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NATIONAL PEANUT BOARD / SOUTHEAST PEANUT RESEARCH INITIATIVE

FINAL REPORT for WORK DONE UNDER RESEARCH AGREEMENT APPA-RIA16-PID 488 BID 1549

PROJECT PERIOD:	1 January 2017–30 June 2018, no cost extension to 12/31/2018
INSTITUTION:	Auburn University
PROJECT TITLE:	The Role of Rhizobia in Peanut Drought Resistance
RES. AGR. NO.:	APPA-RIA16-PID 488 BID 1549
PROJECT LEADER:	Drs. Yucheng Feng and Charles Chen Auburn University, Auburn, AL
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Final Report for Year 1:

Summary

Drought stress is one of the major environmental factors affecting peanut productivity; its effect can be economically devastating when occurring at critical growth stages. Breeding drought-tolerant and high-yielding peanut varieties will help mitigate the drought impact. Rhizobia are soil bacteria that can form symbiosis with legumes and result in the fixation of atmospheric N_2, thereby reducing or eliminating the need for N fertilization. Our on-going study showed that drought stress negatively affected symbiotic N_2 fixation in peanut and the impact of middle-season drought was greater than that of late season. In spite of the observed negative impact, some high-yielding, drought-tolerant genotypes can obtain more N from N_2 fixation than others. In this project, we seek to understand the role that rhizobia play in peanut drought resistance. We conducted greenhouse experiments using three peanut genotypes (two drought-tolerant and one drought-susceptible), subjected to middle-season and late-season drought treatments. A total of 87 bacterial isolates were obtained from root nodules, 15% (13 isolates) of which were positive for ACC deaminase activity. The 13 ACC deaminase positive strains were identified by 16S rRNA gene sequencing and further characterized phenotypically. Three of the 13 ACC-deaminase positive isolates were able to nodulate peanut plants. We are in the process of further characterizing these three isolates and generating ACC-deaminase deficient mutants to determine the role of rhizobia in peanut drought resistance.

Project Title: The Role of Rhizobia in Peanut Drought Resistance

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Objectives:

1. Determine if there is a correlation between drought tolerance and concentrations of ACC in peanut roots.
2. Determine the ACC deaminase activity in root nodules and rhizobia isolated from root nodules of various peanut genotypes.
3. Determine the ACC deaminase activity in the rhizosphere of various peanut genotypes.

Procedures:

In Year 1 of the project, we conducted greenhouse experiments using three peanut genotypes, subjected to middle-season and late-season drought treatments. The experiment was a 3 (genotypes) x 2 (harvest dates) x 4 (treatments) arrangement with three replications. The three genotypes used were Tifrunner, C76-16, and AU-587. Both middle-season and late-season drought treatments lasted for two weeks followed by a one-week recovery period. Plants harvested after each recovery period were used to isolate rhizobia. Roots were excavated and two nodules per plant were used for rhizobial isolation. Crushed root nodule suspensions were streaked on yeast extract-mannitol-Congo red agar (YMA); single colonies were selected and streaked on fresh YMA plates repeatedly until pure cultures of rhizobia were obtained. To identify the isolates, bacterial DNA were extracted using Qiagen DNeasy UltraClean Microbial kits. DNA samples were purified and sent to a commercial lab for sequencing of 16S rRNA genes.

To determine ACC deaminase activities, the rhizobial isolates were grown in a minimal medium containing ACC as a sole nitrogen source to induce the expression of ACC deaminase genes. ACC deaminase activity of each bacterial isolate were determined by measuring the production of alpha-ketobutyrate using a spectrophotometer. Rep-PCR were performed on ACC deaminase positive strains to identify similar strains using BOX A1R primer. Presence of ACC deaminase structural gene, *acdS,* in the isolates were determined by PCR using previously published primers (*forward* 5'-GGCAAGGTCGACATCTATGC-3' and *reverse* 5'-GGCTTGCCATTCAGCTATG-3'). The reaction mixture contained 12.5 µl of GoTaq Master mix, 2.5 µl of extracted DNA, 1 µl of each primer (10 µM) and 0.5 µl of BSA (20 mg/ml) and 7.5 µl of PCR grade water. The thermocycling conditions were as follows: 5 min. of initial denaturation at 95°C, 35 cycles of 1-min denaturation at 95°C, 40-s primer annealing at 60°C, and 2 min of elongation at 72°C, followed by a final elongation of 5 min at 72°C.

Other phenotypical characteristics determined for the isolates included growth curves, production of indole acetic acid, and resistance to streptomycin. Nitrogen fixation potential was

screened by growing inoculated peanut in Leonard jars (Fig. 1) for 35 days. We also conducted extensive literature search in order to develop the analytical method for ACC quantification and initiated method development for analyzing ACC by HPLC.

Results and Discussion

We conducted greenhouse experiments using three peanut genotypes (Tifrunner, C76-16, and AU-587), subjected to middle-season and late-season drought treatments. A total of 87 bacterial isolates was obtained from root nodules. Thirteen of the isolates (15%) were positive for 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. We have identified all 13 ACC deaminase positive isolates by 16S rRNA gene sequencing, four of which were identified as *Bradyrhizobium japonicum* (Table 1). Three isolates were identified as *E. coli*, which may be erroneous. Other methods of identification will be used to verify the classification. Phenotypical characteristics of these isolates, i.e., ACC deaminase activity, production of indoleacetic acid (IAA), and resistance to streptomycin are shown in Table 1. Among the ACC deaminase positive isolates, three were sensitive to streptomycin and only two produced the target PCR product using the primer set previously reported. The nitrogen fixation potentials of all 13 isolates have been evaluated using Leonard jars. Three of the 13 ACC-deaminase positive isolates were able to nodulate peanut plants, none of which contained the PCR product targeted by the *acdS* primer set used. Further experiments will be conducted to develop new primers for the *acdS* gene.

