

Registration of FC1740 and FC1741 Multigerm, Rhizomania-Resistant Sugar Beet Germplasm with Resistance to Multiple Diseases

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Abstract

FC1740 (Reg No. GP-293, PI 681717) and FC1741 (Reg No. GP-294, PI 681718) sugar beet germplasm (*Beta vulgaris* L.) were developed by the USDA-ARS at Fort Collins, CO, Salinas, CA, and Kimberly, ID, in cooperation with the Beet Sugar Development Foundation, Denver, CO. These germplasm are diploid, multigerm sugar beet populations in normal cytoplasm, segregating for self-sterility (*Sf:S⁵S⁵*), genetic male sterility (*A:aa*), and hypocotyl color (*R:rr*). FC1740 and FC1741 have excellent resistance to rhizomania (*Beet necrotic yellow vein virus*). FC1740 was selected as homozygous resistant to markers linked to both *Rz1* and *Rz2* genes for rhizomania resistance. FC1741 was selected as homozygous to the marker linked to the *Rz2* gene for resistance. Both germplasm also have resistance to beet curly top (*Beet curly top virus*) and Fusarium yellows (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *betae* (D. Stewart) W. C. Snyder & H. N. Hans. and other *Fusarium* spp.), as well as moderate resistance to *Aphanomyces* root rot (*Aphanomyces cochlioides* Drechs.), Rhizoctonia crown and root rot (*Rhizoctonia solani* Kuhn.) or sugar beet root aphid (*Pemphigus* spp.). These germplasm provide sources from which to select disease-resistant, multigerm pollinator parents with either or both of the *Rz1* and *Rz2* sources of rhizomania resistance. Because they are from the same population, they also are useful as controls of known genetic background in comparing entries screened for rhizomania resistance conditioned by *Rz1* or *Rz2*.

RHIZOMANIA is now in every major production area of the United States and is present in most sugar beet (*Beta vulgaris* subsp. *vulgaris*, L.) growing areas worldwide (Pavli et al., 2011). Rhizomania can cause major losses in root yield, concentration of sucrose in the root, and juice quality (which measures interference with extraction of sucrose) and may reduce storability (Campbell et al., 2008; Strausbaugh et al., 2008a; Wintermantel, 2009; Biancardi and Lewellen, 2016). *Beet necrotic yellow vein virus* (BNYVV) is vectored by the plasmodiophorid *Polymxa betae* Keskin, which is ubiquitous in soils (Rush, 2003). Because the resting spores of the vector are long lasting in the soil, the only reliable methods for pathogen control are soil fumigation, biotechnology-based resistance, and natural genetic plant resistance to the virus. Soil fumigation is expensive and is being phased out because of environmental concerns, and biotechnology also is expensive to develop and is not accepted universally. Therefore, genetic resistance remains the most realistic method of disease management, and development of resistant commercial hybrids is the preferred method by which this can be achieved (Pavli et al., 2011; Panella and Biancardi, 2016). Determination of rhizomania resistance is generally confirmed by a combination of disease severity in the field on the basis of a widely established scale recognized by the sugar beet industry (Wisler et al., 1999; Richardson, 2012) and by determination of virus titer in sugar beet plants. The latter is usually performed with an enzyme-linked immunosorbent assay (ELISA) using an antiserum specific to BNYVV, a highly reliable and cost-effective method (Wisler et al., 1999), but it can also be determined using reverse transcription polymerase chain reaction (PCR) or reverse transcription, real-time quantitative PCR if necessary (Morris et al., 2001; Webb et al., 2015).

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Abbreviations: BCT, beet curly top; BNYVV, *Beet necrotic yellow vein virus*; BSDF, Beet Sugar Development Foundation; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RB-BNYVV, resistance (*Rz1*-mediated) breaking strains of *Beet necrotic yellow vein virus*; SNP, single-nucleotide polymorphism.

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Journal of Plant Registrations 12:257–263 (2018).

doi:10.3198/jpr2017.07.0042crg

Received 5 July 2017.

Accepted 4 Dec. 2017.

Registration by CSSA.

