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 Spin-Aid herbicide. In our trials, ten spinach breeding populations had 0%, and another five populations had < 10% downy mildew incidences, as compared to the susceptible control ('Viroflay') with 98% disease incidence. Our results show that the recurrent selection method was very effective to increase the downy mildew resistance in the spinach populations. Plants with resistances to downy mildew, leafminers, and Spin-Aid 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. The predominant downy mildew races in California are Race 10 - 17 at present. Resistances against Race 10 - 17 have been found to exist in many spinach cultivars and germplasm accessions. But the resistance can be overcome by the newly appeared pathogen isolates, yet to be named. Durable resistance against downy mildew is greatly needed. 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 34 populations were planted again at our USDA-ARS research station in Salinas, CA on September 10, 2019 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 several times a week in the evening to favor downy mildew development. Leaf samples with sporulation of the pathogen were collected and sent to Dr. Jim Correll's lab at University of Arkansas to identify pathogen races using differential hosts. Diseased and total plants in each population were counted to record disease incidence (plants with downy mildew symptom/total plants). 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.

Results and Discussion

We got good downy mildew pressure in our breeding field. Through differential tests, the downy mildew pathogen present in the field appeared to be a novel isolate UA201621A. Downy mildew disease incidences (%) of selected spinach breeding populations and the susceptible control cultivar Viroflay are shown in Table 1. Ten populations had 0%, and another five populations had < 10% downy mildew incidences, as compared to the susceptible control ('Viroflay') with 98% disease incidence. Last year (2018), only one population had 0% and another five populations had <10% downy mildew incidence. These populations will be evaluated again in 2020 to confirm the results. Resistant plants with few downy mildew lesions were selected from each of the 34 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.

Downy mildew incidences from sixteen populations with data from both 2018 and 2019 seasons are shown in Figure 1. The 2019 populations are progenies of the plants selected from the 2018 populations. Of the 16 populations, 12 populations (75%) showed decreases of downy mildew incidences, 3 populations had a small increase, and only one population had a large increase in disease incidence. Six of the 16 populations dropped downy mildew incidences to 0%. These results show that the recurrent selection method was very effective to increase the downy mildew resistance in the spinach populations. 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 2019.

Populations	Diseased plants /Total plants	Disease Incidence %
18-19-67	4/59	6.8
10-11-80	4/61	6.6
10-11-88	5/77	6.5
17-18-5	2/33	6.1
10-11-85	4/74	5.4
18-19-87	0/20	0.0
18-19-68	0/23	0.0
18-19-83	0/28	0.0
10-11-67	0/42	0.0
15-16-B	0/42	0.0
17-18-14	0/46	0.0
18-19-86	0/50	0.0
18-19-88	0/63	0.0
17-18-1	0/92	0.0
18-19-41	0/102	0.0
Viroflay	96/98	98.0

