

NIFA SCRI project 2023-05687 SPINACH REPORT (2023-2024)

Project Title: Development of germplasm resources and molecular breeding tools to combat endemic and emerging diseases in US spinach production

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Non-Technical Summary

Spinach (*Spinacia oleracea* L.) production is threatened by several diseases that individually or together severely reduce yield and quality. The most effective approach to improving productivity, marketability, and sustainability in spinach cultivation is by developing resistant cultivars. Traditionally, spinach breeding has relied on the time-consuming phenotypic selection that delays cultivar release. Alternatively, molecular breeding offers an advantage by accelerating the development of disease-resistant cultivars. The project builds upon the achievement of a previously awarded NIFA-SCRI project (2017-51181-26830), which focused on molecular breeding through molecular characterization of resistance to the three predominant diseases of spinach: downy mildew (DM), white rust (WR), Fusarium wilt (FW), which laid the foundation for genetic tools, including SNP markers and genetic maps. Building upon this solid groundwork of molecular breeding initiative in spinach, the current project takes on a renewed focus by concentrating on quantitative trait loci mapping, marker validation, and the strategic integration of these findings into cultivar development. This project aims to expedite the development of spinach cultivar resistance to DM, WR, FW, and two emerging leaf spot diseases, namely *Stemphylium* leaf spot (SLS) and anthracnose leaf spot (ALS). This project aims to generate improved genome-enabled resources to introduce resistant traits into the cultivar development pipelines to effectively manage prevalent and emerging diseases in spinach. The project's long-term goal is to develop new disease-resistant cultivars by utilizing advanced genetic tools and selection methods delivered from this project effort that will serve as a valuable resource for spinach improvement. The breeding process will be expedited by integrating newly developed molecular breeding tools benefitting both public and private breeders and growers. The project will address disease threats and contribute to effective disease management strategies by leveraging genetics, genomics, and innovative breeding techniques. This project will address the following areas of the USDA-SCRI program: (1)

“Research in plant breeding, genetics, genomics, and other methods to improve crop characteristics”; and (2) “Efforts to identify and address threats from pests and diseases,” including “emerging and invasive species.”

Goals / Objectives

Spinach (*Spinacia oleracea* L.) is an economically important vegetable crop worldwide with an estimated annual value of \$11.8 billion. The US is the second-largest spinach producer globally while leading in the fresh market segment with an annual product value of around \$500 million. Over the past three decades, the increasing interest in health-conscious diets has led to a consistent demand for spinach. Despite this increase in demand and production, the commercial cultivation of spinach faces a significant threat from several diseases. These diseases, individually or in combination, reduce yield and quality. Downy mildew (DM), white rust (WR), Fusarium wilt (FW), Stemphylium leaf spot (SLS), and anthracnose leaf spot (ALS) are five key diseases threatening spinach production in the US and worldwide. The spinach industry seeks continued development of improved, locally adapted germplasm and cultivars with resistance to major pathogens. Thus, the overarching objectives of this project are to develop spinach germplasm with resistance to these five key pathogens and to develop molecular tools for effectively introducing these traits into the breeding pipeline for cultivar development. The practical long-term goal of this proposal is to generate molecular breeding tools to expedite disease resistance breeding efforts and the continuous release of resistant spinach cultivars against endemic and emerging diseases suitable for both conventional and organic production markets that can dramatically reduce fungicide use for disease control. To meet this goal, the project aims to develop efficient molecular breeding methods and phenotyping tools to accelerate the cultivar development process and serve the industry's needs. The specific research objectives of this proposal are to:

1. Characterize and validate molecular markers for resistance to endemic and emerging spinach diseases.

Hypothesis: *Genetic diversity in spinach germplasm collections includes sources of resistance to these five diseases that can be used for cultivar development.*

2. Introgress disease resistance into spinach breeding lines for cultivar development.

Hypothesis: *Combining molecular and disease phenotyping approaches will expedite cultivar development in spinach.*

3. Develop a spinach grower-oriented outreach program based on experiential learning and economic decision tools for disease management in spinach.

Hypothesis: *Interactive and demonstrative methods can increase the adoption of management practices to reduce production risks and increase production profits.*

The proposed efforts encompass identifying genomic regions that govern disease-resistant mechanisms, optimizing practical genetic tools to improve selection processes, and developing resistant spinach cultivars. In summary, this project endeavors to extend our ongoing efforts to make advances in spinach breeding, develop genomic and phenotyping tools, generate new knowledge, and expand genetic resources to expedite disease-resistant cultivar development to support sustainable and economically viable spinach production in the US.

