Annual Leafy Greens Research Report

For the Period April 1, 2019 to March 31, 2020

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 low Cadmium (Cd) accumulation. Our current emphasis is on open-pollinated cultivars, with the possibility of developing inbreds and hybrids as the project progresses. During this funding period we conducted four breeding trials to select for field level downy mildew resistance and for desirable phenotypic characteristics, under conventional and organic management. Desirable families were intercrossed for generation advancement and are ready for screening in 2020 trials. A field trial in high Cd soils confirmed accessions with low Cd uptake from greenhouse trials and were crossed to our best populations to initiate breeding for low Cd accumulation. We installed a growth chamber with a mist system to enable us to screen for downy mildew year-round, screen against specific isolates and when no downy mildew is present in the field.

Objectives:

1. To screen and evaluate breeding populations in conventional and organic fields in the Salinas Valley (spring-fall) and Imperial Valley (in winter), and continue to develop the breeding program pipeline for cultivar delivery,
2. To develop superior downy mildew screening methods in the growth chamber, including a detached leaf assay,
3. To verify low cadmium accumulating accessions and create biparental populations segregating for cadmium uptake to enable genetic mapping of genes associated with Cd uptake as a first step to incorporate the trait into cultivars, and
4. To genotype bulked samples of our breeding populations using genotyping-by-sequencing (GBS) to generate genome wide markers and find marker-trait associations for several traits of interest.

Objective 1: *To screen and evaluate breeding populations in conventional and organic fields in the Salinas Valley (spring-fall) and Imperial Valley (in winter), and continue to develop the breeding program pipeline for cultivar delivery.*

Four breeding trials were planted during the 2019-2020 reporting period; three in the Salinas Valley (two spring and one fall), and one in the Imperial Valley (winter). Concurrent spring trials evaluating 26 populations with 530 families were conducted at Jacobs Farms in Corralitos under organic management and at the USDA-ARS station in Salinas under conventional management (Figure 1). A fall trial planted at Jacobs Farms in Corralitos under organic management was unsuccessful due to greenhouse and field challenges. However, the same material was successful in a winter trial planted in El Centro (Imperial Valley) at the UC ANR Desert Research and Extension Center (UC DREC) under conventional management. In order to improve field uniformity and selection ability, we are transplanting our nurseries using the PaperPot system (Figure 2).

Selections from the spring trial were made first among populations, then among families within desirable populations, and then within desirable families. Selection criteria were based on visual vigor and other characteristics (leaf texture, leaf shape, leaf color, etc.). Unfortunately, we were unable to select for downy mildew resistance due to low disease pressure. Remnant seeds from selected families were used for intercrossing in greenhouse isolators in fall/winter for 2019-2020 (Figure 3). The failure of the fall organic trial resulted from poor germination of seedlings in the PaperPot transplant trays due to an uneven mist-system and insufficient water at Jacobs Farms. The trial was still transplanted in the hope that we could gather some data from it, but in the end, it was simply not uniform enough to make any meaningful selections.

We successfully repeated the same trial in our winter nursery in the Imperial Valley at the UC Desert Research and Extension Center (DREC), Holtville, CA (Figure 4) .This trial consisted of two replications of a synthetic population (JOM Syn-2) derived from seven F1 parents and intercrossed for two generations. This trial was an augmented design (Figure 4) with 118 half-sib families in blocks of 8 with 2 check entries per block. This design allows for adjustment of traits based on spatial variation relative to nearby checks that are systematically placed throughout the trial. The F1 parents were also included. Each family received a visual score of desirability, based on leaf morphology, vigor, color, and freedom from any disease or insect damage. We observed minimal disease pressure, but stands were excellent. Data for total plot weight, percentage of plants per plot, and plant weight were collected (Figure 4). Selections were based primarily on visual score and plant weight. Two populations are being advanced from these evaluations; JOM Elite Cycle 1 population intercrossing the very best 20 families and JOM Broad Cycle 1 population intercrossing the best 50 families for generation advancement and increased recombination to combine alleles/genes for desirable traits.

Figure 1. 2109 Spring-Summer trials. A. Under organic management at Jacobs Farms in Corralitos, and B. Under conventional management at the USDA-ARS station in Salinas.

Nine populations based on Spring 2019 field selections were selected and intercrossed and are ready for 2020 trials and downy mildew screening in the growth chamber. An additional 24 bulked or first generation populations are also ready for field evaluation. The two JOM populations will be ready for evaluation in the Fall or Winter 2020.