In Year 1 of the project, we also worked on method development. We completed method development for quantifying ACC deaminase activity and initiated method development for analyzing ACC by HPLC and for detecting ACC deaminase genes.

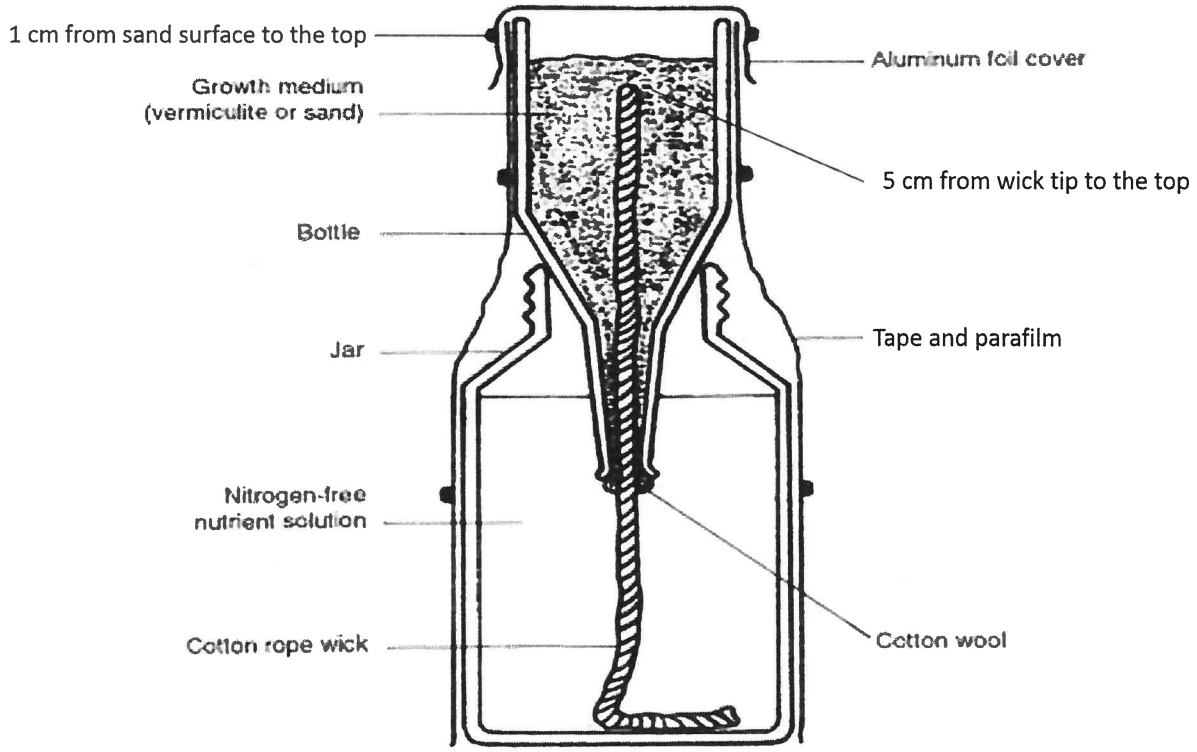

Figure 1. Diagram of a Leonard jar (Somasegaran and Hoben, 1994).

Table 1. Selected characteristics of the bacterial isolated obtained from peanut root nodules

Pot #	Peanut Genotype	Treatment	16S Identification	ACC deaminase activity (umols/mg/h)	Streptomycin		Non-Streptomycin		IAA		PCR product
					R1	R2	R1	R2	mean	SD	
2	587	Irrigated	*Bradyrhizobium japonicum*	*1.162 ± 0.13*	+	+	+	+	7.90	2.12	-
6	587	Drought	*Rhizobium sp. / Agrobacterium sp.*	*0.522 ± 0.30*	+	+	+	+	0.70	0.47	-
7	Tifrunner	Drought	*Labrys sp.*	*1.031 ± 0.11*	+	+	+	+	16.38	0.78	+
9	587	Drought	*Bradyrhizobium japonicum*	*0.910 ± 0.25*	+	+	+	+	4.55	3.93	-
10	Tifrunner	Irrigated	*Mesorhizobium sp. strain PZS_B02*	*0.780 ± 0.30*	+	+	+	+	2.73	0.38	-
23	Tifrunner	Drought	*E. coli*	*0.201 ± 0.09*	-	-	+	+	1.67	0.76	-
27	Tifrunner	Irrigated	*E. coli*	*0.897 ± 0.17*	+	+	+	+	1.31	1.30	-
28	C76-16	Irrigated	*Burkhoderia sp.*	*0.417 ± 0.09*	+	+	+	+	0.89	0.56	-
31	Tifrunner	Drought	*Bradyrhizobium sp.*	*0.831 ± 0.12*	+	+	+	+	1.50	1.36	-
32	C76-16	Drought	*Mesorhizobium sp.*	*1.010 ± 0.12*	-	-	+	+	0.14	0.15	-
56	C76-16	Drought	*Caulobacter sp.*	*0.173 ± 0.10*	+	+	+	+	41.13	7.01	-
60	587	Irrigated	*Bradyrhizobium sp.*	*0.315 ± 0.11*	+	+	+	+	1.11	1.34	-
66	C76-16	Irrigated	*Rhizobium sp.*	*0.877 ± 0.03*	+	+	+	+	5.49	0.50	+
69	Tifrunner	Irrigated	*E. coli*	*1.301 ± 0.20*	-	-	+	+	2.56	2.59	-