Breeding for Multiple Disease Resistance in Sugarbeet: Registration of FC220 and FC221

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ABSTRACT

FC220 (Reg. No. GP-263, PI 651015) and FC221 (Reg. No. GP-264, PI 651016) sugarbeet (*Beta vulgaris* L.) germplasm were released from 05-FC1030-15 (Sp) and 05-FC1030-16 (Sp) seed lots, respectively, and tested under those designations and as 03-FC1030-15 and 03-FC1030-16, respectively. They were developed by the USDA–ARS, at Fort Collins, CO, and at Salinas, CA, in cooperation with the Beet Sugar Development Foundation, Denver, CO. FC220 and FC221 are multigerm sugarbeet germplasm in fertile cytoplasm, segregating for self-fertility and hypocotyl color. Both have resistance to root-rotting strains (AG-2-2) of *Rhizoctonia solani* Kühn and are segregating for the *Rz1* gene, which confers resistance to some strains of *Beet necrotic yellow vein virus*, the causal agent of rhizomania. These germplasm have moderate resistance to *Aphanomyces cochlioides* Drechs., which causes aphanomyces root rot (aphanomyces black root). FC221 is moderately resistant to curly top caused by *Beet severe curly top virus*. FC220 is resistant to the sugarbeet root aphid (*Pemphigus* sp.). Both germplasm are moderately susceptible to cercospora leaf spot, caused by *Cercospora beticola* Sacc. FC220 and FC221 have favorable yield characteristics when evaluated as lines or in experimental hybrids under rhizomania conditions at Salinas, CA. Under these conditions, FC220, as a line and in hybrid combinations, had a slight to significantly higher sucrose concentration than FC221.

In 2007–2008, according to F.O. Licht (2008), the United States is number 5 in sugar production, at 7725 t, behind Brazil (33,000), India (29,500), China (14674), and Thailand (7870). For sugarbeet (*Beta vulgaris* L.) sugar only, which constitutes over half of the combined U.S. sugar crop value, the United States (4372) ranks number 1, ahead of France (4239) and Germany (4155). The National Agricultural Statistics Service forecasted that 502.2 hundred thousand hectares of sugarbeet will be harvested in FY 2008 (Haley and Jerardo, 2007). Sugarbeet is grown in 12 states from the Imperial Valley of California to Michigan in the Great Lakes Region and is an important component of a domestic sugar and sweetener industry. Across the growing region, disease pressures can have an enormous impact on yield and profitability (Whitney and Duffus, 1986). The only public breeders are USDA-ARS breeders, who develop germplasm, which is released to commercial seed companies as sources of disease resistance for their proprietary hybrid development programs. The crop is biennial, and the breeding system is complex, which makes breeding disease-resistant germplasm a long-term (8–15 yr) effort (Panella and Lewellen, 2007). Evaluating germplasm for agronomic characteristics and disease resistance requires a strong collaborative effort among public breeders and private companies. The development of FC220 (Reg. No. GP-263, PI 651015) and FC221 (Reg. No. GP-264, PI 651016) illustrates how this collaborative effort is accomplished. FC220 and FC221 were developed to combine rhizomania (caused by *Beet necrotic yellow vein virus* [BNYVV]) resistance and other resistances from Salinas, CA, germplasm with the resistances (especially rhizoctonia root rot [caused by *Rhizoctonia solani* Kühn]) from Fort Collins, CO, germplasm. These germplasm were developed primarily as populations from which to select disease-resistant, multigerm pollinator parents.

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Abbreviations: BNYVV, *Beet necrotic yellow vein virus*; BSDF, Beet Sugar Development Foundation; CLS, cercospora leaf spot; CMS, cytoplasmic male sterile; DI, disease index; HS, half-sib; MR, mother roots; MS, male sterile; PF, pollen fertile.

