

CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2007 to March 31, 2008

LEAF LETTUCE BREEDING

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SUMMARY:

Backcross programs are underway that emphasize the identification and introduction of genes for disease resistance, particularly to downy mildew, corky root, lettuce mosaic virus and anthracnose, into the four leaf lettuce types. Resistance for downy mildew is being introduced from fifteen new sources into cultivated genotypes suitable for California and will ultimately be combined with resistance to LMV, anthracnose and corky root. Utilization of multiple new sources of resistance and introduction of different resistances into the different lettuce types will likely increase the longevity of individual resistances and minimize the chances that changes in the pathogen will render all cultivars of different lettuce types susceptible simultaneously. Genetic studies are in progress to determine the genetic basis for the resistances and to identify molecular markers to increase the efficiency of generating resistant varieties.

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PROJECT TITLE: **BREEDING LEAF LETTUCE**

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OBJECTIVES

- 1) To develop advanced green leaf, red leaf, romaine and butterhead breeding lines with resistance to multiple diseases and superior horticultural characteristics suitable for California.
- 2) To introgress disease resistance genes, particularly *Dm* genes for downy mildew resistance from wild species.
- 3) To understand the genetic basis of disease resistance.
- 4) To use molecular markers linked to disease resistance genes to accelerate breeding progress.

PROCEDURES AND RESULTS

Breeding Strategy

The program parallels the strategy used for the UC Crisphead Lettuce Program where crosses and early generations are being grown at Davis; later generations are being trialed and selected at several different lettuce growing areas in collaboration with Dr. Ryan Hayes at the USDA, Salinas and with Richard Smith cooperating with California growers. Backcross or modified single-seed descent strategies are being employed for most of the early generations. We are selecting for type, color, slow bolting, and yield as well as disease resistance in the four leaf lettuce plant types. As far as possible we use different sources of resistance for each plant type. When resistant advanced lines of the desired plant type have been generated for individual resistances, they will be intercrossed to create lines with multiple disease resistances for lettuce downy mildew (DM), corky root (CR), anthracnose (ANT) and lettuce mosaic virus (LMV). Additionally we are screening germplasm to identify genetic resistances for Verticillium and Fusarium wilts and Botrytis rot which will be incorporated into the breeding program.

Recurrent parents for backcross programs

Cultivars representing each type were selected on the basis of one or more of the criteria listed below to be the recurrent parents in backcross programs to introgress resistance genes.

- 1) Horticultural type and performance in California representative of the morphological variation within each type.
- 2) Public domain rather than commercial cultivars to avoid issues of being 'essentially derived' from proprietary material.
- 3) Presence of additional disease resistance genes.

For the past seven years, we have used Valmaine and Parris Island Cos as the recurrent parents for the romaine type, Salad Bowl and Grand Rapids for the green leaf type, Lola Rosa and Ruby for the red leaf type, and Bibb and Buttercrunch for the butterhead type. As the first set of introgressions nears completion, we have recently revised our recurrent parents with input from several people and selected new lines that more closely represent current horticultural types and top-performing leafy lettuce cultivars. We are now using Green Towers, Two Star, Red Tide, and Margarita for romaine, green leaf, red leaf, and butterhead types, respectively. We welcome suggestions and further input on the field performance of these and other potential recurrent parents. The recurrent parents used in the final generations of backcrossing will be adjusted to reflect the industry standards at the time.

Sources of downy mildew resistance

We are emphasizing the development of lines with resistance genes from diverse sources to provide protection against pathotypes of downy mildew present in California. In order to maximize the diversity and durability of resistance genes present in the lettuce crop, resistances from different accessions are being introgressed into each leaf type as well as into the crisphead

type. This strategy should fragment the selection on the downy mildew population and prevent cycling of virulent strains of the pathogen between the different lettuce types.

All of the donor lines used in the program provide resistance to a broad range of California isolates of lettuce DM. In 2008, we released two red leaf lines with resistances introgressed from donor CGN14278 and a green leaf line with resistance introgressed from donor CGN14271 (see release notes below). Please contact Oswaldo Ochoa if interested in the releases (oechoa@ucdavis.edu). The most advanced breeding lines in progress are Romaine types with introgressed novel sources of resistance to downy mildew. These lines are now in the BC₆ and BC₇ generations and are being trialed in Salinas. Additional breeding lines for leafy types are at various stages ranging from initial F₁ crosses to the BC₅ generation (Table 1).

Table 1. Status of introgression of novel sources of DM resistance into leafy lettuce types.

