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 Race 10 of downy mildew pathogen and Verticillium wilt disease were found in the USDA spinach genebank. The results suggest that improvements for genetic resistance to these diseases seem feasible in spinach. Indeed, research is currently in progress to incorporate the resistance traits identified in this study into elite cultivars in our spinach breeding program. We are also breeding spinach for resistance to leafminers, herbicides, and heat stress. We previously found that beet necrotic yellow vein virus (BNYVV, normally a sugarbeet pathogen) is a new threat to spinach production in California. Our new experiment results suggest that BNYVV is not transmitted through seeds.

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Objective 1. Screening for Resistance to Downy Mildew Race 10 in the USDA Spinach Germplasm Collection.

Procedures.

Plant materials. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, CA. The 135 accessions from the USDA spinach collection (seeds provided by the North Central Regional Plant Introduction Station, Iowa State University, Ames, IA) and 7 commercial cultivars were screened for resistance to downy mildew in a growth chamber test. The USDA collection includes accessions of cultivated spinach (*Spinacia oleracea*), and wild species *S. turkestanica* and *S. tetrandra*. Cultivar 'Tarpy' that has resistance to Race 1-7 was the susceptible control and 'Lazio' served as the resistant control. The experimental design was a randomized complete block with two replications. In each replication, 8 seeds of each accession was planted in Sunshine Plug 5 Growing Mix (Sun Gro Horticulture, Inc., Bellevue, Wash.) in plastic transplanting trays (128 cells, 3 x 3 x 5 cm in length x width x height) in a greenhouse.

Inoculations. Downy mildew Race 10 inoculums was increased on cultivar 'Tarpy'. The inoculums was also tested against a set of downy mildew race differentials. Only fresh inoculums were used to screen germplasm. Leaves with pathogen spores were put into a flask with refrigerated distilled water and shaken vigorously. The spore suspension was poured through two layers of cheesecloth to remove debris and adjusted to 3.0×10^5 spores/ml. Spores were sprayed onto three-week-old spinach seedlings of different accessions with a hand-held

mister until runoff. Plants were incubated in a dew chamber at 18°C and 99% relative humidity for 48 hours, and then in a growth room for 5 days. Plants were then returned to the dew chamber maintained at 18°C and 99% relative humidity for 24 hours to induce sporulation.

Disease Evaluations. After 24 hours in a dew chamber to induce sporulation, cotyledons and true leaves of the plants were rated qualitatively and quantitatively with the aid of an Opti Visor magnifier. Cotyledons were evaluated for the presence or absence of sporulation. True leaves were rated for the percentage of leaf area with symptoms and sporulating lesions. Disease incidence (% diseased cotyledons and true leaves) and severity (% diseased leaf area) of each genotype were calculated.

Results and Discussion.

There were significant differences in both incidence and severity of the disease among the genotypes tested. Of the 142 genotypes evaluated, disease incidence ranged from 0 to 100% (Fig.1). Similarly, disease severity on true leaves had a range of 0 to 90% (Fig. 2). These results show that there is large genetic variation in resistance to downy mildew Race 10 in the USDA spinach germplasm collection we screened.

Eight accessions, PI 174384, PI 174388, PI 177557, PI 179043, PI 179597, PI 181808, PI 181923, and PI 205234, had no disease incidence and severity, just like the resistant control 'Lazio' (Table 1). These genotypes may be potential source of resistance against the downy mildew Race 10. However, these results are preliminary and the experiments need to be repeated to confirm the findings. We will test the putative resistant accessions again with more replications and controls in future experiments.