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Methods

Early Generation Population Development

Background

Breeding material in the USDA-ARS program at Fort Collins was increased either in the field in a mother root nursery or in the greenhouse. In the mother root nursery, seed was planted between early May and mid-June on 56-cm centers with plots 4 m long after the alleyways were cut. The nursery was hand thinned to 20 to 25 cm between plants and was furrow irrigated. Roots were lifted, sorted, the leaves trimmed, and washed by hand. Roots were placed in crates, stored in a cold room for vernalization at 5°C in >90% humidity for 90 to 180 d in darkness (Hogaboam, 1982; Lexander, 1987; Owen et al., 1940). The vernalization period varies based on nonbolting tendency from 90 d (Fort Collins germplasm) to 120 d (Salinas germplasm) (Steinrücken, 2005). After vernalization, roots were either potted in the greenhouse and allowed to flower or, if a larger quantity of seed was desired, planted the next spring in a field isolation plot. Field-grown populations were separated by at least a 200 m to minimize cross-pollination (Archimowitsch, 1949; Cureton et al., 2006).

For greenhouse production, plants were grown for 8 wk in Cone-tainers (Stuewe & Sons, Inc., Corvallis, OR), transplanted to larger pots, and vernalized (in the pot) in a cold room at 5°C for 90 to 120 d under 24-h fluorescent lighting. Following vernalization, the plants were placed either on benches or in pollen tight cages, under long-day conditions (18 h with metal halide lamps), and allowed to flower and set seed (Gaskill, 1952a,b; Hecker and Gaskill, 1975). During anthesis, the plants in greenhouse chambers were blown daily ("blown down") with a filtered leaf blower to spread the pollen throughout the chamber, enhancing random mating.

In controlled crosses, either genetic male sterile (*aa*) or green hypocotyl color (*rr*) plants were used as females (Owen, 1952). In sugarbeet, hypocotyl color is conditioned by two genes, *Y* and *R*. When *Y* is recessive (*yy*) (giving a white root), any dominant *R* allele will cause the hypocotyl to be red, whereas a homozygous, recessive individual at the *R* locus will have green hypocotyl color (Keller, 1936;Owen and Ryser, 1942). Seedlings with red hypocotyls from green-hypocotyl females are true F_1 hybrids.

Sugarbeet normally is governed by a complex, gametophytic self-incompatibility system ($S_aS_bS_cS_d$), which prevents self-pollination but allows almost any two plants to cross-pollinate (Larsen, 1977; Owen, 1942). However, self-fertility can be achieved through a dominant, self-incompatibility suppressor gene (*Sf*) (Owen, 1942). Self-fertility often is used with genetic male sterility (*aa*) in population improvement programs to allow testing for performance of selfed-progeny families, to develop inbred lines, and still allow random mating (Bosemark, 1971; Doggett and Eberhart, 1968; Owen, 1954).

Development of 19991030, 19991031, and 19991032 at Fort Collins

Reciprocal crosses between 'FC709-2' (PI 599668) and '2915', which is a developmental population of 'C931' (PI 636340), were made in the greenhouse in 1994(Lewellen, 2006; Panella, 1999c). When used as a female, male sterile individuals of C931 were used. When FC709-2 was used as a female, hypocotyl color was used as a marker. Seed of the F_1s of the reciprocal crosses was planted in the field in spring 1995; roots were dug on 3 October and vernalized. The flowering plants were random mated (FC709-2 is self-sterile, and C931 is self-fertile), and the seed was harvested in bulk. In early spring 1996, 118 of 120 roots (six from FC709-2*rr*/2915*R*_ and 114 from 2915aa/FC709-2) were harvested to produce seed, designated as 19961004.

