Annual Leafy Greens Research Report

For the Period April 1, 2022 to March 31, 2023

Project Title: Breeding Baby Leaf Spinach for California Growers

Project Investigator(s): E. Charles Brummer; ecbrummer@ucdavis.edu
University of California, Davis, One Shields Ave., Davis, CA

Cooperating Personnel: Allen Van Deynze, Allison Krill-Brown, and Daniel Pap
University of California, Davis, CA and Steve Klosterman, USDA-ARS, Salinas, CA

Abstract:
The long-term goal of our spinach breeding program is to develop publicly available 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. In this funding cycle, we conducted four field trials to select for field level downy mildew (DM) resistance, improved yield and color under lower nitrogen levels, and desirable phenotypic characteristics. Superior families were intercrossed for generation advancement and populations with undesirable characteristics were dropped from the program. Several new populations were developed. Our growth chamber downy mildew screening enables us to screen for downy mildew resistance year-round. Inoculating seedlings with DM in the greenhouse prior to transplanting has increased incidence of DM in the field and improved selected for resistance.

Objectives:

1. To develop, improve, and evaluate spinach breeding populations in the Salinas Valley (spring-fall) and Imperial Valley (winter) and to use the growth chamber and greenhouse for year-round selection and intercrossing.
2. To identify seed growers and to produce sufficient seed of advanced experimental cultivars to enable solid seeded, small plot and strip trials.
3. To accelerate generation time using speed breeding to enhance genetic gain for broad spectrum downy mildew resistance.
4. To integrate genetic markers into the breeding program to enhance efficiency.

Objective 1: To develop, improve, and evaluate spinach breeding populations in the Salinas Valley (spring-fall) and Imperial Valley (winter) and to use the growth chamber and greenhouse for year-round selection and intercrossing.

Procedures:

Breeding

Our generalized breeding pipeline (Figure 1) begins with germplasm evaluation and population development and evaluation, leading to the creation of new, improved experimental populations that could possibly be new cultivars. We are developing improved populations, not hybrid

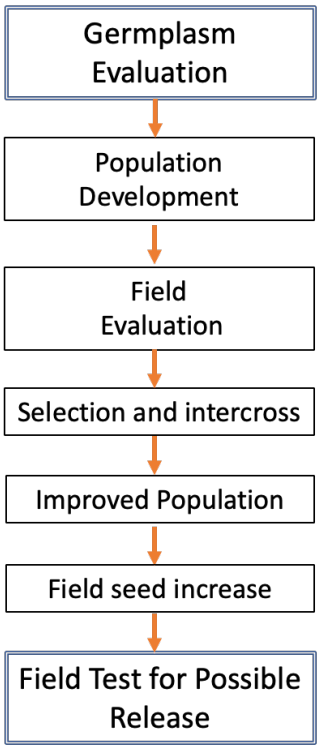

Figure 1. The UCD Spinach Breeding Pipeline

cultivars, in this program and consequently, improved populations can be re-selected to further enhance overall population performance, such as better leaf yield or decreased downy mildew incidence and severity across multiple cycles of selection. We conduct evaluations throughout the year in the field, greenhouse, and growth chamber for abiotic and biotic stress tolerance, plant morphology, and vigor. We created new populations from single crosses and improved populations by intercrossing selected plants in the greenhouse under long days (15-16 hours). Our hybridizations are done with positive pressure airflow under pollination bags or in isolators to prevent cross-contamination. We moved our hybridizations to a greenhouse with better climate control several yeaers ago, and this has allowed us to complete two cycles of breeding or seed increase within a given population per year and has also improved seed production.

DM screening

We have developed several screening methods for evaluating DM resistance at different stages of the breeding pipeline (Figure 2). Our most successful and reliable DM screening methods are in the growth chamber and field, but we also have used a detached leaf assay (DLA) in early generations using a protocol developed by Jim Correll's group as a +/ – screening tool. For that protocol, leaf samples are sanitized and inoculated in Petri dishes on 2% water agar surface with lids on. A dome is used to maintain high humidity. After incubating the Petri dishes at 15°C for 12 days, sporulation on both adaxial and abaxial surface can be identified.

Field screening allows us to evaluate many breeding populations at once. Seedlings were inoculated with a minimum of $1x10^5$ sp / ml *Peronospora effusa* spore solution the night before transplanting in the field. They were inoculated again in the field ~ 2 weeks post transplanting. Inoculating twice insured higher disease pressure and coverage. Although all plants were inoculated at the same rate and time, disease pressure throughout the field was not always homogenous due to differential development within and among populations, possibly related to variation in irrigation. Disease assessment in the field was based on plant counts for incidence (% of plants infected) and a score (ranging from 1 to 5) for severity (% of leaf area infected), with 1 showing no symptoms and 5 showing the most severe symptoms. We use field disease ratings to initially identify new populations to select further and to select individual families within populations for advancement or screening in the growth chamber.

