Identification, Distribution, and Testing for Resistance to Rhizomania in *Beta maritima*

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ABSTRACT

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Many plants with a high level of resistance to rhizomania were found in 17 of 63 (27%) accessions of *Beta maritima* tested in either the greenhouse, the field, or both. Resistance to rhizomania was estimated by disease reaction or by enzyme-linked immunosorbent assay (ELISA) values for beet necrotic yellow vein virus (BNYVV) from plants that were grown in infested soil. Some resistant plants grown in the greenhouse and in the field were virus-free, as measured by ELISA. The number of plants within each accession that was free of the virus ranged from a few plants to all plants. Resistant accessions were from Denmark, England, France, and Italy. All plants tested were susceptible to the fungus *Polymyxa betae*, the vector of BNYVV. Successful crosses were made between sugar beet (*B. vulgaris*) and *B. maritima*. Resistance appeared to be dominant because F_1 plants (resistant × susceptible) were all resistant or segregated for resistant plants. A significant correlation ($r = 0.77$) occurred between the mean greenhouse and field ELISA (BNYVV) values from 15 resistant types. Also, significant correlations based on a disease index (DI) were found among three greenhouse tests, between DIs from the greenhouse and field ELISA, and between greenhouse DIs and field root symptoms. Other correlations, greenhouse DIs versus greenhouse ELISA, greenhouse ELISA versus field DIs, and field DIs versus field ELISA, were not significant. These data suggest that plants of *B. maritima* with resistance to rhizomania can be selected either in the greenhouse or the field and that this resistance can be transferred to sugar beet. This is the first detailed report of rhizomania resistance in *B. maritima*.

Additional keyword: breeding

Rhizomania, one of the most serious diseases of sugar beet (*Beta vulgaris* L.), is caused by beet necrotic yellow vein virus (BNYVV), whose vector is *Polymyxa betae* Keskin, a soilborne fungus. The disease symptoms are: constriction of the taproot, proliferation of roots (bearding), tumorlike obtrusions on the taproot, internal necrosis, and an enlarged crown. Foliar symptoms are usually limited to yellowing and an upright posture, but occasionally veinal yellowing and necrosis will occur if the virus becomes systemic. The disease was first reported in the United States in 1983 (4), but was reported previously in many sugar beet production areas of the world (10,12). Crop rotation is not an effective means of control because the viruliferous fungus remains viable in the soil for many years (6,12). Soil fumigants are an effective control for 1 yr, but they are expensive and, therefore, have not been widely accepted (7,9,10,12).

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Development of resistance through breeding efforts has been slow, and only recently have resistant cultivars been used successfully in Europe to control losses from rhizomania. However, these cultivars are not adapted to California (8) because they lack resistance to other diseases and are prone to bolting during extended cold periods. Some evidence suggested that resistance to rhizomania in European cultivars might have originated from cultivars resistant to *Cercospora*, perhaps from a *B. maritima* parent via an interspecific cross (1,3). This observation stimulated an interest in evaluating the *B. maritima* accessions in the *Beta* collection at Salinas, CA. A preliminary report of this research has been published (11).

MATERIALS AND METHODS

Greenhouse tests. Seed of 61 accessions of *B. maritima* (e.g., WB 41, wild beet accession 41) of European origin were germinated in sand and the plants were transplanted at the first true-leaf stage to soil infested with viruliferous *P. betae*. Time of germination was variable; therefore, the age of plants differed among accessions at transplanting. Each plant was placed in a 250-ml Styrofoam cup of infested soil. A small hole in the bottom of each cup allowed for drainage. Three tests were conducted, each arranged in a randomized complete block design with two replications of four plants per replication. The greenhouse was maintained at 26 C or higher, and soils were kept wet by frequent irrigations. In the first test some plants died, so in subsequent tests the soil in the pots was drenched with fenaminosulf at 90 mg a.i./L and pentachloronitrobenzene at 300 mg a.i./L of water immediately after transplanting to control damping-off. Soil for each test was from a sugar beet field in which rhizomania was uniformly distributed. However, the soil was thoroughly mixed before potting. Plants were grown for 2 mo and then evaluated for disease severity using a disease index (DI) scale of 0–4, where 0 = no top symptoms; 1 = leaves upright and yellow; 2 = leaves upright, yellow, and stunted; 3 = many leaves dead; and 4 = plants dead.

