Annual Leafy Greens Research Report

For the Period April 1, 2017 to March 31, 2018

Project Title: Breeding Baby Leaf Spinach for California Growers

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Abstract:

The long-term goal of our spinach breeding program is to develop publicly released germplasm and cultivars for the baby leaf market that have broad-spectrum resistance to downy mildew and that are commercially acceptable for quality and production characteristics. We conducted two nurseries in the field to select for field level downy mildew resistance in the spring/fall season in 2017. These selections were transported to isolators in Davis for intercrossing and generation advancement and are now (spring/summer 2018) growing in field nurseries for continued field selections. We have built more isolators with capacity of 100 to 200 plants each in Davis to enable intercrossing of field selections, population advancement, and seed increases. We have also started a complex population development with several parental backgrounds to hopefully bring in different suites of broad spectrum resistance alleles. We have established capabilities to screen differentials for race typing at USDA and are developing capabilities for growth chamber screening of breeding lines. We are finalizing the purchase of a growth chamber to enable us to develop and carry out growth chamber based screening protocols for downy mildew year-round to accelerate our selections especially in the off-season and when no downy mildew is present in the field.

Objectives:

1. To develop additional segregating populations and organize populations into a structured breeding program.
2. To continue screening and evaluating breeding populations in the field in Salinas.
3. To optimize and continue screening germplasm in the growth chamber.

Procedures:

Objective1:

We have spent considerable time for the past year evaluating germplasm in the field, identifying promising cultivars or germplasm for parents of new populations, and making crosses in the greenhouse. During the period of April 2017 to March 2018, we have developed more than 30 new breeding populations, including F2 populations from breaking down hybrid cultivars (13), and populations derived from crosses of two (20) or more (3) hybrid cultivars. The larger populations include up to eight different hybrids and are broad-based populations segregating for numerous major and minor resistance genes as well as other traits. We chose parents for the new populations based on field-based observations we made in 2016 and 2017 of about 100 hybrid and OP cultivars at the USDA-ARS station in Salinas (and public trials) under natural infection of downy mildew. We have also evaluated some of our new breeding populations in the field and advanced selections from them. Seed from untested populations are being evaluated in field nurseries in spring/summer 2018 season. During fall 2017, we increased eleven low cadmium accessions that we selected from previous screening tests that will be evaluated for phenotypic traits in the current nursery.

Figure 1. Isolator chambers in greenhouse in Davis

To continue with population development and seed increases, we have fabricated ten more isolators with capacity for 100 plants each, and two isolators with capacity for 200 plants each in the greenhouses in Davis (Figure 1). These isolator chambers give us flexibility to conduct small-scale seed increases of populations, develop large numbers of half-sib families for testing and selection, and intercrossing of field nursery selections. We have been able to access isolators in Salinas at certain times of the year, but these new isolators give us flexibility to make crosses at any time.

We also experimented with large, field-based isolators (Figure 2) that we hoped would enable us to intercross several hundred plants at a time in the field. One of the major limitations was that the humidity in the chambers was too high, which increased disease incidence curtailing seed

production. We used a double layer of row cover material to minimize pollen flow, and perhaps a different type of fabric would provide better air movement and still exclude pollen. We may re-evaluate this approach with alternate management conditions.

Figure 2. Field isolators in Salinas.

<u>Objective2:</u>

We were unable to plant our spring nursery until 11 May 2017 due to wet weather. We evaluated 11 populations in spring/summer 2017 field nursery, including eight open pollinated populations, two F_2 populations and one F_3 population derived from a seed increase of an F_2. We also had about

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Figure 3. Field design with viroflay spreader rows. X= Viroflay, o= entry plot

70 elite and older cultivars for observation. Entries were planted in between Viroflay spreader rows designed to give uniform and severe mildew infection (Figure 3.)