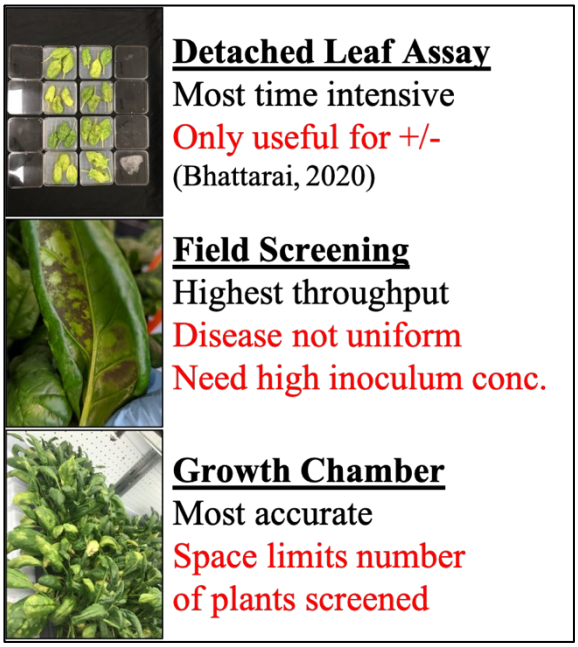

Figure 2. Downy Mildew Screening methods used in the UCD Spinach Breeding Program

The growth chamber was used year-round to maintain our primary DM isolate, to increase spores for field inoculation, and for screening assays on breeding populations. New cultures were established on young plants approximately every 20 days to ensure high levels of spore production for various screening assays. The growth chamber is the most homogeneous environment for disease pressure and allowed us to make single plant selections. The limitation of the growth chamber is that we can only evaluate 216 plants at a time. Plants are grown in 18 cell flats with at least one R and one S check in each flat and are inoculated 2-3 weeks post seeding at a rate of ~2x10^5 spores / ml. Individual plant disease assessments are made ~10-14 days post inoculation and are based on a 1-5 rating scale for severity. Plants rated as a 1 or 2 are selected for advancement. When we initially screen populations, a rating of 1 probably means the plant has a major gene for resistance – while this is obviously a desirable phenotype, this does not represent broad spectrum resistance. However, in subsequent selections within populations known to not have a major resistance gene to the isolate being used, plants with a rating of 1 are the most desirable for selection. In early generations when we are unsure about the presence of a major gene, plants rating "2" have very minor or delayed symptoms suggesting they carry possible broad-spectrum resistance.

We do not have resources to maintain multiple different DM isolates at one time. Co-PI Klosterman and his group at the USDA-ARS regularly trap spores and race type local isolates and this information ensures that isolates we are using reflect those currently seen in the field. Three isolates collected from fields of spinach growing regions in recent years have been used in our screening, *Pe:19, 'Thermal',* and *'Spence2020*' (Figure 3). We are currently working with spores collected at Spence Farm in Salinas in 2020 (*'Spence2020'*) for growth chamber and field screening; this isolate continues to be dominant in the region.

Race	Viroflay	NIL 5	NIL 3	NIL 4	NIL 6	NIL 1	NIL 2	Pigeon	Calendonia	Meerkat	Hydrus
Pe: 18	+	+	+	+	-	-	+	+	+	+	-
Pe: 19	+	+	-	+	+	+	+	+	-	+	+
Spence2020	+	+	-	+	+	+	+	+	-	+	-
Thermal	+	+	+	+	+	(-)	-	-	(-)	-	-

Legend: + = susceptible, - = resistant, (-) = intermediate resistance

Figure 3. Race typing of the downy mildew isolates used for screening.

Breeding field trials

We have tried various field trial planting methods and arrangements and feel we have finally found a system that is reliable and able to overcome variation among and within beds that has frustrated some of our field trials in the past. Our breeding trials are all transplanted using the Paper Pot system. We use paired row plots, with rows one foot apart. Each plot is two feet long with a two inch spacing between plants (Figure 4). Rows are rated visually for DM symptoms and harvested by hand ~30 days post transplanting for yield assessment. Field trials were conducted at the Spence Farm in the Salinas Valley in the spring and fall, on organic land at the UC Desert Research and Education Center (DREC) in the Imperial Valley in the winter, and in a low nitrogen field in Davis in the fall. All trials were inoculated with a local DM isolate prior to transplanting and again in the field ~10-14 days after transplanting to ensure high disease pressure. Entries were rated for incidence and severity of DM symptoms, desirable characteristics, bolting, plant count and yield.