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A single dominant gene for resistance, initially called the “Holly” gene, was found at Tracy, CA, in 1983, whereas concurrently in Europe, a resistant source incorporated originally into the hybrid ‘Rizor’ (developed by SES vanderHave) was used (De Biaggi, 1987; Lewellen et al., 1987; Biancardi et al., 2002). These sources of resistance, which confer strong resistance to rhizomania, have been shown to be the same gene (Stevanato et al., 2015), named *Rz1*, (Lewellen, 1988), and this is the only major-gene resistance that has been discovered within the commercial sugar beet germplasm (Scholten and Lange, 2000; Biancardi et al., 2002; Panella and Biancardi, 2016). FC1740 (Reg No. GP-293, PI 681717) and FC1741 (Reg No. GP-294, PI 681718) contain resistance conditioned by *Rz1* and *Rz2* or just *Rz2*, respectively.

Because single dominant resistance genes often are overcome by mutations in the pathogen, other genetic resources were screened, especially sea beet (*Beta vulgaris* subsp. *maritima*), which is easily crossed with cultivated beet (Panella and Lewellen, 2007; Biancardi et al., 2012a). In the United States, this major emphasis on discovering sources of resistance took place at the ARS station in Salinas, CA (Whitney, 1989; Lewellen, 1997; reviewed by Panella and Lewellen, 2007). The release of 11 sources of resistant sugar beet germplasm, many from sea beet backcrossed to C37, provided the tools that geneticists and breeders needed to begin to understand the genetic control of resistance (Lewellen et al., 1985; Lewellen, 1997). Among the most promising sources of resistance were WB 41 and WB 42 (PI 546385), sea beet collections from Denmark (Lewellen, 1991; Amiri et al., 2003; Capistrano-Gossmann et al., 2017). The source of resistance advanced from WB 42, which sometimes exhibited a higher level of rhizomania resistance than *Rz1*, was called *Rz2* and was located on chromosome 3, 20 to 35 cM from *Rz1* (Scholten et al., 1996; Scholten et al., 1999). It was hoped that because *Rz1* and *Rz2* were at different loci, there would be increased resistance when they were combined, and this was observed in independent studies (Amiri et al., 2003; Gidner et al., 2005; Pavli et al., 2011; Panella and Biancardi, 2016).

Five rhizomania-resistant, single genes from *B. vulgaris* subsp. *maritima* have been described, but only two of these are well supported genetically, *Rz1* and *Rz2*. The bulk of populations carrying known single gene sources from *B. vulgaris* subsp. *maritima* contain *Rz1*, *Rz2*, or both (Biancardi et al., 2002; Panella and Lewellen, 2007; Biancardi et al., 2012b). Another single-gene resistance locus, *Rz3*, maps to chromosome 3 and is linked to *Rz1* and *Rz2* (Gidner et al., 2005). *Rz3* was discovered in *B. vulgaris* subsp. *maritima* accession WB 41 from Denmark. Although the rhizomania resistance response varied when evaluated in a genetic background as a heterozygote, plants with *Rz1* and either *Rz2* or *Rz3* combined (as heterozygotes) exhibited lower virus titer than *Rz1* alone (Gidner et al., 2005). Grimmer et al. (2007) identified a major QTL, which they called *Rz4*, from germplasm R36 (used as a parent in C79-8) (Lewellen, 1997), which is a composite population of many *B. vulgaris* subsp. *maritima* accessions. *Rz4* also maps to chromosome 3; however, it appears distinct from the loci of *Rz1*, *Rz2*, or *Rz3* (Grimmer et al., 2007). C79-11 formed the basis of a mapping population to discover another potential resistance gene, *Rz5*, which also was identified by Grimmer and colleagues (Lewellen, 1997; Grimmer et al., 2008). Both *Rz4* and *Rz5* are located on a chromosome 3 near *Rz1*, suggesting that these genes may be allelic.

Methods

FC1740 and FC1741 Population Development

The population from which these germplasm were selected began as a polycross in the field. Forty-eight plants each of C79-1, C79-2 ... C79-11 (PI 593660–593670, which were predominately red hypocotyl) were planted in a field plot with ‘C37’ (PI 590715), which is homozygous for green hypocotyl (Lewellen et al., 1985; Lewellen, 1997). C37 was released as a parental line because of its adaptation to western US growing conditions (Lewellen et al., 1985). It is also rhizomania susceptible and self-sterile (Lewellen et al., 1985). The 11 germplasm in the C79 series represent different sources of resistance to rhizomania and were backcrossed into C37 one to six times, depending on the source (Lewellen, 1997). C37 provided a needed common genetic background for comparison of these sources in a common recurrent sugar beet parent, especially necessary for those sources that came from sea beet (*B. vulgaris* subsp. *maritima*). Seed was harvested from the C37 plants, and seed from the C79-*x* populations was harvested in bulk.