Seed from 19961004 was planted in the field in spring 1996. Roots were dug on 9 September and vernalized. Male sterile and pollen fertile plants were marked as the flowers opened, and 47 male sterile (*aa*) roots were harvested for seed, having received pollen from 55 pollen fertile plants. The seed, designated as 19971015, was bulked. In spring 1997, seed from this population was planted in the field, and roots were dug 20 August, vernalized, and allowed to set seed in the greenhouse. Seed from individual roots was harvested, producing half-sib families. Forty-four half-sib families had sufficient seed to be tested in the USDA-ARS Beet Sugar Development Foundation (BSDF) nursery at Fort Collins for resistance to rhizoctonia root and crown rot and in the BSDF nursery at Kimberly, ID, for resistance to curly top (caused by *Beet severe curly top virus*).

The 1998 USDA-ARS BSDF rhizoctonia crown and root rot screening nursery was a randomized, complete-block design with five replicates (Panella, 1999b). In 1998 one-row plots, replicated five times, were planted in Windsor, CO, on 21 May. Plots were 4.5 m long with 56 cm between rows and 20- to 25-cm within-row spacing. Rhizoctonia-resistant line FC703 and highly susceptible FC901/C817 were included as controls, as was highly resistant FC705/1 (Gaskill et al., 1967; Hecker and Ruppel, 1977; Hecker and Ruppel, 1985). Inoculation was with dry, ground, barley-grain inoculum of *Rhizoctonia solani* AG2-2 isolate 'R-9' ("B-6" that was used by Pierson and Gaskill [1961]).

Each root was rated for rot on a scale of 0 (no damage) to 7 (dead) at harvest. Analyses of variance using Proc ANOVA of SAS for Windows 9.1 (SAS Institute, Cary NC) were performed on disease indices (DIs), percentage healthy roots (undamaged classes 0 and 1 combined), and percentage of roots in classes 0 through 3 (those most likely to be harvested and taken to the factory). Percentages were transformed using arcsin-square root to normalize the data for analyses (Panella, 1998; Ruppel et al., 1979).

The curly top nursery at Kimberly was planted on 8 and 9 June 1998 (Panella, 1999a; Strausbaugh et al., 2007). Planting was late (early June) to maximize the number of viruliferous leafhoppers available for transfer of the virus to plants in the 8- to 10-leaf stage. Plots were 4 m long, two-rowed with 56 cm between rows and 25–30 cm within-row spacing replicated two times. Viruliferous beet leafhoppers (*Circulifer tenellus* Baker) were released on 15 July. One week before the beet leafhoppers were released in the nursery, they had been placed onto curly top–infected plants to ensure that they were viruliferous when placed in the field. The field was sprayed with Thiodan EC (350 g a.i. endosulfan L^{-1}; Aventis CropScience, Research Triangle Park, NC), an insecticide, at a

rate of 190 mL 100 L^{-1} on 23 August to kill the leafhoppers. Plots were visually evaluated and rated on a DI scale of 0 to 9 (no symptoms to dead) (Mumford, 1974; Murphy, 1942).

Remnant seed from selected families was planted in the greenhouse in early 1999. Male sterile plants (MS) and pollen fertile plants (PF) were marked as the flowers opened and harvested separately. Remnant seed from six families, selected for resistance to both rhizoctonia root rot (6 family mean DI = 3.91) and curly top (6 family mean DI = 3.75), was bulked and planted as a population. In this population, 131 MS plants, designated as 19991030MS, were harvested and the seed bulked. Another 183 PF plants, designated as 19991030PF, were harvested and the seed bulked.

Remnant seed from another 11 families, selected for resistance to rhizoctonia root rot (11 family mean DI = 3.51), were harvested and the seed bulked. In this population, 101 MS plants, designated as 19991031MS, were harvested and the seed bulked. Another 160 PF plants, designated as 19991031PF, were harvested and the seed bulked.

Remnant seed from a different six families, selected for resistance to curly top (6 family mean DI = 3.83), was bulked and planted as a population. In this population, 74 MS plants, designated as 19991032MS, were harvested and the seed bulked. Another 162 PF plants, designated as 19991032PF, were harvested and the seed bulked.