Figure 4. Transplanting using the paper pot system (left), harvesting rows for yield (right).

Cadmium Screening

We had previously screened germplasm for low cadmium uptake in a soil-based greenhouse assay. While that method worked – results were consistent with field assays – the soil structure was poor and plants did not perform well. Based on research in other crops, we investigated a hydroponic system that could provide better control of Cd and also promote better plant growth. In fall 2022, we developed a protocol for growing spinach in a hydroponic system manufactured by Sananbio, using the nutrient film technique (NFT). Spinach cultivars representing high and low cadmium accumulators were used as checks in each trial. We first optimized plant culture for the system, using Grodan A-OK 1" starter plugs as the most suitable medium for the NFT system and Hoagland's No. 2 nutrient solution with EC 1.6 dS/m. The pH was maintained between 5.6-6.0 using potassium carbonate. In the fall and winter of 2022/2023, cadmium concentrations were tested to assess differences in Cd uptake between genotypes. ICP-OES was used to confirm solution nutrient and Cd composition at time zero for each trial. Trial 1 tested a wide range of Cd concentrations (0uM, 2uM, 4uM, 5uM, 6uM, 8uM, 10uM, and 20uM) based on levels used in previous studies. Given that we observed toxicity with these levels, Trial 2 had Cd concentrations 0uM, 0.25uM, 0.5uM, 0.75uM, 1uM, 1.25uM, 1.5uM, and 2uM. Treatments 0.25 uM and 0.5 uM had few or no visible signs of toxicity, similar to spinach grown in our trials in the Salinas Valley in fields with high cadmium; thus, this level of Cd in hydroponics appears to be suitable for future experiments. Fresh and dry weights were recorded. Plants were washed in RO water and dried in an oven at 37.8°C for 7 days.

Analysis

Statistical analysis was done using typical linear modeling to compute mean values and mean separations were done using Fisher's Protected LSD. Analyses were conducted in R or JMP.

Results and Discussion:

In this reporting cycle, we developed 30 new populations; the small amount of seed we have in hand will be increased under next year's project to produce enough seed for field testing (Table 1). We increased seed for 11 F_1 or F_2 populations to produce enough seed for evaluation in fall 2023 trials. We evaluated 20 F_2 or F_3 populations in the field, along with 18 advanced populations. Across all field trials, in addition to the populations themselves, we evaluated 632 families from these populations.

Table 1. UC populations developed or advanced over the past three years.

	Populations		
Generation or Cycle	2020	2021	2022
Single crosses	20	12	30
F_1 and F_2 populations	21	22	11
F_2 and F_3 populations	10	25	20
Cycle 1	6	6	8
Cycle 2	4	3	4
Cycle 3	1	1	6
Total	**62**	**69**	**79**

We conducted four evaluation trials during the 2022-2023 reporting period, two in the Salinas Valley, one in the Imperial Valley, and one in Davis, CA (Table 2). Downy mildew (DM) infection was present in both Salinas trials but was not present in Davis or El Centro, although all trials were inoculated prior to transplanting. Populations evaluated in Salinas were rated for DM incidence and severity (Table 3). Several populations that were susceptible to DM and/or had other undesirable traits in the spring trial were discarded, but several others were tested again in the fall trial.

We typically keep seed from individual female plants separate – these represent half-sib families, so that we can evaluate families and select the best families for the next cycle. However, we also bulk seed equally across families to represent the population and test these bulked populations as an experimental cultivar. Ten populations were tested in both spring and fall trials and showed consistent levels of resistance to DM.

Table 2. Spinach evaluations during the reporting period (2022-2023) and the number of breeding populations (Pop), families within populations (Fam), commercial cultivars (Cult), and germplasm accessions (Germ) evaluated.

Season	Location	Type	Pop	Fam	Cult	Germ	Traits	Notes
Spring 22	Salinas	Conven-tional	12	274	40	-	DM, quality, bolting	Inoculated with DM isolate 'Spence2020'
Fall 22	Davis	Nitrogen Use Eff.	11	106	4	36	Survival, greenness	Damage from crows, frost, and flooding
Fall 22	Salinas	Conven-tional	37	234	12	-	DM, quality, yield, bolting	Inoculated with DM isolate 'Spence2020'
Winter 23	El Centro	Organic	4	139	20	30	Yield, quality	Poor growth/management

We have identified several experimentals that are particularly interesting as potential cultivars or as source populations for subsequent selection. These are noted in boldface in Table 3. Two of these experimentals (in italics) had no DM symptoms; we need to confirm that these do not have a major resistance gene. The two UCSP2006 populations – Cycle 2 and Cycle 3 – are perhaps our best populations, with very low incidence and severity of DM. Cycle 3 did not show further improvement in DM resistance and may have lost a bit in production. Further analysis is needed of these populations, but regardless, UCSP2006 Cycle 2 has significant merit.

