ABSTRACT

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

Project Title: Spinach Breeding and Genetics

Project Investigator: Beiquan Mou, Agricultural Research Service, U.S. Dept. of Agriculture, Salinas, CA

Summary:

Our emphasis is on problems facing the spinach industry in California, including coastal, desert, and interior valley. New or existing diseases, insects, or pathogens continue to appear or evolve to pose new challenges for growers, shippers, researchers, and the industry. Changes in production practices and marketing approaches also demand new genetic solutions. The spinach breeding and genetics program aims to incorporate valuable traits into spinach cultivars including resistances to downy mildew, Verticillium wilt, and Stemphylium leaf spot diseases, leafminer insect, and herbicides, and nutritional improvement in oxalic acid content. Horticultural traits, adaptation, and yield are also important. The most economical means of disease and insect control is through the use of genetic resistance. This is especially true for organic growers who must rely on a combination of plant resistance, organically certified pesticides and cultural practices to control diseases and insects. The use of resistant cultivars may reduce expenses for chemicals, energy, and labor associated with pesticide applications and minimize potential adverse effects of pesticide use. In this study, a wide range of genetic variation and sources of resistance to Verticillium wilt disease were found in the USDA spinach genebank. These resistant spinach genotypes could potentially serve as source of resistance to this disease. The results suggest that improvements for genetic resistance to the disease seem feasible in spinach. We are also breeding spinach for resistance to downy mildew, leafminers, and herbicides. We have conducted biochemical analyses (carotenoids, phenolics, sugars, proteins, etc.) of 16 leafminer resistant and susceptible spinach genotypes to study the mechanism of resistance. Leafminer stings per unit leaf area were negatively correlated with contents of water, chlorophyll, and lutein. Leafminer mines per unit plant weight were negatively associated with water content.

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Objective 1. Screening for Resistance to Verticillium wilt in the USDA Spinach Germplasm Collection.

Procedures.

Plant materials. A total of 102 accessions from the USDA spinach collection that have not been screened were examined for resistance to Verticillium wilt in a greenhouse test. The experimental design was a randomized complete block with four replications. In each replication, 8 seeds of each accession were planted in Sunshine Plug 5 Growing Mix in plastic transplanting trays (128 cells, 3 x 3 x 5 cm in length x width x height) in a greenhouse in winter to control day length.

Inoculations. In collaboration with Dr. Krishna Subbarao's lab, three replications were inoculated with a Race 2 isolate So923 from spinach while the other replication was used as uninoculated checks. Seedlings were inoculated at 2, 3, and 4 weeks after sowing by saturating the soil in each plug tray well with a 3-ml suspension containing 10^6 conidia/ml in sterile, distilled water. Seedlings were incubated for another week after last inoculation and then transplanted into 0.5-liter (16 oz) foam-insulated cups filled with a pasteurized sand: potting soil mixture (3:1, vol/vol). One week after transplanting, day length was extended to 19 hr/day by

supplemental lighting to promote bolting, as symptoms of Verticillium wilt on spinach mainly develop after bolting stage.

Evaluations. Starting from three weeks after last inoculation, severity of symptoms were rated weekly using a scale of 0 to 4: 0 = no symptoms, 1 = lower leaves with patches of yellow areas, 2 = middle leaves with patches of yellow areas, and 3 = upper leaves with patches of yellow areas. After final rating, roots were cleaned of sand and cut longitudinally to evaluate disease severity as the % brown discoloration of vascular tissue in the roots, crown, and lower stem, characteristic of Verticillium wilt. The growth period of the inoculated plants was compared with the uninoculated control. To confirm the presence of the pathogen, *V. dahliae* was re-isolated from diseased tissue. Roots, crown, and lower stems were placed on NP-10 medium and examined microscopically for development of conidiophores and/or microsclerotia of *V. dahliae*. To examine the seed transmission of the pathogen, mature seeds from each plant were harvested separately and assayed for *V. dahliae* by plating 20 seeds on NP-10 medium. The seeds were observed under a microscope for microsclerotia and/or conidiophores and conidial characteristics of *V. dahliae*. In collaboration with Dr. Steve Klosterman's lab, the seeds were also analyzed with a real-time PCR assay by using primers derived from the β-tubulin gene of *V. dahliae* coupled with SYBR green dye.

Results and Discussion.

The Verticillium disease incidence (% diseased plants) varied greatly among the 102 genotypes, ranging from 0 to 100% (Fig. 1). There were also large variation in disease severity among different accessions and cultivars, which ranged from 0 to 3 (Fig. 2). These results suggest that there are significant genetic differences in Verticillium disease resistance among the genotypes.

