

**UNITED STATES DEPARTMENT OF AGRICULTURE**  
Agricultural Research Service  
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**NOTICE OF RELEASE OF SIX F6:8 AND ONE F4:5 ICEBERG BREEDING LINES  
OF LETTUCE GERMPLASM WITH RESISTANCE TO BACTERIAL LEAF SPOT  
CAUSED BY *Xanthomonas campestris* pv. *vitians***

The Agricultural Research Service, United States Department of Agriculture announce the release of six F6:8 and one F4:5 iceberg breeding lines of lettuce (*Lactuca sativa* L.) with resistance to bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *vitians*. These breeding lines were selected from the cross (Saladcrisp x Iceberg) x Salinas 88. In replicated field and greenhouse testing, the level of resistance to BLS in these lines was significantly greater than Salinas 88 and equivalent to or better than Saladcrisp and Iceberg. They have partially covered heads with medium-dark-green and crisp textured leaves, leaf margins have variable degrees of leaf margin serration. Maturity is variable among the lines, but all are later than Saladcrisp. The seeds of each line are black in color. All breeding lines are susceptible to Lettuce mosaic virus. These breeding lines are the first western shipping type iceberg lettuces with resistance to BLS. They should be used as parents for further development of BLS resistant cultivars, or selected for adaptation to specific production environments.

**Introduction**

Bacterial leaf spot (BLS) of lettuce, caused by *Xanthomonas campestris* pv. *vitians*, is an important disease of lettuce in California (Barak et al., 2001, Bull and Koike, 2005), and is sporadic but problematic throughout North America (Carisse et al., 2000; Pernezny et al., 1995; Sahin and Miller, 1997; Toussaint, 1999). The pathogen causes small angular leaf spots which are initially water-soaked and later become necrotic (brown to black) and papery. The leaf spots coalesce, forming large necrotic regions (Bull and Koike, 2005). The symptoms reduce the quality and yield of the lettuce and increase the potential for postharvest losses (Carisse et al., 2000). Even in mild infections, outer diseased leaves must be removed during harvest. The resulting smaller heads do not command a premium price in the market place compared to non-diseased plants.

Chemical applications, including those suitable for certified organic production, may reduce BLS and fit into a comprehensive management strategy (Bull and Koike, 2005, Carisse et al., 2000, Pernezny et al., 2002, Sahin and Miller, 1997). However, the effectiveness of the chemicals depends on their application prior to symptom development. Occurrence of the disease is sporadic and unpredictable, and in most seasons these preemptive applications would be unnecessary, serving only to increase production costs. Therefore, host resistance remains one of the most efficient and cost-effective tools to manage BLS of lettuce.

We are reporting the release of BLS resistant F4:5 breeding line RH04-0157-3, and six F6:8 breeding lines selected from RH04-0157-3 for improved horticultural characteristics (RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, and RH07-0387M). All lines were selected from the cross (Saladcrisp x Iceberg) x Salinas 88. The parents were chosen to develop a western shipping type iceberg lettuce with resistance to BLS. The parents Iceberg and Saladcrisp were selected due to putative moderate resistance to BLS. Iceberg is a red-tinged Batavia type heirloom lettuce cultivar; its origins are unknown. Saladcrisp is a small sized early maturing crisphead type lettuce with highly serrated or “frilly” leaf margins. It was developed by the New York State Agricultural Experiment Station, and is not suitable for coastal California production and marketing. Salinas 88 is a western shipping type crisphead lettuce adapted to coastal California. It possesses the mo12 allele for Lettuce mosaic virus (LMV) resistance (Ryder, 1991).

### **Development of Breeding Lines and Disease Resistance Assessment**

In 2001, F2 plants from Iceberg x Saladcrisp were selected for crisphead type horticultural characteristics and reduced disease in a field experiment inoculated with *X. campestris* pv. *vitians*. The resulting F3 families were evaluated for the same characters in an inoculated field experiment, and a disease free plant from F3 family 01-1233 was selected and backcrossed to Salinas 88. Using the pedigree method, families and individual plants were selected from the F2 through the F4 generation for horticultural characteristics similar to Salinas 88 and reduced BLS.

