**REQUEST FOR FUNDING OF RESEARCH**

**2019-2020 FUNDING CYCLE**

**TITLE: Evaluation of a novel drought-tolerant inoculant on soybean yield in the Mid-South**

**INVESTIGATORS:**

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**PROGRAM AREA (check all that apply):**

\_\_\_ Management of weeds, to include resistance management and economics

\_\_\_ Irrigation/water management

\_\_\_ Quality of harvested seed—Phomopsis/Seed rot

\_\_\_ Disease Management/Control

\_\_\_ Fertility needs (especially P and K) for optimum and economical yield

\_\_\_ Insect management/Control, especially late-season populations

\_\_\_ Harvest aids

\_\_\_ Iron Chlorosis

\_\_\_ Nematode management/control

\_\_\_ Rotations using soybeans

\_V\_ Research Validation or Demonstration

\_\_\_ Producer Communications

\_\_\_ Variety Trials

\_\_\_ Economics

\_V\_ Other (*Inoculants, N2-fixation*)

**PROJECT STATUS:**

New \_V\_\_ *(length of project–1 of 3)*

Renewal \_\_\_\_ (Year of )

Stand alone \_V\_ or cross-commodity \_\_\_ (*additional funding from other sources?*)

**2019 FUNDING REQUEST** \_$60,000\_\_

|  |  |  |  |
| --- | --- | --- | --- |
| **BUDGET FOR 2019 MSSB RESEARCH PROJECT** | | | |
| CATEGORY *(see below for guidelines)* | | ORIGINAL |  |
| A. | Personnel (No Permanent Salaries) |  |  |
|  | 1. Salaries (See Addendum B) | $18,000 |  |
|  | 2. Wages (hourly workers) |  |  |
|  | 3. GRA (include tuition) | $9,100 |  |
| B. | Fringe Benefits (% *used to calculate*) | $1,800 (10%) |  |
| C. | Travel | $11,100 |  |
| D. | Contractual Services | $8,000 |  |
| E. | Subcontracts | $9,000 |  |
| F. | Commodities | $2,000 |  |
| G. | Publication Costs | $1,000 |  |
| H. | Other Costs *(Define)* |  |  |
| TOTAL COST | | $60,000 |  |
| (MSSB does not allow indirect costs/overhead charges) | | | |
| **Personnel**: Show number of hours x hourly rate for each category. GRA cost to include tuition and books. MSSB does not pay salaries of principal investigators or cooperating scientists (USB Compliance Manual, Sect. 17, Part 2-D).  **Fringe Benefits:** Amount and rate for indicated salaries, wages, and GRAs must be shown.  **Travel**: Out-of-pocket and per diem expenses (hotel and meals) for site visits, travel to and from meetings (e.g. airfare, vehicle mileage not to exceed the IRS rate), etc.  **Contractual Services**: External lab fees, consultants, etc.  **Commodities**: Expenses related to the conduct of the project (e.g., seed, fertilizers, pesticides, lab supplies).  **Additional Details**: Provide for each budget category in addendum if necessary.  **Reminder**: All payments for project activities are on a reimbursable basis based on an itemized invoice submitted to MSSB each quarter along with a progress report.  **Budget Transfers**: The MSSB follows USB guidelines, which state “The PI may transfer funds amongst budget categories only with MSSB’s prior written consent if (i) the amount transferred exceeds 10% of any one general budget category (per annual period) or (ii) the funds transferred are travel related. PI shall request permission from MSSB for all budget reallocations and account for them in his/her financial report.”  **Additional Instructions:** See Addendum B. | | | |

**TECHNICAL SUMMARY**

It has been well established that the microorganism *Bradyrhizobium japonicum* has a beneficial impact on soybean plants. By using this mutualist partner as an agricultural inoculant, the rewards can be measured with crop production expressed as soybean yield and quality. However, more than 95% of the bacteria die before they can form a symbiotic relationship in the field due to heat and desiccation. Therefore, isolating and evaluating *Bradyrhizobia* strains for drought-tolerance could help mitigate the negative effect of environmental stressors when applied in the field. Soybean crop production can then be evaluated when drought-tolerant inoculants are used compared to commercial inoculants.

