CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

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ISOLATE MAINTENANCE AND INOCULUM PRODUCTION IN SUPPORT OF THE *SCLEROTINIA* RESISTANCE BREEDING PROGRAM IN LETTUCE

PRINCIPAL INVESTIGATOR: **Krishna V. Subbarao**
Department of Plant Pathology
University of California, Davis

COOPERATING PERSONNEL:
Bullo Erena Mamo
Department of Plant Pathology
University of California, Davis

Ivan Simko
USDA-ARS, Salinas

ABSTRACT

Lettuce drop caused by *Sclerotinia* spp. is a serious disease that causes significant yield losses. Host plant resistance provides an effective control and is being explored intensively. As lettuce drop resistance is often confounded with premature bolting, previous attempts to identify resistance were largely unsuccessful. Recently, a high level of lettuce drop resistance was identified in the slow-bolting cultivar Eruption. Resistance in Eruption is quantitatively inherited with 3-4 genes with minor effects. Eruption is currently being used as a source of resistance in breeding programs. The genetic basis of this resistance is largely unknown. Eruption and other genotypes with different growth habits and levels of reaction to drop were therefore characterized for disease, plant architecture, cell wall components, and microclimate conditions under leaf canopy. Lettuce drop resistance in Eruption is not due to bolting. Analyses of stem strength as a host resistance/susceptibility factor, cell wall composition of resistant and susceptible lettuce, the effect of relative humidity and temperature is in progress. Sclerotial inoculum to infest one-half acre of field plot for screening breeding materials was provided.

OBJECTIVES:

A. Identify cell wall polymers associated with resistance to lettuce drop.
B. Develop innovative techniques of screening for lettuce drop resistance to advance resistance breeding.

C. Produce large quantities of sclerotia inoculum for the infestation of the field to screen breeding material.

PROCEDURES: Development of a feasible resistance-based lettuce drop control method requires a thorough understanding of the lettuce-*Sclerotinia* spp. interactions. One approach of accomplishing this is through systematic evaluation of host, host-pathogen interactions, and environmental factors. Thus, six lettuce genotypes were evaluated for lettuce drop, host plant architecture, and microenvironment conditions under host plant canopy in the greenhouse and field at the USDA Salinas research station during several seasons (Figure 1). The cell wall components of these materials are currently being analyzed.

Peeled potatoes cut into small pieces were dispensed into wide-mouthed jars, covered with aluminum foil, and autoclaved twice. Mycelial plugs from four isolates collected in the Salinas Valley were aspectically transferred into each jar and covered with aluminum foil again. The jars were incubated on laboratory benches for about 3 wk. The sclerotia produced in the jars were then harvested, washed multiple times on a sieve, and dried for several days. The sclerotia were then milled to produce sclerotia of uniform size for distribution in the seed rows using planters.

Figure 1. Seedlings of lettuce genotypes planted in the greenhouse to evaluate for lettuce drop and architectural traits. The pots were spaced out on benches and arranged according to the experimental design after the plants grew up.

RESULTS AND DISCUSSION: The six lettuce genotypes with different canopy architectures also differed in their reaction to lettuce drop (Table 1). The lettuce cultivars Salinas, Reine des Glaces (RG), and Eruption are slow bolting, as reflected by their low bolting scores. However, they differ in that Eruption is resistant to lettuce drop as it has one of the lowest disease ratings (0.28). Da Ye Wo Sun (DYWS) and PI 251246 are rapid-bolting genotypes but they differ in reaction to

Sclerotinia spp., indicating that bolting *per se* is not the responsible factor for lettuce drop resistance. PI 251246 is resistant to lettuce drop, whereas, DYWS is susceptible. On the other hand, 11-G99 bolts early and is tolerant to lettuce drop during spring seasons.

Evaluation of the lettuce genotypes for different lettuce drop phenotypes indicated that they significantly differ from each other (Table 2). The controls Brave Heart and Green Forest were susceptible to lettuce drop as expected. Eruption and PI251246 are consistently resistant to lettuce based on the disease rating and severity index, as opposed to the other genotypes. In contrast, PI251246 and 11-G99 did not collapse and their root systems did not 'rot' when inoculated at the flowering stage. The lettuce drop resistance in these genotypes increases with plant age and the mechanism is different from that in Eruption. DYWS showed medium disease levels due to its relatively faster rate of bolting.

Relationship between lettuce drop and bolting. As indicated above, lettuce drop, and the rate of bolting are negatively associated as rapid bolting accessions usually exhibit lower disease incidence. Correlation analysis indicated that the lettuce drop phenotypes are associated with plant architectural characteristics (Table 3). The collapse rating, stem base degradation length, root degradation severity, and days to mortality were negatively correlated with days to flowering, stem diameter, plant height, and stem strength. Collapse rating was positively correlated with days to flowering and stem diameter, indicating that late flowering and materials with larger stem circumference tend to be susceptible to lettuce drop. Collapse rating, stem base degradation length, and root degradation severity were negatively correlated with stem strength, indicating that lettuce genotypes with strong stem have low drop measurement values.