*** Target Audience**

Spinach Producers, Growers, and Seed Companies: The target audience reached includes spinach producers, growers, and seed companies through field days and other communications. Seed companies have been utilizing our molecular markers associated with disease resistance to improve their breeding programs.

Scientists: Our research methods, including new QTL and association mapping results, SNP marker discoveries, as well as our published articles, abstracts, presentations, and reports, are valuable resources for the scientific community. These contributions facilitate further research and development in plant science and breeding technologies.

Students: Several students have been trained in both classical and molecular breeding techniques. This includes hands-on experience in traditional breeding methods such as crossing, advancing generations, and variety development. Additionally, students have learned molecular breeding technologies, including QTL and association mapping, genome-wide association studies (GWAS), genomic prediction, SNP discovery and genotyping, next-generation sequencing methods like whole genome sequencing (WGS), whole genome resequencing (WGR), and genotyping-by-sequencing (GBS). They have also been trained in the application of bioinformatics tools in breeding programs and in the use of marker-assisted selection (MAS) and genomic selection (GS) for crop improvement.

***Products**

Publications

2024

1. Alatawi, I., H. Xiong*, B. Mou*, K. Chiwina, W. Ravelombola, Q. Luo, Y. Xiao, Y. Tian, and A. Shi*. 2024. Genomic insights of leafminer resistance in spinach through GWAS approach and genomic prediction. Horticultural Plant Journal, <https://doi.org/10.1016/j.hpj.2024.03.012>.
2. Clark, K.J., C. Feng, A.G. Anchiet, A. Van Deynze, J.C. Correll, and S.J. Klosterman. 2024. Dual transcriptional characterization of spinach and *Peronospora effusa* during resistant and susceptible race-cultivar interactions. BMC Genomics 25:937. <https://doi.org/10.1186/s12864-024-10809-x>
3. Klosterman, S.J., K.J. Clark, A.G. Anchiet, S.L. Kandel, B. Mou, M.T. McGrath, J.C. Correll, and N. Shishkoff. 2024. Transmission of spinach downy mildew via seed and infested leaf debris. Plant Disease 108: 951-959. <https://doi.org/10.1094/PDIS-06-23-1225-RE>
4. Olaoye, D., G. Bhattarai, C. Feng, J.C. Correll*, and A. Shi*. 2024. Evaluation of downy mildew resistance in spinach. Euphytica 220, 38. <https://doi.org/10.1007/s10681-023-03289-9>
5. Spawton, K.A., L.A. Stein, L.J. du Toit. 2024a. Evaluation of spinach cultivars for resistance to *Stemphylium* leaf spot (*Stemphylium vesicarium*) and white rust (*Albugo occidentalis*). HortScience 59 (1), 51-63. <https://doi.org/10.21273/HORTSCI17373-23>
6. Spawton, K.A. and L.J. du Toit. 2024b. Characterization of *Stemphylium* species associated with *Stemphylium* leaf spot of spinach (*Spinacia oleracea*). Plant Disease, <https://doi.org/10.1094/PDIS-10-23-2223-RE>
7. Spawton, K.A. and L.J. du Toit. 2024c. Prevalence of FRAC group 11 fungicide resistance in *Stemphylium vesicarium* isolates, but not *S. beticola* isolates, causing *Stemphylium* leaf spot of spinach (*Spinacia oleracea*). Plant Disease, Published Online:12 Jun 2024; <https://doi.org/10.1094/PDIS-11-23-2328-RE>
8. Xiong, H*, K. Chiwina, W. Ravelombola, Y. Chen, I. Alatawi, Q. Lou, T.M. Phiri, B. Mou*, and A. Shi*. 2024. Genomic insights into oxalate content in spinach: A genome-wide association study and genomic prediction approach. Horticultural Plant Journal, Available online 14 September 2024, <https://doi.org/10.1016/j.hpj.2023.12.015>.