Previously, we isolated three drought-tolerant strains in Texas and the two strains (TXVA-1107-3003 and TXEA-1109-2000) showed outstanding performance in nodulation, nitrogen fixation, and enhancing plant growth and production. To improve the inoculants’ performance and optimize the benefits of biological nitrogen fixation in the Mid-South, we will evaluate the effects of the drought-tolerant inoculant on soybean yield at drought-prone sites in six states including AR, LA, MO, MS, TN, and TX. The objective of this proposal is to provide farmers in the Mid-South with i) documented performance data for the novel inoculant (i.e., TXVA-1107-3003) in soybean production under drought and ii) biological information (e.g., molecular data, whole-genome sequencing, RNA-Seq, etc.) to support release of the drought-tolerant inoculant.

For the first year trial (year 2019), three inoculation treatments (drought-tolerant inoculant, commercial inoculant, and no inoculation) will be compared under irrigated vs. non-irrigated conditions at drought prone sties in the Mid-South. At the first sampling, the number of nodules per plant will be counted and nodule size will be measured. A second harvest of plants will be performed to evaluate final soybean seed production. The climate and weather factors (e.g., precipitation, temperature, and humidity) for each location will also be monitored.

At the completion of the proposed research, we expect to provide positive effects of the drought-tolerant inoculant on soybean profitability and aid Mid-South producers in better understanding of the potential benefits for biological nitrogen fixation. We believe that providing such information will allow soybean producers to advance the management of soybean plants and inoculants for economical and ecological benefits.

**OUTLINE OF RESEARCH**

**I. RATIONALE/JUSTIFICATION**

The soil bacterium *Bradyrhizobium japonicum* is an agriculturally important microorganism because it is the endosymbiont of the soybean plant which is widely used in the food industry, and soybean oil accounts for more than 50% of biodiesel production in the U. S. The increase in the demand for soybean production has led to several practical approaches to achieving increased crop yields, ranging from utilizing early planting dates to the development of genetically engineered herbicide-tolerant and/or insect-resistant soybeans. However, little has focused on the fundamental improvement of nitrogen fixation by *B. japonicum* whose cultures have been applied as inoculants (i.e., biological fertilizers) to the field.

Heat and dry conditions in summer are the major environmental stresses in Mid-South (e.g., AR, LA, MO, MS, TN, and TX) soybean fields that inoculants must overcome to establish successful nodulation on soybean plant roots. However, Mid-South researchers and producers rarely evaluate desiccation and heat tolerance of inoculants in the fields where the average temperature and dry index are higher than those of the major soybean producing states (i.e., IA, IL, etc.). For example, those states produce almost 200 times more soybean crops than that of the state of Texas: in 2015, IA, IL, and TX produced 553.70, 544.32, and 2.99 million bushels of soybeans, respectively. Therefore, improving *Bradyrhizobium* inoculants’ performance under dry and heat stress conditions will benefit Mid-South soybean producers in terms of better nitrogen fixation which ultimately leads to enhanced soybean crop yield.

The use of drought-tolerant inoculants has profound economic benefits by reducing the financial cost of nitrogen fertilizers. Currently, average U.S. farm prices of the nitrogen fertilizers are as follows: anhydrous ammonia, $847; nitrogen solution (30%), $410; urea, $592; ammonium nitrate, $544 per ton, where price has increased in each category since 2011. In addition, enhancement of biological nitrogen fixation has significant ecological benefits by reducing the use of nitrogen fertilizers that are a significant source of greenhouse gas emissions and cause degradation of water sources ranging from groundwater, where derivatives of nitrogen cause “blue-baby” syndrome, to oceanic systems where increased nitrogen leads to hypoxia (lack of oxygen) and large scale “dead zones” (e.g., in the northern Gulf of Mexico).

With the use of a novel drought-tolerant inoculant, soybean crop production could be improved in the Mid-South. We previously identified drought-tolerant *Bradyrhizobia* strains by using a newly developed molecular marker system in our laboratory. These strains were then tested for their symbiotic efficiency and effect on soybean production in South Texas. The use of these drought-tolerant inoculants improved soybean yield as compared to no treatment and the commercial inoculant. **By applying these drought-tolerant inoculants in the Mid-South states, we expect to improve soybean crop yields.**

***A summary of the previous work related to the proposed research:***

Among 30 strains isolated in Texas, we were able to identify and select the three native, drought-tolerant Texan strains (i.e., TXCP-01, TXVA-1107-3003, and TXEA-1109-2000 isolated from Delta, Victoria, and Lubbock counties, respectively). In addition, we have been able to publish work on stress biology of *Bradyrhizobium* inoculants in a number of journals since 2011, including Nature Communications, Applied and Environmental Microbiology (AEM), the #1 cited microbiology journal in the world.