Type	Donor line		Status
Romaine	00G950	<i>L. saligna</i>	BC ₇ Field Trial 2008
	CGN5916	<i>L. serriola</i>	BC ₅
	CGN14263-19	<i>L. serriola</i>	BC ₂
	CGN5147	<i>L. saligna</i>	BC ₃
	W84	<i>L. saligna</i>	F ₂
Red leaf	00G952	<i>L. saligna</i>	BC ₅
	CGN14278	<i>L. serriola</i>	2007 Releases UC07100, UC07101
	PI509523	<i>L. saligna</i>	F ₂
Green leaf	CGN5882	<i>L. saligna</i>	BC ₅
	PI491000	<i>L. saligna</i>	BC ₅
	CGN14271	<i>L. serriola</i>	2007 Release UC07103
	CGN14263-11	<i>L. serriola</i>	BC ₃
	PI509525	<i>L. saligna</i>	BC ₃
Butterhead	CGN14263-21	<i>L. serriola</i>	BC ₃
	CGN5267	<i>L. saligna</i>	BC ₁

Genetic basis of resistance to downy mildew

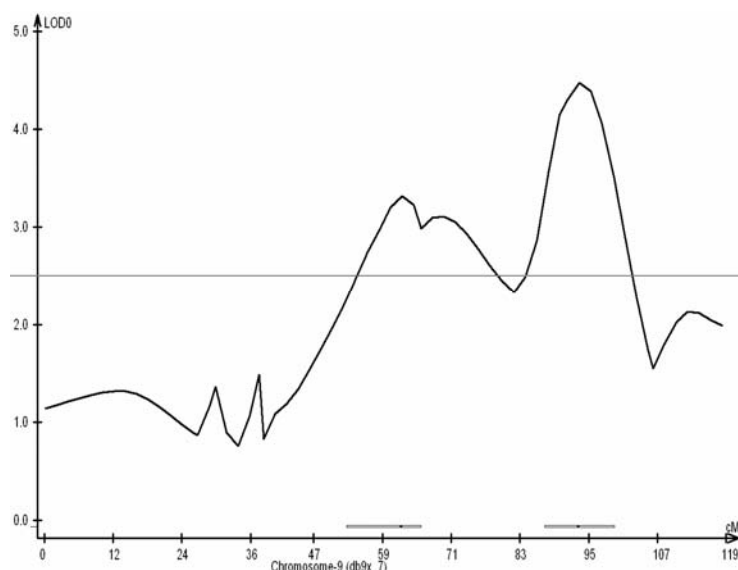
The backcross strategy to introduce resistance genes from wild donors into recurrent parents provides informative populations to determine the genetic basis of resistance. In each generation, we select for resistant individuals (with the resistant locus heterozygous) and discard susceptible individuals that are homozygous for the susceptible allele. Genomic regions not associated with the resistance become increasingly homozygous with each generation of backcrossing, while genomic regions associated with the resistance gene remain heterozygous. We are now in the process of identifying the genomic regions associated with resistance to downy mildew from over twenty resistant donor lines from both the leaf and crisphead breeding programs.

Populations derived from early generation backcrosses are being phenotyped and genotyped in order to map the resistance phenotypes. DNA is extracted from resistance and susceptible plants and pooled into groups of susceptible and resistant individuals. Genotyping by bulked segregant analysis (BSA) is carried out using the Illumina GoldenGate[®] SNP assay to identify markers that are different between the bulks and are therefore linked to resistance. This also identifies markers unlinked to resistance that can be used to select against the remaining donor genome in subsequent generations. Phenotyping has been completed for eleven populations segregating for resistance. As BSA identifies markers tightly linked to resistance, these markers are further analyzed on individuals of the entire population in order to determine the precise genetic position of the resistance gene and provide markers suitable for Marker Assisted Selection (MAS). Genotyping and mapping of resistances have thus far been completed for the resistances introgressed from three donor lines (Leafy CLRP report 2007; Variation CLRP report 2008).

Identification and genetic analysis of resistance to *Botrytis cinerea*

A screen for resistance to 15 different isolates of *Botrytis cinerea* was conducted on ten lettuce accessions that represented the parents of mapping populations; a wide range of levels of rot caused by *B. cinerea* were observed (Leafy CLRP report 2007). Cultivars Salad Bowl and Diana were identified as the most resistant, showing greatly delayed and reduced rot to nine of the 15 isolates. Genetic analysis of resistance against two different isolates of *Botrytis* was conducted using 90 F₃ families derived from a cross between cultivar Salad Bowl and *L. serriola* acc. CGN14263. We have identified at least three QTL affecting resistance to *B. cinerea*, with one major QTL region in chromosome 7 explaining approximately 25% of the phenotypic variation (Fig. 1). Further analyses are being conducted to validate and fine map these QTL as well as provide molecular markers to select for resistance.