In 2005, 11 F5 breeding lines from (Saladcrisp x Iceberg) x Salinas 88 were evaluated for resistance to BLS and horticultural characteristics in a replicated field experiment. Included in the experiment were the parents Salinas 88, Saladcrisp, and Iceberg, the resistant cultivars Little Gem, Holborn Standard, and Batavia Reine des Glaces, and the susceptible controls Sniper and Vista Verde. The field experiment was planted on August 31 and maintained using standard cultural practices for coastal California lettuce production. *Xanthomonas campestris* pv. *vitians* strains Xav 98-12, BS339, and BS347 were maintained and inoculated onto seedlings using the methods described by Bull et al., 2007. All strains originated from the Salinas Valley of California, as previously reported (Barak and Gilbertson, 2003; Bull and Koike, 2005). Lines were evaluated for disease severity and incidence on November 1, approximately one week before harvest maturity. Disease incidence was calculated as the proportion of diseased plants in each replicate. The disease severity of 10 randomly selected plants was determined by scoring the location of lesions on each plant (0 = no disease, 1 = disease on lower frame leaves, 2 = disease on middle frame leaves, 3 = disease on upper frame leaves, 4 = disease present on top cap leaves) as well as the lesion severity (0 = no disease through 4 = large patches of coalesced lesions). The mean lesion severity and location score was calculated for each treatment replicate, then multiplied together to obtain a 0 through 16 disease severity score. Data were analyzed using Proc Mixed of SAS as a randomized complete block design. All the breeding lines from (Saladcrisp x Iceberg) x Salinas 88 had 100% disease incidence, and were not significantly different from any other check cultivars except Little Gem (5% incidence). Nevertheless, four breeding lines demonstrated resistance to BLS expressed as reduced symptom severity (disease severity ranging from 2.05 to 2.76; Figure 1). These lines had significantly lower disease severity than Salinas 88 (disease severity = 5.75), Sniper (disease severity = 10.54), and Vista Verde (disease severity = 13.28), but were not significant different from Little Gem (disease severity = 0.02), Batavia Reine des Glaces (disease severity = 0.73), Holborn Standard (disease

severity = 1.43), Iceberg (disease severity = 1.60) and Saladcrisp (disease severity = 1.79). Among the BLS resistant lines, the horticultural characteristics of the resistant line RH04-0157-3 were superior to the remaining lines, and included segregants that more closely resembled Salinas 88. Five single plant selections (RH04-0157-3-1 through 5) were made from RH04-0157-3 for plants that most closely resembled Salinas 88. The remaining lines were discarded.

RH04-0157-3 and five F5:6 derivative lines (RH04-0157-3-1 through 5) were evaluated for BLS resistance in a greenhouse experiment with three replications using the methods of Bull et al., (2007). The susceptible control Vista Verde and parents Iceberg, Saladcrisp, and Salinas 88 were included. For each replication, a row of 9 cells was planted to each breeding line or single cultivar. The susceptible cultivar Vista Verde was planted around the borders of each flat to insure uniform microclimatic conditions. Seedlings were germinated in a growth chamber, and were acclimated to the greenhouse for one week prior to inoculation with  $1 \times 10^9$  CFU/ml *X. campestris* pv. *vitians* strains Xav 98-12, BS339, and BS347 mixed in equal proportions. The inoculum was sprayed onto the leaves of three-week-old plants until run-off, with each plant receiving approximately 1ml, using a hand held spray bottle. Plants were incubated in the greenhouse on a misting table for a total of 28 days. The top three leaves of each plant were evaluated for disease severity and incidence 7, 14, 21 and 28 days after the initial inoculation and were inoculated again at 7, 14, and 28 days. A rating of 0 was given for plants with no disease; 1 for plants with few lesions of < 3 mm; 2 for plants with lesions > 3 mm; and 3 for plants with coalesced lesions. The data was analyzed as a randomized complete block using Proc Mixed of SAS. RH04-0157-3 and its five F5:6 derivative lines were resistant to BLS, all having disease severity scores less than one at all four assessment dates (Table 1). By assessment date two, all breeding lines had significantly less disease than Vista Verde, and five breeding lines had significantly less disease than both Vista Verde and Saladcrisp (assessment date two disease severity values: RH04-0157-3 = 0.28, RH04-0157-3-1 = 0.43, RH04-0157-3-2 = 0.68, RH04-0157-3-3 = 0.72, RH04-0157-3-4 = 0.88, RH04-0157-3-5 = 0.63; Iceberg = 0.36, Salinas 88 = 1.06, Vista Verde = 1.53, Saladcrisp = 1.87). Comparisons of disease severity means between Iceberg, Salinas 88, and the breeding lines were not significant. By assessment date four, all breeding lines had significantly less disease than Vista Verde, Saladcrisp, and Salinas 88. Two breeding lines had significantly less disease than Iceberg (assessment date four disease severity values: RH04-0157-3 = 0.68, RH04-0157-3-1 = 0.59, RH04-0157-3-2 = 0.76, RH04-0157-3-3 = 0.82, RH04-0157-3-4 = 0.82, RH04-0157-3-5 = 0.92; Iceberg = 1.47, Saladcrisp = 1.75, Salinas 88 = 1.88, Vista Verde = 2.42). RH04-0157-3 and its sub selections were not significantly different for disease severity, suggesting that RH04-0157-3 is no longer segregating for BLS resistance (data not shown).