2023

9. Batson, A. M., J. Woodhall, and L.J. du Toit. 2023. Real-time PCR assays for races of the spinach

Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *spinaciae*. Plant Disease 107:2633-2642. <https://doi.org/10.1094/PDIS-11-22-2658-RE>

10. Bhattarai, G.*, A. Shi*, B. Mou*, and J. Correll*. 2023. Skim resequencing finely maps the downy mildew resistance loci *RPF2* and *RPF3* in spinach cultivars Whale and Lazio. Horticulture Research, uhad076, <https://doi.org/10.1093/hr/uhad076>
11. Spawton, K.A., T.L. Peever, L.J. du Toit. 2023. Genome resource for *Stemphylium beticola*, one of the causal agents of Stemphylium leaf spot of spinach (*Spinacia oleracea*). PhytoFrontiers™ 3 (4), 870-873, <https://doi.org/10.1094/PHYTOFR-03-23-0035-A>

Submission/preparation

12. Bhattarai, G., B. Liu, J.C. Correll*, **A. Shi***. 2024. Genetic characterization of leaf spot (*Stemphylium vesicarium*) resistance in spinach diversity panel and standardizing machine learning models to optimize genomic selection (manuscript in pre-review).
13. Liu, B., G. Bhattarai, A. Shi, and J.C. Correll. 2024. Evaluation of resistance of USDA spinach germplasm and commercial cultivars to *Stemphylium vesicarium* (Preparation for HortScience).
14. Gyawali, S., G. Bhattarai, L.J. du Toit*, J.C. Correll, and **A. Shi***. 2024. Genome wide association studies (GWAS) of Fusarium wilt resistance in wild spinach (*S. turkestanica*) (Submitted to Molecular Breeding)
15. Rameneni, J.J., A.S.M.F. Islam, C.A. Avila, A. Shi. 2024. Improving genomic prediction of vitamin C content in spinach using GWAS-derived markers. BMC genomics. *In revision*

Abstract/Meeting presentation:

1. Alatawi, I., H. Xiong, G. Bhattarai, K. Chiwina, H.M. Alkabkabi, B. Mou, and A. Shi. 2024. Genome-wide association study and genome prediction of tallness trait in spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
2. Alkabkabi, H.M., H. Xiong, G. Bhattarai, K. Chiwina, I. Alatawi, B. Mou, and A. Shi. 2024. Genome-wide association study and genome prediction of bolting trait in spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
3. Avila, CA., ASM Faridul Islam, Samuel Zapata, Larry Stein. Spinach seed for grain consumption: feasibility and potential for genetic improvement. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
4. Bhattarai, G., A. Shi, C. Kik, L. du Toit, R. van Treuren, and S. Gyawali. 2024a. Insights into the Genetic Diversity and Population Structure of Wild and Cultivated Spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
5. Bhattarai, G., A. Shi, B. Mou, and J. Correll. 2024b. VGBR 1 - Progress and Insights into Downy Mildew Resistance Mapping Efforts in Spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
6. Chiwina, K., H. Xiong, B. Mou, and A. Shi. 2024. Genome-wide association study and genome prediction of Verticillium wilt resistance in spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
7. Joshi, V., Shi, A. Formiga, B. Analin, and M. Colley. 2024. Phenotypic landscape of the photosynthetic performance and seed productivity of spinach germplasm under organic condition. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.
8. Villavicencio, X., S. Zapata, A. Xicay. 2024. Machine learning algorithms for vector autoregressive models to evaluate the linkages across the value chain in Geotemporal separated markets." Selected

Paper. 2024 Southern Agricultural Economics Association Annual Meeting. Atlanta, GA, February 06, 2024.

9. Villavicencio, X., S.D. Zapata, and A. Xicay. 2024b. An Application of Machine Learning Methods to Assess Price Transmission in Specialty Crops in the U.S. International Food and Agribusiness Management Association (IFAMA). Almería, Spain, June 17-20, 2024.
10. Villavicencio, X., S.D. Zapata, and A.E. Xicay. 2024c. Assessing the Geotemporal Resilience of the U.S. Specialty Crop Value Chain. Agricultural and Applied Economics Association Annual Meeting. New Orleans, LA, July 29, 2024.
11. Xiong, H., A. Shi, H. Alkabkabi, I. Alatawi, and K. Chiwina. 2024. Genome-Wide Association Study Identifies Key SNPs Associated with Mineral Element Accumulation in Spinach. ASHS 2024 Annual Conference 23 - 27 September, Honolulu, Hawaii.

* Other Products

* Accomplishments

What was accomplished under these goals?