Root sap from plants with a zero disease rating were tested for the presence of BNYVV by enzyme-linked immunosorbent assays (ELISA) in the second and third tests. Plants and soil were removed from the cups 2 mo after transplanting. The bottom half of the soil ball of each plant was excised, washed to remove the soil from root tissue, and 0.5 g of roots were blended in 2 ml of phosphate-buffered saline, pH 7.4. ELISA tests were the double-antibody sandwich method described by Clark and Adams (2), except that the coating globulin was used at 1 μg/mg and the enzyme-conjugated (alkaline phosphatase) globulin was used at 1:800. The use of the bottom one-half of the soil ball was nondestructive. Therefore, additional tests could be made by repotting the plant and the remaining soil ball and allowing the plant to regrow.

Four 1-cm-long sections of fibrous root from four greenhouse-grown plants that showed resistance (DI = 0) to rhizomania by the ELISA test were placed on a microscope slide to make a semipermanent mount. These unstained roots were viewed at 100× for the presence of cytosori of *P. betae*.

Field tests. Fifteen accessions of *B. maritima* that showed resistance in the three greenhouse tests and two additional

Table 1. Mean rhizomania disease indices of 61 wild beet (*Beta maritima*) accessions and a sugar beet control when grown in viruliferous fungus-infested soils for 2 mo in the greenhouse

B. maritima accessions	Disease index[x]	
	Mean	Range
280	3.8 a[y]	3–4
278	3.7 ab	2–4
266	3.7 ab	2–4
277	3.7 ab	2–4
281	3.5 abc	3–4
252	3.5 abc	1–4
283	3.5 abc	2–4
245	3.4 abcd	1–4
65	3.3 abcd	1–4
267	3.3 abcd	2–4
303	3.3 abcd	2–4
68	3.3 abcd	1–4
304	3.2 abcd	2–4
244	3.1 abcde	1–4
309	3.1 abcde	1–4
253	3.0 abcdef	1–4
268	3.0 abcdef	1–4
255	2.8 abcdefg	1–4
284	2.8 abcdefg	1–4
254	2.8 abcdefg	1–4
256	2.8 abcdefg	1–4
270	2.7 abcdefgh	0–4
311	2.7 abcdefgh	1–4
242	2.5 abcdefghi	1–4
243	2.5 abcdefghi	1–4
251	2.3 bcdefghij	1–4
257	2.3 bcdefghij	0–4
282	2.3 bcdefghij	0–4
71	2.3 bcdefghij	1–4
70	2.3 bcdefghij	1–4
306	2.3 bcdefghij	1–4
97	2.3 bcdefghij	1–4
69	2.1 cdefghijkl	0–4
172d	2.1 cdefghijkl	0–4
250	2.0 defghijklmn	0–4
172a	2.0 defghijklmn	0–4
173	2.0 defghijklmn	1–4
185	2.0 defghijklmn	0–4
66	1.8 efghijklmno	0–4
67	1.8 efghijklmno	0–4
73	1.5 fghijklmnop	0–3
275	1.5 fghijklmnop	0–4
169	1.5 fghijklmnop	0–4
191	1.3 hijklmnop	0–4
182	1.3 hijklmnop	0–4
181	1.3 hijklmnop	0–4
179	1.3 hijklmnop	0–4
310	1.1 ijklmnop	0–4
180	1.0 jklmnop	0–4
177	1.0 jklmnop	0–4
187	0.8 klmnop	0–3
184	0.8 klmnop	0–4
258	0.8 klmnop	0–4
190	0.7 mnop	0–4
319	0.6 nop	0–4
249	0.5 op	0–4
178	0.5 op	0–4
42	0.5 op	0–4
318	0.4 op	0–4
151	0.3 p	0–4
41	0.2 p	0–1
8717 (check)	1.210	1–4

[x] Disease index on a scale of 0–4, where 0 = no top symptoms; 1 = leaves upright and yellow; 2 = leaves upright, yellow, and stunted; 3 = many leaves dead; and 4 = plants dead.

[y] Numbers followed by the same letter were not significantly different ($P = 0.05$) according to Duncan's multiple range test. Three tests each with eight plants per accession.

[z] Inoculated beet control.

accessions (WB 51 and WB 52) were evaluated in the infested field from which the inoculum was obtained for the greenhouse tests. Two replications, 6.1 m long, of each accession were planted, and plants were thinned to a 20-cm spacing 1 mo after planting. Normal cultural practices for beet production were used. Ten plants from each replication were harvested, rated for disease, and tested for BNYVV by ELISA. *B. maritima* roots were scored either plus or minus for the proliferated root symptom of rhizomania because of the fibrous root system of *B. maritima*.