The three drought-tolerant strains were tested for their performance in nodulation and plant growth. After performing symbiotic efficiency tests at Rio Farms in TX, the two strains (i.e., TXVA-1107-3003, and TXEA-1109-2000) proved successful in enhancing plant growth and production. Plot layout is visualized in Figure 1. Plants inoculated with the isolated strains showed significant differences in average plant dry weight and average seed production as compared with the commercial strain (Table 1). For nodule number, plants inoculated with the strains TXVA-1107-3003 and TXEA-1109-2000 had significantly more nodules than the commercial strain (Table 1).

**II. OBJECTIVES**

To evaluate and improve inoculants’ performance and optimize the benefits of biological nitrogen fixation under drought conditions, it is important to fully understand how a novel drought-tolerant *Bradyrhizobium* inoculant responds to this stress. The ***objective of this proposal*** is to provide farmers in the Mid-South with i) documented performance data for the novel inoculant (i.e., TXVA-1107-3003) in soybean production under drought and ii) biological information (e.g., molecular data, whole-genome sequencing, RNA-Seq, etc.) to support release of the drought-tolerant inoculant. By addressing this objective, we will provide Mid-South soybean producers with empirical and economic benefits of the drought-tolerant *Bradyrhizobium* inoculant.

To accomplish the objective of this proposal, we will pursue three specific objectives:

1. **Evaluate the effects of the TXVA inoculant on soybean yield (one variety, maturity group [MG] IV) at drought-prone sites in AR, LA, MO, MS, TN, and TX (Year 1).**
2. **Evaluate the effects of the TXVA inoculant on soybean yield of five varieties (from MG III, IV, V and drought-resistant varieties) at drought-prone sites in AR, LA, MO, MS, TN, and TX (Year 2).**
3. **Provide the Mid-South soybean industry with fact sheets that will assist farmers to obtain and apply the efficient and drought-tolerant *Bradyrhizobium* inoculant (Year 3).**

***We are well positioned to undertake this proposed research,*** because Chang’s group has isolated several drought-tolerant rhizobia in TX and developed a novel molecular marker system to evaluate drought tolerance of rhizobial inoculants. In addition, collaborators (Drs. Leandro Mozzoni, Trey Price, Pengyin Chen, Rusty Smith, Vince Pantalone, Avat Shekoofa, and James Grichar) in the Mid-South will help test these inoculants on soybean plants in drought-prone fields. Chang’s group has many years of experience in testing drought-tolerant inoculants in soybean fields. In 2016 and 2017, field trials were performed at Rio Farms in the lower Rio Grand Valley of TX, in which drought-tolerant inoculants were evaluated for their impact on soybean growth and yield.

**III. APPROACH AND EXPERIMENT CONDUCT:**

**Objective #1. Evaluate the effects of the TXVA inoculant on soybean yield (one variety, MG IV) at drought-prone sites in AR, LA, MO, MS, TN, and TX.**

Drought-tolerant (TXVA-1107-3003) and commercial (Cell TechTM) inoculants will be applied to one soybean variety during planting time for each location. For planting, buffer rows and alleys will be implemented between treatments to prevent cross-contamination of inoculants. Inoculants (cell density [e.g., 109 cells/ml], growth requirements [e.g., mid-log phase], application [e.g., 1 liter per acre], etc.) will be adjusted and standardized across all treatments and locations. Plot layout is described as follow: For one set of the experiment, soybeans will be planted approximately 2 inch apart (e.g., 6-8 seed/ft) down 2 × 30-foot rows with at least 10-foot alleys. Each row is ca. 24-36 inch apart, and one row will be left unplanted between treatments to avoid cross-contamination. There will be 3 treatments (TXVA, Cell Tech, and no inoculation) with irrigation vs. non-irrigation, which results in a total of 6 treatments. In addition, there will be three sets (i.e., three biological replicates) per treatment for the 2019 trial.