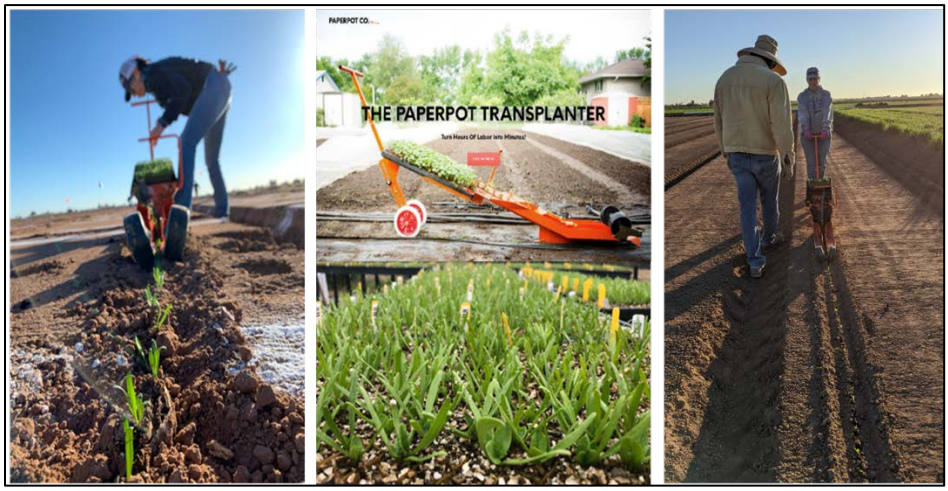
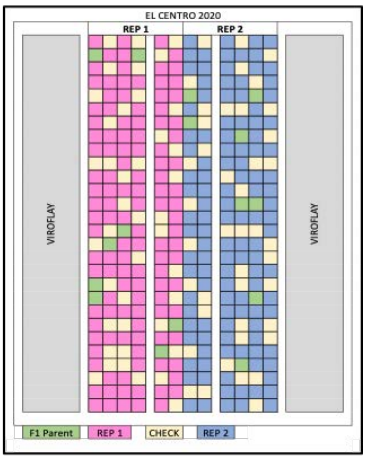


Figure 2. PaperPot transplanter and two-week old spinach seedlings, ready to plant.

Figure 3. Breeding populations planted in greenhouse isolators for intercrossing and generation advancement.



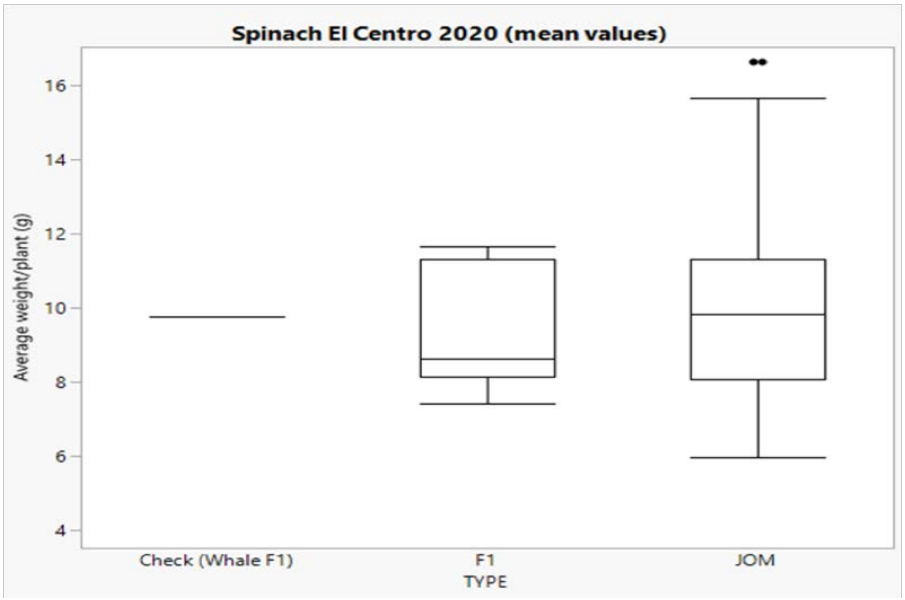


Figure 4. 2020 Winter breeding trial in the Imperial Valley at the UC Desert Research and Extension Center (DREC), Holtville, CA, using an augmented experimental design (top right). (Bottom) Average weight per plant of breeding families (JOM), F1 parental hybrids of the JOM population and the Check (Whale F1). Middle lines represent mean, boxes represent 1 standard deviation and T-bars represent range. The JOM population has broader segregation than the seven parental hybrids.

