Romaine Lettuce Breeding Lines with Resistance to Lettuce Dieback Caused by Tombusviruses

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The disease lettuce dieback was first observed in the mid-1980s, but the causative agent was identified only recently (Liu et al., 1999; Obermeier et al., 2001). Reports of the disease have increased over the last 10 years in the lettuce production areas of both California and Arizona. Together, these regions account for over 95% of the lettuce grown in the United States [U.S. Dept. of Agriculture (USDA), 2002]. Symptoms of lettuce dieback include chlorosis, mottling, and necrosis in older leaves, as well as stunting and death (Obermeier et al., 2001). In severely affected fields, the disease has caused complete crop losses. The most economically significant damage has occurred in romaine lettuce, and no commercial romaine cultivar has been shown to be resistant to the disease. In the United States, romaine lettuce is a rapidly growing market segment, and in 2001 accounted for 19.3% of value of all lettuce produced, up from 11.3% 5 years ago (USDA, 2002). High incidences of disease have also occurred in commercial fields of some red and green leaf lettuce cultivars, however, the disease has not been observed on any modern crisphead (iceberg) cultivars.

Lettuce dieback is caused by at least two members of the soilborne virus family Tombusviridae: lettuce necrotic stunt virus (LNSV) and tomato bushy stunt virus (TBSV) (Obermeier et al., 2001). Neither virus has a known vector, and reliable methods of cultural control have not been identified (Wisler and Duffus, 2000). As a result, genetic resistance remains the most effective way to control the disease. With the increasing frequency of reports of lettuce dieback and the rising demand for romaine lettuce, there is an urgent need for dieback resistance in a romaine type lettuce. In this paper, we describe romaine lettuce germplasm that is resistant to lettuce dieback.

Origin

Cultivars of all major types (romaine, leaf, crisphead, and butter) and several Plant

Introduction (PI) accessions were grown in dieback-affected commercial fields to evaluate reaction to lettuce dieback (Wisler and Duffus, 2000; E.J. Ryder and R. C. Grube, unpublished results). No modern crisphead cultivars developed symptoms. Some of the heirloom crisphead, leaf, and butterhead cultivars tested were resistant, while others were susceptible. A total of 22 romaine cultivars were tested, and all were susceptible. Based on the results of these trials, the heirloom crisphead cultivar Iceberg and the modern crisphead cultivar Salinas were used as susceptible and resistant controls, respectively, in all subsequent trials.

Out of 37 PI accessions with romaine growth habit that were tested, three (PI 491209, PI 491214, and PI 491224) were asymptomatic in all fields in which they were grown. PI 491224 was most similar in appearance to commercial romaine cultivars and was chosen for further evaluation and selection. PI 491224 was free of lettuce dieback symptoms in six trials conducted over 3 years in four commercial growers' fields located in Chualar, King City, Salinas, and Arroyo Grande, Calif. Virus was detected in lettuce plant sap by mechanical inoculation onto indicator plant species, by reverse transcription-polymerase chain reaction (RT-PCR), or by both, using the methods described by Obermeier et al. (2001). The RT-PCR primers were specific for a genomic region that is highly conserved among tombusviruses to permit detection of a wide range of TBSV-related viruses. Strains of TBSV or LNSV were detected in symptomatic plants of susceptible genotypes from each trial.

In 1999, 24 plants of PI 491224 were grown in the greenhouse for seed production, and most developed tipburn and *Botrytis* lesions. Tipburn is a physiological disorder thought to be associated with low calcium levels in foliar tissue. Because tipburn lesions render heads unmarketable, breeding materials are routinely evaluated for susceptibility to this disorder. Two tipburn-free plants were selected and were self-pollinated to produce 17 lines, which were evaluated in field plantings in 2001 in the absence of dieback. Tipburn-free plants were selected from three of these lines, 01-778, 01-781, and 01-789. These lines were chosen on the basis of horticultural characteristics and low incidence of tipburn. Plants with large heads and upright and open growth habit were selected from 01-778 and 01-781, whereas plants with large, closed heads suitable

for romaine hearts were selected from 01-789. In 2002, progeny from these lines were tested for resistance to lettuce dieback in commercial field trials and were evaluated for horticultural traits in a disease-free trial. Selections were made within these progenies based on head type, shape, and absence of tipburn, and seeds from selected plants were combined to generate 01-778M, 01-781M, and 01-789M.

Description

The breeding lines 01-778M, 01-781M, and 01-789M are dark green romaine lettuces with slight blistering. Lines 01-778M and 01-781M have an open growth habit, with erect leaves that do not tend to curve inwards. The line 01-789M has a larger percentage of closed heads, but is otherwise similar in appearance to 01-778M and 01-781M.

Disease resistance. Breeding lines 01-778M, 01-781M, and 01-789M were evaluated for resistance to lettuce dieback in two experiments conducted in Oceano and Chualar, Calif., in 2002. Several commercial romaine cultivars and resistant and susceptible controls were included in both experiments. In each experiment, genotypes were direct-seeded in two replicates of 20 to 60 plants each. In Oceano, plants were seeded on 26 Mar. 2002 in five rows on beds 3.1 m long and 1.8 m wide. In Chualar, plants were seeded on 19 Apr. 2002 in two rows on beds 3.1 m (cultivars) or 4.6 m (breeding lines) long and 1.0 m wide. At harvest maturity (105 d in Oceano, 78 d in Chualar), the number of plants that were stunted and chlorotic or that had necrotic lesions characteristic of lettuce dieback were counted. Disease incidence (DI) was calculated as the percentage of the total number of plants that showed symptoms. A few symptomatic and asymptomatic plants were sampled to confirm presence or absence of tombusviruses, either by mechanical inoculation of sap onto indicator plant species or by RT-PCR.

