CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2009 to March 31, 2010

BREEDING LEAF LETTUCE

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SUMMARY:

Backcross programs continue 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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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.

Previously we used cultivars 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. Last year we 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 types (Breeding Crisphead Lettuce Report, 2010). This strategy should diversify the selection pressure 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. The most advanced breeding lines in progress are romaine types with novel sources of resistance to LDM introgressed from the donor UC00950 (*L. saligna*). These lines are BC_7S_1 generation. Lines homozygous for resistance are being selected. Additional breeding lines for leafy types are at various stages ranging from initial F_1 crosses to the BC_4 generation (Table 1). New sources of resistance, mostly *L. saligna* accessions, have been identified and resistance is being introgressed into all types.

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

Lettuce type	Donor line and species		Status
Romaine	UC00950	*L. saligna*	BC_7S_1
	05G1419	*L. serriola*	BC_1
	CGN5322	*L. saligna*	BC_2
	LB101	*L. saligna*	F_2 &BC_1
	LB134	*L. saligna*	F_2 & BC_1
Red Leaf	PI509523	*L. saligna*	BC_4
	LB153	*L. saligna*	F_2 & BC_1
	LB136	*L. saligna*	F_2 & BC_1
Green Leaf	05G1411	*L. saligna*	BC_1
	LB158	*L. saligna*	F_2 & BC_1
	LB165	*L. saligna*	F_2 & BC_2
Butterhead	05G1421	*L. serriola*	BC_2
	LB100	*L. saligna*	F_2 & BC_1
	LB143	*L. saligna*	F_2 & 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 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 (bulks) of susceptible and resistant individuals. Genotyping by bulked segregant analysis (BSA) is then 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. Markers tightly linked to resistance 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). In past year, two resistances from *L. serriola* were mapped to chromosome 4 along with *Dm4*, *Dm7*, *Dm11* and *Dm44*.

Identification of Markers Tightly Linked to *cor*

We are continuing backcross programs to combine *cor* with downy mildew resistances from the novel sources. We are also continuing our efforts to identify markers tightly linked to the recessive resistance gene *cor*, which confers 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 close to *cor* when analyzed in multiple crosses, possibly indicating an elevated rate of recombination in this genomic region. We are currently using several strategies to identify markers tightly linked to *cor* and, ultimately, to clone *cor*. 1) 12 BACs identified through PCR analysis of BAC pools using markers close to the *cor* gene have been sequenced. 2) 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 have been tested for co-segregation with *cor* in recombinants derived from cvs. Green Lakes and Diana. This has provided ~20 genes that are very tightly linked to *cor*. We are currently fine mapping the position of *cor* relative to these genes by identifying a large number of recombinants in the region using flanking molecular markers.

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* race 1 in a RIL population derived from a cross between a F_1 of cv. Valmaine x cv. Salinas 88 and cv. Salinas. After replicated field trials and genetic analysis three QTLs were identified on linkage groups 1, 2, and 7. The Valmaine alleles in LG1 and LG2 conferred resistance, whereas the Salinas allele in LG7 was responsible for the increase in resistance (See Fig. 2 in CLRGP 2007-2008 report).

The same RIL population was analyzed by Ivan Simko (USDA, Salinas) to map a gene for resistance to lettuce dieback (*Tvr1*). Cv. Salinas is resistant to dieback as are many crisphead cultivars and cv. 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 absolutely co-locate and we have identified lines resistant to dieback that were also resistant to *Fusarium*. These lines were phenotyped for resistance during Summer 2009 in the *Fusarium*-infested field at UC Davis and we confirmed those lines carrying both resistances (Table 2). These lines can now be used to breed romaine and crisphead lines that are resistant to both diseases.

Table 2: Results of 2009 *Fusarium* field trial of lines resistant to dieback.