Variation among plant architectural characteristics. Statistically significant variations were observed among the lettuce genotypes in the growth architectures (Table 4). The controls Brave Heart, Green Forest, and Hearts Delight had statistically similar low bolting scores to the test genotypes Salinas and Eruption. PI251246 differed significantly from the rest of the genotypes in the rate of bolting. When compared on stem strength, PI251246 and 11-G99 had the highest stem strength that was more than twice that of the other genotypes. In addition to the rate of bolting, Eruption also has statistically similar stem strength values with Salinas, RG, and DYWS, indicating that both stem strength and bolting may not play a significant role for lettuce drop resistance in Eruption.

The difference in lettuce drop among cultivars with different growth habits is usually attributed to the possible modulation of microclimate conditions under the leaf. Thus, we assessed the moisture and temperature levels under the plant canopy in lettuce drop field experiments. However, the results did not fully explain the variations in lettuce drop among lettuce accessions. In conclusion, the mechanism of lettuce drop resistance in Eruption differs from that in PI 251246 or 11-G99. The former is most likely due to traditional defense signaling systems; whereas, lettuce drop resistance in the latter appears to be plant architecture-mediated. This work shows the value of a comprehensive analysis of lettuce-*Sclerotinia* spp. interactions and suggest that stem strength could be targeted in breeding for lettuce drop resistance.

Table 1. Lettuce genotypes used in this project and their rate of bolting along with lettuce drop incidence

Genotype	Salinas	Reine des Glaces	Eruption	Da Ye Wo Sun	11-G99*	PI 251246
Horticultural type/species	Crisp	Batavia	Latin	Stem	*L. serriola* L.	Oil
Bolting score (1 to 7)	1	1	1.3	4	3.2 (2)	6
Disease rating	0.68	0.91	0.28	0.61	0.4	0.27

***** 11-G99 bolts relatively rapidly during spring and slow in the fall seasons

Table 2. Mean values of lettuce drop measurement phenotypes: disease rating, disease severity, collapse rating, stem degradation length, root degradation severity, and days to mortality

Genotype	Disease rating	Disease severity index*	Collapse rating	Stem base degradation length	Root degradation severity	Days to mortality
PI251246	0.27 d	41.49 d	0.00 e	0.00 d	0.06 d	14.90 c
11-G99	0.40 cd	100.00 a	0.00 e	0.02 d	0.00 d	18.29 ab
Eruption	0.28 d	83.34 bc	0.41 cd	1.09 c	0.78 ab	8.60 f
Da Ye Wo Sun	0.61 abcd	93.33 ab	1.17 a	0.21 d	0.13 d	7.44 fg
Salinas	0.68 abc	91.11 ab	1.18 a	0.99 c	1.00 a	6.60 g
Reine des Glaces	0.91 ab	93.80 ab	0.75 b	1.67 a	0.53 c	9.13 ef
Brave Heart	0.65 abc	n.d	n.d	n.d	n.d	n.d
Green Forest	0.72 abc	n.d	n.d	n.d	n.d	n.d

*n.d, not determined

Table 3. Correlation coefficients for lettuce drop phenotypes and plant developmental characteristics measured in the greenhouse

Lettuce drop phenotypes	Plant developmental characteristics			
	Days to flowering	Stem diameter	Plant height	Stem strength
Collapse rating	0.64**	0.60**	-0.32 [x]	-0.58*
Stem base degradation length	0.53*	-0.06[x]	-0.77***	-0.57*
Root degradation severity	0.67**	-0.05[x]	-0.68**	-0.51*
Days to mortality	-0.69**	-0.58*	0.68**	0.70**

*** p <0.001; ** p <0.01; * p <0.01; [x] No correlation

Table 4. Mean values of lettuce drop measurement phenotypes: bolting score, days to flowering, plant height, stem diameter, and stem strength

Genotype	Bolting score	Days to flowering	Plant height	Stem diameter	Stem strength
PI251246	6.33 a	49.50 g	101.34 c	8.07 f	8790.8 a
11-G99	2.58 c	106.55 f	162.53 a	7.32 f	7471.2 ab
Eruption	1.29 d	149.30 d	52.62 f	12.66 d	3480.2 cd
Da Ye Wo Sun	3.75 bc	132.67 e	115.84 b	21.76 a	3338.4 cde
Salinas	1.00 d	152.33 cd	80.35 d	9.92 e	3013.4 cdef
Reine des Glaces	1.00 d	123.43 e	51.10 f	9.93 e	2654.2 cdef
Brave Heart	1.00 d	n.d	n.d	n.d	n.d
Green Forest	1.21 d	n.d	n.d	n.d	n.d
Hearts Delight	1.13 d	n.d	n.d	n.d	n.d

*n.d, not determined