Plants from each *B. maritima* accession free of the virus (equal to or less than the mean of healthy plant juice), as determined by ELISA, were vernalized to initiate seed stalks. Flowering plants were crossed with two sugar beet lines, one susceptible and self-sterile line (Y941) and the other susceptible and self-fertile (C719). A flowering stalk from one plant each of sugar beet and *B. maritima* was encased in a white paper bag for pollination. In the case of the self-fertile sugar beet, the plant of *B. maritima* was encased in a nylon mesh bag to facilitate cross-pollination but isolate the selfed sugar beet seed produced. Plants from seed of these crosses were grown in infested soil in the greenhouse and tested by ELISA as described above.

RESULTS

Greenhouse tests. Means for DI (on a scale of 0–4) for the accessions of *B. maritima* ranged from 0.2 to 3.8, with significant differences among accessions (Table 1). There also was a significant difference among the three tests, with the highest DI from the undrenched test (test 1). Means were 2.32, 2.08, and 1.97 for tests 1, 2, and 3, respectively (LSD = 0.26, $P = 0.05$). Correlations between DIs for the first, second, and third tests were significant at $r = 0.69$, 0.65, and 0.70, respectively. Cultivar means and ranges for ELISA readings of a selected group of *B. maritima* accessions from the second and third tests in the greenhouse are shown in Table 2.

Field tests. Mean ELISA readings from root samples of *B. vulgaris* and *B. maritima* grown in the field in rhizomania-infested soil were significantly different, with the sugar beet cultivar US H11 the most susceptible (Table 3). The ELISA readings for entries ranged from 0.0 to 1.5. Mean readings ranged from 0.011 to 0.693. ELISA values for roots from healthy sugar beet controls varied from 0.002 to 0.047 (mean 0.010, $N = 18$).

Correlations among tests. A significant correlation ($r = 0.77$) between the field and greenhouse ELISA evaluations for the 15 accessions common to both tests was obtained. Significant correlations also were found between greenhouse DIs and field ELISA ($r = 0.53$) and greenhouse DIs and field evaluations (percentage with symptoms, $r = 0.50$). Greenhouse DIs versus greenhouse ELISA, greenhouse ELISA versus field DIs, and field DIs versus field ELISA were not significantly correlated. All of the *B. maritima* accessions in the rhizomania infection tests examined for *P. betae* were found to be infected with this fungus. The resistant populations of *B. maritima* were collected from Denmark, England, France, and Italy (Table 3).

Crosses between selected resistant *B. maritima* and sugar beet were fruitful and transmitted rhizomania resistance to the F_1 offspring. The F_1 were either nearly all

Table 2. Enzyme-linked immunosorbent assay (ELISA) means and ranges for 15 wild beet (*Beta maritima*) accessions and the number of plants tested when grown in viruliferous fungus-infested soil in two greenhouse tests

B. maritima accessions	ELISA (A_{405nm})		Plants tested (no.)[z]
	Mean	Range	
41	0.162	0.001–0.973	15
42	0.030	0.001–0.166	14
151	0.013	0.002–0.041	15
169	0.195	0.038–0.717	8
177	0.124	0.005–0.473	11
179	0.248	0.000–0.624	9
180	0.317	0.017–0.613	13
184	0.534	0.034–1.999	11
187	0.249	0.002–1.548	13
190	0.474	0.010–0.271	8
191	0.012	0.000–0.030	15
249	0.254	0.036–1.999	15
258	0.592	0.065–1.999	13
318	0.564	0.099–1.999	12
319	0.610	0.062–1.999	11
8717 (sugar beet check)	0.413	0.082–1.369	13
Uninoculated (sugar beet check)	0.031	0.001–0.065	9

[z] Number of *B. maritima* tested is variable because some died and others were observed as susceptible based on symptoms and were not tested.

resistant or had about one-half resistant plants (Table 4).

DISCUSSION

A preliminary report was previously published (11), but these are the first extensive experimental results that show the occurrence of rhizomania resistance in *B. maritima*. Seventeen of 63 accessions tested were found to have virus-free plants (plants equal to or less than the mean ELISA for uninoculated plants), as determined by ELISA. Fujisawa and Sugimoto (5) reported differences to infection by *P. betae* in *B. maritima*, but not to the virus.

It is of interest that resistance was found to be widespread in *B. maritima* in accessions from Europe (Denmark, England, France, and Italy). Perhaps a common ancestral source or common selection pressure existed during the evolution of this resistance.

The greenhouse evaluation of plants based on symptoms provides a simple method of screening genotypes for resistance. Therefore, ELISA testing is needed only where resistance is observed.