**Project locations:** Below are experiment locations in each state.

*AR: Arkansas Agricultural Experiment Station, Monticello, AR.*

*LA: LSU AgCenter, Macon Ridge Research Station, Winnsboro, LA*

*MO: University of Missouri-Delta Center, Portageville, MO*

*MS: USDA-ARS, Stoneville, MS*

*TN: West Tennessee Research & Education Center, Jackson, TN*

*TX: Texas A&M AgriLife Research Site-Yoakum, TX (29.1729 N, 97.0815 W)*

At approximately stage R1 of soybean growth, samples will be collected to evaluate nodulation, plant height, and plant dry weight. Mature nodules can be observed ca. 4 - 6 weeks after infection. Sampling will be carried out by shaking to remove all the loosely attached soils while monitoring the integrity of fine roots. We will cut plant roots containing nodules, immediately wash them with sterilized deionized distilled water, and then place them into 30% glycerol. The number of nodules per plant will be counted and nodule size will be measured. A second harvest of plants will be performed to evaluate final soybean seed production. The climate and weather factors (e.g., precipitation, temperature, and humidity) for each location will be monitored when evaluating plant growth and production.

**Objective #2. Evaluate the effects of the TXVA inoculant on soybean yield of five varieties (from MG III, IV, V and drought-resistant varieties) at drought-prone sites in AR, LA, MO, MS, TN, and TX.**

Based on the result of Objective #1, we will plan to evaluate the performance of the TXVA inoculant in biological nitrogen fixation, nodulation, and crop production on five different varieties (MG III - VII). We will also include drought-tolerant soybean varieties to examine if there is a synergetic effect on plant growth and production. For each soybean variety, the same experimental procedure and sampling strategy will be applied as described in Objective #1.

*We will determine economic return of soybean production expense for native vs. commercial inoculation in the Mid-South.* Results obtained in this objective will be used to compare soybean performance including yield (bushels/acre) and oil content as well as nodulation properties such as nodule numbers per plant, nodule distance from the ground, and N-fixing ability between soybeans with commercial strains and those with the drought-tolerant inoculant. We will also calculate and determine the economic return of soybean production expense per acre.

**Objective #3. Provide the Mid-South soybean industry with fact sheets that will assist farmers to obtain and apply the efficient and drought-tolerant *Bradyrhizobium* inoculant.**

To provide the soybean industry in the Mid-South with fact sheets, we will obtain biological information of the drought-tolerant inoculant. Biological information will include free-living phenotype, kinetics of nodulation, and nitrogenase activities. We will also consider whole-genome sequencing of the inoculant strain.

*a) Free-living phenotype:* It has been known that extracellular polysaccharide (EPS) production is induced by desiccation stress. We will compare EPS production between the drought-tolerant inoculant (TXVA-1107-3003) and the type strain *B. japonicum* USDA110. Another phenotype we will compare is biofilm formation, since the hallmark of biofilms is EPS. Differential production of EPS is likely to influence biofilm forming ability. The standard microtiter-based biofilm assay using crystal violet will be conducted to compare biofilm formation between the two strains. Not only are nodulation properties of bacteria affected by the amount of EPS, but they also affected by different compositions. EPS-I and EPS-II produced by *S. meliloti*, a nitrogen-fixing alfalfa symbiont, are good examples. Therefore, we will compare glycosyl compositions of EPS between the drought-tolerant inoculant and the type strain. Compositional analysis will be done at Complex Carbohydrate Research Center (CCRC), The University of Georgia.

*b) Kinetics of nodulation:* We will compare the nodulation abilities of the drought-tolerant inoculant and the type strain. Briefly, soybean seeds will be surface-sterilized with 20 % (v/v) bleach and 0.01 M HCl and rinsed with sterile ddH2O. The washed soybean seeds will be placed on pre-soaked Wattman paper in petri dishes, and germinated in the dark at room temperature for 2 or 3 days until the length of root will be between 2 and 3 cm. The germinated soybean seeds will be aseptically placed in pouches and inoculated with 1 ml of the cell suspension (OD600 = 0.1) of the drought-tolerant inoculant or the type strain. The position of the root tip will be marked on the surface of the pouch. Kinetics of nodulation will be assessed at several time points (e.g. every week after inoculation) by counting the number of nodules and measuring the distance of all nodules from the root tip mark to see if delayed nodulation occurs. This experiment will be done in a non-destructive manner due to the transparent nature of plastic pouches.

*c) Measuring nitrogenase activity:* We will compare the nitrogenase activity of isolated soybean mature nodules infected with the drought-tolerant inoculant or the type strain. To quantify the nitrogenase activity, we will apply the sensitive acetylene reduction assay (ARA) on a specific mass of mature nodules. ARA is based on the reduction of the acetylene (C2H2) to ethylene (C2H4) by the nitrogenase. More specifically, isolated nodules will be exposed to atmosphere containing 10% acetylene. Gas will be collected from 10 min to 1 h (10, 20, 40 and 60 min) and analyzed by gas chromatography to measure ethylene content. This technology is available at the Chang’s Lab (UT-Arlington). We will perform these experiments on three independent biological replicates.

