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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE **Final report**
QUARTERLY PROGRESS REPORT FOR WORK DONE UNDER RESEARCH AGREEMENT

INSTITUTION: University of Georgia

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

RES. AGR. NO.: PROJECT LEADER: Scott Jackson
GACCP Budget No.: GA-175

EXPIRATION DATE: Dec 31, 2017 NPB CONTACT: Bob Parker/Maria Mehok
NPB Budget No.:

Objectives

The aim of this project is to 1) establish a practical method to generate artificial tetraploid peanut species; 2) create new peanut accessions, provide disease resistance resources for peanut breeders, and increase the genetic diversity of peanut cultivars.

Project accomplishments

1). *Growth and identification of real diploid hybrid*

A total of 34 putative hybrid plants derived from five combinations of different diploid wild peanut species were planted in the greenhouse in Athens. As the real hybrids showed similarity for many phenotypic traits such size and shape of leave and plant height (Fig 1a), thus we identified real F1 hybrids based on the flower color (Fig 1b) and/or pollen staining (pollen of parents were staining but pollen of F1 hybrids were abnormal and not staining). 17 real hybrids were identified using these methods (Table 1).

Table 1. Summary of identification of real diploid hybrids

Crosses	No. of plants	No. of true hybrids	Phenotyping methods	
			Flower color	Pollen staining
A.valida PI468154/A.stenosperma V 10309	10	9	yes	yes
A.magna PI468337/A.stenosperma V 10309	5	2	yes	yes
A.magna K30097A.stenosperma V 10309	5	3	yes	yes
A.magna/A.duranensis	3	3	yes	yes
A. gregoryi V6389/A.villosa V12812	11	0	yes	
Total	34	17		

2). *Induction of chromosome duplication*

In 2016 and 2017, a total of 947 cuttings of real hybrids from two crosses (Table 2) using 0.2% colchicine solution from May to September. As of Nov 10, more than 55 pegs were found from the treated cuttings (Fig 1c,d) and two pods were harvested for both crosses. It is expected that more pods will be harvested from the cuttings.

Table 2. Summary of cutting treatment

Crosses	No. of treated cuttings	No. of survival hybrids	No. of pegs	No. of pods	No. of seedlings*
A. valida X A. stenosperma (PI468154X V 10309)	903	687	>300	43	102
A. magna x A. stenosperma (K30097 x V10309)	225	176	>20		
A. magna X A. duranensis	909	745	>30	2	6
Total	2037	1608	> 350	45	108

Note: * means germinated seedlings before harvest.

Figure 1. Plants (a) and flowers (b) of two diploid wild peanut species. The flower of F1 and male parent is yellow but the female parent has orange flower. Cuttingsmain treated with colchicine were maintained in trays filled with white sand (c). One peg has emerged from treated cuttings that suggested successful chromosome duplication of diploid hybrid peanut (d).

3). *Growth and phenotypic variations of synthetic tetraploid wild species*

In the spring and summer, 53 tetraploid wild plants (48 from Valste and 5 from Magdu) were phenotyped. Variations in branch angles are detected between different individuals

(Figure 2), Interestingly, differences in flower size are found, and some flowers show distinct patterns of flower color and unique flower shape such as rose flower (Figure 3).