Nomenclature becomes confusing, because the notation used at Salinas uses just the year of the decade as a prefix (e.g., 540 could be a 1995 or 2005 seed production); therefore, we have added the year in brackets ([199]540, etc.). Of the seed from C37, 79 sugar beet roots were selected for resistance to rhizomania, percentage sucrose, root yield (weight), and red hypocotyl (indicating that they were hybrids), and the seed produced was designated as R[199]440-1. Seed of R[199]440-1 was planted, and individual roots were selected for percentage sucrose, yield, and resistance to rhizomania, with seed designated as R[199]540-1. The seed from the C79 populations was used to produce plants that were tested for resistance to rhizomania, harvested, and designated as R[199]40. Plants of R[199]40 were grown, roots were tested for resistance to rhizomania, and the 20 harvested plants were designated as R[199]540. R[199]40 plants were also grown and crossed to C37, roots were tested for resistance to rhizomania, and the plants were designated as R[199]551. Roots from these three populations were combined (R[199]540-1 [78 mother roots], R[199]540 [20 mother roots], and R[199]551 [46 mother roots]), grown, selected for percentage sucrose, yield, and resistance to rhizomania, and harvested for seed to form population R[199]740. This population was planted, and individual roots were selected for percentage sucrose, yield, and resistance to rhizomania, with seed designated as R[199]940. This seed remained in storage until a strain of BNYVV that had mutated to overcome the *Rz1* source of resistance was found (Liu et al., 2005).

The *Rz1* single-gene resistance was the major source of resistance to rhizomania and was deployed worldwide; therefore, the breeding program in Salinas began working with R[199]940 once more. Seed of R[199]940 was planted, individual roots were selected for percentage sucrose, yield, and resistance to rhizomania—both to the common strain (BNYVV) and the resistance-breaking strain (RB-BNYVV)—and harvested seed was designated as R[200]540. Another round of selection for percentage sucrose, yield, and resistance to rhizomania (BNYVV and RB-BNYVV) produced R[200]740. The smallest population size during the selection cycles was 20 mother roots. When R.T. Lewellen retired in 2008,

R[200]740 was shared with other ARS sugar beet breeders. In Fort Collins, CO, researchers noted its excellent performance under severe rhizomania disease pressure, and seed was increased.

Marker-Assisted Selection

There are two dominant, single-gene sources of resistance to rhizomania that are currently deployed in commercial sugar beet hybrids to manage rhizomania in the field—*Rz1* and *Rz2* (Biancardi et al., 2002; Panella and Biancardi, 2016). In locations where rhizomania has been able to overcome the resistance of *Rz1* to provide sugar beet with resistance to RB-BNYVV strains, *Rz1* and *Rz2* are combined in commercial hybrids. Single-nucleotide polymorphism (SNP) markers linked to *Rz1* and *Rz2* (Stevanato et al., 2012, 2014; Panella et al., 2015a) were used to select individuals. Seed of an increase of R[200]740—20101079—was planted, and using markers linked to *Rz1* and *Rz2*, the genotype of 570 plants was inferred. FC1740 was based on 210 vegetative plants selected that had homozygous-resistant *Rz1* and *Rz2* SNP markers (inferred genotype: *Rz1Rz1Rz2Rz2*), of which 54 plants flowered after vernalization to produce seed designated as 20131001HO. FC1741 was selected from the same population using *Rz1* and *Rz2* SNP markers homozygous resistant for *Rz2* and homozygous susceptible for *rz1* (inferred genotype: *rz1rz1Rz2Rz2*) and designated 20131002HO (193 roots selected, of which 71 flowered). Male sterile plants of 20131001HO (FC1740) were increased without selection as 20141006 (129 mother roots in the greenhouse) and 20141007 (350 mother roots in a field isolation plot). Male sterile plants (insuring cross pollination) of 20131002HO (FC1741) were increased without selection as 20141008 (126 mother roots in the greenhouse) and 20141009 (392 mother roots in a field isolation plot). Seed for release was obtained from seed productions 20141007 and 20141009.