Six putative resistant accessions, PI 224959, PI 274051, PI 274060, PI 303138, PI 339545, and PI 531452, had no disease symptoms, low seed infection % from NP-10 plate assay, and low pathogen copy numbers in quantitative PCR tests (Table 1). These spinach genotypes could potentially serve as source of resistance to Verticillium wilt disease. However, these are preliminary experiment results and need to be confirmed in further testing. These and other resistant accessions from previous screenings are being tested against another Race 2 isolate as well as a Race 1strain in greenhouse. Nevertheless, these results are encouraging and suggest that the development of Verticillium-resistant spinach cultivars is feasible.

Table 1. Verticillium disease incidence and severity, seed infection % (tested on NP-10 plates), and pathogen copy number in seed (determined by quantitative PCR) of selected accessions of the USDA spinach germplasm collection.

Genotype	Incidence %	Severity 0 – 3	NP-10 positive %	Pathogen qPCR copy#
PI 263873	45.9	3.0	31.8	236.7
PI 648942	46.5	2.5	49.5	135.3
PI 224959	0.0	0.0	0.0	0.0
PI 274051	0.0	0.0	5.0	4.4

PI 274060	0.0	0.0	6.3	8.2
PI 303138	0.0	0.0	17.6	91.3
PI 339545	0.0	0.0	14.3	153.6
PI 531452	0.0	0.0	3.6	3.9

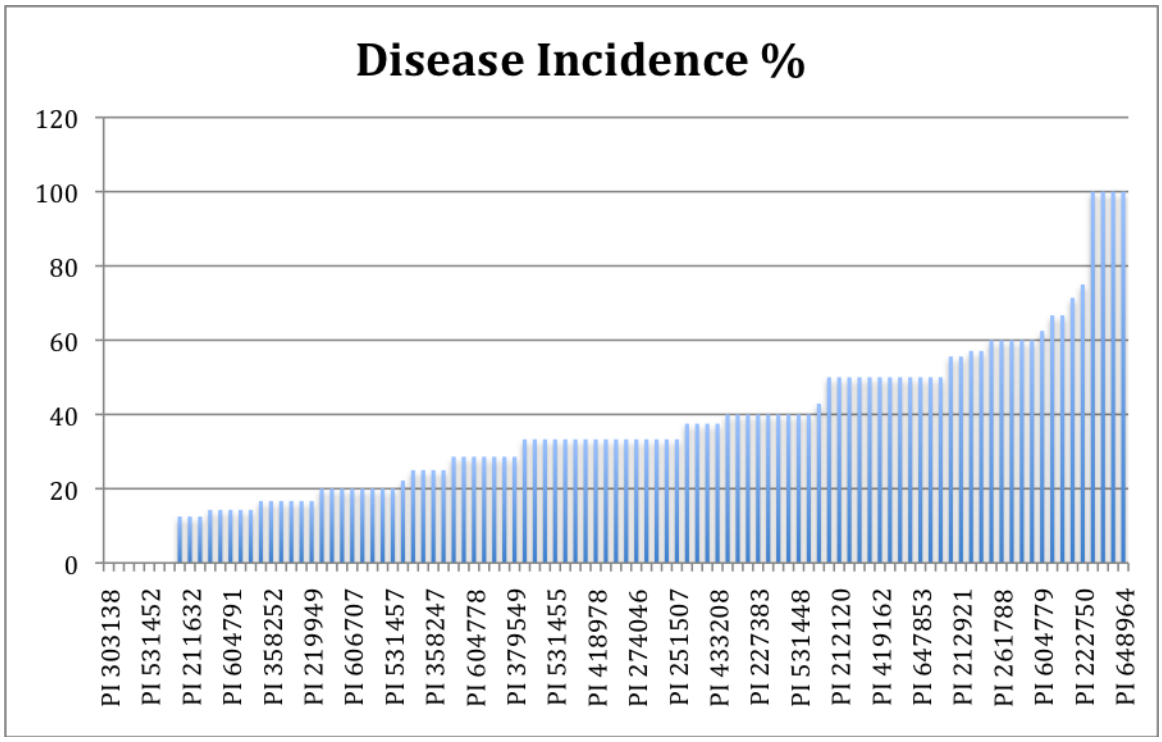

Fig. 1. Verticillium wilt disease incidence of 102 accessions from the USDA spinach germplasm collection.