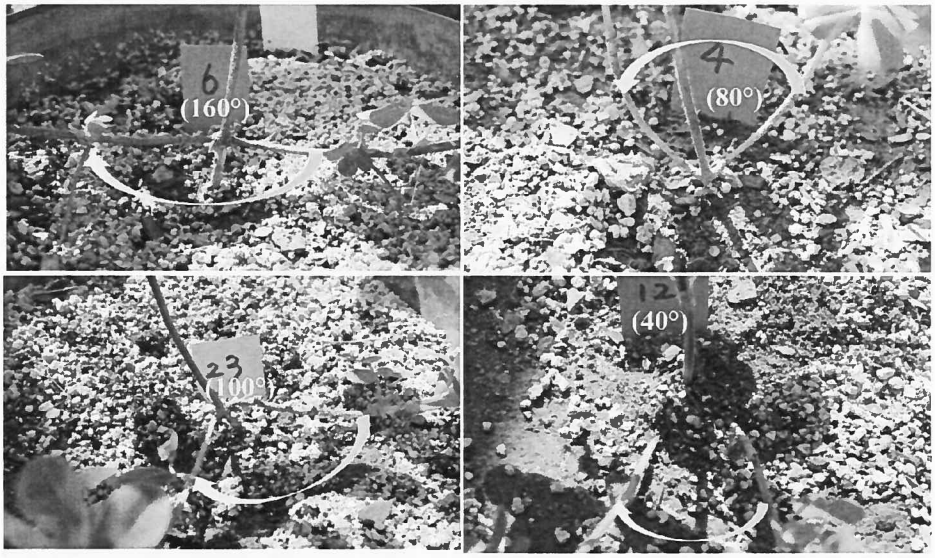

Figure 2. Variations in branch angles of tetraploid wild plants from Valste.

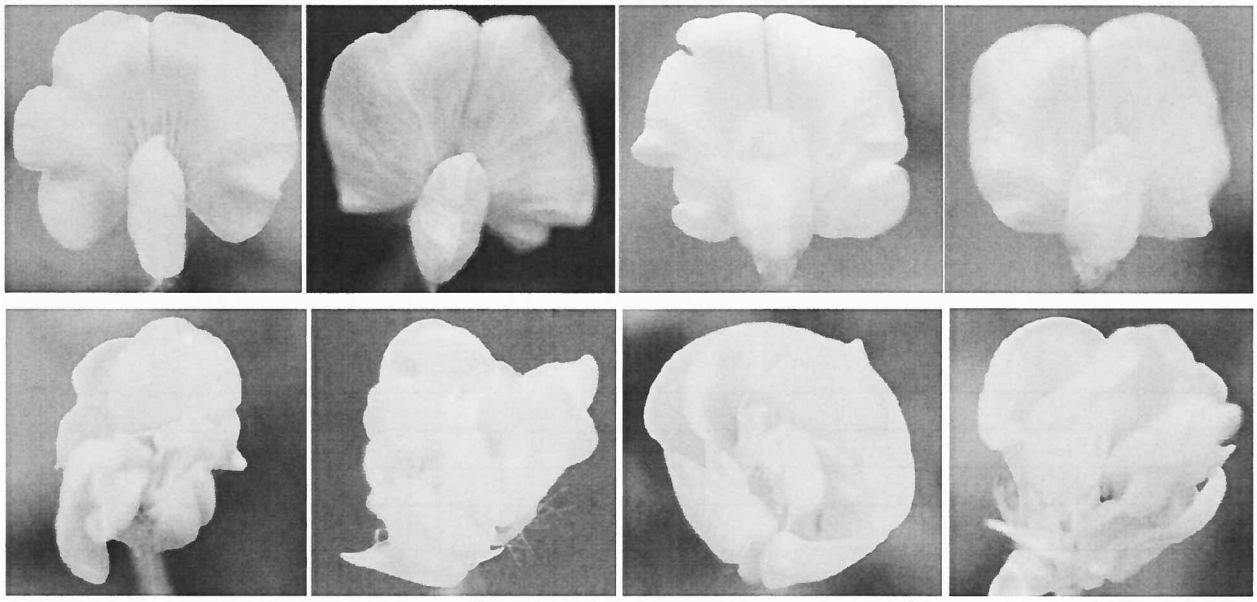

Figure 3. Variations in flower shape and color of tetraploid wild plants from Magdu.

4) Pollen staining

Using the acetocarmine solution, I investigated the pollen viability of tetraploid wild individuals and their parents, all tetraploid plants investigated show more than 90% staining rate (Table 3, Fig 4). Combining the pegs emerging from the synthetic wild plants, this data suggest that the new wild materials are likely allotetraploid plants.