DI was moderate in both trials, with the susceptible control 'Iceberg' exhibiting mean DI of 52.4% in Oceano and 36.0% in Chualar (Table 1). Commercial cultivars differed in susceptibility to dieback, with 'Heart's Delight' and 'Coastal Star' showing significantly lower DI than 'Green Towers', 'Darkland', and 'Parris Island Cos' in both trials. All romaine cultivars exhibited some symptomatic plants, however, with mean DI ranging from 5.3% to 31.7% in Oceano, and from 2.3% to 32.2% in Chualar. No symptomatic plants were observed among any of the breeding lines or the resistant control 'Salinas'. TBSV-related tombusviruses were detected in symptomatic plants from each trial. Tombusviruses were not detected in asymptomatic plants from either trial.

Morphological characters. The breeding lines were grown alongside the unselected PI 491224 and several commercial cultivars in 2002 for comparison of horticultural characteristics at the USDA/Agricultural Research Service (ARS) Spence Field location in Salinas, Calif. Each genotype was direct-seeded in two rows on a single bed 15.2 m long and 1.0 m wide. Ten representative plants from a single

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Table 1. Incidence of lettuce dieback observed in romaine breeding lines and cultivars evaluated in two infested fields in 2002.

Genotype	Type/description	Oceano, Calif.		Chualar, Calif.	
		Incidence ^a (%)	SD	Incidence (%)	SD
PI 491224	PI accession	0 a	0	0 a	0
01-778M	Breeding line	0 a	0	0 a	0
01-781M	Breeding line	0 a	0	0 a	0
01-789M	Breeding line	0 a	0	0 a	0
Salinas	Iceberg cultivar	0 a	0	0 a	0
Heart's Delight	Romaine cultivar	5.3 a	7.5	2.3 a	3.2
Coastal Star	Romaine cultivar	11.4 a	0.5	8.9 ab	12.6
Clemente	Romaine cultivar	14.7 ab	9.0	NT ^b	NT
Gladiator	Romaine cultivar	13.8 ab	13.4	15.0 bc	9.5
Green Towers	Romaine cultivar	27.3 bc	0	32.2 d	17.3
Darkland	Romaine cultivar	31.7 c	9.5	26.3 cd	15.9
Parris Island Cos	Romaine cultivar	NT	NT	31.8 d	18.0
Iceberg	Batavia cultivar	52.4 d	13.1	36.0 d	25.2

^aMeans within a column followed by the same letter are not significantly different using Duncan's multiple range test at $P = 0.05$.

^bNT = not tested.

replicate of each genotype were evaluated for the following morphological traits at harvest maturity (105 d): 1) plant height, measured on bisected heads from butt to the tip of the longest leaf; 2) total number of leaves ≥ 6.4 cm; 3) core length, measured on bisected head; 4) total head weight; 5) presence of tipburn lesions; and 6) formation of tight hearts.

Plant heights and total head weights of the three breeding lines were not significantly dif-

ferent from those of commercial cultivars tested (Table 2). The breeding lines had significantly more leaves than all cultivars tested. This may be due to earlier maturity. In summer plantings, the breeding lines exhibited some premature bolting (data not shown). In the spring trial in Spence Field, however, measurements of core length were no greater than those of most cultivars (Table 2). Although readily detached, older leaves of the breeding lines did not snap as

crisply from the bases of plants as did those of commercial cultivars. There was no indication that the breeding lines were more susceptible to tipburn than commercial romaine cultivars, though our results are preliminary due to small sample sizes. The breeding lines did not form solid hearts as readily as most commercial cultivars. Some heart formation was observed in line 01-789M, however, suggesting that this line may be useful in the development of cultivars suitable for heart production.

In this germplasm release announcement, we have described romaine lettuce germplasm that is resistant to lettuce dieback, a soilborne disease caused by two or more related tomosviruses. The breeding lines described were completely free of dieback symptoms in fields in which some proportion of all commercial romaine cultivars grown developed symptoms. The mechanisms of resistance, reactions to other diseases, and adaptation and performance in a wide range of commercial production conditions have not yet been ascertained.

Seed availability

Limited samples of seed are available from the authors 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.

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Table 2. Head characteristics of romaine breeding lines and cultivars evaluated at the USDA/ARS Spence Field site in Salinas, Calif., May 2002.

Genotype	Plant height (cm) ^a	No. of leaves ≥ 6.4 cm	Core length (cm)	Head wt (g)	Tipburn (no./10)	Formed hearts (no./10)
PI 491224	26.7 a	41.6 b	6.8 ab	1026 a	2	0
01-778M	30.6 cd	57.1 c	7.8 bc	1331 bc	1	0
01-781M	30.1 b-d	53.4 c	7.9 bc	1238 a-c	1	0
01-789M	32.5 d	53.2 c	8.1 c	1366 bc	3	3
Clemente	27.5 ab	38.4 ab	6.8 ab	1336 bc	2	6
Darkland	30.2 cd	38.9 ab	7.8 bc	1474 c	5	5
Gladiator	28.3 a-c	39.3 ab	7.6 a-c	1263 bc	1	0
Green Towers	28.7 a-c	37.3 ab	6.5 a	1171 ab	3	3
Heart's Delight	31.9 d	38.7 ab	7.8 a-c	1424 bc	5	7
King Henry	32.0 d	38.0 ab	7.3 a-c	1396 bc	4	8
Parris Island Cos	30.4 cd	37.4 ab	7.8 bc	1384 bc	4	7
Valmaine	30.4 cd	36.0 a	7.4 a-c	1427 bc	8	5
cv (%)	8.0	10.8	17.0	19		

^aMean values were obtained by measuring ten representative mature heads from each genotype. Means within a column followed by the same letter are not significantly different using Duncan's multiple range test at $P = 0.05$.