CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2008 to March 31, 2009

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 (LMV) 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 (LDM), 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. 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, Tropicana, Red Fox, 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 lettuce downy mildew (LDM) 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 LDM 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 LDM. In 2008, we released two red leaf lines with resistances introgressed from donor CGN14278 (UC07100 and UC07101) and a green leaf line with resistance introgressed from donor CGN14271 (UC07103) (CLGR report 2007-2008) (Table 1). The most advanced breeding lines in progress are romaine types and red leaf types with introgressed novel sources of resistance to LDM from donors CGN5916 (*L. serriola*), UC00_950 and UC00_952 (*L. saligna*). These lines are BC_7S_1 generation. Individuals homozygous for the resistance are being selected. Additional breeding lines for leafy types are at various stages ranging from initial F_1 crosses to the BC_5 generation. New sources of resistance, mostly *L. saligna* accessions, have been identified (see Crisp breeding report) and are being introgressed into all the types.

Table 1. Status of introgression of novel sources of LDM resistance into leaf lettuce types.

Lettuce Type	Donor line and species		Status
Romaine	UC00_950	*L. saligna*	BC_7S_1
	CGN5916	*L. serriola*	BC_7S_1
	CGN14263	*L. serriola*	BC_3
	W84	*L. saligna*	BC_1
Red Leaf	CGN14278	*L. serriola*	Released 2008
	CGN14278	*L. serriola*	Released 2008
	UC00_952	*L. saligna*	BC_7S_1
Green Leaf	CGN14271	*L. serriola*	Released 2008
	05G1411	*L. saligna*	BC_3
	W37	*L. saligna*	BC_1
	CGN5882	*L. saligna*	BC_2
Butterhead	05G1421	*L. serriola*	BC_2

Genetic Basis of Resistance to Downy Mildew

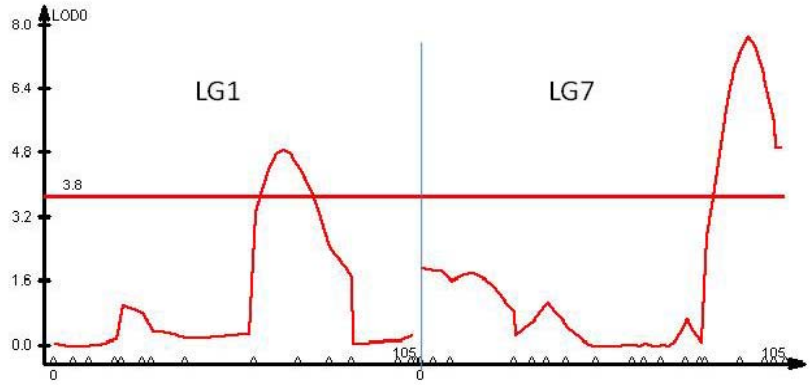
The backcross strategy to introduce resistance genes from wild donors into recurrent parents provides informative populations to determine the genetic basis of resistance. In each generation, we select for resistant individuals (with the resistance locus heterozygous) and discard susceptible individuals 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 resistant 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. 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 *Dm43*, *Dm44* and *Dm45* (Variation CLRP report 2008). The precise position of markers for other resistances from *L. serriola* donors are still being validated in the complete populations (CLGRB Variation Report 2008-2009).

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* was observed (Leafy CLRP report 2007). Cultivars Salad Bowl and Diana were identified as the most resistant, showing greatly delayed and reduced rot in response to nine of the 15 isolates. Genetic analysis of resistance against two different isolates of *Botrytis* was conducted using 90 F_3 families derived from a cross between cultivar Salad Bowl and *L. serriola* acc. CGN14263. An improved genetic map was constructed for this population by incorporating Illumina GoldenGate SNP markers. The combined map had 368 markers (AFLP, SSR and SNP) in eleven linkage groups. Using this map we identified two major QTLs for resistance to *B. cinerea* on linkage groups 1 and 7 (Figure 1) that explain 20 and 33% of the phenotypic variation, respectively. Further analyses are being conducted to validate and fine map these QTL as well as provide molecular markers to select for resistance. The genetic information on these QTLs will be useful to assure that susceptibility to *Botrytis* is not introgressed while breeding for other resistances mapped to the same chromosomal region.

Figure 1. Two major QTL on linkage groups 1 and 7 associated with resistance to *Botrytis cinerea* segregating in the Salad Bowl x CGN14263 population. The horizontal line at the LOD score of 3.8 is the significance threshold determined by permutation analysis.



Identification of Markers Tightly Linked to *cor*

We are continuing our efforts to identify tightly linked markers linked to the recessive resistance gene *cor*, conferring resistance to corky root rot. This gene was originally mapped in F_3 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. We are currently using several strategies to identify markers tightly linked to and ultimately to clone *cor*. 1) BACs identified through PCR analysis of BAC pools with markers flanking the *cor* gene have been sequenced and are being assembled to provide a contig across the region. 2) Candidate genes identified as mapping to the *cor* region using the ultra-high density map generated by genotyping the Salinas x UC96US23 RIL population with the lettuce Affymetrix® genotyping chip (CLRP Variation Report 2008) are being tested for co-segregation with *cor* in recombinants derived from Green Lakes and Diana. This has provided ~20 genes that are very tightly linked to *cor*. We are continuing backcross programs to combine *cor* with downy mildew resistances from the novel sources.