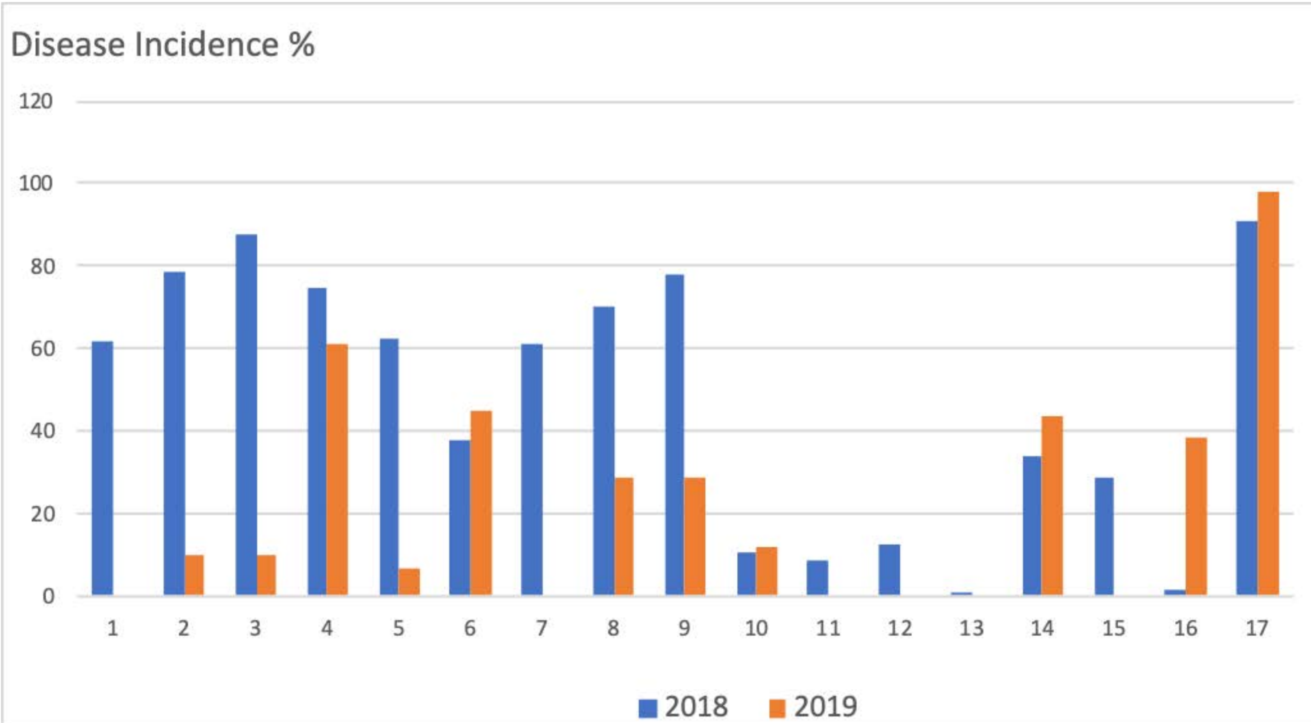

Figure 1. Downy mildew incidence (%) in selected spinach breeding populations from 2018 to 2019 generations. The 2019 populations are progenies of the plants selected from the 2018 populations. Population 17 is the susceptible control cultivar Viroflay.

Objective 2. Breeding for Resistance to Spin-Aid 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. In 2008-09, Helm Agro suspended the production of RoNeet, the primary herbicide for spinach. The prospect of losing effective herbicides is a constant threat for California spinach industry. Herbicide-tolerant spinach would allow weed control with the herbicide without injuring the crop, thus providing growers another tool or alternative for weed management.

We worked on the tolerance to linuron herbicide in spinach in the past, but decided to switch to tolerance to Spin-Aid herbicide because it is already registered for spinach crop. Both Linuron and Spin-Aid are Photosystem II inhibitors, and spinach may have similar tolerance mechanisms. Spin-Aid is a selective post-emergence herbicide that can provide effective control of more than 10 broadleaf weeds. Differences in tolerance to this herbicide have been observed in spinach varieties. However, the herbicide has been registered only for processing and seed crops of spinach, and red (table) beets, but not for fresh market spinach yet (Bayer CropScience, 2009). The herbicide can cause temporary growth retardation and/or chlorosis or tipburn, and fresh market spinach may not have enough time to recover from the injury due to its short crop cycle. Therefore, more tolerance to this herbicide is needed in fresh market spinach cultivars.

Procedures.

Nine breeding populations were planted at a 1-foot spacing in the field along with 16 commercial cultivars on 8/28/2019. Spin-Aid herbicide was sprayed at 3 pints/acre on 9/19/2019 at 4-leaf stage. The field was sprayed with Spin-Aid again on 10/8/2019 at 9 pints/acre.

Results and Discussion

The first spray of Spin-Aid at 3 pints/acre controlled weeds well and did not result in visible injury on spinach plants. That made it difficult to select more herbicide-tolerant plants. We had to spray the field again with the herbicide at 9 pints/acre 19 days later as older plants have higher level of tolerance to the herbicide. Most plants survived the second herbicide spray with various degrees of leaf damage and stunting. Plants with little leaf damage and stunting in each population were selected, dug up, and transplanted into isolators to produce seeds for future testing and selection. We will repeat the experiment next year to confirm the results. We do need to continue the improvement of herbicide tolerance levels in our breeding populations to meet the production requirement.

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. A breeding effort is needed because there is a lack of leafminer resistace in commercial spinach cultivars.

Procedures.

A recurrent selection method was used to increase the level of resistance to leafminer stings and mines in five populations of different leaf types space-planted at 1-foot apart in an USDA-ARS research field in Salinas on 8/28/2019. Plants with fewer stings and/or mines were selected from the 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 2019-20:

Kandel, S., A. Hulse-Kemp, K. Stoffel, S. Koike, A. Shi, B. Mou, A. van Deynze, and S. Klosterman. 2020. Transcriptional analyses of differential cultivars during resistant and susceptible interactions with *Peronospora effusa*, the causal agent of spinach downy mildew. Scientific Reports (in press)

Kandel, S.L., K.V. Subbarao, A. Shi, B. Mou, and S.J. Klosterman. 2019. Evaluation of biopesticides for managing downy mildew of spinach in organic production systems, 2017 and 2018. Plant Disease Management Reports 13: V171.

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)

Mou, B. 2019. 'USDA Red' spinach. HortScience 54: 2070-2072. https://doi.org/10.21273/HORTSCI14308-19 (Cover story)