Project Title: Spinach Breeding and Genetics

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Abstract:

Spinach production in California and Arizona involves exceptionally high seeding rates, resulting in dense crop canopies and high humidity that favor the development of various foliar diseases such as downy mildew (*Peronospora effuse*). Leafminer (*Liriomyza langei*) is the most important insect pest in spinach in central California. Spinach growers have few herbicides available and need more weed control options. Herbicide-resistant crops enable growers to control weeds with herbicides without injuring crops. We conducted recurrent selections to breed spinach for resistance to downy mildew, leafminers, and linuron (Lorox) herbicide. Three breeding populations had 0 – 2% downy mildew incidence, while the susceptible control cultivar 'Virofly' showed 91% disease incidence. A breeding population had about twice the survival rate of the most tolerant commercial cultivar after the spray of linuron herbicide in the field. Plants with resistances to downy mildew, leafminers, and linuron herbicide were selected and transplanted into our isolators to produce seeds for the next round of selection. Although progress has been made, we need to confirm the results and continue the improvement in breeding experiments next year.

Objective 1. Breeding for Downy Mildew Disease Resistance

Almost all spinach seeds planted in California and Arizona are F_1 hybrids. Spinach grown for processed frozen, standard fresh market, and fresh market "baby leaf" (harvested at the four- to five-leaf stage) products are planted at 1.0 to 1.5, 2.7 to 3.5, and 3.5 to 4.0 million viable seeds per acre, respectively. Such a high seeding rate makes the seed cost very high for spinach growers. Therefore, there are considerable interests in seeds of cheaper, open-pollinated (OP) cultivars. However, the current OP cultivars on the market do not have the resistance against all of the predominant downy mildew races. We proposed to continue our program to breed for spinach OP cultivars with resistance to all prevalent downy mildew races. The “field resistance” is supposed to be non-race-specific, so we hope that the downy-mildew resistance in the OP cultivars from our field selections is more durable than the current commercial cultivars.

Procedures

To breed for open-pollinated cultivars, plants of several spinach F_1 hybrid cultivars with resistance to the predominant downy mildew races 10 - 17 were planted together to open-pollinate with each other. Plants were isolated in groups according to leaf type (flat or savoy) and plant type (oriental or Western). We selected the cultivars involved to maximize the diversity of genetic resistance to downy mildew. Seeds harvested were space-planted in the field between beds of susceptible plants to be naturally infected with downy mildew. Resistant plants were selected and transplanted into isolators and allowed to open-pollinate with each other in isolation. About 200 seeds harvested from 26 populations were planted again at our USDA-ARS research station in Salinas, CA in the fall of 2018 at 3-inch spacing on 40-inch beds along with resistant and susceptible controls in the field with two replications. The plots were between 80-inch beds of susceptible cultivar Viroflay which provided natural inoculants of downy mildew pathogen. ‘Viroflay’ was planted twice, 4-weeks before and at the planting of the main experiment. The field was kept wet with sprinkler irrigations two to three times a week in the evening to favor downy mildew development. We also spread spinach leaves with downy mildew spores collected from another field onto our plots to increase pathogen inoculum. Leaf samples with sporulation of the pathogen were collected to identify pathogen races using differential hosts. Diseased plants in each population were counted to record disease incidence. Resistant plants were selected again from each population and transplanted into isolators for seed production. This cycle of recurrent selection will continue until a satisfactory level of resistance is achieved. Then the populations will be tested in the field for downy mildew resistance, uniformity, horticultural traits, and yield.

We also planted 15 seeds from each of the 386 accessions of the USDA spinach germplasm collection at our USDA-ARS research station in Salinas, CA in the fall of 2018 at 3-inch spacing on 40-inch beds with two replications. Then we collected the downy mildew disease incidence and severity (% diseased area of the leaf with most mildew on the plant) data for each plant. This work is in collaboration with Drs. Ainong Shi and Jim Correll at the University of Arkansas for association mapping of the downy mildew resistance genes.

Results and Discussion

Through differential tests, the downy mildew pathogen present in our breeding field appeared to be Race 17. Downy mildew disease incidences (%) of selected spinach breeding populations and the susceptible control cultivar Viroflay are shown in Table 1. ‘Viroflay’ had 91% disease incidence. Five populations had less than 10% disease incidence. One population, 17-18-27, showed 0% downy mildew incidence, but it had only 25 plants due to poor germination. These populations will be evaluated again in 2019 to confirm the results. Resistant plants with few downy mildew lesions were selected from each of the 26 populations and transplanted into our isolators for open-pollination. Seeds produced have been harvested and are being cleaned, and will be used for the next round of selection in the field.

We also planted the USDA spinach germplasm collection (386 accessions) in the field in two replications and recorded the downy mildew incidence and severity. The data were sent to Dr. Ainong Shi at University of Arkansas to develop molecular markers for downy mildew resistance. We also made crosses between female-only ‘Viroflay’ and other downy mildew resistance differentials ‘Resistoflay’, Califlay’, ‘Clermont’, ‘Campania’, ‘Boeing’, ‘Lion’, ‘Lazio’, ‘Whale’ in isolators to develop resistance mapping populations. These populations were sister-crossed to develop F2 populations. We are also making crosses between ‘Lazio’ and ‘Lion’ in isolators.

These results show that we are making progress in the breeding for downy mildew resistance in spinach. However, the results need to be confirmed in trials next year.

Table 1. Downy mildew disease incidence in selected spinach populations along with the susceptible control cultivar Viroflay in a field at the USDA-ARS station in Salinas, CA in Fall 2018.

