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United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034419

Project Title

Development of Genomic Resources for Pythium Tolerance in Spinach

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-67013-45988	**Cumulative Award Amt.** $615,000.00
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Program Code [A1141] Plant Health and Production and Plant Products: Plant Breeding for Agricultural Production	

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(N/A)

Non Technical Summary

Spinach (Spinacia oleracea L.) is an economically important leafy vegetable and one of the fastest-growing crops in the United States due to increasing consumer demand for nutritious, health-promoting foods. However, damping-off and root rot caused by Pythium species represent major constraints to spinach production in both open-field systems and Controlled Environment Agriculture (CEA), including greenhouse, hydroponic, and vertical farming systems. Tolerance to Pythium in spinach is quantitatively inherited, and no commercial cultivar currently exhibits complete resistance. This project aims to accelerate spinach genetic improvement by integrating traditional breeding approaches with advanced genomic tools. Genome-wide association studies (GWAS) and genomic selection (GS) will be used to identify genetic variation, genomic regions, and molecular markers associated with tolerance to multiple Pythium species. These resources will enable breeders to more efficiently select and develop spinach cultivars with improved disease tolerance. The specific objectives are to: (1) evaluate and screen spinach germplasm for tolerance

to multiple Pythium species; (2) identify single nucleotide polymorphism (SNP) markers associated with Pythium tolerance through GWAS; and (3) develop and validate genomic prediction models to accelerate breeding progress. The anticipated outcomes include improved spinach germplasm and genomic resources that support sustainable production in both open-field and CEA systems. This project aligns with the AFRI priority area Plant Breeding for Agricultural Production.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	20%
Applied	60%
Developmental	20%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1430	1081	25%
202	1430	1081	25%
202	1430	1080	25%
201	1430	1080	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

[1081] Breeding

[1080] Genetics

Keywords

Disease Resistance, GWAS, Plant Breeding, Pythium, SNP, Spinach, Tolerance, Spinacia oleracea

Goals / Objectives

Spinach (Spinacia oleracea L.) is a globally important vegetable crop valued at approximately $39.6 billion, with U.S. production of 367,433 tons in 2020, ranking second worldwide (Rameneni et al., 2025). Its high nutritional value--

including vitamins (A, C, K, and folate), essential minerals (iron and calcium), antioxidants, and dietary fiber--has driven steadily increasing consumer demand. In the United States, most fresh-market spinach is produced in open-field systems, primarily in California and Arizona. However, growing demand for locally produced, year-round, and pesticide-free leafy greens has accelerated adoption of Controlled Environment Agriculture (CEA), including hydroponic and vertical farming systems, which improve resource-use efficiency and reduce environmental impacts. Despite these advantages, most existing spinach cultivars were developed for open-field production and are poorly adapted to indoor CEA conditions, limiting productivity and sustainability. In addition, spinach production in both field and CEA systems is severely constrained by damping-off and root rot caused by Pythium species.The overarching goal of this project is to accelerate public spinach breeding by identifying genetic variation and developing genomic tools for tolerance to Pythium species. This work will support sustainable spinach production in both open-field and controlled environment agriculture while reducing reliance on chemical disease control.ObjectivesScreen spinach germplasm for Pythium toleranceEvaluate 480 diverse spinach genotypes--including USDA-GRIN accessions, commercial cultivars and hybrids, and Arkansas breeding lines--to identify tolerant germplasm and generate high-quality phenotypic data.Identify genomic regions and SNP markers associated with toleranceConduct genome-wide association studies (GWAS) using high-density SNP data from whole-genome resequencing to identify genomic regions and breeder-ready SNPs for marker-assisted selection (MAS).Develop and validate genomic prediction modelsApply genomic selection to estimate genomic estimated breeding values (GEBVs) for Pythium tolerance and evaluate multiple prediction models to accelerate selection in public breeding programs.ImpactThis project will deliver Pythium-tolerant germplasm, SNP markers, and validated genomic prediction models, enabling faster and more precise development of disease-tolerant spinach cultivars for both soil-based and CEA production systems. The outcomes will enhance sustainability, strengthen food security, and improve the competitiveness of U.S. spinach production.

