



Agricultural Marketing Service

U.S. DEPARTMENT OF AGRICULTURE

AWARD YEARS 2021 AND PRIOR

This annual Performance Progress Report (PPR) must illustrate the progress made toward the completion of your project. If this project is completed at the time of the annual PPR submission, the project report should be marked as final. Please note character limits (abbreviated "char.") throughout.

PROJECT INFORMATION

Project Title (250 char.)	Diagnose seed quality and composition of pulse crops at South Dakota State University
Organization Name (250 char.)	South Dakota State University
Project Start Date	09/30/2021
Project End Date	9/29/2024

ORGANIZATION POINT OF CONTACT

Full Name	Senthil Subramanian
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BACKGROUND INFORMATION

PROJECT PROGRESS

Please provide the total percentage of work completed on the project.	80%
Is this a final report? Please select the box to the right if the answer is "yes".	☐

PROJECT BACKGROUND (REQUIRED FOR FINAL REPORTS)

Provide enough information for the reader to understand the importance or context of the project and any key findings. This section may draw from the background and justification contained in the accepted project plan. As the project has been completed, write this section in past tense. If this is an annual report, please skip this section. (1200 char.)

Seed health is very critical for sustainable production of pulse crops in South Dakota. Seed infection can reduce germination, and seedlings that develop from infected seeds may appear diseased and have poor growth. Besides compromising seed health, there is no information about the impact of disease-causing pathogens on the composition and functionality of pulses. This project proposed to address a critical knowledge gap on seed-borne diseases and microbes in field pea, an important pulse crop in the region by leveraging on-going crop performance trials to collect information on seed borne diseases and the effect on seed quality and using the information to develop diagnostic methods for seed-borne diseases. The larger goal in the project was to assist crop producers

in adopting best management practices to protect their crop from seed-borne diseases.

OBJECTIVE(S) (REQUIRED)

Please provide the approved project's objectives from the accepted project plan. Use the boxes to indicate if the objective is in progress, has been completed, or is not (and will not be) completed. At the final report, if "no" is selected for any of the listed objectives, you must expand upon this in the Challenges and Developments Section. Additional objectives may be added as needed.

+	#	Objective (2,000 char.)	Objective Completed?
-	1	Objective 1. Assist the SDSU Seed Testing Laboratory to detect and obtain data on seed pathogens, specifically fungal and bacterial, using DNA-based diagnostics and traditional plant pathology techniques (e.g. culture-based methods).	Yes
-	2	Objective 2. Screen the commercial varieties (that are evaluated by Dr. Chris Graham as part of the crop performance trials in South Dakota) of peas, chickpeas and lentils for prevalent seed-borne diseases.	Yes
-	3	Objective 3. Determine the impacts of plant disease causing pathogens on the composition and functional properties of pulses through composition analysis and functionality assessment.	Yes
-	4	Objective 4. Educate specialty crop growers on tools to minimize the impact of fungal and bacterial pathogens on their farm.	Yes

OUTCOMES

Please only report on the outcomes and indicators that are selected and approved for this project. Use the check boxes to indicate which outcomes and indicators are selected. Select the "N/A" box for any indicators that are not applicable.

1. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH INCREASED SALES

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2. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH INCREASED CONSUMPTION

☐

3. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH INCREASED ACCESS AND AWARENESS

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4. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH GREATER CAPACITY OF SUSTAINABLE PRACTICES OF SPECIALTY CROP PRODUCTION RESULTING IN INCREASED YIELD, REDUCED INPUTS, INCREASED EFFICIENCY, INCREASED ECONOMIC RETURN, AND/OR CONSERVATION OF RESOURCES

☒

Please provide the numbers of plant/seed releases (e.g., cultivars, drought-tolerant plants, organic, enhanced nutritional composition, etc.) for this reporting period.

Indicators	Value	N/A
1. Number of plant/seed releases	2	☐

Please select the applicable indicators and provide data for the adoption of best practices and technologies resulting in increased yields, reduced inputs, increased efficiency, increased economic return, and conservation of resources for this reporting period.

Indicators	Value	N/A
2.a. Number of growers/producers indicating adoption of recommended practices		☒
2.b. Number of growers/producers reporting reduction in pesticides, fertilizers, water used/acres	36	☐
2.c. Number of producers reporting increased dollar returns per acre or reduced costs per acre	36	☐
2.d. Number of acres in conservation tillage or acres in other best management practices		☒

Please provide the total number of adults reached and select the applicable indicators for this reporting period.