1. Characterize and validate molecular markers for resistance to endemic and emerging spinach diseases.

1.1 DOWNY MILDEW (DM, Pfs)

Downy Mildew Evaluation: (i) Seventy spinach genotypes, including advanced breeding lines and released commercial cultivars/hybrids, were evaluated for resistance to new races and novel strains of downy mildew (*Peronospora effusa*, syn. *P. farinosa* f. sp. *spinaciae*, Pfs) at two locations: the Salinas Valley in California and Yuma in Arizona, during the winter seasons from 2012 to 2024. Over 30 new spinach breeding lines and cultivars/hybrids demonstrated resistance to downy mildew in each experiment. (ii) Thirty-nine commercial spinach cultivars were evaluated for resistance to a recently identified race, Pfs race 19. Additionally, the progenies of F1-derived NIL1 and NIL3, as well as F2 populations derived from *Califlay* and susceptible Viroflay, were evaluated for resistance to Pfs race 5. The results indicated that resistance conferred at the *RPF1* or *RPF3* loci was completely dominant and controlled by a single dominant gene (Olaoye et al., 2024).

Fine Mapping of *RPF2* and *RPF3*: The *RPF2* locus, derived from the resistant differential cultivar *Lazio*, was mapped to a region between 0.47 Mb and 1.46 Mb on chromosome 3, where a gene encoding a CC-NBS-LRR plant disease resistance protein was identified. Furthermore, combined analysis of progeny panels from *Lazio* and *Whale*, segregating for the *RPF2* and *RPF3* loci, narrowed down the resistance region on chromosome 3 to between 1.18–1.23 Mb and 1.75–1.76 Mb (Bhattarai et al., 2023).

Gene Expression: Differentially expressed gene (DEG) analysis in resistant spinach interactions with R13-NIL1 and R19-NIL3 revealed DEGs from the protein kinase-like and P-loop containing families, which play roles in plant defense (Clark et al., 2024).

1.2 WHITE RUST (WR):

White rust resistance was evaluated in 79 spinach cultivars during the 2021 field trial, 87 cultivars in 2022, 63 cultivars in 2023, and 114 breeding lines at the White Rust Nursery in Crystal City, TX. White rust symptoms were observed in most of the tested spinach lines; however, the majority of the lines were not fully infected by the pathogen and exhibited a resistant reaction, as determined by a 1-10 rating scale, likely due to insufficient pathogen epidemic pressure during those years (Spawton et al., 2024a; Avila & Stein, 2024).

1.3 FUSARIUM WILT (FW, *Fos*)

Fusarium wilt resistance was evaluated in 84 *Spinacia* genotypes (68 *S. turkestanica* and 16 *S. oleracea*) using an inoculum comprising a mix of isolates from races 1 and 2. Of the 68 *S. turkestanica* accessions, 17 showed high levels of resistance at medium inoculum density, and 8 exhibited resistance at high inoculum density. Twelve SNPs were significantly associated with Fusarium wilt resistance, distributed across 10 QTL regions located on chromosomes 1, 3, 4, and 6. SNP S6_38110665 on chromosome 6 was validated across multiple GWAS models and demonstrated a major effect (-2.48 to -2.79) in reducing Fusarium wilt severity (Gyawali et al., 2024).

Real-time PCR assays were developed for detecting the races of Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *spinaciae* (Batson et al., 2023).

1.4 STEMPHYLIUM (SLS) AND ANTRACHNOSE (ALS) LEAF SPOTS

Stemphylium leaf spot (SLS) field evaluation: SLS was evaluated in 79 spinach cultivars during the 2021 field trial, 87 cultivars in 2022, and 63 cultivars in 2023. Between 11% and 27% of the cultivars were identified as resistant to Stemphylium leaf spot (Spawton et al., 2024a).

Stemphylium leaf spot greenhouse evaluation: Fifteen lines were observed to be resistant, and 42 SNP markers were significantly associated with SLS resistance. A high genomic prediction accuracy (r-value) of up to 0.79 was estimated (Bhattarai et al., 2024; Liu et al., 2024).

Anthrachnose leaf spot (ALS) evaluation: Accessions and breeding lines identified with resistance to ALS are currently being increased in isolation chambers at the Texas A&M AgriLife Research and Extension Center by Dr. Avila. The anthracnose pathogen was collected from the field and cultured under lab conditions for inoculation screening trials.