Project Methods

This project will evaluate spinach (Spinacia oleracea L.) germplasm for tolerance to multiple Pythium species, identify associated genomic regions, and develop genomic prediction models to accelerate breeding of resistant cultivars. Plant MaterialsA total of 480 spinach genotypes will be evaluated, including 400 USDA-GRIN accessions, 50 commercial cultivars and hybrids, and 30 Arkansas breeding lines. This panel has been used previously for trait evaluation and genomic studies (Bhattarai et al., 2022; Shi et al., 2022). Newly produced or properly stored seeds with ?70% germination will be used to ensure data quality.Pythium SpeciesFour spinach-pathogenic Pythium species will be tested: P. aphanidermatum, P. dissotocum, P. ultimum, and P. debaryanum. Isolates include AZ3A (P. aphanidermatum, Yuma, AZ), TPs-1 (P. dissotocum, Bowery Farming hydroponics), and one isolate each of P. ultimum and P. debaryanum. Species identity will be confirmed using ITS and Cox gene sequencing.Phenotyping for Pythium ToleranceSoil-Based Greenhouse EvaluationExperiments will follow a completely randomized design with four replicates.Germination stage: Thirty seeds per genotype will be sown in Pythium-infested soil and 30 seeds in non-infested soil. Germination will be recorded at 9 and 15 days after planting. Tolerance will be quantified using the Relative Disease Index (RDI). Genotypes with <70% germination under control conditions will be excluded.Seedling stage: Thirty-day-old plants will be inoculated with Pythium using colonized PDA plugs and incubated under high humidity. Disease severity will be rated on a 0-3 scale.Indoor Hydroponics EvaluationHydroponic experiments will be conducted in closed-loop deep-water culture systems at the University of Arkansas and in controlled grow rooms at 80 Acres Farms. For each genotype and Pythium species, tolerance will be assessed using relative chlorophyll index (RCI), relative root-weight index (RRI), relative shoot-weight index (RSI), root lesion development, and pathogen re-isolation.Phenotypic Data AnalysisAnalysis of variance (ANOVA) and mixed linear models will be used for individual and combined analyses. Highly tolerant genotypes will be selected for pre-breeding, germplasm release, or use as breeding parents.Genotyping and GWASAll 480 genotypes have been whole-genome resequenced at 10× coverage, generating more than 2.35 million high-quality SNPs. Phenotypic data from soil-based and hydroponic trials will be used for GWAS using MLM in TASSEL 5 and MLM, FarmCPU, and BLINK in GAPIT3. Linkage disequilibrium and haplotype blocks will be analyzed using Haploview. Significant SNPs will be identified for marker-assisted selection (MAS) and genomic selection (GS).Genomic PredictionGenomic prediction will be conducted using random SNP sets

(5-50,000 SNPs) and GWAS-derived SNP marker sets. Models evaluated will include Bayesian methods (BA, BB, BL, and BRR), BLUP-based methods (cBLUP, gBLUP, GAGBLUP, rrBLUP), and machine-learning approaches (Random Forest (RF) and SVM). Prediction accuracy will be assessed using five-fold cross-validation repeated 100 times and estimated as the Pearson correlation between genomic estimated breeding values (GEBVs) and observed phenotypes.Expected Outcomes, Feasibility, and SafetyThis project is expected to identify more than 30 Pythium-tolerant spinach lines, SNP markers, and validated genomic prediction models to accelerate breeding for both soil-based and controlled-environment systems. Preliminary data demonstrate feasibility, and collaboration with 80 Acres Farms provides sufficient hydroponic capacity. No hazardous chemicals harmful to humans or the environment will be used.