Development of 20001004 at Fort Collins

Reciprocal crosses between 'FC902' (PI 590655) and 'R278' were made in the greenhouse in 1994 (Smith and Gaskill, 1979). R278, from the USDA-ARS breeding program at Salinas, was released as 'C78' (PI 593671) (Lewellen, 1997). It is a source of rhizomania resistance, segregates for genetic male sterility, and should be self-sterile. FC902, from the USDA-ARS breeding program at Fort Collins, is mostly self sterile with a small percentage of plants (~11%) that are genetic male sterile. It has moderate resistance to cercospora leaf spot (CLS; caused by *Cercospora beticola* Sacc.) and curly top. When R278 was used as a female, 36 MS individuals were harvested and seed was designated as 19941013H2. When FC902 was used as a female, seed was harvested from six green hypocotyl plants and designated as 19941014H2.

Plants from F_1 seed from both reciprocal crosses were used as males to cross with genetic MS (*aa*) plants of '4918' in the greenhouse in 1994. 4918 is an increase of 'C918' (PI 578079) from the USDA-ARS breeding program at Salinas, which is similar in pedigree to 'C931' (PI 636340) (Lewellen, 2006). It is a germplasm with resistance to both rhizomania and to curly top and is highly self-fertile.

Seed of the reciprocal crosses was planted in the greenhouse and vernalized. Seed from 53 plants of 4918*aa*/19941013H2 (199561001) and 55 plants of 4918*aa*/19941014H2 (19951002) was harvested. Equal amounts (by weight) of seed from both 19951001 and 19951002 were mixed and planted in the greenhouse in early 1996. Fifty-seven vernalized plants flowered and set seed designated as 19961002.

Another population was started in 1994 to bring higher sucrose into a CLS background. In a population cross, 'FC607'*rr* (PI 590837) was the CLS-resistant female and pollen was provided by 'MonoHy T6', 'MonoHy A4', and 'MonoHy A7'—obsolete hybrids from the Great Western Sugar Company—and 'SR 87' (PI 607899), a smooth root germplasm (Saunders et al., 2000;Smith and Ruppel, 1980) In late 1995, seed from this cross was planted in the greenhouse, plants vernalized, and allowed to random mate. This seed production was designated as 19961001.

Seed of the 19961001 and 19961002 was planted in the field in spring of 1997, roots of 961001 were dug on 30 September and those of 19961002 were dug on 20 August and vernalized for 90 and 120 d, respectively. The cross was made based on hypocotyl color—19961002*rr*/19961001—and seed designated as 19981008H3. Seed of 19981008H3 was planted in pots in the greenhouse during summer 1998. Seed harvested from 96 plants was designated as 19981036. Seed of 19981036 was planted in the field in spring 1999. Roots were dug on 17 August and vernalized for 120 d. The flowering plants were bulk increased and the seed harvested. Seed from 94 plants was designated as 20001004.

In 2000 seed of 20001004, 19991030MS, 19991030PF, 19991031MS, 19991031PF, 19991032MS, and 19991032PF was sent to Salinas.

Final Population Development and Selection at Salinas

Part of the seed was planted in the Spence field nursery (Salinas) under rhizomania conditions. After 120 d, mother roots (MR) were dug and visually evaluated for response to rhizomania. There were 161 MR selected (48 MR of 20001004, 27 MR of 19991030MS, 23 MR of 19991030PF, 10 MR of 19991031MS, 5 MR of 19991031PF, 24 MR of 19991032MS, and 24 MR of 19991032PF). The roots were vernalized in the cold room. The remaining seed of these populations had been sent to Medford, OR, and planted in August in the field to produce stecklings (Kockelmann and Meyer, 2006). Forty stecklings from each of the Fort Collins populations and the selected MR were transplanted together in March in a field isolation plot and allowed to set seed. In the isolation plot, the 161 MR were planted in rows down wind from the rows of Oregon stecklings. During anthesis, the genetic male sterile (*aa*) plants within both groups were tagged.