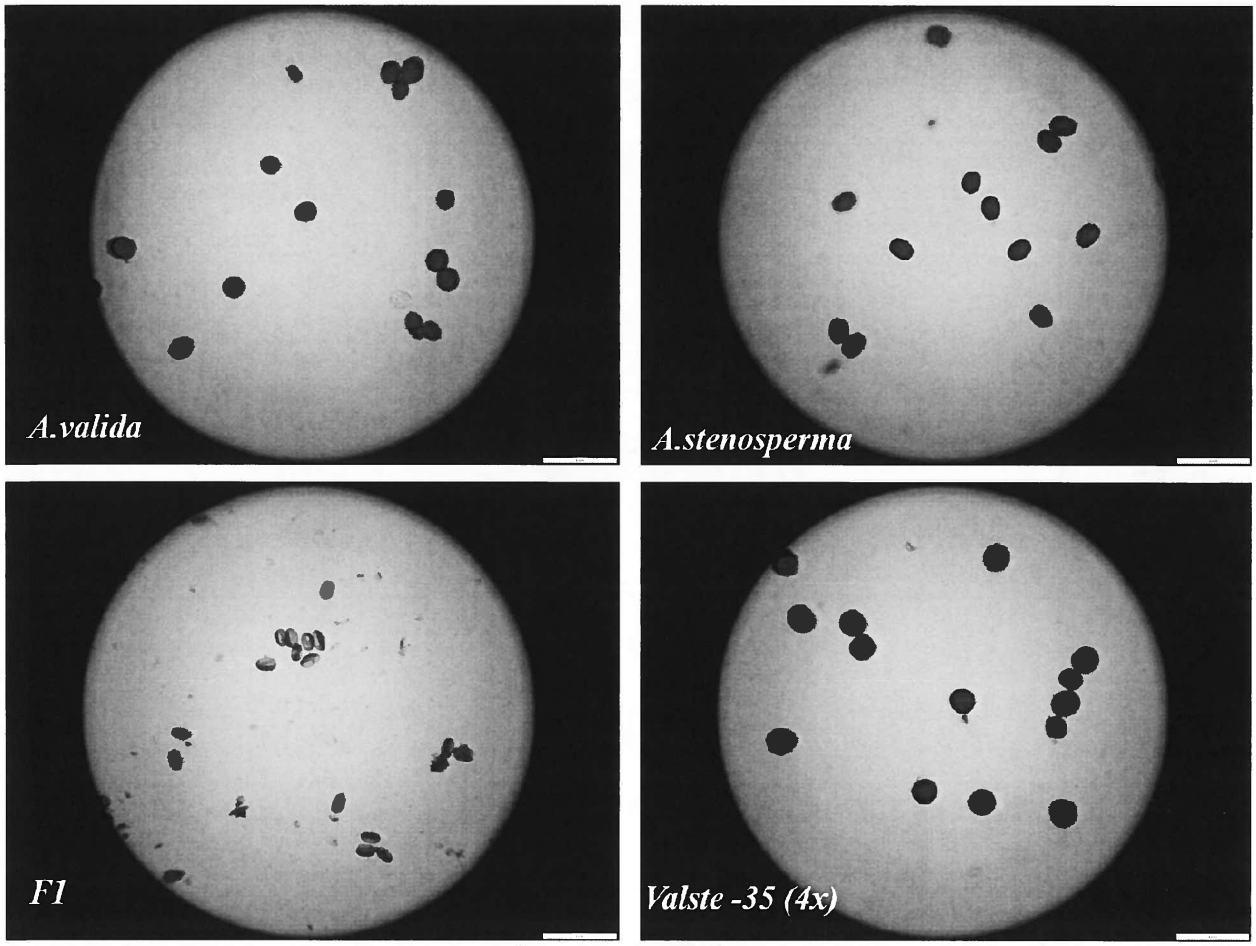

Figure 4. Pollen staining of diploid parents, F1 and tetraploid wild plants.

Table 3. Pollen staining of tetraploid plants

Plant	Total pollen	Staining pollen	Staining rate (%)
A. valida 468154	2394	2230	93.1
A. stenosperma V10309	1771	1707	96.4
A. valida/ A. stenosperma	847	0	0
Valste -1 (4X)	1581	1447	91.5
Valste -2 (4X)	1842	1720	93.4
Valste -3 (4X)	1573	1453	92.4
Valste -6 (4X)	1718	1630	94.9
Valste -9 (4X)	1795	1623	90.4
Valste -35 (4X)	1665	1558	93.6
TifNV-high O/L	1715	1633	95.2

5). *Crosses between the new synthetic tetraploids and 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),

Table 4. 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

6). Ongoing work

A. cytogenetic analysis of roots of synthetic wild peanuts.

B. sequence analysis of affymetrix genechip.

We have extracted DNAs from diploid wild peanuts and 48 tetraploid plants and sent to affymetrix genechip analysis.