Populations	Diseased plants /Total plants	Disease Incidence %
17-18-25	15/106	14.2
17-18-23	15/118	12.7
17-18-21	13/120	10.8
17-18-22	7/81	8.6
10-11-67	7/89	7.9
17-18-15	2/118	1.7
17-18-24	1/81	1.2
17-18-27	0/25	0.0
Viroflay	81/89	91.0

Objective 2. Breeding for resistance to linuron herbicide.

Few herbicides are registered for leafy vegetables in California and they are subject to cancellation due to their relatively small acreage, marginal profitability of these products, and regulatory challenges. Linuron (Lorox) is an herbicide that can provide effective control of more than 20 broadleaf weeds and grasses. Differences in tolerance to this herbicide have been observed in spinach varieties. Herbicide-tolerant spinach would allow growers to control weeds with the herbicide without injuring the crop.

Procedures.

Twelve breeding populations were planted at a 1-foot spacing in the field along with 15 commercial cultivars. Linuron herbicide were sprayed (1.0 lb a.i./a) right after planting.

Surviving tolerant plants were counted for each breeding population and cultivar. Survivors were transplanted into isolators to produce seeds for future testing and selection.

Results and Discussion

The stand count of the cultivars and selected breeding populations after the linuron herbicide spray is shown in Table 2. Among commercial cultivars, Viroflay had the highest survival rate at 3.3% after the linuron spray. A breeding population 17-18-12 had the highest survival rate of 6.3%, showing about twice the survival rate of the most tolerant commercial cultivar. We will repeat the experiment next year to confirm the results. Surviving tolerant plants were transplanted into isolators to produce seeds for testing and selection in 2019.

The 6% survival rate still sounds pretty low. That is partly because we chose a high herbicide dose to reduce the number of survivals we have to select and transplant into our isolators. However, we do need to continue the improvement of herbicide tolerance levels in our breeding populations to meet the production requirement.

Table 2. Number and % of survivors for 15 spinach commercial cultivars and selected breeding populations after the spray of linuron herbicide at 1.0 lb a.i./a in a field of the USDA-ARS station in Salinas, CA in fall, 2018.

Genotype	Survivors/Seeds planted	% Survived
Polar Bear	0/100	0
Spring Field	0/100	0
Bordeaux	0/100	0
Red Deer	0/100	0
Melody	0/100	0
Tarpy	0/100	0
Cypress	0/100	0
Whale	0/100	0
Unipack 277	0/100	0
Space	0/100	0
Eagle	0/100	0
Seven R	0/100	0
Crocodile	13/750	1.7
Swan	4/150	2.7
Viroflay	5/150	3.3
16-17-10	21/600	3.5
17-18-31	38/700	5.4
17-18-12	44/700	6.3

Objective 3. Breeding for leafminer resistance.

The predominant species of leafminers in central California is *Liriomyza langei.* They have a wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds. Damages caused by adult sting and larval mining of leaves reduce photosynthetic capacity and product quality, render spinach leaves unmarketable, and provide an entrance for disease organisms. Chemical control is not long-lasting, and it is well documented that leafminers can develop a high degree of resistance to insecticides. Insecticides for adult flies do not provide economic control against a moving target. Chemicals against leafminer larvae may be limited by pre-harvest spray interval (PHI) for a short-season crop like spinach or plant back restrictions for rotational crops. Plant resistance may be the most economical means to control leafminers.

Procedures.

A phenotypic recurrent selection method was used to increase the level of leafminer resistance against stings and mines. Plants with fewer stings and/or mines were selected from five resistant populations in the field and placed in glass isolators on the station to open-pollinate with each other. Seed harvested from these plants will be planted in the field next season. Leafminer-resistant plants will be selected for another cycle of hybridization. This cycle will continue until a satisfactory level of resistance is reached. Starting with 27 yellow clover aphid-resistant plants selected from a wide diversity of germplasm, Gorz and co-workers (1979) used phenotypic recurrent selection to improve resistance to aphids in red clover. Through five cycles of testing and selection for yellow clover aphid resistance and three such cycles for pea aphid resistance, a synthetic variety ('N-2') was developed that had a high level of resistance to both aphids.

Results and Discussion.

Plants with fewer leafminer stings or mines were selected and transplanted into isolators to produce seeds for further rounds of evaluation and selection. When populations reach certain resistance levels, they will be placed in resistance trials in the field along with resistant and susceptible controls. The experiment design will be a randomized complete block with eight replications. Each plot will be consisted of 10 plants of a genotype, with 30 cm between plants and 35 cm between rows on double-row beds of 1-m center. Leafminer stings will be counted in a 20-cm^2 leaf area with the highest sting density on each plant five weeks after planting. Mines per plant and plant weight excluding roots will also be recorded. Mines per plant will be divided by plant weight to generate mines per unit plant weight. Per plant values will be averaged and analysis will be conducted on the basis of plot means.

Although we have released two spinach germplasm with resistance to leafminer mines, we have not found a satisfactory level of resistance to leafminer stings. More than 800 spinach germplasm accessions and cultivars have been screened; however, only some differences in leafminer sting density were observed. We plan to elevate the level of resistance to stings through recurrent selection. We also need to increase the level of resistance to leafminer mines and breed the resistance into different types of spinach.

Publications relevant to this project in 2018-19:

Kandel, S., B. Mou, N. Shishkoff, A. Shi, K. Subbarao, and S. Klosterman. 2019. Spinach downy mildew: Advances in our understanding of the disease cycle and prospects for disease management. Plant Disease 103: 791-803. https://doi.org/10.1094/PDIS-10-18-1720-FE (Cover story)

Fletcher, K., S. Klosterman, L. Derevnina, F. Martin, L. Bertier, S. Koike, S. Reyes-Chin-Wo, B. Mou, and R. Michelmore. 2018. Comparative genomics of downy mildews reveals potential adaptations to biotrophy. BMC Genomics 19:851. https://doi.org/10.1186/s12864-018-5214-8