#### **Selection within RH04-0157-3 for Horticultural Characters**

While RH04-0157-3 has a high level of resistance to bacterial leaf spot, it is variable for multiple horticultural characteristics. To derive lines that are more uniform for head type, selections were made from lines RH04-0157-3-1, RH04-0157-3-2, and RH04-0157-3-4. Lines RH04-0157-3-3 and RH04-0157-3-5 were discarded due to excessively serrated leaf margins. Subsequent field experiments identified six lines as having the most desirable horticultural characteristics. Seed was increased from these lines, which are being released as RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, and RH07-0387M. We have not directly tested these six breeding lines for resistance to bacterial leaf spot.

### **Morphological Description**

The released breeding lines have partially covered heads with medium-dark-green and crisp textured leaves. All lines have larger heads, less serrated leaf margins, and are later maturing than Saladcrisp. These lines are most similar to Salinas 88. However, they are intentionally variable for head size, leaf serration, and maturity to facilitate selection of BLS resistant iceberg lettuces with adaptation to diverse markets and production conditions.

These lines were tested for resistance to a California isolate of LMV using aphid inoculation of 12 plants per breeding line in a greenhouse experiment; all lines were susceptible to LMV. They have not been characterized for their resistance or susceptibility to any other disease and physiological defects that affect lettuce.

### **Availability**

Limited samples of seed are available for distribution to all interested parties for research purposes, including the development and commercialization of new cultivars. Samples will also be deposited in the National Plant Germplasm System. It is requested that appropriate recognition be made if the breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to Dr. Ryan Hayes, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905.

Table 1 and Figure 2 available upon request

Ryan J. Hayes, Edward J. Ryder, and Carolee T. Bull  
Agricultural Research Service, United States Department of Agriculture, USDA-ARS, Salinas, CA 93905.

### **References**

Barak, J.D., Koike, S.T., and Gilbertson, R.L. 2001. Role of crop debris and weeds in the epidemiology of bacterial leaf spot of lettuce in California. *Plant Disease* 85:169-178.

Barak, J.D., and Gilbertson, R.L. 2003. Genetic diversity of *Xanthomonas campestris* pv. *vitians*, the causal agent of bacterial leaf spot of lettuce. *Phytopathology* 93:596-603.

Bull, C.T., and Koike, S.T. 2005. Evaluating the efficacy of commercial products for management of bacterial leaf spot on lettuce. Online. *Plant Health Progress* doi:10.1094/PHP-2005-1121-01-RS.

Bull, C.T. Goldman, P.H., Hayes, R.J., Madden L.V., Koike, S.T., and Ryder, E.J. 2007. Genetic diversity of lettuce (*Lactuca sativa*) for resistance to bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians*. *Plant Health Progress*. doi:10.1094/PHP-2007-0917-02-RS.