Objective 2: *To develop superior downy mildew screening methods in the growth chamber, including a detached leaf assay*.

The goal of this objective is to refine a growth chamber-based screening system for year-round screening against multiple races for broad based resistance against downy mildew. We have installed a PGC Flex Conviron growth chamber in April 2019 at UCD in order to have a controlled space dedicated to our breeding program for year-round downy mildew screening (Figure 5). A misting system was installed in Feb 2020, which is necessary to promote sporulation of the downy mildew pathogen. We have been able to develop, test, and refine downy mildew screening methodologies in the growth chamber.

Figure 5. Three tier CONVIRON growth chamber installed at UCD for downy mildew screening.

The growth chamber conditions are set to maintain stable *in vitro* downy mildew cultures and examine viability of spores following inoculation. We can screen 154 spinach individual varieties or breeding lines for downy mildew resistance per cycle (3 weeks/cycle) simultaneously (Figure 5). The chamber is currently operating under a short daylength schedule to promote vegetative growth, with an average temperature of 16℃ and average relative humidity of 91%, which enables downy mildew infection and sporangial germination. We also have used darkened humidity domes to introduce a dew-chamber-like condition in part of the growth chamber to minimize contamination (Figure 6, top). We have recently acquired a new downy mildew isolate from Dr. Alex Putman (UCR extension) and are currently conducting race typing with differential sets (Figure 6, middle and bottom). We will screen this downy mildew isolate on our breeding population following culture increase.