Seed from each *aa* plant was harvested separately. From each *aa* half-sib (HS), an equal amount of seed was composited to produce the FC1030 synthetic. Seed of each HS also was retained for progeny tests. The two lines FC220 and FC221 are descended from two HS families arbitrarily labeled 01-FC1030-15 and -16, which had no relationship to their individual positions in the seed plot or any other trait, except seed weight. (By weight, seed was labeled from heaviest amount to least amount.)

Half-sib progeny families (32 families) of 01-FC1030 were evaluated in 2002 for yield under rhizomania and CLS conditions. To determine the rhizomania DI, plots were partially topped, lifted, laid out on the soil surface, and roots individually scored for rhizomania (DI = average score of each plant within the entry; where 1 = normal root to

9 = very severe rhizomania or dead). Two classes of percentage resistant were calculated. The percentage resistant (0–3) was calculated [= (total roots in classes 0 + 1 + 2 + 3)/total roots scored], and the percentage resistant (0–4) also was calculated (= roots in classes 0–4/total roots scored).

The foliar score is a visual rating before harvest, in which the canopy of each plot was scored for leaf color. This rating is on a scale of 1 to 5, where 1 = dark green, 2 = green, 3 = light green or mixed green to yellow, 4 = mostly yellow, and 5 = uniformly yellow in the manner of susceptible varieties under rhizomania. When the canopy or foliar score did not capture the plant-to-plant segregation or variability within a plot, that is, the segregation for rhizomania, we also attempted to score the plots for segregation for green versus yellow, where 1 = all dark green, 2 = about 25% yellowish plants, 3 = 50% yellowish plants, 4 = 75% yellowish, and 5 = all yellowish. The scores for foliar color and segregation were similar. A concern and problem for both of these rating methods is that although the plants were segregating for yellowing caused by rhizomania, they also were segregating and variable for yellowing/leaf chlorosis that resembled Mg, Mn, Fe, Zn deficiency. The relationship between the light yellowing generally attributed to rhizomania and the Mg yellowing is not known. It also is difficult with these scoring methods to separate the color due to natural canopy intensity of green from the effects of rhizomania.

After testing, 01-FC1030-15 was selected for high sucrose concentration and moderate resistance to CLS (Table 1). Simultaneously, 01-FC1030-16 was selected because of high sugar yield (the product of root fresh weight and percentage sucrose) and moderate resistance to CLS (Table 1). In August 2002, remnant seed of each family was planted in the Medford steckling nursery, dug in March 2003, and transplanted to greenhouse isolation chambers. At this point, a genetic-cytoplasmic male sterile (CMS) population, 'C790-15CMS', also was put in the isolation chambers to produce a test-cross hybrid (Lewellen, 1994; Owen, 1945). Seed harvested from a bulk increase of these families was designated as 03-FC1030-15 and 03-FC1030-16, and seed from the crosses to the CMS female was designated as 03-FC1030,15H50 and 03-FC1030-16H50.

In 2004 these half-sib families were tested as the above designations, and in August 2004, seed was planted in the MR nursery at Salinas under rhizomania conditions and in the steckling nursery at Medford under disease-free conditions. Roots from 03-FC1030-15 (20 MR and 16 stecklings) and 03-FC1030-16 (17 MR and 32 stecklings) were transplanted in separate isolation chambers again with C790-15CMS for bulk increase and production of test-cross hybrids. These increases were designated as 05-FC1030-15(Iso) and 05-FC1030-16(Iso). Test-cross seed from the CMS female was designated as 05-FC1030-15H50 and 05-FC1030-16H50.