RIL	*Fusarium* 2009	*Fusarium* 2008	TVR1	LMV	Lettuce type
295	0.28 [a]	0.34	R	mo1 [b]	Iceberg
44	0.58	0.38	R	mo1	Romaine
193	0.62	0.25	R	mo1	intermediate
171	0.62	0.4	R	mo1	intermediate
275	0.64	0.43	R	Mo1	
192	0.65	0.41	R	Mo1	
76	1.5	0.6	R	mo1	
33	1.51	0.55	R	mo1	
50	1.64	0.3	R	mo1	
5	1.85	0.58	R	mo1	
105	2.18	0.5	R	Mo1	
115	2.32	0.6	R	mo1	
151	2.62	2.81	R	mo1	
149	2.71	2.94	R	mo1	
260	2.75	2.78	R	Mo1	
262	2.89	2.91	R		
166	2.98	2.94	R	mo1	

[a]: 20 plants scored using 2 reps on a 0 to 3 scale. 0 = no disease. 3 = 100% plants diseased.
[b]: Presence of resistance to LMV determined using a SNP marker for the *mo1* gene.

We included in our field trial released lines that had cvs. Valmaine and Lolla Rosa as recurrent parents in their background since both of these cultivars are resistant to *Fusarium*. Most of the lines had very good resistance. We also included some of our new recurrent parents (Green Towers, Red Fox and Red Tide). Green Towers was resistant, Red Fox had an intermediate response and Red Tide was highly susceptible. In order to determine the genetic basis of the resistance in Lolla Rosa and Green Towers and to compare it to the resistance from Valmaine and Salinas we have initiated crosses among all these cultivars (Table 3). Progeny of these crosses will be tested in the *Fusarium*-infested field this summer.

Table 3. Crosses made to analyze the genetic basis of resistance to *Fusarium*.

Type	Resistance	Cross	Type	Resistance	Generation
Iceberg	Partial res.	Salinas x Green Towers	Romaine	Resistant	F_2
Red Leaf	Resistant	Lolla Rosa x Salinas	Iceberg	Partial res.	F_2
Red Leaf	Resistant	Lolla Rosa x Green Towers	Romaine	Resistant	F_1
Red Leaf	Resistant	Lolla Rosa x Red Tide	Red Leaf	Susceptible	F_1

Screening for resistance to *Verticillium* wilt

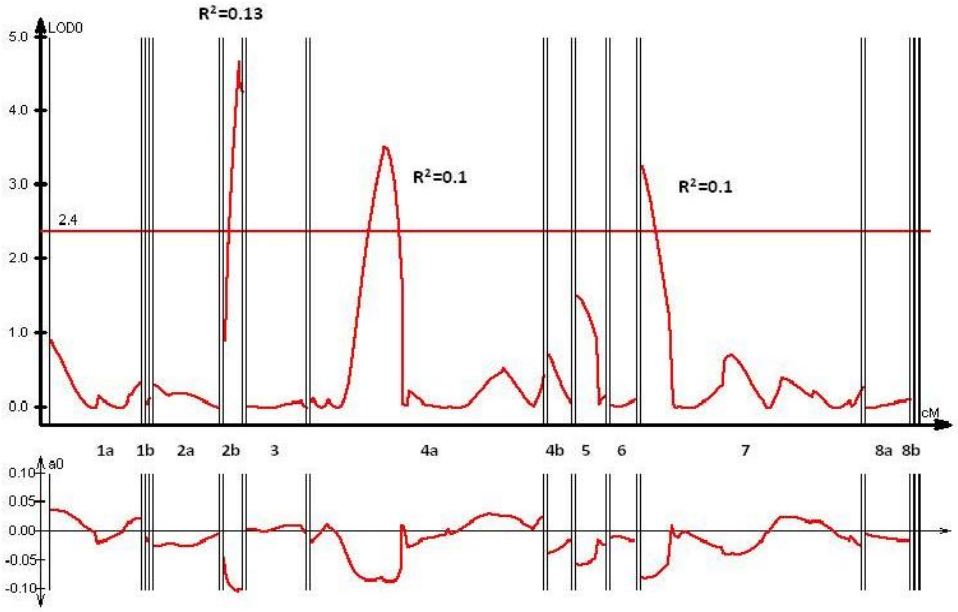
In conjunction with the crisphead breeding program and in collaboration with Krishna Subbarao (UC Davis), we have screened 333 cultivated and wild accessions for resistance to *Verticillium dahliae*, Race 2 and are continuing to screen additional accessions (Crisphead CLGRB Report 2009- 2010). Resistance is being evaluated by both visual scoring of symptoms as well as quantitative PCR (qPCR) measurements of *V. dahliae* DNA present in plants sampled 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* present 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 progeny that are now being analyzed for resistance.