Data Analyses

Data were analyzed in SAS using the mixed model procedure (Proc Mixed), and Dunnett's one-tailed *t* test was used to compare each entry with the resistant control and the most susceptible entry (LSD, $\alpha = 0.05$). In the curly top evaluation nursery, a randomized complete blocked design with three replicates was used for the experimental design. The plots were visually evaluated and rated on a disease index scale of 0 (no symptoms) to 9 (dead). In the Fusarium evaluation, a randomized complete blocked design with three replicates was used for the experimental design. Reaction to Fusarium was scored on the basis of stand persistence and foliar yellowing of plots. The disease rating used to evaluate the lines was based on a visual rating from 1 (completely healthy) to 9 (all dead or missing) (P. O'Boyle, Betaseed, personal communication, 2015). In the Aphanomyces evaluation nursery, a disease index based on a visual 1 to 9 rating scale of stand persistence and plant health was used to evaluate Aphanomyces root rot (*Aphanomyces cochlioides* Drechs.) damage. A rating of 1 is a complete stand of healthy beets. A rating of 9 has no surviving plants. A randomized complete blocked design with three replicates was the experimental design. Foliar ratings were taken one to three times during the growing season, and root symptoms were evaluated in late August (P. O'Boyle, personal communication, 2015).

In the rhizomania evaluation nursery, the foliar rating was the percentage of susceptible plants with foliar yellowing symptoms, and this rating was performed on 13 and 20 July in 2013 and 2015, respectively. This is the preferred rating for comparing performance of entries. Root rating is a disease severity rating in which 10 roots per plot were evaluated using a scale of 0 to 9 (0 = healthy, and 9 = dead) and an index value for each plot established using the following formula: $[(0A + 1B + 2C + 3D + 4E + 5F + 6G + 7H + 8I + 9J)/90]100$ where *A* through *J* are the number of plants in categories 0 to 9, respectively. Data were analyzed in SAS using the general linear model procedure (Proc GLM), and Fisher's protected LSD ($\alpha = 0.05$) was used for mean comparisons.

FC1740 and FC1741 were tested for resistance to *Cercospora* leaf spot (caused by *Cercospora beticola* Sacc.) in artificial epiphytotics at the ARS-Beet Sugar Development Foundation (BSDF) joint nursery at the Saginaw Valley Bean and Beet Research Farm in Michigan, and at the Betaseed nursery at Rosemount or Randolph, MN (as previously described in Panella et al., 2008). Both germplasm also were tested for resistance to Rhizoctonia crown and root rot (caused by *Rhizoctonia solani* Kühn) at Fort Collins in 2013, 2014, and 2015, as previously described (Panella et al., 2008).

Characteristics

Agronomic and Morphological Description

FC1740 has a fertile cytoplasm, is predominately multigerm (>95%), and is segregating for genetic male sterility (*aa*) and self-sterility (*S^f:S^s*) introduced through the Salinas parent germplasm. Self-fertility (*S^f*) and genetic male sterility (*aa*) are used in sugar beet prebreeding programs, which gives the breeders the ability to test selfed progeny families and develop inbred lines, while allowing the possibility of random mating (Panella et al., 2008). FC1740 (20141006) was 22% male sterile, and 20141007 had a 100-seed weight of 0.62 g. FC1741 has a fertile cytoplasm, is predominately multigerm (>95%), and is segregated for genetic male sterility (*aa*) and self-sterility (*S^f*) because it was introduced through the Salinas parent germplasm. FC1741 (20141008) was 75% male sterile, and 20141009 had a 100-seed weight of 0.66 g. FC1740 (20141007) segregated for hypocotyl color (59% red), as did FC1741 (20141009), which had 50% red hypocotyls.

