraploid wild accessions, we planted 43 and two plants from ValSte and Magdu which were generated from the crosses between *A. magna x A. duranensis* and *A. valida x A. stenosperma*, respectively. More than 1,000 pods were harvested from ValSte plants, and 294 pods were harvested from the plants of Magdu. 36 seedlings from Magste derived from *A. magna x A. stenosperma* were transplanted in the spring of 2017 and are expected to grow more seeds.

2. Identification of real tetraploid wild accessions and phenotypic variation

We investigated the morphological characters of the induced putative tetraploids, and found the tetraploids seemed more vigorous than the parents (Figure 1A). The tetraploids produced bigger and yellow flower which is as same as the male parent, *A. stenosperma* (Figure 1B). We also investigated the staining of mature pollen grain in the diploid wild

Table 1. Summary of cutting treatments

Genotypes	Number of treated cuttings	Number of survival cuttings	Number of pegs (pods)
30076 x 10602 *(A. ipaensis x A. diogoi)*	35	18	NCSU, no peg
30076 x 410 *(A. ipaensis x A. stenosperma)*	211	61	NCSU, 2 pegs
30076 x 10309 *(A. ipaensis xA. stenosperma)*	79	3	NCSU, no peg
30076 x 9530 *(A. ipaensis x A. correntina)*	78	13	NCSU, no peg
30092 x 10602 (A. magna x A. diogoi)	24	13	NCSU, no peg
30097 x 10017 *(A. magna x A. cardenasii)*	110	52	NCSU, no peg
30092 x 10017 *(A. magna xA. cardenasii)*	28	3	NCSU, no peg
9484 x 10602 *(A. batizocoi xA. diogoi)*	38	1	NCSU, no peg
9484 x 9530 *(A. batizocoi xA. correntina)*	25	5	NCSU, no peg
A. magna x A. duranensis	232	171	UGA, 12 pods and 22 seedlings
468154 x V10309 *(A. valida x A. stenosperma)*	633	571	UGA, 356 pods and 27 seedlings
K30097 x V10309 *(A. magna x A. stenosperma)*	225	176	UGA, 62+5 pods and 44 seedlings
Total	1718	1087	435 pods, 93 seedlings

and putative tetraploids, both diploid wild species and the synthetic wilds were highly fertile (Figure 1C). The putative tetraploids produced mature pods and seeds which weights are similar to that of *A. valida* but much smaller than that of cultivated peanut TifNV-High O/L (Figure 1D-E). The root tips of synthetic accessions were used to conduct cytogenetic analysis, the results indicated the real tetraploid as 40 chromosomes were found in metaphase for both ValSte and MagDu (Dr. Ana claudia Araujo, personal communication).

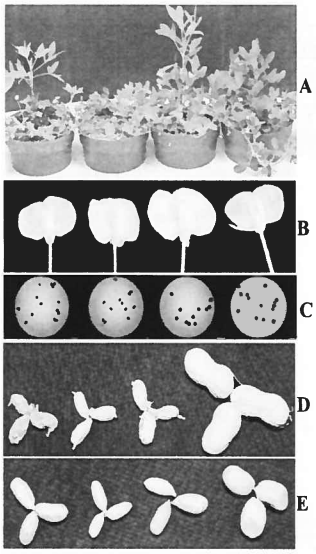

Figure 1. Plant (A), Flower (B), Pollen staining (C), Pods (D) and Seeds (E) of A. valida, A. stenosperma, ValSte (4X) and TifNV (from left to right)

3. Introgression of disease resistance gene from wild species into cultivated peanut

To introduce the disease resistance from wild species into cultivated peanuts, four crosses were made using the two new synthetic tetraploids (Valste and Magdu) and cultivated peanuts (TifNV-high O/L and TifGP-2) in 2017. Totally, 571 flowers of cultivated peanuts were pollinated and 120 potential hybrid pods were obtained (Table 2).

Table 2. Crosses between new synthetic tetraploids and cultivated peanuts

Crosses	Pollinated flowers	Pods	Success rate (%)
TifNV-high O/L/Valste	205	54	26.3
TifNV-high O/L/Magdu	77	31	40.2
TifGP-2/Valste	163	16	9.8
TifGP-2/Magdu	126	19	15.1
Total	571	120	21.0

In the spring of 2018, we planted 96 potential hybrid seeds from the crosses between cultivated peanuts and synthetic tetraploid wilds,and identified 17 real hybrids using molecular markers (KASP) and phenotypic traits including flower colors and sizes (Fig. 2, Table 3). All real hybrid seedlings were transplanted and used as male parents to make backcrosses with TifNV HO/L.

Table 3. Summary of real hybrid

Genotype	Plant date	Number of planted seed	Number of real hybrid	Note
TifNV/Magdu	03/07/2018	20	1	KASP and phenotype
TifNV/Valste	03/13/2018	21	4	KASP and phenotype
TifNV/Valste	05/09/2018	39	8	phenotype
TifGP-2/Valste	04/18/18	15	4	phenotype
Total		96	17	

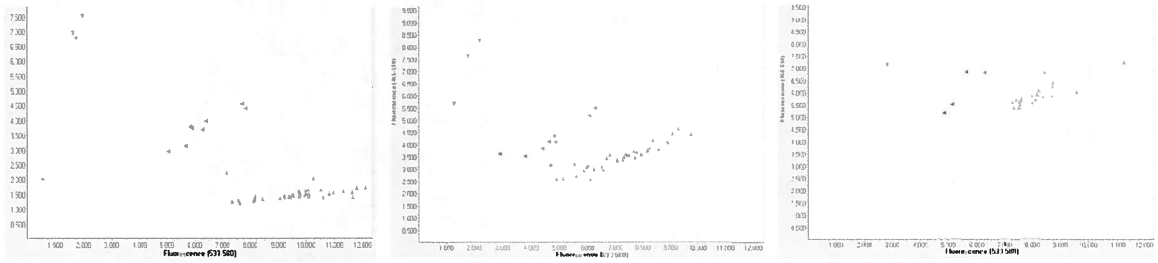

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Fig. 2. Molecular identification of real hybrid of TifNV/ValSte using three KASP markers. The red pots indicated real hybrids, two replicates for each sample.