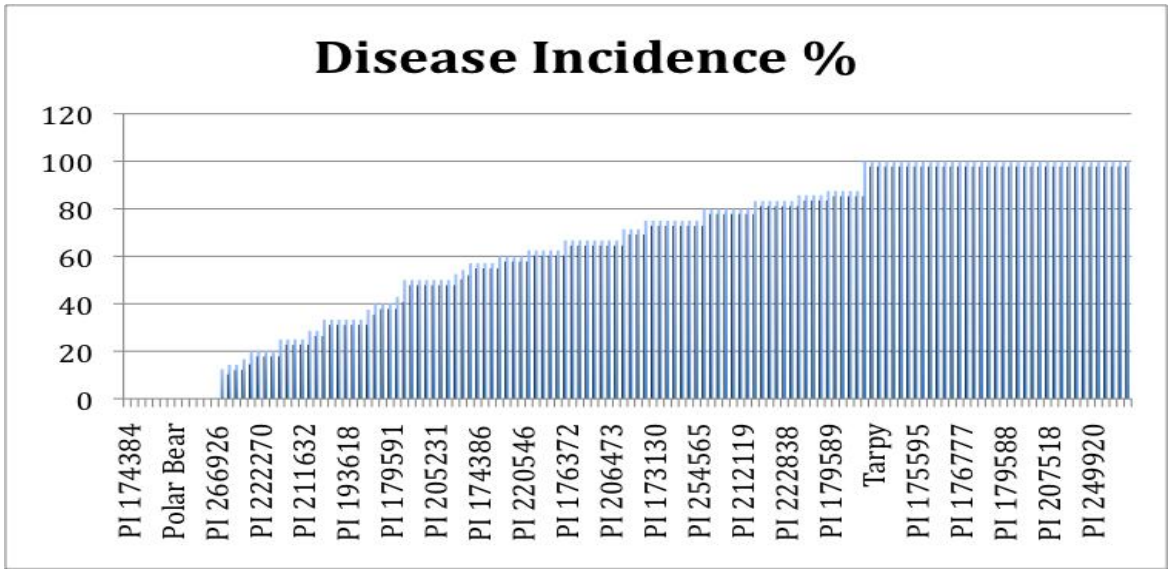

Fig. 1. Downy mildew Race 10 disease incidence % on cotyledons and true leaves of the USDA spinach germplasm collection.

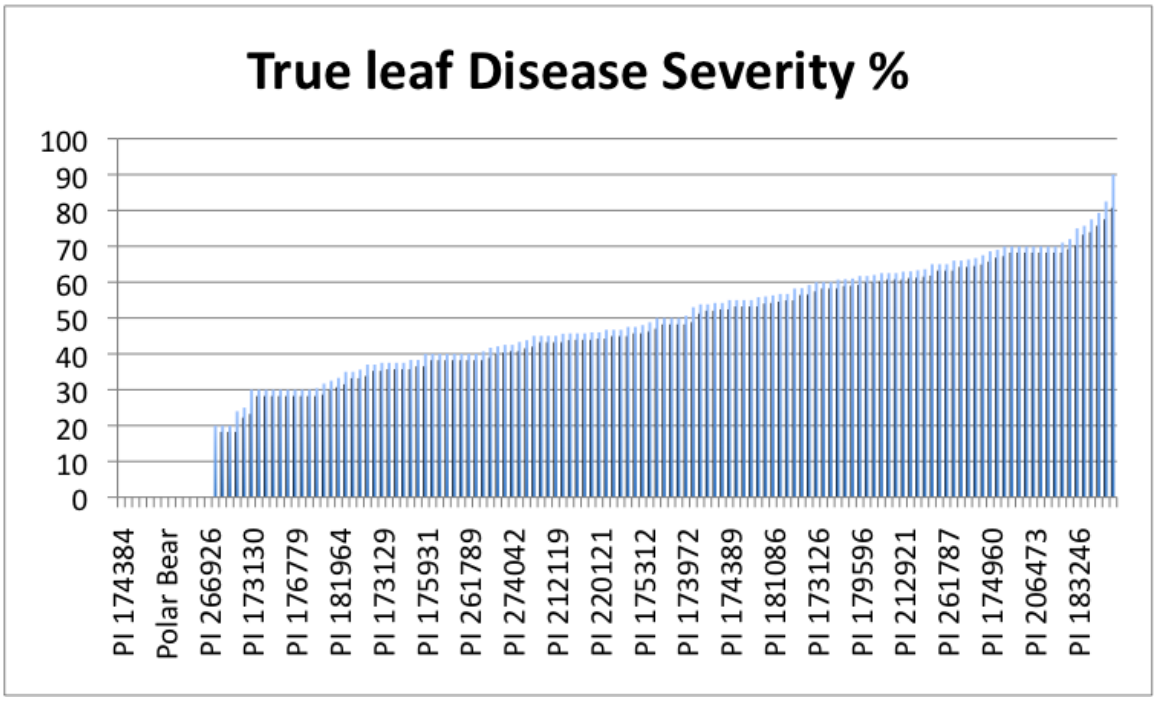

Fig. 2. Downy mildew Race 10 disease severity % on true leaves of the USDA spinach germplasm collection.