Disease Resistance

Beet Curly Top

Beet curly top (BCT) disease is caused by *Beet curly top virus*, which is transmitted by the beet leafhopper (*Circulifer tenellus* Baker) (Bennett, 1971; Strausbaugh et al., 2008b). Although there was no selection for resistance to BCT during the development of these germplasm, both showed excellent resistance to this pathogen. C37 traces back to US22/3, so at least some of the BCT resistance likely came from US22/3 through C37. C37 tested moderately resistant to BCT (Lewellen, unpublished data, 2006), but the level of resistance to BCT in these germplasm seems higher than C37 alone would have imparted (Lewellen et al., 1985). Because the C79 population (R40) carrying the rhizomania sources was developed from many *B. vulgaris* subsp. *maritima* accessions (Lewellen, 1997), it is possible that this source added to the level of BCT resistance seen in these germplasm.

FC1740 and FC1741 were tested at the joint ARS-BSDF curly top nursery at Kimberly, ID, in 2013, 2014, 2015, and 2016 (Table 1), as previously described (Panella et al., 2008; Panella and Strausbaugh, 2014, 2015, 2016). The most important rating is the final rating, in which the disease expression is at its peak (Mumford, 1974). FC1741 was never significantly different from the most resistant control (Table 1). FC1740 was not significantly different from the most resistant control in 2013 and 2014 (Table 1). In 2015 and 2016, it was significantly less resistant than the most resistant control, but also more resistant than the most susceptible line in the test (Table 1). Both germplasm show very good resistance to BCT.

Fusarium Yellows

Fusarium yellows [caused by *Fusarium oxysporum* f. sp. *betae* (D. Stewart) W.C. Snyder and H.N. Hansen] is common throughout the sugar beet growing areas in the United States (Hill et al., 2011). A Fusarium screening nursery was planted by Betaseed in Glyndon, MN, in 2015 and 2016. Scores are from the last, most severe evaluation of the nursery (Table 2). There were significant differences among entries. FC1740 and FC1741 were never significantly different from the most resistant

control (Table 2). Both germplasm showed very good resistance to Fusarium yellows.

Aphanomyces Root Rot

FC1740 and FC1741 were evaluated for resistance to Aphanomyces root rot in a field nursery in Shakopee, MN, by Betaseed (Panella et al., 2008).

In both years that the germplasm were evaluated, 2015 and 2016, they scored moderately susceptible when foliar ratings were compared (Table 2). FC1740 and FC1741 were significantly more susceptible than the resistant control and significantly more resistant than the susceptible control. This demonstrated their intermediate response to Aphanomyces root rot and that these germplasm may have potential for selection of a higher level of resistance to *A. cochlidioides*.

Rhizomania

There are two major dominant resistance genes, *Rz1* and *Rz2*, incorporated into commercial sugar beet germplasm for management of rhizomania disease caused by BNYVV (Biancardi et al., 2010; De Biaggi et al., 2011). In some locations, rhizomania has been able to overcome the resistance of *Rz1* (RB-BNYVV), which was the first resistance gene deployed (Biancardi et al., 2002). In

Table 1. Reaction of germplasm lines in the beet curly top (BCT) field nursery in Kimberly, ID. FC1741 was not different from the most resistant control in all 4 yr. In 2013 and 2014, FC1740 was not significantly different from the most resistant control; however, FC1740's performance was intermediate in 2015 and 2016, significantly different from the most resistant control, but also more resistant than the most susceptible control.

Entry	BCT rating			
	2016	2015	2014	2013
	0-9†			
Beta G6040 (resistant control)	3.6	4.1	4.7	4.2
HM PM90 (resistant control)	3.7	4.0	4.1	4.4
20141009 (FC1741)	4.4	5.3	–	–
20141009 (FC1741)	4.4	–	–	–
20131002pfHO (FC1741)	–	–	5.1	4.3
20141007 (FC1740)	5.1	5.7	–	–
20141007 (FC1740)	5.2	–	–	–
20131001pfHO (FC1740)	–	–	5.0	4.3
SV2012RR (susceptible control)	6.5	7.4	7.2	–
Monohikari (susceptible control)	–	–	7.0	6.3
20131010H11 (most susceptible entry)	7.1	–	–	–
20121034 (most susceptible entry)	–	7.9	7.9	–
20111009 (most susceptible entry)	–	–	–	6.7

† Scale: 0 = healthy, 9 = dead.