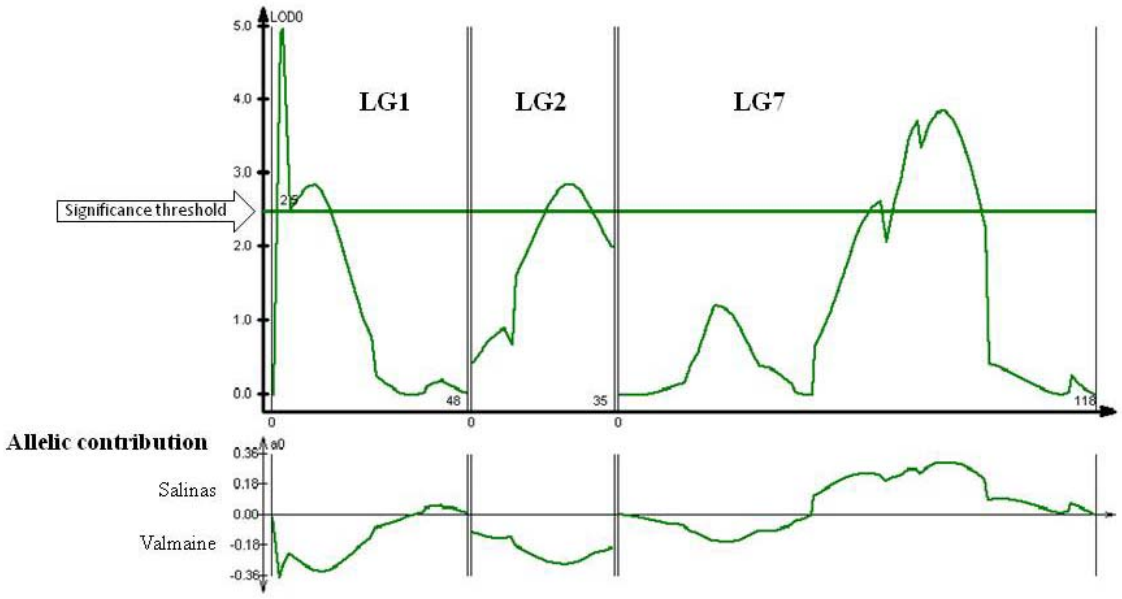
Genetic Analysis of Resistance to *Fusarium* wilt

In collaboration with Dr. Thomas Gordon (Dept. Plant Pathology, UC Davis), we have analyzed resistance against *Fusarium oxysporum* in a RIL population derived from a cross between a F_1 of cv. Valmaine x cv. Salinas 88 and cv. Salinas. Phenotyping for resistance was conducted during summer 2007 and 2008 in an artificially infested field at UC Davis. We phenotyped 68 RIL families plus Valmaine and Salinas in 2007. In 2008, we phenotyped 79 additional RIL families plus 10 RIL families that had been phenotyped in 2007 and that had shown a spectrum of resistance were re-phenotyped. The re-phenotyped RIL families, Valmaine, and Salinas showed consistent results in both years. 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 for both resistance and susceptibility observed in the RIL population indicated that the resistance in each parent is conferred by different genes. We genotyped the 147 RIL families with Illumina GoldenGate® SNP markers. Seventy-six markers were polymorphic and used to construct a linkage map. Three QTLs were identified on linkage groups 1, 2, and 7. The Valmaine allele in LG1 and LG2 conferred resistance, whereas the Salinas allele in LG7 was responsible for the increase in resistance (Fig. 2).

The same RIL population has been analyzed by Ivan Simko (USDA, Salinas) to map a gene for resistance to lettuce dieback. Cv. Salinas is resistant to dieback as are many crisphead cultivars and Valmaine, like many romaine types, is susceptible. A single gene for resistance to dieback was identified as mapping in LG2 close to the location of the QTL for *Fusarium* resistance. Since *Fusarium* resistance at this location was conferred by the Valmaine allele and the dieback resistance comes from Salinas, there was the concern that backcrossing to transfer resistance to *Fusarium* into crispheads from romaines or resistance to dieback resistance into romaines from crispheads could inadvertently introduce susceptibility to the other disease. However, further genetic analysis showed that the two resistances did not co-locate and we have identified multiple lines resistant to dieback that were also resistant to *Fusarium*. These lines will be phenotyped for *Fusarium* resistance this summer in the infested field at UC Davis to

confirm their resistance. These lines can then be used to breed romaine and crisphead lines that are resistant to both diseases.

Figure 2. QTLs for resistance to *Fusarium* wilt in LG1, LG2 and LG7.



Screening for resistance to *Verticillium* wilt

In conjunction with the crisphead breeding program and in collaboration with Krishna Subbarao (UC Davis), we have screened 333 cultivated and wild accessions for resistance to *Verticillium dahliae*, Race 2 and are continuing to screen additional accessions (Crisphead CLGRB Report 2009). Resistance is being measured by both visual observation of symptoms as well as quantitative PCR (qPCR) measurements of *V. dahliae* DNA present in plant samples taken at various developmental stages. Although differences in symptoms have been observed, no fully resistant accessions have been identified so far. Visual assessments do not always correlate with the levels of *V. dahliae* in the plant as detected by qPCR, demonstrating that there can be significant amounts of the pathogen present in asymptomatic plants. A few accessions of *L. serriola* have been identified that show delayed development of symptoms. These lines have been intercrossed to generate F_2 populations that are now being analyzed for resistance.