Carisse, O., Ouimet, A., and Toussaint, V. 2000. Evaluation of the effect of seed treatments, bactericides, and cultivars on bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians*. *Plant Disease* 84:295-299.

Pernezny, K., Raid, R.N., Stall, R.E., Hodge, N.C., and Collins, J. 1995. An outbreak of bacterial spot of lettuce in Florida caused by *Xanthomonas campestris* pv. *vitians*. *Plant Disease* 79:359-360.

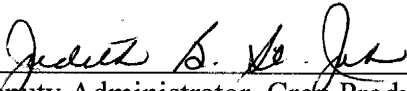
Pernezny, K., Nagata, R., Raid, R.N., Collins, J., and Carroll, A. 2002. Investigation of seed treatments for management of bacterial leaf spot of lettuce. *Plant Disease* 86:151-155.

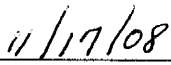
Ryder, E.J. 1991. 'Salinas 88' Lettuce. *Hortscience*. 26:439-440.

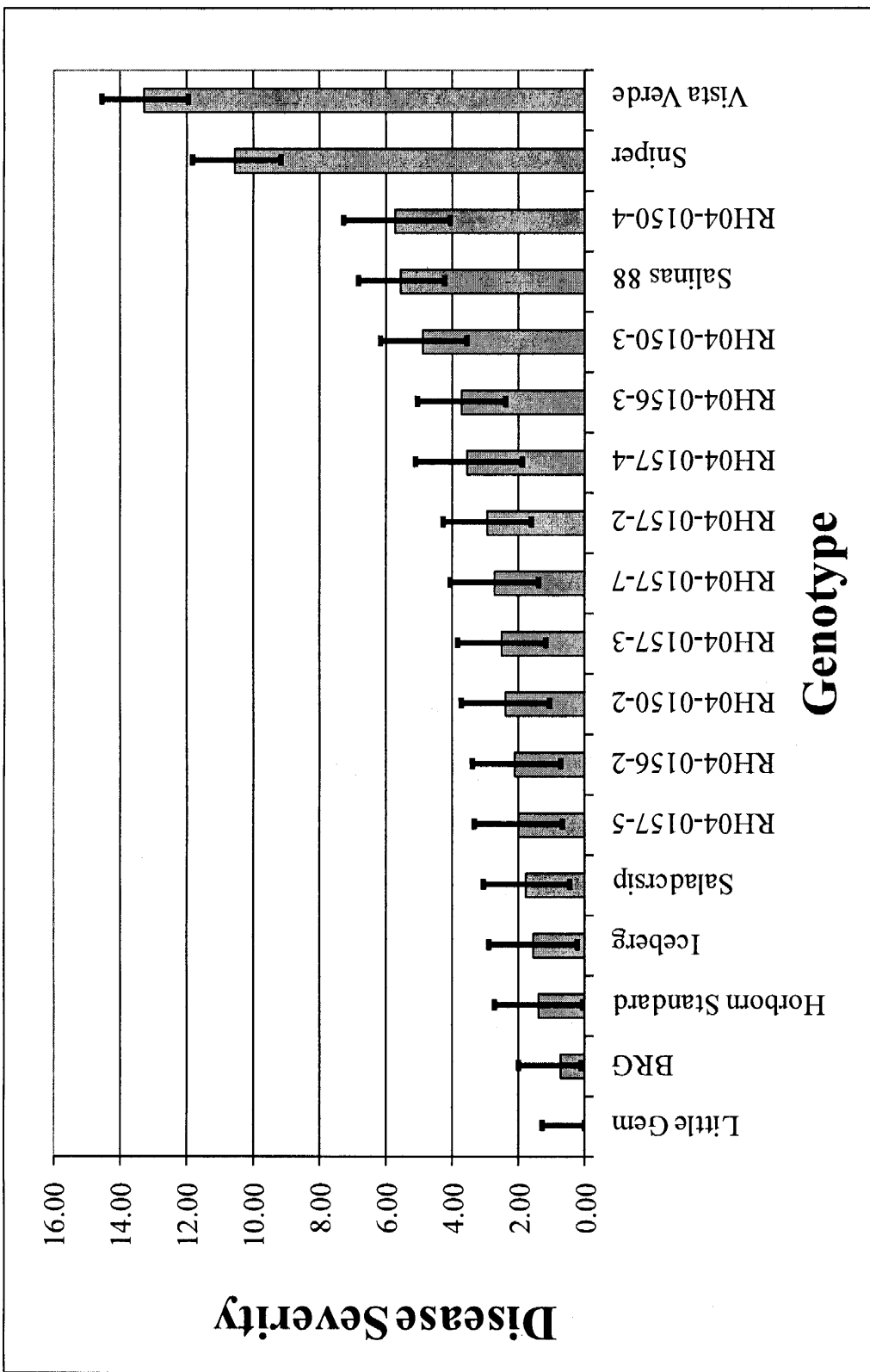
Sahin, F., and Miller, S.A. 1997. Identification of the bacterial leaf spot pathogen of lettuce, *Xanthomonas campestris* pv. *vitians*, in Ohio, and assessment of cultivar resistance and seed treatment. *Plant Disease* 81:1443-1446.