**PROJECTED IMPACT OF RESULTS ON MID-SOUTH SOYBEAN PRODUCTION**

Based upon empirical evidence with the use of drought-tolerant inoculants, an increase of soybean production and yield (e.g., ca. 20-30%) can be expected under drought stress conditions. Not only will evaluating and isolating drought-tolerant inoculants provide positive effects on soybean profitability, but also aid Mid-South producers in better understanding the potential benefits for biological nitrogen fixation. Soybean farmers in the Mid-South will have experience and knowledge in the practical and optimal use of inoculants and the use of more efficient strains could improve yield. Our plan to optimize drought-tolerant rhizobia and determine the economic return will provide farmers with resources to cultivate a successful agriculture practice. Therefore, providing such information will allow them to advance in the management of soybean plants and inoculants for economical and ecological benefits.

**EXPECTED END PRODUCT(S)**

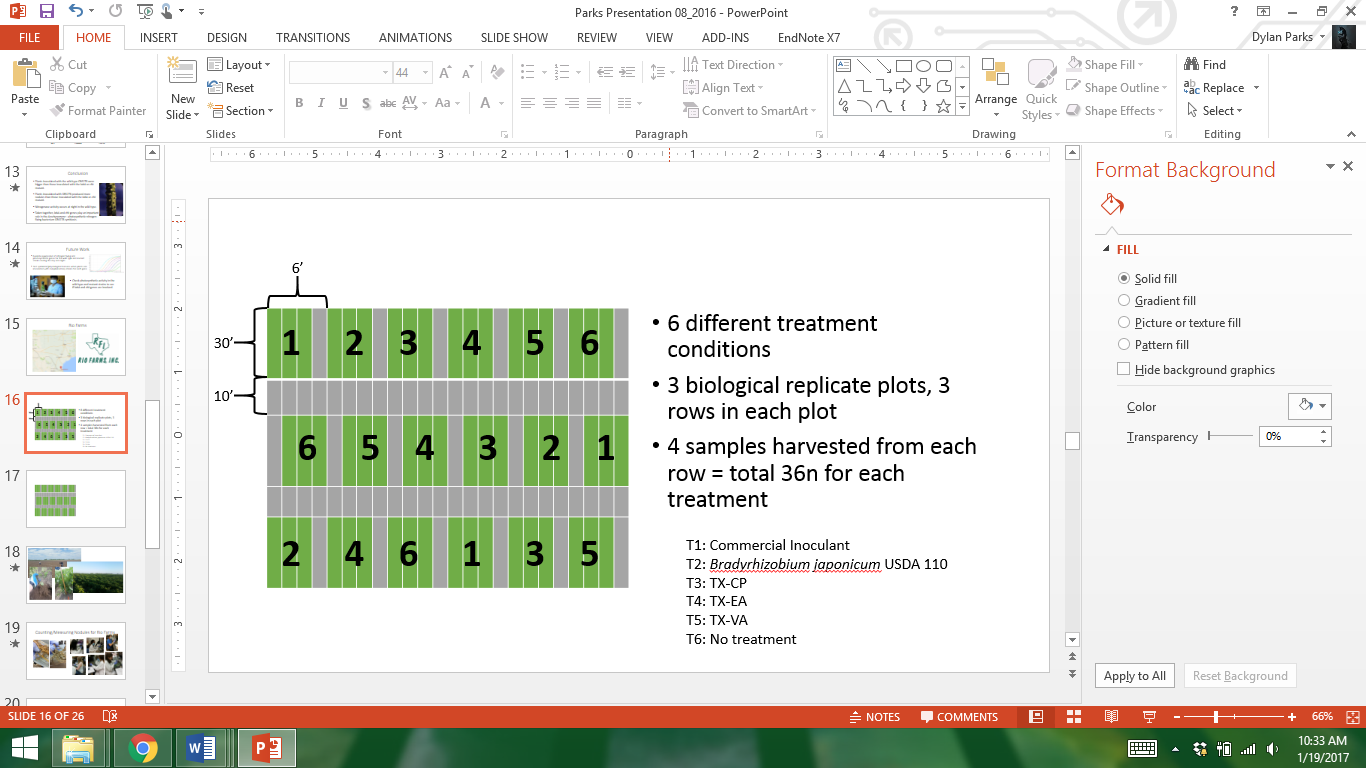
Expected outcomes for the proposed research:

* PowerPoint presentations at UTA Annual Celebration of Excellence by Students (ACES).
* Project presentations at related conferences
* Journal Publications
* Poster Presentations at UTA Annual Celebration of Excellence by Students (ACES).