Indicators	Value	N/A
3. Number of habitat acres established and maintained for the mutual benefit of pollinators and specialty crops		☒

5. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH MORE SUSTAINABLE, DIVERSE, AND RESILIENT SPECIALTY CROP SYSTEMS ☒

Please provide data for the applicable indicators for this reporting period.

Indicators	Value	N/A
1. Number of new or improved innovation models (biological, economic, business, management, etc.), technologies, networks, products, processes, etc. developed for specialty crop entities including producers, processors, distributors, etc.		☒
2. Number of innovations adopted		☒
3. Number of specialty crop growers/producers (and other members of the specialty crop supply chain) that have increased revenue expressed in dollars		☒
4. Number of new diagnostic systems analyzing specialty crop pests and diseases	2	☐
5. Number of new diagnostic technologies available for detecting plant pests and diseases	2	☐
6. Number of first responders trained in early detection and rapid response to combat plant pests and diseases		☒
7. Number of viable technologies/processes developed or modified that will increase specialty crop distribution and/or production		☒
8. Number of growers/producers that gained knowledge about science-based tools through outreach and education programs		☒

6. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH INCREASING THE NUMBER OF VIABLE TECHNOLOGIES TO IMPROVE FOOD SAFETY ☐

7. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH INCREASED UNDERSTANDING OF THREATS TO FOOD SAFETY FROM MICROBIAL AND CHEMICAL SOURCES

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8. ENHANCE THE COMPETITIVENESS OF SPECIALTY CROPS THROUGH ENHANCING OR IMPROVING THE ECONOMY AS A RESULT OF SPECIALTY CROP DEVELOPMENT

☐

ADDITIONAL APPROVED OUTCOME (IF APPLICABLE)

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OUTCOME REMARKS (REQUIRED)

Please explain any quantifiable results, along with an update on their progress and data collection efforts for each of the outcomes and indicators selected in the accepted project plan. It is understood that the results may not yet be final at the time that this report is submitted; however, please provide an update on progress to date. If you do not have any data on the indicator(s) listed above, you must explain why in this section. (2,000 char.)

Microbiota structure was analyzed on 12 pea varieties each with 4 replicates. DS-Admiral, Durwood, Nettle 2019, AAC Profit, Salamanca, CDC Saffron, and AAC Carver belonged to the yellow seed-coated variety, while CDC Striker, SW Arcadia, AAC Comfort, CDC Greenwater, and Greenwood belonged to the green seed-coated variety. A total of 258 epiphytes and 129 endophytic isolates were obtained from seeds harvested at Dakota Lakes and Sturgis. Results: (1) variety influenced the diversity of both epiphytes and endophytes across the two sites, (2) green-seeded varieties had significantly more endophytes, while yellow-seeded varieties had significantly more epiphytes, (3) Dakota Lakes exhibited significantly more endophytes than Sturgis, while R2A media supported significantly more isolates than TSA (statistical analysis; 95% confidence). These findings suggest that plant variety, geographical site, elevation, and media type all play significant roles in shaping the assemblage of pea seed-associated microbiota. To assess the role of these microbiota on seed and seedling health, their ability to produce IAA, fix nitrogen, solubilize phosphate, and produce siderophores were evaluated. Isolates from green pea varieties produced more IAA (better seedling growth potential) than yellow varieties. Conversely, yellow varieties had more microbes with siderophore producing capability (better nutrient absorption potential) (4.1). Both varieties had microbiota capable of growing under minimal nitrogen and phosphate solubilizing potential. DNA-based taxonomic identification of the isolates has been completed and data is being evaluated. Based on yield trial data feedback and extension communications during farm visits, 36 reports (cumulative) of reduced fungicide use and higher yields were noted (a combination of variety and environment is expected to have contributed to this) (4.2b,c). The DNA data and microbial load data add 2 diagnostic systems (PCR and sequencing) and technologies (4.4,5)

NARRATIVE

ACCOMPLISHMENTS (REQUIRED)

If this is an annual report, list your accomplishments for this reporting period. If this is a final report, your accomplishments should be cumulative and include all accomplishments previously reported on and updated to include activities conducted this past reporting period. Indicate how these accomplishments associated with each of your project's objective(s), outcome(s), and/or indicator(s). This listing should identify your project's partners and their contributions. Additional accomplishments can be added as needed.