Because the field and greenhouse methods of identification of resistance are nondestructive, several tests can be made on each plant, or individual plants can be selected and used for breeding purposes. By the examination of a fibrous root sample from each *P. betae* root system the reliability of the test is increased and escapes can be identified.

The segregating F_1 distribution suggests that the factor (5) for resistance is dominant and simply inherited, as determined by ELISA. Although uninoculated and inoculated checks were included in each ELISA test, it was not easy to discriminate among plants with ELISA values slightly greater than the mean of healthy plants and those with ELISA values equal to or less than the mean of healthy plants. Therefore, to reduce the possibility of accepting a susceptible plant as resistant, both inoculated and uninoculated plants should be used in all tests as controls in ELISA evaluations. Additionally, plants with questionable readings (i.e., greater than the mean of healthy plants) can be repotted, allowed to grow for 6–8 wk, and retested.

The data on F_1 families for sugar beet × *B. maritima* crosses suggested that resistance was dominant and simply inherited. However, more recent observations suggest that it may not be simply inherited because F_2 populations do not fit expected ratios (*unpublished data*).

The reason some correlations were not significant could be attributed to the fact that differences between tolerance to the disease and resistance based on symptoms are confounded. Thus, some plants appear healthy and free of the virus while others appear healthy but do not have a reduced virus content (perhaps tolerant).

Table 3. Enzyme-linked immunosorbent assay (ELISA) means and ranges and the percentage of plants with symptoms for 17 field-grown wild beet (*Beta maritima*) accessions to evaluate rhizomania resistance

B. maritima accessions	Place of origin	ELISA (A_{405nm}) Mean[x]	ELISA (A_{405nm}) Range	Symptoms (%)[y]
US H11[z] (check)	United States	0.693 a	0.108–1.509	100
319	France	0.590 ab	0.011–1.406	25
184	England	0.531 ab	0.058–1.165	65
180	Denmark	0.450 ab	0.003–1.476	60
179	England	0.443 abc	0.010–1.524	45
318	France	0.423 abc	0.009–1.261	40
187	England	0.403 abc	0.004–1.329	65
258	Italy	0.355 abc	0.008–1.398	30
169	Italy	0.267 abc	0.017–0.861	95
41	Denmark	0.228 bc	0.002–1.405	50
177	Denmark	0.222 bc	0.013–1.368	40
249	France	0.206 bc	0.000–1.020	0
190	England	0.147 bc	0.006–0.874	65
42	Denmark	0.147 bc	0.001–1.124	40
52	Denmark	0.078 c	0.000–0.938	30
51	Denmark	0.060 c	0.001–0.717	0
151	Denmark	0.015 c	0.002–0.044	35
191	Denmark	0.011 c	0.001–0.022	25
US H11 (uninoculated)		0.010	0.002–0.047 ($N = 18$)	

[x] Numbers ($N = 20$) followed by the same letter were not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Percentage of fibrous roots that showed adventitious roots.
[z] Commercial sugar beet.

Table 4. Enzyme-linked immunosorbent assay (ELISA) means and ranges and the percentage of plants resistant to rhizomania for F_1 hybrids between *Beta vulgaris* and *B. maritima*

B. vulgaris × accession[y]	ELISA (A_{405nm}) Mean	ELISA (A_{405nm}) Range	Plants tested (no.)	Resistant (%)[z]
Y941 × 41-4	0.135	0.004–0.704	36	55
Y941 × 42-8	0.111	0.026–0.429	33	28
Y941 × 151-6	0.005	0.000–0.013	18	100
Y941 × 151-7	0.028	0.002–0.097	51	96
Y941 × 151-8	0.006	0.000–0.020	62	100
Y941 × 169-3	0.009	0.000–0.052	21	95
Y941 × 190-4	0.061	0.008–0.287	34	56
Y941 × 258-1	0.005	0.000–0.011	28	100
Y941 × 318-6	0.080	0.002–0.456	25	48
Y941 (uninoculated check)	0.016	0.000–0.036	12	
C36 (uninoculated check)	0.036	0.015–0.072	15	
Y941 (inoculated check)	0.372	0.085–0.858	12	

[y] Crosses between *B. vulgaris* and an individual selected plant resistant to *B. maritima* (e.g., 41-4 = accession 41, plant 4).
[z] Percentage of plants less than or equal to the ELISA mean absorbance values of the uninoculated sugar beet cultivars Y941 and C36.

The incorporation of resistance from *B. maritima* into sugar beet should provide additional sources of resistance (8). However, to confirm that these sources are different, detailed genetic studies will be essential. Other approaches to disease control would be to seek resistance to the vector (5) and to both the fungus and the virus.

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