The UCSP2010 germplasm has been selected for DM resistance across multiple cycles and several of the populations that we know do not have major resistance genes look promising, including UCSP2010 4RC2 and 41MR-RC3. Other populations of note are UCSP2139, UCSP2294, and UCSP2103. All three look good in terms of DM symptoms and are prime candidates for further growth chamber evaluation and selection to decrease DM further and to enhance yield and other characteristics.

A low N population (UCSP2206) consisting of 90 families, along with 4 commercial cultivars and 36 selected germplasm lines were planted in the fall in Davis in a low nitrogen (<50ppm N) field.

Table 3. Plant yield and downy mildew (DM) ratings in the Salinas Spring and Fall 2022 trials for various breeding germplasm and commercial cultivars.

		Spring 2022	Fall 2022		
		DM		DM	
Entry	Cycle	Severity	Plant Yield	Incidence	Severity
		Score (1-5)*	g/plant	% plants	Score (1-5)
UCSP2002	C1		3.09	36	3.33
UCSP2002	C2		4.04	25	3.33
UCSP2003	C1		4.88	36	2.83
UCSP2006	C1		2.46	0	1.00
UCSP2006	**C2**		**4.47**	**1**	**1.33**
UCSP2006	**C3**		**3.79**	**2**	**1.50**
UCSP2010	3R C2	1.40	4.13	4	1.67
UCSP2010	**4R C2**	**2.71**	**4.16**	**11**	**2.17**
UCSP2010	5S C2	4.33	4.63	53	3.83
UCSP2010	**41 MR-R C3**		**4.83**	**7**	**1.50**
UCSP2010	42 MR-MR C3		4.16	27	2.33
UCSP2010	43 MR-S C3		4.63	44	3.17
UCSP2010	44 MR(R) C3		4.19	11	1.83
UCSP2108	***C0***	***1.38***	***4.13***	***0***	***1.00***
UCSP2201	***C0***		***5.32***	***0***	***1.00***
UCSP2139	**C0**	**1.54**	**3.88**	**9**	**2.17**
UCSP2197	C0		3.46	14	3.33
UCSP2194	**C0**		**4.33**	**18**	**2.67**
UCSP2103	**C0**	**2.28**	**4.05**	**17**	**2.83**
UCSP2186	C1		4.48	32	3.33
UCSP2187	C0		4.02	27	4.17
UCSP2109	C0		4.06	33	4.17
UCSP2106	C0	4.54	3.37	50	2.83
UCSP2116	C0	4.13	1.62	46	4.67
UCSP2192	C0		3.98	44	3.83
UCSP2185	C0	5.00	3.75	46	4.00
UCSP2203	C0		4.43	55	3.67
UCSP2193	C0	4.25	3.76	61	4.00
UCSP2011	C1		3.22	67	4.83
Banjo	Cultivar		4.40	0	1.00
Carmel	Cultivar		4.74	0	1.00
Flamingo Improved	Cultivar		5.29	0	1.00
Kiowa	Cultivar		5.09	0	1.00
Sioux	Cultivar		5.10	0	1.00
Sun Angel	Cultivar		4.83	0	1.00
Whale	Cultivar		4.62	0	1.00
Magnetic	Cultivar		3.49	31	3.17
Gazelle	Cultivar		4.22	63	5.00
Green Beret	Cultivar		4.23	83	4.50
Red Kitten	Cultivar		2.54	53	3.59
Corvair	Cultivar		4.08	54	4.33
Tundra	Cultivar		4.41	94	4.67
Mean		3.06	4.01	29	2.82
LSD (0.05)		NA	1.36	29	1.40

* DM ratings: 1=no symptoms, 5 = most severe symptoms

UCSP2206 was created during the last project period by intercrossing selected plants from 10 F_2 breeding populations grown in a low N trial. Selection was based on greenness, vigor, and plant

type. This year's NUE trial unfortunately had major damage from crows, frost, and flooding with only ~ 30% survival. Data were collected for survival, greenness, and quality, which will be used to make selections in the future. We also grew 73 families from UCSP2206 in the DREC winter organic trial, which also had major issues with management, irrigation, and low fertility. Data were collected for plant count and yield.