Genome sequencing: Two isolates of *S. beticola*, St0030 and St1145, were sequenced to generate draft genome assemblies (Spawton, 2023).

Pathogenicity and fungicide resistance: The isolates of *S. beticola*, *S. drummondii*, and *S. vesicarium* were found to be pathogenic to spinach (Spawton et al., 2024b). DNA mutation assays and in vitro assays demonstrated that FRAC group 11 fungicide resistance is widespread in spinach isolates of *S. vesicarium* but not in *S. beticola* (Spawton et al., 2024c).

2. Introgress disease resistance into spinach breeding lines for cultivar development

We conducted recurrent selections to breed spinach for resistance to downy mildew. Crosses were made among cultivars carrying different downy mildew-resistant genes to combine their resistances. Progenies from 34 crosses, along with resistant and susceptible controls, were planted in replicated field trials at the USDA-ARS station in Salinas, CA, to test their resistance. In our trials, two spinach breeding populations showed 0% downy mildew incidence, while five other populations had less than 10% incidence, compared to the susceptible control ('Viroflay') with 97% disease incidence. Our results demonstrate that the recurrent selection method was highly effective in increasing downy mildew resistance in spinach populations. Plants with resistance to downy mildew were selected and transplanted into isolation chambers to produce seeds for the next round of selection.

A total of 836 breeding lines were evaluated at the Texas A&M AgriLife Research and Extension Center in Weslaco, with selection performed for leaf characteristics and overall disease resistance.

Other 200 new spinach lines were grown and harvested during the winter seasons of 2022-2023 and 2023-2024. These 200 new spinach lines were planted in October 2024 at the Alma (Kibler) Station for evaluation and selection.

3. Develop a spinach grower-oriented outreach program based on experiential learning and economic decision tools for disease management in spinach.

An enterprise crop budget was developed for fresh spinach production in Texas, and data related to the marketing, production, and trade of fresh spinach in the U.S. were collected, compiled, and tabulated (Villavicencio et al. 2024a,b,c).

4. Others

Transmission of downy mildew: An experiment demonstrated that oospores can serve as a source of inoculum for downy mildew (DM), providing further evidence of direct seed transmission of the pathogen to spinach seedlings via seedborne oospores, in addition to the airborne sporangia involved in the disease cycle of *Peronospora effusa* (Klosterman et al., 2024).

Oxalate content: Nine significant SNPs (four on chromosome 1 and five on chromosome 5) were associated with oxalate content, and genomic prediction models demonstrated notable predictive abilities, yielding accuracies of up to 0.51 for GEBV estimation (Xiong et al., 2024).

Leafminer tolerance: Three SNP markers on chromosomes 1, 3, and 4 were identified as associated with leafminer resistance, and a high prediction ability ($r = 0.79$) was estimated for GEBV using the cBLUP model (Alatawi et al., 2024).

Vitamin C content: Sixty-two SNP markers distributed across all six spinach chromosomes were associated with vitamin C content, with prediction ability (PA) exceeding 40%. Using GWAS-derived significant SNP markers further increased PA, with a high r -value of up to 0.82 (Rameneni et al., 2024).

What opportunities for training and professional development has the project provided?

1. Postdoc, research associate, research assistant, and graduate students hiring

One Post-Doctoral Research Associate positions was filled in Dr. du Toit's laboratory at Washington State University and another will be hired at Dr. Avila's research program at Texas A&M AgriLife, respectively. A program associate, a breeding assistant and a PhD student were also hired in Dr. Shi's lab.

An undergraduate student will complete a 15-week professional internship (August-December 2024) at the Texas A&M AgriLife Research and Extension Center in Weslaco, TX. The student will work under Dr. Zapata's supervision to evaluate the economic feasibility of resistant varieties and cultivars developed in this project.

Four undergraduate students from the University of Texas – Rio Grande Valley, Texas A&M – Kingsville, and the University of Texas at Austin participated in a 10-week summer 2024 internship at Dr. Carlos Avila's laboratory at the Texas A&M AgriLife Research and Extension Center as part of the Cross-Border Threat Screening and Supply Chain Defense (CBTS) program. Participants were trained in disease identification using conventional and molecular methods, PCR disease diagnostics, and PCR marker-assisted selection.

2. Outreach:

A spinach field day took place at San Juan Bautista, CA, on November 8, 2023, hosted by Dr. Correll at the University of Arkansas and co-hosted by ENZA.

