Project Title: Spinach Breeding and Genetics

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Abstract:

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. No accession was resistant to all three Race 1 and Race 2 isolates of *Verticillun dahliae* from spinach, although some accessions were resistant to two isolates. It seems that the two isolates of Race 2 pathogen had different virulence against these spinach accessions. Some accessions had no disease incidence, severity, seed infection, and/or pathogen copies in seeds. Some accessions showed no disease symptoms, but still had seed transmission of the pathogen. The susceptible control and commercial cultivars exhibited high levels of disease incidence %, severity, seed infection % on NP-10 plates, and pathogen copy numbers. These results are consistent with the

results from previous preliminary screening experiments using a Race 2 isolate. These accessions are being tested again in 2013 against the Race 1 and Race 2 isolates of the pathogen. If confirmed, these spinach genotypes could potentially serve as source of resistance to Verticillium wilt disease. Breeding efforts are needed to develop spinach cultivars with resistance to both Race 1 and Race 2 isolates, even different isolates of the same race. Nevertheless, these results are encouraging and suggest that the development of Verticillium-resistant spinach cultivars is feasible. We also conducted experiments to breed spinach for resistance to downy mildew, leafminers, and linuron herbicide.

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

Procedures.

Plant materials. We retested the 19 putative resistant accessions of the USDA spinach germplasm collection that were identified in preliminary screening experiments from 2009 to 2011, plus 4 resistant accessions identified by Dr. Jim Correll, along with 8 susceptible and 9 cultivar controls, to confirm their resistance to *Verticillium dahliae* Race 2 and test their resistance to Race 1. The experimental design was a randomized complete block with nine 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, three replications were inoculated with a Race 1 isolate So302 from spinach, and two replications were inoculated with a new Race 2 isolate So925 from spinach, while the other replication was used as uninoculated checks. Seedlings were inoculated at 3, 4, and 5 weeks after sowing by saturating the soil in each plug tray well with a 3-ml suspension containing $2x10^6$ 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 or wilting, 2 = middle leaves with patches of yellow areas or wilting, 3 = upper leaves with patches of yellow areas or wilting, 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*. 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 genotypes tested, ranging from 0 to 100%. There were also large variation in disease severity among different accessions and cultivars, which ranged from 0 to 3. These results suggest that there are significant genetic differences in Verticillium disease resistance among the genotypes.

Two accessions, NSL6092 and PI 303138, had no disease incidence, severity, and seed transmission (as tested by NP-10 and quantitative PCR assays) when inoculated with a Race 2 isolate So923 (Table 1). Two accessions, NSL81328 and PI 179042, also showed no disease incidence and severity, but had low levels of pathogen transmission through seeds. PI 176774 had low disease incidence and severity ratings but no seed transmission. These results confirmed the conclusions from previous tests that these accessions are resistant to this Race 2 isolate from spinach.

Only two accessions, NSL6097 and PI 179042, showed no disease incidence and severity, but they had seed transmission (as tested by NP-10 and quantitative PCR assays) when inoculated with another Race 2 isolate from spinach, So925 (Table 2). All accessions had certain levels of seed transmission against this isolate. This Race 2 isolate seems more aggressive than the other Race 2 isolate, So923.

Only one accession, PI 303138, had no disease incidence and severity when inoculated with a Race 1 isolate from spinach, So302, but it showed low levels of seed transmission of the pathogen (Table 3). Another accession, NSL6092, had low disease incidence, severity, and seed transmission. Not many accessions were resistant to this Race 1 isolate. That is not surprising, because these accessions were selected against a Race 2 isolate So923 in previous tests.

Disease incidence and seed transmission (as tested by NP-10 assay) of the accessions against the Race 1 isolate and two Race 2 isolates are listed in Table 4. The accessions showed resistance to one or two of the three Race 1 and Race 2 isolates of *Verticilliun dahliae* from spinach, but not to all three isolates.

In conclusion, no accession was resistant to all three Race 1 and Race 2 isolates of *Verticilliun dahliae* from spinach, although some accessions were resistant to two isolates. It seems that the two isolates of Race 2 pathogen had different virulence against these spinach accessions. Some accessions had no disease incidence, severity, seed infection, and/or pathogen copies in seeds.