Cadmium

In Trial 1, Cd concentrations ranging from 2uM to 20uM all produced spinach with visible signs of toxicity including chlorosis, necrosis, and stunting (Figure 5). Therefore, we retested concentrations lower than 2 uM in Trial 2 and found that 0.25 uM and 0.5 uM had few signs of toxicity. Thus, we will likely use one of these concentrations for selection and genetic analysis of Cd tolerance. We need to determine if the Cd uptake under hydroponics is similar to that of either the greenhouse or field soil assays, something other crops have found to be true. For the experiments described here, we have two contrasting check cultivars. Samples have been submitted for foliar analysis, bulked by genotype and treatment, and we are currently waiting for the results.

Figure 5. Cd hydroponic trials. (left) Trial 1 showing early signs of chlorosis due to levels being too high. (Right) Trial 2 showing no visible signs of chlorosis. Leaf samples will be tested for Cd concentrations.

Objective 2: To identify seed growers and to produce sufficient seed of advanced experimental cultivars to enable solid seeded, small plot and strip trials.

Procedures:

We need to produce larger quantities of seed to test experimental populations in direct seeded trials under commercial density. We attempted seed increases of 3 breeding populations with growers in Whidbey Island, WA; Vashon Island, WA; and McMinnville, OR – long day environments under which seed production should be ideal for spinach. We also attempted to increase one population at the UC ANR Intermountain Research and Extension Center (IREC) in Tulelake CA. Approximately 1000 seeds were used for each increase, which were seeded directly into the field.

Results and Discussion:

We had poor results from our seed growers. Three of the four sites failed to produce seed due to a combination of disease and/or insect pressure. One site did produce ~5lbs of seed (Whidbey Island, WA), but the germination for this seed was <10%.

Identifying growers for small seed increases is not trivial; most of the best seed growers already grow for companies and because spinach is outcrossing, isolation is needed between different seed increases. However, in the renewal of this project, we have contracted with 2 new growers in WA for summer 2023 seed increases. For these increases, they have transplanted rather than direct seeded and have successfully grown the plants to bolting at the time of writing. So, we anticipate better results in 2023 than we had in 2022.

Objective 3: To accelerate generation time using speed breeding to enhance genetic gain for broad spectrum downy mildew resistance.

Procedures:

Speed breeding for our program is primarily focused on shortening the generation time (the time from "seed to seed"). While we can select in 30 days for baby leaf traits, we need to wait for several months to obtain seed from selected plants when intercrossed in the greenhouse. Therefore, we are interested to determine if flowering would occur faster if plants were grown under LED lights in a growth chamber compared to the greenhouse and to determine if certain light spectra accelerated flowering more than others.

We have conducted several trials with basically the same format. Seeds of several spinach cultivars were sown in pots. After seeds germinated and the plants were at a similar developmental stage, the pots were placed in the greenhouse or placed in a growth chamber with three different LED light treatments: white light; 80% red and 20% blue; and 60% red and 40% blue (Figure 6). All three growth chamber treatments were under 22 hour light:2-hour dark treatment; the greenhouse grown plants had our typical 16 hour photoperiod. The plants were monitored for bolting and seed set. Germination tests were conducted after the seeds were harvested.

Results and Discussion:

Across all trials, plants in the growth chambers bolted and produced seed 17-30 days earlier than plants in the greenhouse. Thus, by simply moving plants to growth chambers with 22 hour photoperiod, we can accelerate seed production. In the

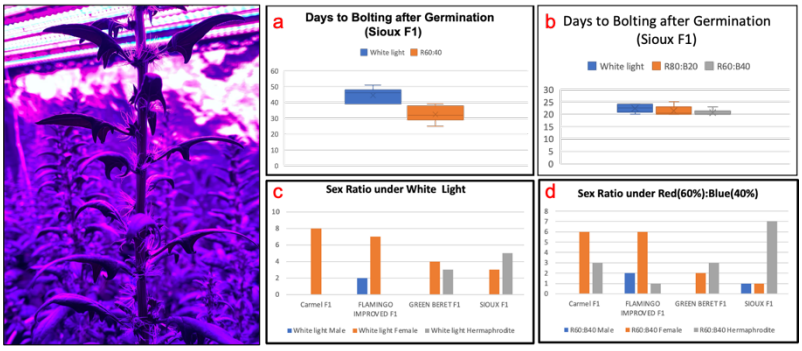

Figure 6. (left) Spinach plants flowering under a 60:40 red:blue light spectrum. (right) a and b: the cultivar Sioux flowered slightly earlier under the 60:40 treatment than under white light. We observed more male or hermaphrodite plants under 60:40 than white light.

chambers, changing from white light to a 60:40 red:blue spectrum decreased time to bolting (Figure 6), although the effect was not strong in all cultivars. Another effect of altered light spectra was that more male and hermaphrodite plants were observed under the 60:40 treatment than white light. This desirable results means that we can produce seed in populations that, under greenhouse conditions, would have been all female. The seed viability in all treatments was over 80%.