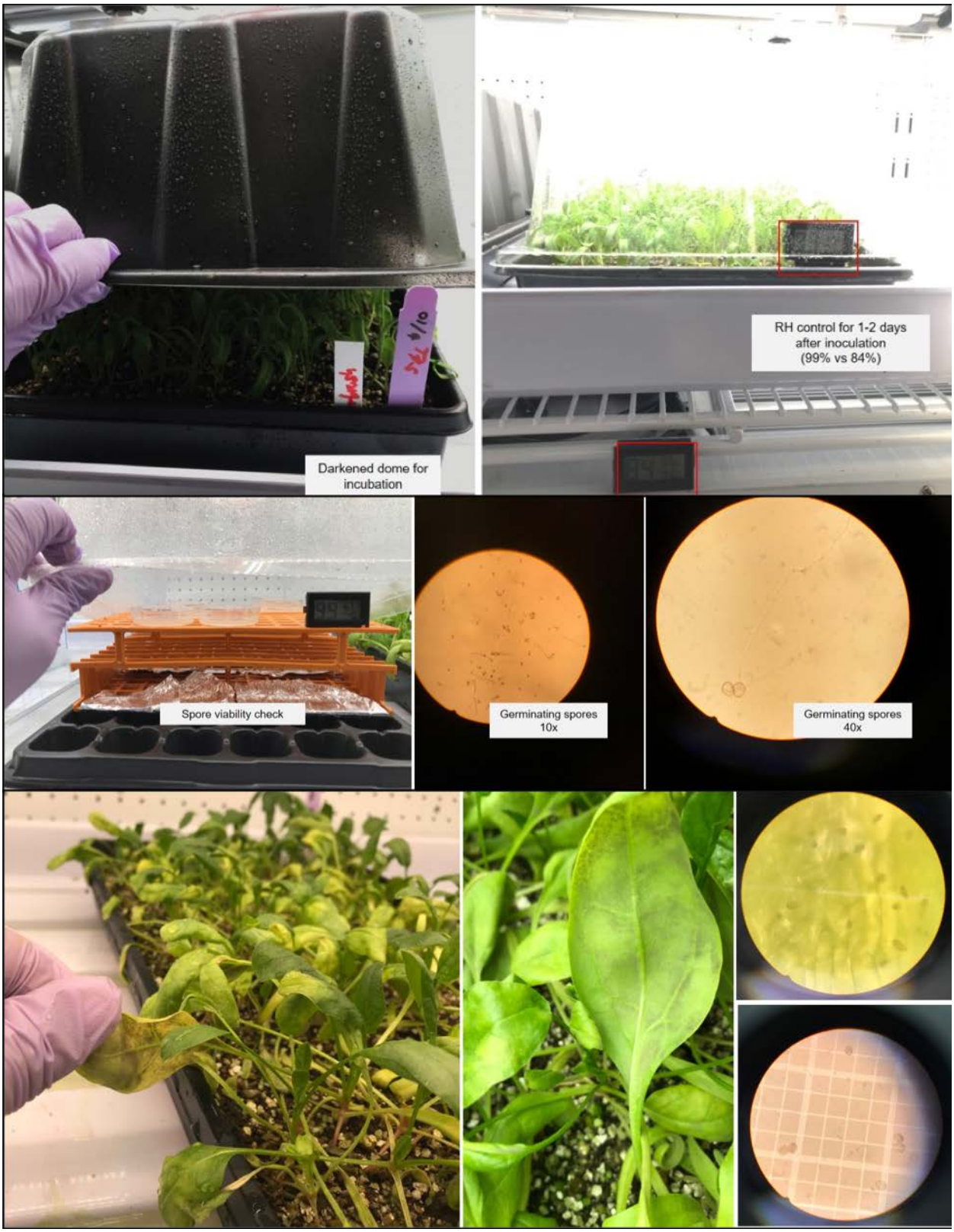


Figure 6. (Top) Domes used for dark incubation and humidity control. (Middle) Spore samples to check viability. (Bottom) Severe symptoms and sporulation detected 12 days after inoculation

Objective 3: *To verify low cadmium accumulating accessions and create biparental populations segregating for cadmium uptake to enable genetic mapping of genes associated with Cd uptake as a first step to incorporate the trait into cultivars.*

Our main goal for this objective is to develop low cadmium-accumulating germplasm. Based on the greenhouse results of 2016 and 2017, 11 low cadmium-accumulating accessions were selected for replicated trials in Salinas and San Juan Bautista in summer 2018. From these trials, four accessions with the most desirable phenotypic appearance were selected and validated in a replicated field trial in 2019 that included two controls, in a high cadmium soil (7.2 ppm) north of Kings City, CA, (Figure 7). Results from the validation trial showed that several accessions accumulated less than or equal to the low accumulating check (Figure 4). Three of the four desirable accessions with low cadmium accumulation were crossed in winter 2018-2019 to three hybrid cultivars with high cadmium accumulation to generate three biparental populations for genetic mapping and marker development for the trait.

One of these bi-parental populations (NSL28218 x Camaro) was selected for testing in high cadmium soil in the greenhouse. This population was chosen because the low Cd parent, NSL28218, was the lowest accumulating accession in the field trial described above. F_1 plants will be self-fertilized to develop inbred family seed for further testing. In order to self these plants, sex expression DNA markers were used to select for female or monoecious plants, silver thiosulfate was used to induce male pollen, and single plant isolators were created (Figs 9-11).

For the first round of testing, 60 F_1 plants from this population were successfully selfed, DNA was isolated for genotyping, and leaf samples collected and evaluated for Cd concentration. Genotype and Cd accumulation data will enable genetic mapping of Cd uptake. Due to the suspension of university activities due to Covid-19, we delayed the second round of testing until Fall 2020. Selfed families from these plants will be grown again to confirm Cd status and to continue genetic mapping experiments. Preliminary results indicate field and greenhouse trials are correlated, and thus greenhouse screening of low cadmium accessions and cultivars should be possible.

Concurrently, our labs are in the process of genotyping 1200 genes including 50 Cd regulation genes in about 400 genotypes using exome capture to identify quantitative trait loci involved in Cd uptake (California Seed Association grant). The bi-parental population will also be genotyped at these loci to complement sequencing results with linkage analyses. Markers for this trait will enable the identification of favorable alleles for low Cd accumulation, facilitating their rapid integration into elite breeding germplasm adapted to California. Thirty-three low cadmium-accumulating F_1 breeding populations have been developed.

Figure 7. Field trial in high cadmium soil north of Kings City, CA., with leaf tissue washed and dried for Cd accumulation measurements.