From this nursery, 50-100 plants from three top populations that showed promise and that also showed segregation for downy mildew reaction and other traits, were selected and moved into field isolators in Salinas for intercrossing and population advancement. From the commercial cultivars planted for observations, 12 top performers were picked to initiate a series a complex

crosses. The larger populations, for the complex crosses, include up to eight different hybrids and are broad-based populations segregating for numerous major and minor resistance genes as well as other traits. These will form the basis of genetic mapping populations for resistance genes. We are also acquiring near isogenic lines to verify resistance gene clusters.

In the 2017 fall nursery, we evaluated 19 experimental populations; four populations derived from pairwise crosses of non-patented F1 hybrids; eight F2 populations derived from self-pollinating non-patented F1 hybrids; and two populations for which several half-sib progenies were evaluated. This trial had moderate DM infection. Selected plants (12 – 80 per population) were moved to Davis and each population was placed in a separate isolation chamber for intercrossing. These populations will be evaluated in our current nursery started in May 2018.

We have been experimenting with other planting systems to achieve uniform plant-to-plant spacing. We have tried the PaperPot system to germinate seedlings in the greenhouse so that when plants are transplanted to the field, plants will be evenly space. However, this system is time consuming, but with better results in plant-to-plant uniformity. We have contacted Tanimura and Antle about the Plant Tape system they have pioneered and hope to work with them in the coming year to modify that system for our needs.

Objective 3:

We were working on establishing a screening method using the growth chamber at UC-ANR in Salinas. We identified a reasonable planting method to screen populations and/or families for mildew reaction (Figure 4.) Up this point, we were able to identify resistant versus susceptible entries, which may be useful as a pre-screening system for later test in the field. With Steve Koike leaving the UC-ANR, and technical difficulties using his growth chamber, we decided to purchase a CONVIRON PGC-FLEX growth chamber to perform downy mildew testing in Davis. This chamber is being purchased with funding that PI Brummer had from other sources.

The CONVIRON PGC-FLEX growth chamber will be equipped with LED lights that will allow us to have a better temperature control for downy mildew screenings. All the paper work has been submitted to the purchasing system at UC Davis, and we hope to have the chamber in place by the end of the summer 2018, and start testing and screening in the coming fall.

Figure 4. Screening downy mildew in the growth chamber in UC-ANR in Salinas. The planting density shown on the right seems to work well for distinguishing among resistant and susceptible entries.

Results and Discussion:

Objective 1.

By the end of the reporting period, we had developed three times more populations than the number of populations in the proposed deliverables (10). These populations include F_2 populations from breaking down hybrid cultivars (13), and populations derived from crosses of two (20) or more (3) hybrid cultivars. Additionally, from observations made in field nurseries during 2016 and 2017, we chose 12 non-patented hybrids under natural field infection that presented less DM symptoms and were commercially acceptable. These 12 hybrids were used as parental lines to initiate complex crosses to obtain populations segregating for major and minor alleles for downy mildew resistance. Three new populations were created, 1) with eight parents, 2) with four parents, and the last one 3) with two parents. In the spring/summer season 2018, a bulk of the first population (1) and a bulk of the third (3) population were planted to determine its segregation for DM and other phenotypic traits. For the second complex population (with four parents), we were not able to harvest seeds due to difficulties in the greenhouse. The isolators were the complex populations are being developed have been move to a more adequate space with more daylight. A second cycle for the first complex population (eight parents) was initiated in the spring 2018 to allow shuffling of alleles to hopefully uncover genes for broad genetic resistance.

Objective 2.

During the reporting period, we planted two field nurseries in the USDA-ARS station in Salinas (summer and fall 2017), and evaluated over 30 experimental populations to select desirable plants that have been naturally challenged with downy mildew. We use phenotypic recurrent selection or half-sib family selection for population development. We evaluate in field for freedom for disease

and visual characteristics, select best plants and eliminate some populations with less desirable characteristics, then, selections are moved to isolation chambers for hybridization (Figure 5.)