+	#	Accomplishment (1,200 char.)	Related to (Objective/ Outcome)
-	1	A total of 258 epiphytes and 129 endophytic isolates were obtained from seeds harvested at Dakota Lakes and Sturgis.	Objective 1: To develop diagnostic tools for seed microbiomes, a basal microbiome analysis is crucial. Given that direct DNA-based methods have not yielded strong detection of pathogens, culture-based methods will provide the basis for development of diagnostic tools.
-	2	Optimization of culture-independent seed microbiome evaluation methods for pea and identification of isolates from culture-dependent method using DNA-based taxonomy.	Objective 1: Direct detection of total microbiome load from pea seeds is a challenge due to the cross-amplification of plant chloroplast DNA. Specialized primer sets that distinguish chloroplast DNA were evaluated under multiple conditions and optimized for use with pea. The primer sets and PCR conditions amplify only bacterial detection amplicons and not plant chloroplast DNA.
-	3	Culture independent evaluation of total microbiome load from 49 pea cultivars evaluated in 2021 growing season.	Objective 2 and 3: Total microbiome load was evaluated using culture-independent PCR-based methods. Amplification of microbial DNA from total seed DNA indicated very low microbial load consistent with no detection of disease occurrence in the field.
-	4	Field trials for 49 pea varieties across two growing seasons to evaluate for disease resistance and identification of specific cultivars with potential resistance.	Objective 2: Forty nine pea varieties were evaluated in two different locations across three growing seasons and three replicates each. The data was used to shortlist 12 pea varieties for microbiota isolation.
-	5	Seed and seedling health promoting characteristics were evaluated successively narrowing down based on ranking (387 isolates for IAA production - seedling vigor; 43 selected for phosphate solubilization, growth on low nitrogen and finally down to 15 isolates for siderophore production)	Objective 3. Statistical analysis indicated the connection between pea variety, location of field trial and growth medium used for isolation. Green seed coat varieties consistently carried more beneficial microbiota with the potential for better seed health.

-	6	DNA-based identification of 43 isolates with highest seed and seedling health potential.	Objectives 1, 2 and 3. Method development for microbe identification and information collection on taxonomies of microbes present on the seed surface and inside the seeds for variety selection as well as the development of DNA-based detection methods and seed treatment cultures.
-	7	Information sharing with producers and resources for the development of educational materials on seed health	Objective 4. Information shared during on-farm tours and farm day programs; Data on microbial load, seed and seedling health potential, connection between characteristics of seed microbiota and seed/seedling vigor will all be shared with extension personnel and producer groups.

CHALLENGES AND DEVELOPMENTS (REQUIRED)

Provide any challenges to the completion of your project or any positive developments outside of the project's original intent that you experienced during this reporting period. Also, provide the corrective actions you took to address these issues. If you did not attain an approved objective, outcome(s), and/or indicator(s), provide an explanation in the Corrective Actions column. For developments, you may put "N/A" under Corrective Actions. Additional challenges/developments may be added as needed.

+	#	Challenge or Development (1,200 char.)	Corrective Action
-	1	Low disease pressure (dry conditions) during ield trials for 49 pea varieties across two growing seasons to evaluate for disease resistance and identification of specific cultivars with potential resistance	Total microbial load was used as an indicator instead of actual disease. 12 pea varieties were selected representing green/yellow seedcoat from the two locations of trial from a single season.
-	2	Failure of cultivation-independent methods to yield clear taxonomic data on microbiota of seeds	Endophytic (inside the seed) and Epiphytic (seed surface) microbiota were isolated using two different culture media and characterized for their potential to promote seed and seedling vigor/health. A selection of 43 isolates were analyzed for taxonomic information.

UPCOMING ACTIVITIES (REQUIRED FOR ANNUAL REPORTS)

Provide a description of the activities that you are planning to complete during the next reporting period. Additional activities can be added as needed. Please note, if this is a final report there should be no upcoming activities associated with the project, and this section should be left blank.