Objective 4: To integrate genetic markers into the breeding program to enhance efficiency.

Procedures:

Sex Markers

We regularly use the male specific sex marker T11A using the protocol published by (Wadlington, 2018) to identify male (YY), androdiecious (XY) and female (XX) plants before bolting in order to more efficiently make crosses as well as determine if speed breeding conditions can be used to modify the androdieciousness of individual plants.

DM markers

Resistance to a majority of the known races of the DM pathogen (*Peronospora effuse*) is controlled by at least six major genes in Spinach (Feng 2018). Three of these loci (RPF1, RPF2, and RPF3) have been characterized and several linked markers have been published. In order to develop cultivars with broad genetic resistance to DM, we need to know if major genes are present in a population. We have previously tested 12 RPF markers representing these three loci, but results were not consistent with the literature for many of them and/or they did not segregate in our breeding lines and therefore were not of use for our breeding program. Only a SNP marker for RPF1 (RFPF1-8) was consistent and has been developed into an IDT PACE^{TM} SNP genotyping assay to identify this major gene resistance in breeding populations.

We identified a quantitative trait locus (QTL) for major gene resistance to 'Spence2020' in our breeding material, which falls within the region of other major RPF loci. We developed and tested several IDT DNA PACE^{TM} SNP genotyping assays based on this QTL that allow for quick and high throughput identification of plants that have this locus, with the objective of creating a population with high resistance that does not derive from a major gene but instead relies on broad spectrum resistance.

We are routinely genotyping our breeding populations using Genotyping-by-Sequencing (GBS) (Poland and Brown, 2012). Sequencing is done at the UC Davis Genome Center using an Illumina NextSeq 500. Bioinformatics is done using the TASSEL5 GBS pipeline (www.maizegenetics.net/tassel) with alignment to the high-quality spinach genome sequence generated by PI Van Deynze (Hulse-Kemp, 2021, phytozome-next.jgi.doe.gov/info/Soleracea_Spov3).

Results and Discussion:

One of our most promising experimental populations for broad spectrum resistance, UCSP2010 is a broad-based population derived from seven 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. This population is currently in its fourth cycle of selection for broad spectrum resistance after multiple field and growth chamber trials using three current DM isolates. Three Cycle 2 subpopulations were created based on growth chamber screening: one susceptible to DM (S), one that shows no symptoms indicating major gene resistance (R), and one with very mild symptoms (MR) indicating no major gene presence but possible broad-spectrum resistance (Table 4).

Using genome-wide (GBS) SNP data and DM growth chamber ratings from UCSP2010 Cycle 2, we identified a major QTL for resistance to the *Spence2020* isolate. This is consistent with major DM resistant loci (RPF) published in the literature (Bhattarai, 2021). The most significant SNP under the identified QTL (SSPOV3_CHR3_1499694300), shows a significant dominant effect on DM resistance (similar to previously reported R genes) and is located close to RPF3. Based on these SNP data, C2-MR, the population with the most potential for broad-spectrum resistance, still contained plants that were heterozygous for the R locus. In order to further advance a population that did not contain the R locus, we screened C2-MR in the growth chamber. The turnaround time for GBS data does not currently allow us to select for this major locus before selection and intercrossing, so we relied on phenotypic response in addition to the genotype information of the female parent. Plants with the marker were used to create a population C3 MR-R(+). Plants without the marker for the major gene were used to generate three populations: C3-MR-R(-) showed no symptoms, C3 MR-MR showed minor symptoms, C3 MR-S showed severe symptoms. Since all of these plants in C2 MR were intercrossed and we only know the genotype of the female parent, it is possible that some plants in the C3 MR-R(-) population could still be heterozygous for the R locus if it was contributed by the male parent. GBS genotype data for the C3 population has recently been received and analysis is currently pending. We also advanced another cycle of the C3 MR-MR populations to C4 to continue to improving broad spectrum resistance in a population we know does not contain the R locus (Figure 7). We used this population instead of C MR-R(-) out of an abundance of caution to ensure we did not have a major gene still segregating.