Table 1. Downy mildew Race 10 disease incidence and severity of selected commercial cultivars and accessions of the USDA spinach germplasm collection.

Genotype	Disease incidence %	Leaf disease severity %
Tarpy	100.0	45.6
Lion	54.2	43.8
Crocodile	50.0	53.8
Lazio	0.0	0.0
PI 174384	0.0	0.0
PI 174388	0.0	0.0
PI 177557	0.0	0.0
PI 179043	0.0	0.0
PI 179597	0.0	0.0
PI 181808	0.0	0.0
PI 181923	0.0	0.0
PI 205234	0.0	0.0

Objective 2. Screening for Resistance to Verticillium wilt in the USDA Spinach Germplasm Collection.

Procedures.

Plant materials. A total of 106 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. Three replications were inoculated with a Race 1 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, 3 = upper leaves with patches of yellow areas, and 4 = all leaves died. 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*.

Results and Discussion.

The Verticillium disease incidence (% diseased plants) varied greatly among the 106 genotypes, ranging from 11 to 100% (Fig. 3). There were also large variation in disease severity among different accessions and cultivars, which ranged from 0.3 to 3 (Fig. 4). These results suggest that there are significant genetic differences in Verticillium disease resistance among the genotypes tested.

Several USDA spinach germplasm accessions, such as PI 176774, PI 171861, PI 179042, PI 179588, and NSL 81328, had low disease incidence or severity (Table 2). These spinach genotypes could potentially serve as source of resistance to Verticillium wilt disease. Again, these are preliminary experiment results and need to be confirmed in further testing.

Nevertheless, these results are encouraging and suggest that the development of Verticillium-resistant spinach cultivars is feasible.

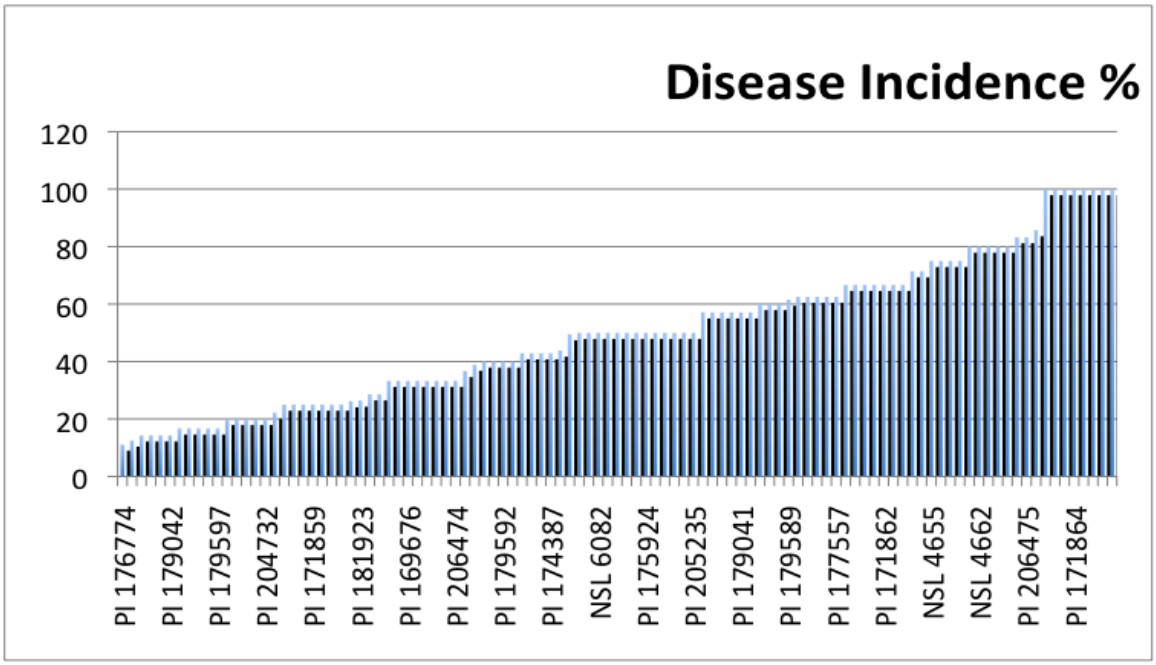

Fig. 3. Verticillium wilt disease incidence of 106 accessions from the USDA spinach germplasm collection.