Table 2. Reactions of germplasm lines in Fusarium yellows field nursery of Betaseed in Glyndon, MN, are shown below. FC1740 and FC1741 were not significantly different from the most resistant control in both years. The reactions of germplasm lines in an Aphanomyces root rot field nursery of Betaseed in Shakopee, MN, also are shown below. FC1740 and FC1741 were significantly more susceptible than the most resistant control and significantly more resistant and the most susceptible control in both years.

Entry	Fusarium rating		Entry	Aphanomyces (Aph) rating	
	2015	2016		2015	2016
	0-9†			0-9†	
Fusarium resistant control 1	2.7	3.7	Aph resistant control 1	1.7	1.5
20141007 (FC1740)	3.0	3.0	Aph resistant control 2	1.7	–
20141009 (FC1741)	2.7	3.8	Aph moderately resistant control 2	4.5	–
Fusarium resistant control 2	3.2	–	20141007 (FC1740)	5.0	4.2
Fusarium moderately resistant control	5.7	4.8	20141009 (FC1741)	5.0	5.2
Fusarium susceptible control 1	7.3	8.2	Aph moderately susceptible control 1	5.5	4.0
Fusarium susceptible control 2	8.2	8.3	Aph susceptible control 1	8.0	7.3
			Aph susceptible control 2	8.3	7.5

† Scale: 0 = healthy, 9 = dead.

these areas, *Rz1* and *Rz2* are stacked in the commercial hybrids to provide sugar beet with resistance to RB-BNYVV strains. FC1740 and FC1741 were evaluated at the USDA-ARS/BSDF rhizomania nurseries in Kimberly in 2013 (Strausbaugh and Panella, 2014) and 2015 (Strausbaugh and Panella, 2016) (Table 3). Neither FC1740 nor FC1741 were significantly different from the most resistant

controls from either commercial cultivars Angelina (*Rz1_Rz2_*) or Beta 4430R (*Rz1_rz2rz2*) in 2013 and 2015. Hybrids of both germplasm were crossed with two females, C869CMS (PI 628755) (segregating for rhizomania resistance markers) and FC708CMS (PI 590846) (very low frequency of rhizomania resistance markers) (Hecker and Ruppel, 1981; Lewellen, 2004). The

Table 3. Relative resistance of FC1740, FC1741, and hybrids in an ARS/BSDF nursery in Kimberly, ID in 2013 and 2015. They were evaluated for resistance to rhizomania using foliar and root symptoms. Their performance based on the foliar symptoms showed a clear separation at the LSD_{0.05} of 21% and 25% in 2013 and 2015, respectively.