**GRAPHICS/TABLES**

**Milestone**

|  |  |  |  |
| --- | --- | --- | --- |
| **Objectives** | **Year 1** | **Year 2** | **Year 3** |
| *1. Evaluate the effects of TXVA and TXEA inoculants on soybean yield (one variety, maturity group [MG] IV) at drought-prone sites in AR, LA, MO, MS, TN, and TX* |  |  |  |
| *2. Evaluate the effects of the TXVA inoculant on soybean yield of five varieties (from MG III, IV, V and drought-resistant varieties) at drought-prone sites in AR, LA, MO, MS, TN, and TX* |  |  |  |
| *3. Provide the Mid-South soybean industry with fact sheets that will assist farmers to obtain and apply an efficient and drought-tolerant Bradyrhizobium inoculant* |  |  |  |



**Figure 1.** Plot measurements at Rio Farms, Inc. in TX. For each biological replicate, plants were planted approximately ½ foot apart down 30’ rows (green) with 10’ alleys (gray). Each row was 18” apart, and one row left unplanted between treatments (gray) to avoid cross-contamination of inoculants. 1, Cell Tech; 2, USDA110; 3, TXCP-01; 4, TXVA-1107-3003; 5, TXEA-1109-2000; 6, no treatment.

**Table 1.** Average of plant dry weight, nodule numbers, and seed production per soybean plant inoculated with various strains.

|  |  |  |  |
| --- | --- | --- | --- |
| **Strains inoculated** | **Avg. plant dry weight (g)** | **Avg. nodule #/plant** | **Avg. seed #/plant** |
| Cell-TechTM | 33.27 ± 1.19 (A) | 92.44 ± 10.76 (A) | 81.93 ± 8.37 (A) |
| USDA110 | 33.72 ± 0.92 (A) | 111.39 ± 14.23 (A) | 79.61 ± 10.46 (A) |
| TXCP-01 | 43.55 ± 1.30 (B) | 51.61 ± 7.15 (C) | 132.58 ± 14.30 (B) |
| TXVA-1107-3003 | 37.58 ± 1.41 (B) | 125.14 ± 9.06 (B) | 147.89 ± 22.50 (B) |
| TXEA-1109-2000 | 46.69 ± 2.73 (B) | 145.17 ± 14.75 (B) | 173.68 ± 15.94 (B) |
| No treatment | 29.79 ± 0.78 (A) | 24.52 ± 6.02 (C) | 48.61 ± 6.81 (C) |

\* Note that the different letters indicate a statistical difference (*P*-value < 0.05).

**APPENDICES**

* **Explanation/justification of budget categories where needed for clarification or detail.**

**A1. Salaries:** A graduate student will get paid $2,000 per month for 9 months: (9 × $2,000 = $18,000). This is the typical rate for either graduate research assistant (GRA) or graduate teaching assistant (GTA) in the Department of Biology at the University of Texas at Arlington (UTA).

**A3. GRA:** Tuition for a graduate student is $9,100 per year.

**B. Fringe Benefits:** 10% of salary for graduate students ($1,800).

**C. Travel:** I plan to attend one meeting with a graduate student. In addition, there will be 3 visits to each experiment site for planting, nodule sampling, and harvesting, respectively. With 6 states, there will be 18 trips.

- Meeting registration fees (2 people, 1 meeting): 2 × $300 = $600

- Airfare (2 people, 1 trip): 2 × $400 = $800

- Truck rental and gas (for 18 trips): 18 × $150 = $2,700

- Hotel (2 people, 20 nights): 2 × 20 × $100 = $4,000

- Meals (2 people, 30 days): 2 × 30 × $50 = $3,000

Thus, $600 + $800 + $2,700 + $4,000 +$3,000 = $11,100

**D. Contractual Services:** $8,000 including external lab. fees ($1,000) and consulting ($7,000)

**E. Subcontracts:** $9,000 (to Drs. Chen, Shekoofa, and Price)

**F. Commodities:** Lab. supplies ($2,000)

**G. Publication Costs:** $1,000