Table 4. Downy mildew incidence and severity in UCSP2010 C2 and C3 populations grown in field trials in spring and fall 2022 in Salinas.

		Severity	
Cycle	Incidence	Spring 2022	Fall 2022
	%	*Score (1-5)*	
C2 R (+)*	4	1.4	1.7
C2 MR (-/+)	11	2.7	2.2
C2 S (-)	53	4.3	3.8
Cycle 3 pops derived from C2 MR without major gene			
C3 MR-R (-)	7		1.5
C3 MR-MR (-)	27		2.3
C3 MR-S (-)	53		3.2

*A "+" indicates that a major gene for resistance is present; "-" that the gene is absent, and "+/-" that the major gene is segregating.

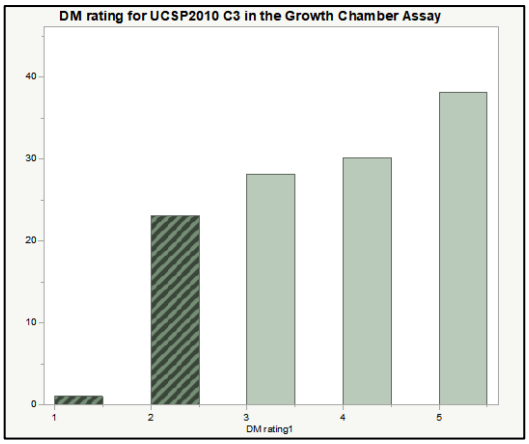

Figure 7. Distribution of DM ratings in the C3 MR-MR plants. Plants with ratings of 1 or 2 were selected for intercrossing to create a Cycle 4 population.

We have developed and tested several PACE markers that allow for quick and high throughput identification of plants containing this marker locus and have successfully identified one that works well (Figure 8). Using this marker, we were able to screen plans of the C3 populations in the greenhouse. As predicted the C3 MR-R(+) and C3 MR-R(-), which both showed no symptoms in screening, contained plants carrying the the R locus. Further, 35% of the C3 MR-R(+) plants did not contain the locus, which explains why there was not complete resistance seen in field trials. The 18% of the C3 MR-R(-) plants which did carry the locus probably reduced the symptoms seen in the field. We increased seed of these C3 populations based on the marker genotypes and will test these populations further in the field.

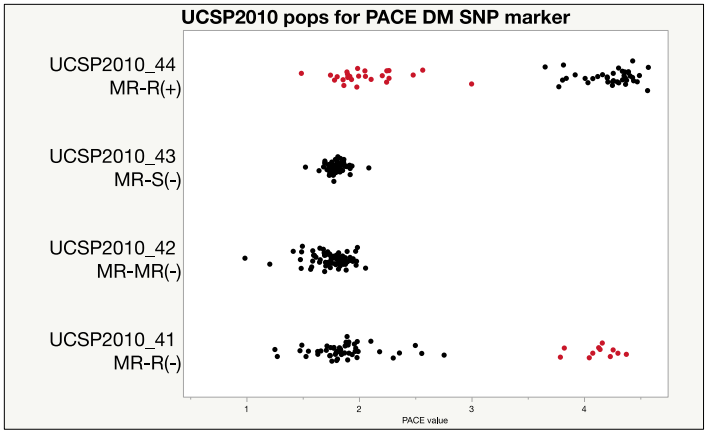

Figure 8. Individual plants of four Cycle 3 populations from UCSP2010 assayed using a PACE SNP marker for a major QTL for DM resistance. Plants are homozygous R- (clusters to the left), homozygous R+ (clusters to the right), with heterozygotes falling in between. Red dots indicate plants that were removed prior to seed increase in order to ensure a population did or did not have the major gene locus.

Whole-genome markers:

We have genotyped numerous individual plants from our breeding populations with genome-wide SNP markers using GBS (Figure 9). These populations show a broad genetic diversity, and in many cases, differ from commonly grown commercial cultivars. These results suggest that we have abundant variation to improve various traits. The marker datra for individual populations will be used in the new project to identify genes associated with Cd uptake, DM tolerance, yield, and other traits.