Stecklings of 03-FC1030-15 (240 stecklings) and 03-FC1030-16 (210 stecklings) from Medford were also planted in two field isolation plots under disease-free conditions. In addition, to produce test-cross seed, stecklings of C790-15CMS (PI 564758) and 'C833-5CMS' (PI 615523) were planted downwind from the pollinator line in each isolation plot (Lewellen, 2002). During anthesis, the genetic MS (*aa*) plants within both HS-derived lines were tagged, and 30 to 40 plants from each of the lines were harvested and bulked, ensuring recombination. The harvested seed was designated as 05-FC1030-15(Sp) and 05-FC1030-16(Sp). Test-cross hybrid seed from C790-15CMS is 05-FC1030-15H50 and 05-FC1030-16H50, and from C833-5CMS is 05-FC1030-15H5 and 05-FC1030-16H5. Seed released as FC220 was from 05-FC1030-15(Sp), and seed released as FC221 was from 05-FC1030-16(Sp).

Altogether, these germplasm went through one cycle of HS selection for rhizoctonia resistance, one cycle of selection for curly top resistance, and one direct cycle for resistance to rhizomania, which was then diluted with nonselected roots, and one cycle of half-sib selection for yield performance under rhizomania and CLS conditions.

Characteristics

Agronomic and Morphological Description

FC220 [05-FC1030-15(Sp)] and FC221 [05-FC1030-16(Sp)] have multigerm seedballs with a fertile cytoplasm (Savitsky, 1952b). The population is segregating for self-sterility (S^s) because self-fertility was introduced by both FC902 and C78. Because the population released was harvested from genetic MS plants, the '*a*' allele should be present in the population at a frequency of at least 0.50 and at equilibrium there should be ≥25% male sterile plants in the population. Both populations segregate for hypocotyl color with FC220 having 36% green hypocotyls (of 200 seedlings counted) and FC221 having 9% green hypocotyls (of 127 seedlings counted). When tested for germination in September 2005, FC220 had 206 sprouts per 100 seedballs and FC221 had 190 sprouts per 100 seedballs (Savitsky, 1952a).

Resistance to Disease and Other Pests

Rhizoctonia Root and Crown Rot

Six of the original seven germplasm components from Fort Collins were chosen on the basis of resistance to rhizoctonia root and crown rot. The developmental and released germplasm were tested for resistance to rhizoctonia at Fort Collins (as described above) during and after selection at Salinas (Table 2). The DI was determined by visually rating each root in a single row plot on a 0 (disease free) to 7 (dead and rotted) scale. Plot means for each of the five replications were used for an ANOVA (SAS Proc MIXED; SAS Institute, Cary, NC), and the LSD means separation with a $P = 0.05$ was used. In all of the tests for resistance to rhizoctonia (Table 2), FC220 and FC221 were not significantly different in DI from the resistant controls except in 2003, when FC221 had significantly less resistance than the resistant controls. Both germplasm have excellent resistance to rhizoctonia root and crown rot.

Curly Top

Some of the parents in this cross (4918, R278, FC902, FC607) were chosen because of their resistance to curly top. Two of the original components of the germplasm were selected at Kimberly for resistance to curly top, and

Table 1. Performance of half-sib progenies from population FC1030 in three tests at Salinas in 2002. These two half-sib families became FC220 (01-FC1030-15) and FC221 (01-FC1030-16).