Figure 1. A major QTL on chromosome 7 associated with resistance to *Botrytis cinerea* segregating in the Salad Bowl x CGN14263 population.



Identification of markers tightly linked to *cor*

The recessive resistance gene *cor*, conferring resistance to corky root rot, was originally mapped in F₃ families derived from a cross between cvs. Green Lakes and Diana (Moreno-Vazquez *et al.*, 2003, Genome. 46:1059-69). There is a paucity of markers in the region when analyzed in multiple crosses, possibly indicating an elevated rate of recombination. Also, no very tightly linked markers have been identified for the *cor* gene. Therefore, there is a need to identify causal or tightly linked genes for *cor*. We are currently implementing several strategies to achieve this. 1) BACs identified through PCR analysis of BAC pools with markers flanking the *cor* gene are being sequenced. 2) Candidate resistance genes recently identified as mapping to the *cor* region are being tested for co-segregation with *cor* in the population derived from Green Lakes and Diana. These candidate genes were positioned in the *cor* region based on the ultra-high density map generated by genotyping the Salinas x UC96US23 RIL population with the lettuce Affymetrix[®] genotyping chip (Variation CLRP Report 2008). 3) Identification of polymorphism using massively parallel BSA by hybridization of resistant and susceptible bulks from the Green Lakes x Diana population to the lettuce Affymetrix[®] genotyping chip; polymorphisms linked to resistance will be tested for co-segregation with *cor* using individuals of this population.

We are continuing backcross programs to combine *cor* with downy mildew resistances from the novel sources.

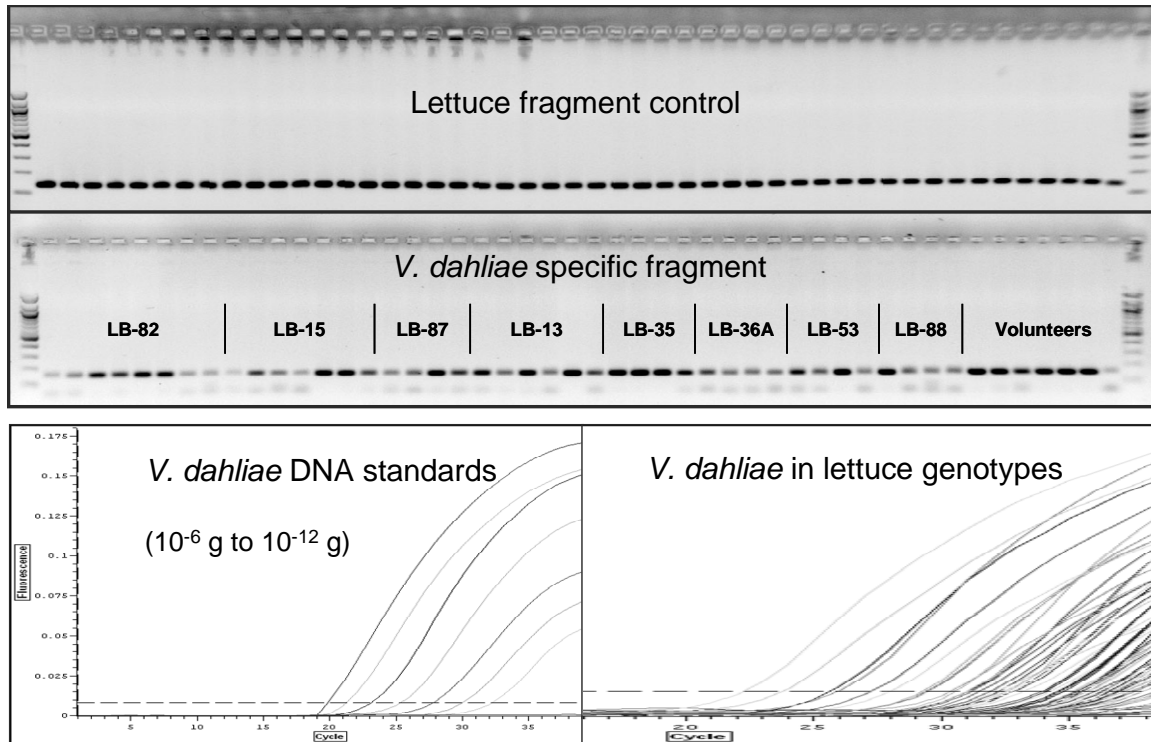
Genetic analysis of resistance to *Fusarium* wilt

In collaboration with Dr. Thomas Gordon (UC Davis), we have analyzed resistance against *Fusarium oxysporum* in the RIL population derived from a cross between cvs. Salinas and Valmaine. Phenotyping has been completed for 68 RIL families. Although the cultivar Valmaine has a high level of resistance compared to the cultivar Salinas, both parental lines have some resistance to *F. oxysporum*; the transgressive segregation observed in the population phenotyped indicates that the resistances from each parent is conferred by genes at different loci. Additional families will be phenotyped in field experiments this summer to provide phenotypes for a total ~150 RIL families. Genotyping of the population will use the Illumina GoldenGate[®] SNP assay for markers in candidate resistance genes (Variation CLRP report 2008).