Source/cultivar†	Description and allelic composition‡	Rhizomania rating	
		Foliar§	Root¶
2013			
Angelina	(<i>Rz1_Rz2_</i>)	0	19
20131002HO5	× C869CMS (<i>Rz1_rz2rz2</i>)	1	20
Beta 4430R	(<i>Rz1_rz2rz2</i>)	2	20
FC1740	20131001pfHO (<i>Rz1Rz1Rz2Rz2</i>)	2	18
20131001HO11	× C869CMS (rest of C869CMS)	2	17
20131001HO10	× C869CMS (<i>Rz1_Rz2rz2</i>)	10	19
20131001HO7	× C869CMS (<i>Rz1Rz1rz2rz2</i>)	11	21
20131001HO8	× C869CMS (<i>Rz1rz1rz2rz2</i>)	12	20
FC1741	20131002pfHO (<i>rz1rz1Rz2Rz2</i>)	13	24
20131001HO9	× C869CMS (<i>Rz1_Rz2rz2</i>)	14	24
FC1740	20131001pfHO (<i>Rz1Rz1Rz2Rz2</i>)	15	25
20131002HO8	× C869CMS (rest of C869CMS)	18	22
Beta G017R	(<i>rz1rz1Rz2_</i>)	32	24
20131001HO6	× FC708CMS (rest of FC708CMS)	32	24
20131001HO3	× FC708CMS (<i>Rz1Rz1rz2rz2</i>)	43	25
20131002HO3	× FC708CMS (<i>rz1rz1rz2rz2</i>)	58	26
20131001HO4	× FC708CMS (<i>Rz1_Rz2rz2</i>)	67	25
Roberta	(<i>rz1rz1rz2rz2</i>)	72	31
20131002HO2	× FC708CMS (<i>Rz1Rz1rz2rz2</i>)	82	25
<i>P</i> > <i>F</i> #		<0.0001	<0.0001
LSD		21	4
2015			
Beta G017R	(<i>rz1rz1Rz2_</i>)	0	19
Beta 4430R	(<i>Rz1_rz2rz2</i>)	0	20
Angelina	(<i>Rz1_Rz2_</i>)	0	21
20131001HO11	× C869CMS (rest of C869)	0	21
FC1740	20141007 (<i>Rz1Rz1Rz2Rz2</i>)	0	23
20131001HO9	× C869CMS (<i>Rz1_Rz2rz2</i>)	0	26
20131002HO5	× C869CMS (<i>Rz1Rz1/rz2rz2</i>)	2	16
20131002HO10	× C869CMS (res <i>Rz1t</i> of C869)	2	27
20131001HO7	× C869CMS (<i>Rz1Rz1rz2rz2</i>)	5	26
20131001HO8	× C869 (<i>Rz1rz1rz2rz2</i>)	6	23
20131001HO10	× C869CMS (rest of C869)	10	27
20131002HO4	× C869CMS <i>Rz1rz1/</i>	12	25
FC1741	20141009 (<i>rz1rz1Rz2Rz2</i>)	12	30
20131001HO6	× FC708CMS (rest of FC708)	12	32
20131002HO8	× C869CMS (rest of C869)	15	30
20131001HO4	× FC708CMS (rest of FC708)	19	24
20131002HO3	× FC708CMS (<i>rz1rz1rz2rz2</i>)	38	30
Roberta	(<i>rz1rz1rz2rz2</i>)	92	51
<i>P</i> > <i>F</i>		<0.0001	<0.0001
LSD		25	7

† Four commercial hybrid cultivars were used as controls (shown in bold): Roberta, Beta 4430R, Beta G017R, and Angelina. The allelic composition of their rhizomania resistance is shown.

‡ Allelic composition is inferred from single-nucleotide polymorphism markers linked to the two rhizomania resistance loci *Rz1* and *Rz2*.

§ The foliar rating is the percentage of susceptible plants on the basis of foliar yellowing symptoms, and this rating was performed on 13 and 20 July in 2013 and 2015, respectively.

¶ Root rating is a disease severity rating in which 10 roots per plot were evaluated using a scale of 0–9 (0 = healthy and 9 = dead) and an index value for each plot established using the following formula: [(0A + 1B + 2C + 3D + 4E + 5F + 6G + 7H + 8I + 9J)/90]100, where A–J are the number of plants in categories 0–9, respectively.

P > *F* was the probability associated with the *F* value.

genetic composition of the cytoplasmic male sterile (CMS) parents was inferred using SNP markers. None of the hybrids with C869CMS, which had a high frequency of the marker linked with *Rz1* and a lower frequency of the marker linked with *Rz2*, were significantly different from either Angelina (*Rz1_Rz2_*) or Beta 4430R (*Rz1_rz2rz2*) in 2013 and 2015 (Table 3). Hybrids with FC708, which had a very low frequency of markers linked with *Rz1* and *Rz2*, had more variable performance (Table 3).

Foliar and root symptoms are variable depending on the severity of the infections; therefore, an ELISA is a dependable and quantifiable method of screening plants, especially seedlings and younger plants (Wisler et al., 1999). Both germplasm sources were subsequently tested in the greenhouse in Salinas using a commercial double antibody sandwich ELISA test kit with antiserum specific to BNYVV (Agdia) per manufacturer's recommendations, and results were compared with controls of known genotypes (Table 4). Results from tests performed in the greenhouse using soils containing traditional BNYVV Pathotype A demonstrated that both germplasm sources performed comparably with the commercial resistant controls (Table 4). FC1740, which has a similar homozygous diploid resistance to rhizomania as that in the commercial cultivars Angelina and B4430R (based on comparisons using the SNP markers), performed comparably with these resistant commercial controls when tested in duplicated greenhouse experiments using soil containing a traditional form of rhizomania normally controlled by the *Rz1* resistance gene alone. Performance of FC1741, which is homozygous for the marker *Rz2* but not for *Rz1*, resembled performance of G017R most closely in Exp. 1. G017R shares the same complement of *Rz* alleles with FC1741 (Table 4).