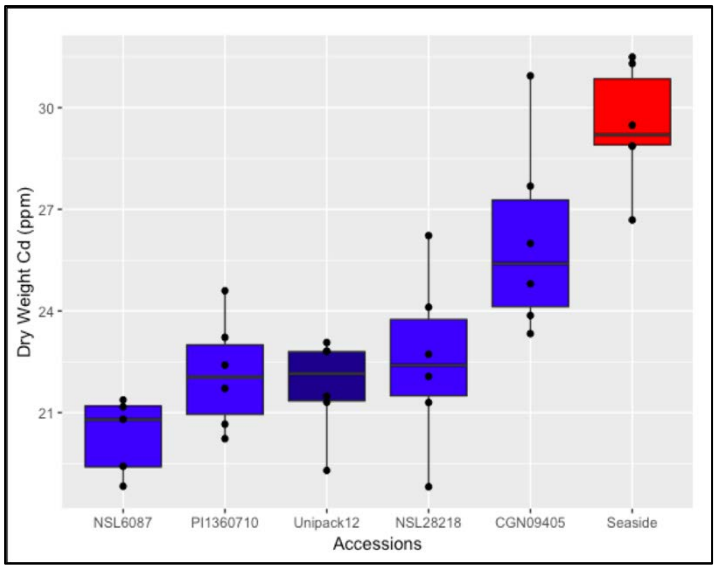


Figure 8. Subset of four selections in validation trials, spring-summer 2019. Unipack 12, low cadmium accumulating check, and Seaside, high cadmium accumulating check.

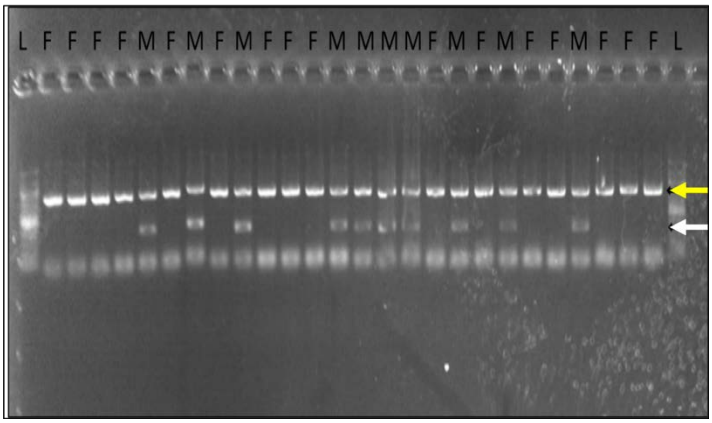


Figure 9. Sex linked marker, T11A (Akamatsu et el 1998), showing autosomal band (yellow arrow) and male specific band (white arrow).

Figure 10. Pollen (red arrow) on genetically female plant after silver thiosulfate application

Figure 11. Single plant isolators made for selfing plants.

Objective 4: *To genotype bulked samples of our breeding populations using genotyping-by-sequencing (GBS) to generate genome wide markers and find marker-trait associations for several traits of interest*

Our most promising experimental germplasm, JOM, is a broad-based population derived from eight elite cultivars. This population includes a diversity of leaf shapes, from oval to elongated, major downy mildew resistance genes to races 1-16, semi-savoy to flat leaves, moderate to dark green, and other traits.

Tissue samples for DNA isolation have been collected from these selections from this population as described in Objective 1. We will extract DNA and use GBS sequencing and/or exome capture as described above to genotype the population. Note the exome capture will assay diversity for all predicted resistance genes (totaling 503 genes) in the spinach genome, as well as genes related to leaf traits, Cd accumulation, and abiotic stress, whereas GBS samples variation

throughout the genome, not just in specific genes. Both platforms sample genes across all 6 chromosomes. We have run a pilot test for GBS on several lines and have shown that we can efficiently genotype samples using this technology yielding 10,000s of SNPs.

Projects leveraged by this grant:

In 2016, we (Van Deynze, PI, with Klosterman and Brummer as co-PI) obtained a CDFA grant "A Platform for Breeding Broad Genetic Resistance to Downy Mildew for Organic Spinach Production." This grant complements the breeding on this project by extending selection to organic conditions. Van Deynze and Brummer are screening 400 lines for diversity in ~1500 genes including those related to resistance to downy mildew with a grant supported by the California Seed Association Spinach fund. An additional project will define the genome sequence of 4 downy mildew races and test a sequence-base assay for new races in the field. A second SCRI grant focuses on understanding genetic variation and breeding: Improving Nitrogen Use Efficiency and Food Safety in Spinach Production, which we (Van Deynze and Brummer) obtained as part of project organized by Texas A&M Univ. Additional grants are in review stages that leverage our breeding program. An additional grant has been submitted to the CDFA to expand the work on breeding for downy mildew as in 2016.

Reference:

Akamatsu T, Suzuki R, Uchimiya H (1998) Determination of male or female of spinach by using DNA marker. Japanese patent JPH1052284, Sakata No Tane KK, Japan