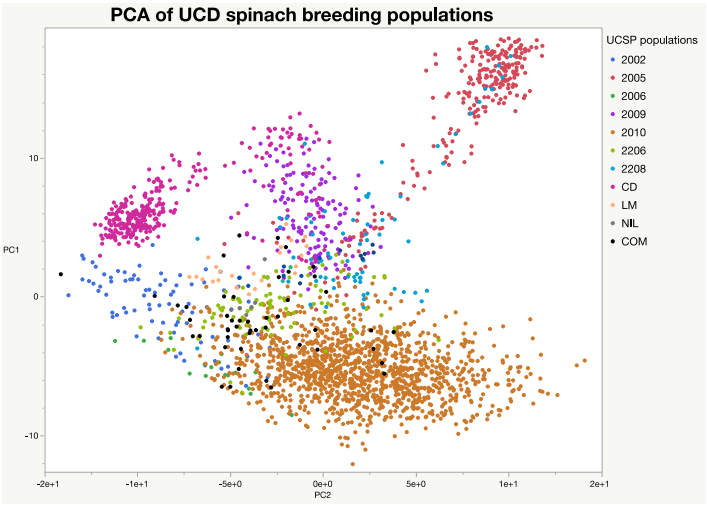

Figure 9. Principal Components Analysis of GBS-SNP marker data of individual plants from various UC breeding populations as well as commercial cultivars (COM) and the nearly isogenic line (NIL) downy mildew differentials. CD = population segregating for Cd uptake; LM = a population from Beiquan Mou with leaf miner resistance.

Projects leveraged by this grant:

We currently have several additional grants supporting our spinach breeding program. We (Brummer, Van Deynze, Klosterman) recently completed a CDFA grant that started in November 2020 “Advanced Breeding for Broad Genetic Resistance to Downy Mildew in Spinach for Organic Production” to expand our breeding to organic ground. We (Brummer, Van Deynze, Krill-Brown, Joshi) received a grant from the Specialty Crop Multi-State Program (SCMP) with a

collaborator at Texas A&M "Understanding and Improving Nitrogen Use Efficiency In Spinach" continuing a project that began three years ago. Finally, we received a small grant from the California Seed Association to identify genes associated with broad spectrum downy mildew resistance.

References:

Akamatsu T, Suzuki R, Uchimiya H (1998) Determination of male or female of spinach by using DNA marker. Japanese patent JPH1052284, Sakata NWadlington WH, Ming R. Development of an X-specific marker and identification of YY individuals in spinach. Theor Appl Genet. 2018 Sep;131(9):1987-1994. doi: 10.1007/s00122-018-3127-1. Epub 2018 Jul 3. PMID: 29971471.

Bhattarai, G., Feng, C., Dhillon, B., Shi, A., Villarroel-Zeballos, M., Klosterman, S. J, & Correll, J. C. (2020). Detached leaf inoculation assay for evaluating resistance to the spinach downy mildew pathogen. *European journal of plant pathology, 158*, 511-520. doi: 10.1007/s10658-020-02096-5

Bhattarai, G., Yang, W., Shi, A. *et al.* High resolution mapping and candidate gene identification of downy mildew race 16 resistance in spinach. *BMC Genomics* **22,** 478 (2021). https://doi.org/10.1186/s12864-021-07788-8

Clark KJ, Anchieta A, da Silva MB, Kandel SL, Choi YJ, Martin F, Correll J, Van Denyze A, Brummer EC, Klosterman SJ. Early detection of the spinach downy mildew pathogen in leaves by recombinase polymerase amplification. Plant Dis. 2022 Mar 6. doi: 10.1094/PDIS-11-21-2398-RE. Epub ahead of print. PMID: 35253491.

Feng, C., K.H. Lamour, B.H. Bluhm, S. Sharma, S. Shrestha, B.D.S. Dhillon, et al. 2018. Genome Sequences of Three Races of Peronospora effusa: A Resource for Studying the Evolution of the Spinach Downy Mildew Pathogen. Mol Plant Microbe In 31: 1230-1231. doi:10.1094/MPMI-04-18-0085-A.

Hulse-Kemp, A. M., Bostan, H., Chen, S., Ashrafi, H., Stoffel, K., Sanseverino, W., … Van Deynze, A. (2021). An anchored chromosome-scale genome assembly of spinach improves annotation and reveals extensive gene rearrangements in euasterids. *The Plant Genome*, *14*(2). https://doi.org/10.1002/tpg2.20101

Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. PLOS ONE 7(2): e32253. https://doi.org/10.1371/journal.pone.0032253

She, H., W. Qian, H. Zhang, Z. Liu, X. Wang, J. Wu, et al. 2018. Fine mapping and candidate gene screening of the downy mildew resistance gene RPF1 in Spinach. Theoretical and Applied Genetics 131: 2529-2541. doi:10.1007/s00122-018-3169-4.

Wadlington WH, Ming R. Development of an X-specific marker and identification of YY individuals in spinach. Theor Appl Genet. 2018 Sep;131(9):1987-1994. doi: 10.1007/s00122-018-3127-1. Epub 2018 Jul 3. PMID: 29971471.