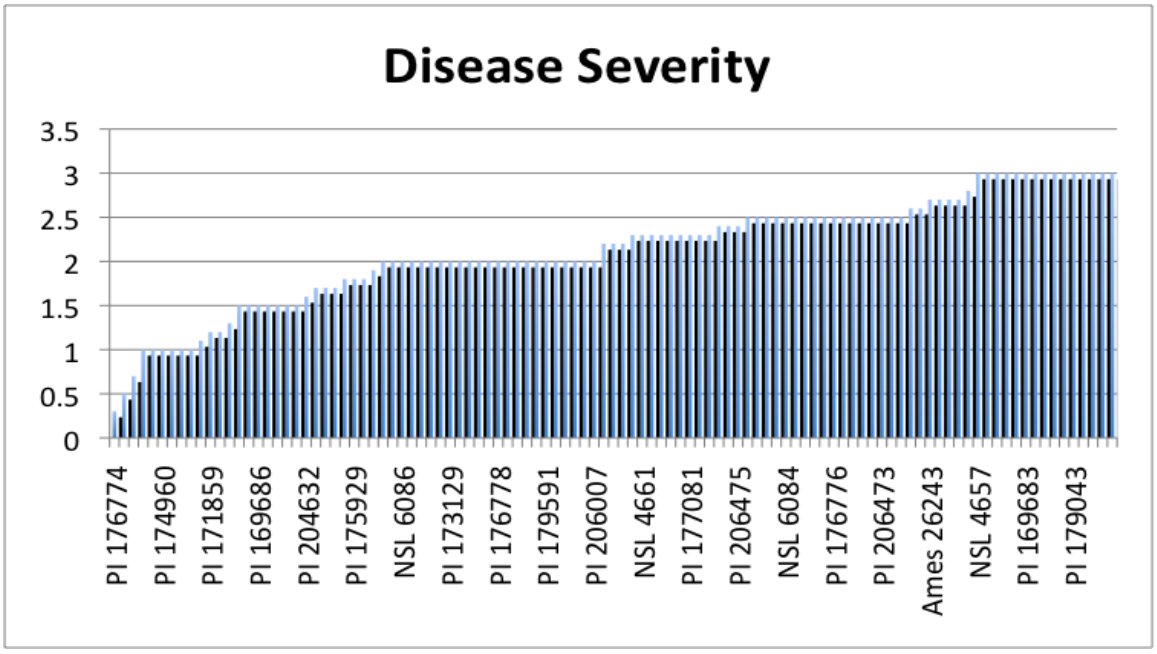

Fig. 4. Verticillium wilt disease severity of 106 accessions from the USDA spinach germplasm collection.

Table 2. Verticillium disease incidence and severity of selected accessions of the USDA spinach germplasm collection.

Genotype	Disease incidence %	Disease severity 0 – 3
PI 173123	75.2 A	2.5 A
Ames 26365	49.5 AB	2.5 A
NSL 81328	33.3 AB	0.5 B
PI 181923	26.4 B	1.5 AB
PI 171859	25.0 B	1.2 AB
PI 204735	19.4 B	1.0 AB
NSL 6087	16.7 B	1.7 AB
PI 179588	16.7 B	0.7 B
PI 179042	14.3 B	1.0 AB
PI 171861	14.3 B	1.2 AB
PI 176774	11.1 B	0.3 B

Other Research Projects:

Leafminer A recurrent selection method was used to increase the level of resistance to leafminers in 12 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. 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 are being analyzed. These genotypes were also grown in replicated experiments in growth chambers for biochemical analysis.

Beet Necrotic Yellow Vein Virus We previously reported that BNYVV is a new threat to spinach production in California. To investigate whether BNYVV is seed-transmitted, seeds were collected from plants of eight spinach cultivars with and without leaf symptoms in each of four replications in two fields infested with two different BNYVV strains. Twenty randomly selected seeds from each harvested plant were tested for BNYVV by ELISA and the results were all negative. These results suggest that BNYVV is not transmitted through spinach seeds.

Publications relevant to this project in 2010-11:

Liu, H. Y., B. Mou, K. Richardson, and S.T. Koike. 2010. First report of *Beet necrotic yellow vein virus* infecting spinach in California. Plant Disease 94(5): 640.