Some accessions showed no disease symptoms, but still had seed transmission of the pathogen. The susceptible control and commercial cultivars exhibited high levels of disease incidence %, severity, seed infection % on NP-10 plates, and pathogen copy numbers. These results are consistent with the results from previous preliminary screening experiments using a Race 2 isolate. These accessions are being tested again in 2013 against the Race 1 and Race 2 isolates of the pathogen. If confirmed, these spinach genotypes could potentially serve as source of resistance to Verticillium wilt disease. Breeding efforts are needed to develop spinach cultivars with resistance to both Race 1 and Race 2 isolates, even different isolates of the same race. 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 inoculated with a Race 2 isolate So923 from spinach.

Genotype	Incidence %	Severity 0 – 3	NP-10 positive %	Pathogen qPCR copy#
NSL6092	0.0	0.0	0.0	0.0
NSL6097	4.2	1.0	0.8	9.8
NSL81328	0.0	0.0	5.0	6.7
PI 176774	15.3	1.7	0.0	0.0
PI 179042	0.0	0.0	1.7	0.0
PI 181923	25.0	0.6	21.0	38.4
PI 204735	15.3	1.0	5.7	9.1
PI 303138	0.0	0.0	0.0	0.0
PI 648948	91.7	3.0	51.0	191.8
Polar Bear	63.5	2.8	35.7	78.3

Table 2. 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 inoculated with a Race 2 isolate So925 from spinach.

Genotype	Incidence %	Severity 0 – 3	NP-10 positive %	Pathogen qPCR copy#
NSL6097	0.0	0.0	20.0	46.6
NSL81328	19.7	2.0	13.7	12.5
PI 167194	30.0	1.0	11.3	44.5
PI 179042	0.0	0.0	38.3	113.1
PI 179588	12.5	2.0	25.0	77.3
Tarpy	75.0	2.9	45.3	113.6
PI 648942	100.0	2.9	56.7	413.4

Table 3. 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 inoculated with a Race 1 isolate So302 from spinach.

Genotype	Incidence %	Severity 0 – 3	NP-10 positive %	Pathogen qPCR copy#
NSL6087	10.3	2.0	40.8	394.2
NSL6092	11.1	0.3	0.0	0.9
PI 171861	27.0	2.8	55.6	169.9
PI 303138	0.0	0.0	17.6	6.4
PI 648948	85.6	2.9	72.1	701.5
Polar Bear	69.4	2.9	77.3	231.1

Table 4. Verticillium disease incidence and seed infection % (tested on NP-10 plates) of selected accessions of the USDA spinach germplasm collection inoculated with three Race 1 and Race 2 isolates from spinach.

	Race 1 (So 302)		Race 2 (So 923)		Race 2 (So 925)	
Genotype	Incidence %	NP-10 Positive %	Incidence %	NP-10 Positive %	Incidence %	NP-10 Positive %
NSL6092	11.1	0.0	0.0	0.0	50.0	---
NSL6097	36.7	---	4.2	0.8	0.0	20.0
NSL81328	66.7	---	0.0	5.0	19.7	13.7
PI 179042	34.9	---	0.0	1.7	0.0	38.3
PI 303138	0.0	17.6	0.0	0.0	34.8	---
PI 648948	85.6	72.1	91.7	51.0	92.9	---
Polar Bear	69.4	77.3	63.5	35.7	58.4	---

Other Research Projects:

Downy Mildew (with Steve Koike) 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. 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 9 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.

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 2012-13:

Mou, B. 2013. Spinach. in: Wehner, T. C. and Mou, B. (Ed.) Vegetable cultivar descriptions for North America, List 27. HortScience 48: 268-269.

Maruthachalam, K., S. J. Klosterman, A. G. Anchieta, **B. Mou**, K. V. Subbarao. 2013. Colonization of spinach by *Verticillium dahliae* and effects of pathogen localization on the efficacy of seed treatments. Phytopathology 103: 268-280.

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.