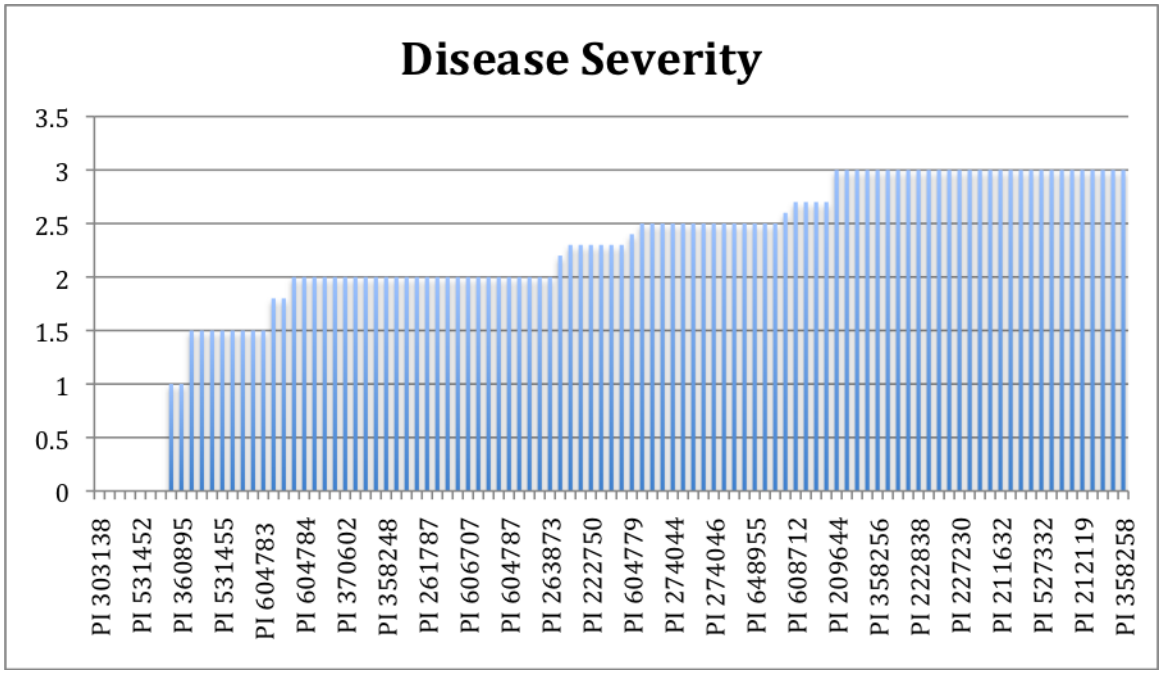

Fig. 2. Verticillium wilt disease severity of 102 accessions from the USDA spinach germplasm collection.

Other Research Projects:

Downy Mildew (with Steve Koike, Jim Correll) Several rounds of inoculum increase were performed on susceptible cultivars to maintain and produce sufficient inoculums of different downy mildew strains for germplasm screening and resistance breeding. We are making crosses to help Dr. Jim Correll's group develop near isogenic lines of different DM resistant genes. Crosses were made among ten cultivars with different DM resistant genes to combine their resistances.

Leafminer A recurrent selection method was used to increase the level of resistance to leafminers in 10 populations of different leaf types. Plants with fewer leafminer stings or mines were selected and transplanted into isolators to produce seeds for further rounds of evaluation and selection. In collaboration with Dr. Jianlong Bi, we have conducted biochemical analyses (carotenoids, phenolics, sugars, proteins, etc.) of 16 leafminer resistant and susceptible spinach genotypes to study the mechanism of resistance. These genotypes were evaluated in a field trial with four replications for leafminer sting and mine damages, and plant weight. Leaf samples were taken from each plot and were analyzed. These genotypes were also grown in replicated experiments in growth chambers for biochemical analysis. Leafminer stings per unit leaf area were negatively correlated with contents of water, chlorophyll, and lutein (Table 2). Leafminer mines per unit plant weight were negatively associated with water content.

Table 2. Phenotypic correlation coefficients of leafminer sting density on spinach leaves and mines per 100 g leaves with concentrations of phytochemicals in 16 leafminer-resistant and susceptible spinach genotypes.

Concentrations	Sting/cm^2	Mine/100 g
Water %	- 0.624**	- 0.522*
Chlorophyll a	- 0.694**	- 0.111
Chlorophyll b	- 0.733**	0.076
Total Chlorophyll	- 0.729**	- 0.053
Lutein	- 0.632**	0.465
β-Carotene	- 0.455	0.198
Flavonoids	0.050	0.285
Protein	- 0.328	0.229
Glucose	- 0.386	0.265
Fructose	0.304	- 0.230
Sucrose	0.002	- 0.247

Herbicide Resistance (with Steve Fennimore's Group) We screened spinach germplasm for resistance to Linuron herbicide in the field and resistant plants were transplanted into isolators to produce seeds for future testing (see Dr. Fennimore's report for details).

Publications relevant to this project in 2011-12:

Mou, B., K. Richardson, S. Benzen, and H.-Y. Liu. 2012. Effects of *Beet necrotic yellow vein virus* in spinach cultivars. Plant Disease 96: 618-622.

Duressa, D., G. Rauscher, S. T. Koike, B. Mou, R. J. Hayes, K. Maruthachalam, K. V. Subbarao, and S. J. Klosterman. 2012. A real-time PCR assay for detection and quantification of *Verticillium dahliae* in spinach seed. Phytopathology 102: 443-451.