Toussaint, V. 1999. Bacterial leaf spot, a new disease of lettuce in Québec caused by *Xanthomonas campestris* pv. *vitians*. *Phytoprotection* 80:121-125. Signature:

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Deputy Administrator, Crop Production and Protection  
Agricultural Research Service, U.S. Department of Agriculture

  
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**Figure 1.** Mean bacterial leaf spot disease severity in 9 F<sub>4.5</sub> breeding lines from Salinas 88 x (Saladcrisp x Iceberg), the resistant cultivars Little Gem, Batavia Reine des Glaces (BRG), Holborn Standard, and Saladcrisp, and the susceptible parent and controls Salinas 88, Sniper, and Vista Verde in a *Xanthomonas campestris* pv *vitians* inoculated field experiment with three replications. Error bars indicate 95% confidence intervals.

Table 1. Disease severity on six breeding lines from (Saladcrisp x Iceberg) x Salinas 88, Iceberg, Saladcrisp, Salinas 88, and Vista Verde in a greenhouse experiment with three replications over four assessment dates.

Line	Generation		Mean Disease Severity <sup>1</sup>				F <sub>6,8</sub> Lines Released
	Tested	Assessment 1	Assessment 2	Assessment 3	Assessment 4	F <sub>6,8</sub> Lines Released	
RH04-0157-3-1	F <sub>5,6</sub>	0.13	0.43 v,sc <sup>2</sup>	0.28 v.sc,s88	0.59 v.sc,s88,1	RH07-0386M, RH07-0387M	
RH04-0157-3	F <sub>4,5</sub>	0.06	0.28 v,sc	0.36 v,sc,s88	0.68 v,sc,s88,1		
RH04-0157-3-2	F <sub>5,6</sub>	0.93	0.68 v,sc	0.67 v.sc	0.76 v,sc,s88	RH07-0379M, RH07-0380M	
RH04-0157-3-3	F <sub>5,6</sub>	0.26	0.72 v,sc	0.61 v.sc	0.82 v,sc,s88		
RH04-0157-3-4	F <sub>5,6</sub>	0.37	0.88 v	0.74 v	0.82 v,sc,s88	RH07-0373M, RH07-0370M	
RH04-0157-3-5	F <sub>5,6</sub>	0.45	0.63 v,sc	0.76 v	0.92 v,sc,s88		
Iceberg		0.03	0.36	1.01	1.47		
Saladcrisp		0.97	1.87	1.67	1.75		
Salinas 88		0.58	1.06	1.32	1.88		
Vista Verde		0.52	1.53	2.10	2.42		

<sup>1</sup>/ Disease severity rated as 0 = no disease, 1 = few lesions < 3 mm, 2 = lesion > 3 mm, 3 = coalesced lesions.

<sup>2</sup>/ Indicated disease severity mean significantly different from Vista Verde (v), Saladcrisp (sc), Salinas 88 (s88), and Iceberg (I) using a 1-tailed test at  $P < 0.01$ .