Screening for resistance to *Verticillium* wilt

In conjunction with the Crisphhead Breeding Program (Crisphhead CLRP Report 2008) and in collaboration with Krishna Subbarao (UC Davis), we have screened 172 cultivated and wild accessions for resistance to *Verticillium dahliae*, Race 2 and are continuing to screen additional accessions. Resistance is being measured by both visual observation of symptoms as well as quantitative PCR (qPCR) measurements of *V. dahlia* DNA present in plant samples taken at various developmental stages (Fig. 2). Although differences in symptoms have been observed, no fully resistant accessions have been identified so far. Results from visual assessments do not always correlate with the levels of *V. dahlia* in the plant as detected by qPCR, demonstrating that there can be significant amounts of the pathogen present in asymptomatic plants (Fig. 2).

Figure 2. Amplification of DNA of *V. dahliae* by PCR and qPCR from asymptomatic plants.



Trials of Breeding Lines

The program continues the strategy of crosses being made and early generations being grown at Davis with later generations being trialed and selected at field trails in. Backcross or modified single-seed descent strategies are being employed for most early generations. Two trials were planted collaboration with Richard Smith (UC Monterrey Cooperative Extension, Salinas). One was at the Banovac Ranch, Willoughby Farms in May 2007 and the second was planted in August 2007 at the Cooper ranch, Royal Packing Co. We continue to select for good color, slow bolting, and yield as well as disease resistance in the different plant types.

RELEASE OF DOWNY MILDEW RESISTANT BREEDING LINES OF LEAF LETTUCE

Please contact Oswaldo Ochoa (oeochoa@ucdavis.edu) or Richard Michelmore (rwmicelmore@ucdavis.edu) to receive samples of these lines.

Two advanced breeding lines of red leaf and one green leaf lettuce are available for use by plant scientists and breeders in public and private institutions. These lines were developed by Richard Michelmore, María José Truco and Oswaldo Ochoa at the University of California, Davis. When this germplasm contributes to a new cultivar, appropriate recognition should be given as to its origin.

These lines have been developed to provide superior disease resistance in a red and green leaf horticultural type by backcrossing resistant genotypes with cv. Lolla Rosa for the red leaf type and cv. Grand Rapids for the green leaf type as the recurrent parent. These lines are now homozygous for the resistance. Field evaluations in Salinas were made during the last two selfed generations. These lines are close to horticultural types suitable for use in the coastal production areas of California. However, there is residual variation in most of these lines and further selections may be required to fix plant type. Trials and selections should be made to determine specific areas and seasons to which these lines are best adapted.

Red Leaf Type:

Line **UC07100** was derived from *L. serriola* CGN14278 by backcrossing to cv. Lolla Rosa as the recurrent parent. Molecular studies have shown the resistance to be located on linkage group 1 away from the cluster of resistance where *Dm5/8* is located (Leafy CLRP report 2007). This resistance is effective against all Californian isolates of Downy mildew.

Line **UC07101** was derived from *L. serriola* CGN14278 by backcrossing to cv. Lolla Rosa as the recurrent parent. Even though this line is derived from the same wild source than line UC07100, molecular studies of the resistance indicate that this line is carrying a different resistance gene. The resistance is on linkage group 1 away from the cluster of resistances where *Dm5/8* is located (Leafy CLRP report 2007). This resistance is effective against all Californian isolates of Downy mildew.

Green Leaf Type:

Line **UC07103** was derived from *L. serriola* CGN14271 carrying downy mildew resistance by backcrossing to cv. Grand Rapids. The resistance is located on linkage group 1 away from the cluster of resistance where *Dm5/8* is located. This resistance is effective against all Californian isolates of Downy mildew.

These resistances have yet to be combined with other resistance genes. The individual resistances in all of these lines protect against all isolates of LDM tested. They will, however, probably be rendered ineffective over time due to changes in the pathogen. To our knowledge, these sources of downy mildew resistance have not been used as parents for existing cultivars grown in California.