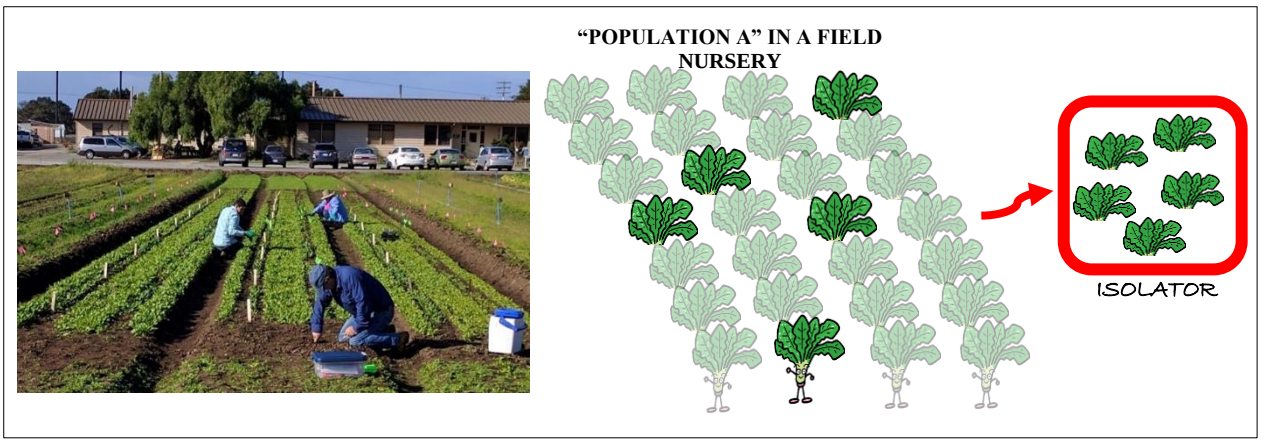

Figure 5. Phenotypic recurrent (mass) selection scheme.

Seeds harvested from isolation chambers are kept separate by maternal parent to plant half-sib families for the next cycle of selection. Some seeds from each family are also bulked to evaluate as a new experimental cultivar. From our 2017 field and greenhouse seasons, we successfully turned cycles and were able to obtain seeds for many newer populations (30) that are planted in the current replicated nursery for a second cycle of selections.

Finally, several accessions were identified to be low accumulators of cadmium in a parallel project funded by the leafy greens board. These populations were increased for seeds in greenhouse during the fall of 2017 and are planted in our current nursery to evaluate them for agronomic and phenotypic traits. The top performers from low cadmium accumulators will be crossed to our best breeding populations. The developed populations will be tested for low cadmium accumulation to determine if the uptake is significantly reduced.

Objective 3:

We are setting up the stage to have in house (in Davis) a growth chamber to screen breeding populations and families for broad resistance to downy mildew. We are finalizing the purchase of a CONVIRON chamber, and are hopeful to start screenings at the end of the fall this year (2018). Up to this point, we were working with Steve Koike establishing a method to pre-screen for downy mildew resistance vs. susceptibility. We identified a reasonable planting method to screen populations and/or families for mildew reaction that will be implementing as soon as we get the growth chamber installed and working.

Leveraging board funding to attract additional funding.

Our group has been awarded a CDFA grant to develop a platform for breeding genetic resistance to downy mildew for organic production. This grant focuses on developing an in-field assay to detect downy mildew pathogen prior to leaf symptoms develop. We will be leveraging the CDFA

grant with the current breeding program to develop a more efficient screening process for broad genetic resistance in chamber screenings and field phenotyping.

In addition, Steve Klosterman is working on a detached leaf assay to more robustly identify resistant genotypes. This assay would enable us to screen plants selected in the field to ensure we have resistant genotypes prior to flowering and seed production. As this test becomes available, we will add it to our selection program. This adds 2 postdocs, Mychele Bastista Desilva and Shiam Handle, and one MS student, Samantha Hilborn, to the spinach breeding program.

We have also been awarded a grant to study nitrogen use efficiency in spinach in collaboration with Texas A & M from the Specialty Crops Research Initiative beginning Fall 2018. This will add one Ph.D student to the program.