Genetic analysis of tip burn resistance in a cross between a romaine and a crisphead type

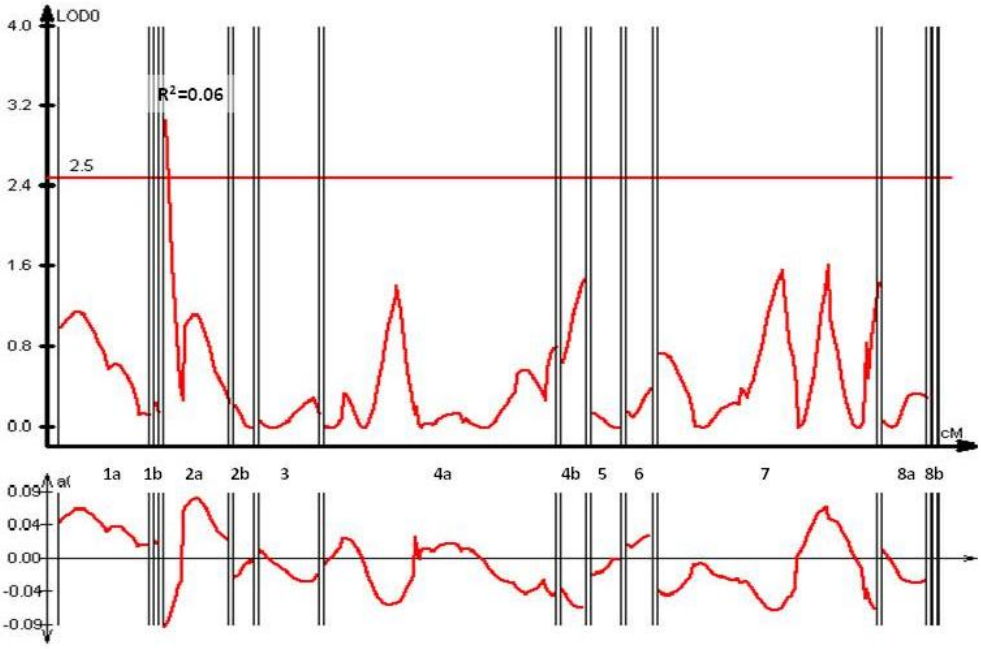
We have analyzed a RIL population developed from a cross between an F_1 of a romaine by crisphead cross (Valmaine x Salinas 88) and cv. Salinas for resistance to tip burn. A total of 211 RIL families were screened for percentage of tip burn occurrence at two different locations by our collaborators Ivan Simko and Ryan Hayes (USDA, Salinas) and by Bill Waycott (Seminis, Monsanto). A map was constructed for the population with 76 SNPs using the Illumina GoldenGate SNP assay. The QTL analysis of the two field trials indicated different chromosomal regions associated with tip burn (Figure 1 a & b). Three QTLs were identified in the USDA trial, one on each of linkage groups 2b, 4a and 7; a single QTL was identified in the Seminis trial on LG 2a. All the QTLs identified explained a small proportion of the phenotypic variation. More evaluations and new populations are necessary to get a better understanding of the genetic basis of tip burn resistance.

Figure 1. QTL analysis of tip burn resistance in progeny from a romaine x crisphead cross.

a) USDA trial



b) Seminis trial



The upper graph of each figure shows the probability (LOD) score (y axis) for each chromosome aligned along the x axis (chromosome nine had no polymorphic markers). The red horizontal bar indicates the significance threshold derived by permutating the data. The lower graph of each figure shows the allelic contribution from each parent (Valmaine above and Salinas below).