+	#	Activity (600 char.)	Anticipated Completion
-			
-			

LESSON LEARNED (REQUIRED FOR FINAL REPORTS)

The recipient or subrecipient is required to provide recommendations or advice that others may use to improve their performance in implementing similar projects. If this is an annual report, please skip this section. (1200 char.)

Research design: The project success relied on environmental conditions being favorable for disease development. The lack of such environment required that the investigators develop alternate goals and objectives to overcome this challenge. Potential collaborations with other geographic regions to collect diseases seeds for evaluation might promote the success of such research.

Procedures and reporting: Annual reporting and expense monitoring exercises were very helpful in keeping up progress. Many research projects are conducted using graduate students with specific academic semester start dates. Flexible project start dates and no cost extension procedures would greatly benefit proper expenditure and timely completion of research.

CONTINUATION AND DISSEMINATION OF RESULTS (REQUIRED FOR FINAL REPORTS)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results. If this is an annual report, please skip this section. (1200 char.)

We are currently analyzing the DNA-base taxonomic data. The student is being supported by a teaching assistantship by the Department. We expect to publish two research articles describing the results from this project. We will share information on pea varieties and seed microbial load as well as the seed/seedling health vigor potential characteristics of seed-borne microbiota with the producers and extension personnel for development of relevant outreach materials.

PROJECT BENEFICIARIES (REQUIRED FOR FINAL REPORTS)

Provide the number of project beneficiaries. If this is an annual report, please skip this section. (12 char.)

200

FEDERAL PROJECT EXPENDITURE

EXPENDITURE (REQUIRED)

You must respond to at least one cost category. Please note, the subtotal and total rows include formulas to auto-calculate totals. The approved budget should come from the accepted project plan or any approved budget amendments.

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds Only)
Personnel	\$37,992.00	\$33,180.07
Fringe Benefits	\$380.00	\$207.66
Travel	\$0.00	\$0.00
Equipment	\$0.00	\$0.00
Supplies	\$5,806.00	\$4,443.03
Contractual	\$0.00	\$0.00

Other	\$15,484.00	\$11,958.00
Direct Costs Sub-Total	\$59,662.00	\$49,788.76
Indirect Costs	\$4,773.00	\$3,983.16
Project Expenditure Total Federal Costs	\$64,435.00	\$53,771.92

DISCUSSION OF EXPENDITURES (REQUIRED)

For 1st Annual Reports, if total project expenditures is less than 30 percent, and for 2nd Annual Reports, if total project expenditures is less than 60 percent, include a statement explaining how the grant funds will be expended and project activities completed as planned by the end date on the grant agreement. Any state approved budget amendments should be included here; Include the line item that the funds are coming from and going to and when the state approved the budget amendment. (1,200 char.)

A Master's graduate student Ms. Diana Djuguna performed the experiments associated with Objectives 1 and 2. Salary funds supported her research assistantship and other expenses are tuition remission costs. Supplies associated with the experiments were charged to the project. The student started only in January 2023 and hence expended approximately 20 months of salary support. Supplies budget was used for laboratory supplies to isolate and grow microbes, characterize their properties, perform molecular work (DNA isolation, amplification and sequence-based identification). Some supplies were also used to print poster materials for sharing of information with other scientists and producer groups.

PROGRAM INCOME (IF APPLICABLE)

If program income was earned on the project, grant recipients are required to report on the source, amount, and how funds were expended. Program income is gross income --earned by a recipient or subrecipient under a grant --directly generated by the grant-supported activity or earned only because of the grant agreement during the grant period of performance. Program income includes, but is not limited to, income from fees for services performed; the sale of commodities or items fabricated under an award (this includes items sold at cost if the cost of producing the item was funded in whole or partially with grant funds); registration fees for conferences, etc.

+	Source/Nature of Program Income (100 char.)	Amount Approved in Budget	Actual Amount Earned
-		\$0.00	\$0.00

USE OF PROGRAM INCOME (IF APPLICABLE)

Describe how the earned program income was used to further the objectives of this project during the performance period. Any income generated must be reinvested back into the project and not set aside or reserved for future expenses after the grant ends. (1,200 char.)

PPR ATTACHMENTS DESCRIPTION (IF APPLICABLE)

Grantors will rely on the data that is submitted in this form when they report to congress. However, if you think additional documents are necessary to support PPR, please attach then and provide a document name and description of document below.

+	Document Name	Document Description
-		