Other Diseases

When tested for resistance to *Cercospora* leaf spot, although FC1740 had a lower rating than FC1741 in general, neither

germplasm exhibited resistance to the disease (data not shown). Neither FC1740 nor FC1741 showed any resistance to *Rhizoctonia* crown and root rot (data not shown) (Panella et al., 2015b, 2016). FC1740 and FC1741 were both screened for resistance to sugar beet root aphid (*Pemphigus betae* Doane) by Betaseed and were susceptible (data not shown).

Availability

Breeder seed of FC1740 and FC1741 is maintained by the USDA-ARS and will be provided in quantities sufficient for reproduction on written request to Sugar Beet Research, USDA-ARS, Crops Research Laboratory, 1701 Center Ave., Fort Collins, CO 80526-2083. Seed of these releases was deposited in the National Plant Germplasm System, where it is now available for research purposes, including development and commercialization of new cultivars. We request that appropriate recognition be made of the source when these germplasm contribute to a new cultivar. United States Plant Variety Protection will not be requested for FC1740 or FC1741.

Acknowledgments

We thank the Beet Sugar Development Foundation and the Western Sugar Cooperative for their support of the USDA-ARS breeding programs at Fort Collins, CO, and Salinas, CA. Tests at Shakopee, Glyndon, and Rosemount, MN were conducted by M. Rekoske, P. O'Boyle, and J. Miller, Betaseed, and we appreciate their cooperation and the support from Betaseed. We thank M. McGrath and L. Hanson with the ARS at East Lansing, MI, for performing *Cercospora* leaf spot evaluations. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

Table 4. Rhizomania resistance enzyme-linked immunosorbent assay (ELISA) results of FC1740, FC1741, and check cultivars Roberta, Beta 4430R, Beta G017R, and Angelina planted in naturally infested or in rhizomania soils with *Beet necrotic yellow vein virus* (BNYVV) in the greenhouse in Salinas, CA, in 2013. Check cultivars were also planted in autoclaved, disease-free soil as an experimental control, but were not included in statistical analyses. The experiment was conducted twice and results are presented separately.

Soil	Source/cultivar†	Allelic Composition‡	Experiment 1 ELISA§	Experiment 2 ELISA
BNYVV	Roberta	<i>rz1rz1rz2rz2</i>	3.23A	5.53A
	Beta 4430R	<i>Rz1rz1rz2rz2</i>	2.75ABC	3.58AB
	Beta G017R	<i>rz1rz1Rz2rz2</i>	2.16ABC	1.88C
	Angelina	<i>Rz1rz1Rz2rz2</i>	1.76C	3.31B
	FC1740	<i>Rz1Rz1Rz2Rz2</i>	1.81C	3.93AB
	FC1741	<i>rz1rz1Rz2Rz2</i>	1.84BC	4.31AB
	<i>P</i> > <i>F</i> ¶			0.0022
ELISA plate diseased control			3.32	6.21
ELISA plate healthy control			1.00	1.00
Autoclaved	Roberta	<i>rz1rz1rz2rz2</i>	0.97	1.52
	Beta 4430R	<i>Rz1rz1rz2rz2</i>	0.99	1.08
	Beta G017R	<i>rz1rz1Rz2rz2</i>	1.02	1.07
	Angelina	<i>Rz1rz1Rz2Rz2</i>	0.97	1.34

† Four commercial hybrid cultivars were used as controls: Roberta, Beta 4430R, Beta G017R, and Angelina. The allelic composition of their rhizomania resistance is shown.

‡ Allelic composition is inferred from single-nucleotide polymorphism markers linked to the two rhizomania resistance loci *Rz1* and *Rz2*, which most likely are not located within these genes.

§ Each ELISA value is the absorbance reading (A_{405nm}) 1 h after addition of substrate divided by the absorbance reading of the ELISA plate healthy control. Values of 3.0 or greater are considered positive for the virus. Within each experiment, lines with the same letter are not significantly different at $p > 0.05$ according to mean separation using the Tukey adjustment to the mixed model.

¶ $P > F$ was the probability associated with the *F* value.

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