Another spinach field day was held at the University of Arizona Experiment Station in Yuma, AZ, on February 21, 2024, co-hosted by Dr. Correll at the University of Arkansas and the University of Arizona. This event attracted over 100 stakeholders.

The third field day occurred on February 20, 2024, at Tiro Tres Farms in Crystal City, TX, coordinated by Dr. Larry Stein, Mr. Er Ritchie II, and the Texas Wintergarden Spinach Board. Approximately 70 participants, including producers, seed representatives, research and extension faculty, and stakeholders, attended the event, where CEUs were offered. Presentations covered a variety of topics, including lawn regulations, research trials by Dr. Stein, fungicide control trials for *Stemphylium*, *Stemphylium* screening

trials by Dr. Lindsey du Toit from Washington State University, spinach seedling diseases, and an overview of the SCRI spinach project. After the presentations, participants were directed to the field to observe the trials.

Additionally, Dr. Correll has hosted and organized multiple stakeholder spinach field days in Salinas, CA, and Yuma, AZ, during winter seasons and hosted the spinach international conferences for over 20 years.

How have the results been disseminated to communities of interest?

Three field days were held in 2023 and 2024, featuring overall presentations about the SCRI spinach project, which are described in detail in the Outreach section.

Dr. Avila organized a workshop at the American Society for Horticultural Sciences (ASHS) annual meeting in Honolulu, HI, in September 2024. This workshop aims to showcase current efforts and strategies for breeding vegetable crops for quality and post-harvest shelf-life. The audience will include members of the scientific community, students, and industry representatives attending the meeting.

Dr. Poudel-Ward organized an Annual Plant Pathology Program workshop on August 29, 2024, which was attended by 72 stakeholders. Additionally, a Western Regional Meeting of the National Association of County Agricultural Agents was attended by 30 extension professionals.

In total, ten articles were published, four manuscripts were submitted, and eleven abstracts/presentations were made at the 2024 International ASHS conference and other meetings.

What do you plan to do during the next reporting period to accomplish the goals?

Field evaluation: Spinach lines will be evaluated under field conditions for downy mildew (DM) resistance in Yuma, AZ, and Salinas, CA; and for white rust (WR), Stemphylium leaf spot (SLS), and Anthracnose leaf spot (ALS) resistance in Crystal City, TX.

Greenhouse/growth chamber evaluation: Spinach lines will also be evaluated under greenhouse/growth chamber conditions for DM resistance at the University of Arkansas, for ALS resistance at Texas A&M, and for Fusarium wilt (FW) resistance in Washington State.

Disease resistance genetics: QTL mapping, genome-wide association studies (GWAS), and genomic prediction (GP) will be conducted to evaluate resistance to the five pathogens (ALS, DM, FW, SLS, and WR).

Spinach line development: We will continue selecting and breeding new spinach lines for: DM resistance at both the USDA facility in Salinas, CA, and the University of Arkansas at Fayetteville, AR; ALS, SLS, and WR resistance at both the University of Arkansas and the Texas A&M AgriLife Research and Extension Center in Weslaco, TX; and FW resistance at Washington State University and the University of Arkansas.

Outreach: Field days will be organized and held in: Crystal City, TX, Salinas, CA, and Yuma, AZ.

Publications and conferences: Ten or more articles will be published in refereed journals, and over ten presentations will be delivered at international, national, and regional conferences, as well as field days.

Changes/Problems

Describe major changes/problems in approach and reason(s) for these major changes. If applicable, provide special and/or additional reporting requirements specified in the award Terms and Conditions.

None.

Estimated Project FTEs for The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	8	5	1	2	16
Professional	2				2
Technical	2				2
Administrative					
Other					
Computed Total					
	12	5	1	2	20

Activities

Research	80 %
Extension	10 %
Education	10 %

Research Effort Categories

Basic	20 %
Applied	60 %
Developmental	20 %

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1430	1080	25
202	1430	1080	25

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1430	1081	25
202	1430	1081	25

Knowledge Area

201 - Plant Genome, Genetics, and Genetic Mechanisms; 202 - Plant Genetic Resources

Subject Of Investigation

1430 - Greens and leafy vegetables (includes endive, lettuce, spinach, turnip-greens, celery, rhubarb, parsley, asparagus)

Field Of Science

1080 - Genetics; 1081 - Breeding