Variety	Yield trial[†]					Nonbolting trial[‡]			CLS resistance evaluation[§]			
	Sugar yield[¶]	Sucrose[#]	RJAP[††]	PM score[‡‡]	% Bolt[§§]	% Bolt 4 Sept. 2002	DM[¶¶] 3 Apr. 2002	PM score	Sugar yield	Sucrose	RJAP	CLS score[##]
	kg ha^{-1}	—— % ——				———— % ————			kg ha^{-1}	—— % ——		
01-FC1030-15	19,972	18.73	84.6	5.0	4.2	70.6	35.6	5.6	13,673	16.13	80.8	2.0
01-FC1030-16	21,056	17.37	87.0	5.0	2.1	67.3	30.1	6.3	19,874	17.87	85.2	2.3
Test mean	18,916.3	17.19	84.0	5.5	1.1	58.4	29.4	5.8	15,752.4	16.56	82.9	2.3
$LSD_{0.05}$	3,808.3	1.40	4.0	1.2	4.6	20.5	38.1	1.0	3,456.0	1.61	4.4	0.8
CV	12.3	5.00	3.9	12.8	247.2	21.4	79.3	10.6	13.4	5.95	3.2	21.4
F value	2.2 NS[†††]	1.67*	1.1 NS	2.7**	1.8*	7.9**	1.3 NS	1.9*	2.6**	1.84*	1.0 NS	1.7*
Dispersion of all progenies[‡‡‡]												
Highest	22,841	18.7	87.0	6.3	9.8	97.0	66.1	6.8	19,874	17.9	85.9	3.3
Lowest	15,714	15.0	80.4	3.5	0.0	12.7	4.2	4.8	11,931	14.5	80.1	1.7

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]Test 4302: disease free, 32 varieties with three replications, randomized complete block (RCB) design, one-row plots, 3.3 m in length, planted 28 Feb. 2002, harvested 2 Oct. 2002.

[‡]Test 1602: Overwinter nonbolting trial, not harvested for yield, 32 varieties with three replications, RCB, one-row plots, 3.3 m in length, planted 7 Nov. 2001.

[§]Test 5902: Inoculated test for resistance to cercospora leaf spot (CLS) (with ground, infested leaves in a talc carrier—techniques used by Betaseed, Shakopee, MN), 32 varieties with three replications, RCB, one-row plots, 3.3 m in length, planted 25 Mar. 2002, harvested 15 Nov. 2002, inoculated 8 Aug. 2002, scored 13 Nov. 20022. Soil naturally infested with *Beet necrotic yellow vein virus* (rhizomania).

[¶]Sugar yield = root yield × % sugar.

[#]Percentage of fresh weight.

[††]RJAP, raw juice apparent purity. Apparent purity = raw juice apparent purity [(% sugar/% total soluble solids)100].

[‡‡]PM, powdery mildew. PM score = powdery mildew (*Erysiphe* sp.) from natural infection scored on a scale of 0–9, where 9 = 100% of leaf area covered with powdery mildew.

[§§]Percentage of the plants that bolted from natural vernalization [(number bolted plants/stand count)/100].

[¶¶]DM, downy mildew. Percentage of plants showing downy mildew (*Peronospora farinosa* Fr.f.sp. *betae* Byford) from natural infection.

[##]CLS, cercospora leaf spot (*C. beticola* Sacc.). CLS score is infection scored on 13 Nov. 2002 on a scale of 0–5, where 0 = no visible spots and 5 = level of symptoms of susceptible B4430R in border and spreader rows.

[†††]NS, not significant.

[‡‡‡]Range or dispersion extremes of means for 32 half-sib families tested.

two more were selected for combined resistance to curly top and rhizoctonia root and crown rot. No further selection was made for resistance, but the HS populations were tested at the BSDF curly top nursery at Kimberly (as above) during development, as were the released germplasm (Table 3). The early generation tests were not randomized, but check varieties were planted multiple times throughout the experiment (Table 3). Although statistical analyses are not appropriate, mean comparisons give some insight into the performance of these germplasm when challenged by *Beet severe curly top virus* and other closely related curtovirus species (Table 3). Plots were visually evaluated and rated on a DI scale of 0 to 9 (0 = no symptoms; 9 = dead). The most important rating is the final rating, in which the disease expression is at its peak. The 2004 test had a very mild infection compared with 2006. The 2006 nursery was planted in a completely randomized block design, and an ANOVA (PROC GLM) was performed with means separation using Duncan's Multiple Range Test ($P = 0.05$). Differences among lines at all three dates were significant ($P < 0.0001$). FC221 performed significantly better than the susceptible check and not significantly different from the resistant checks at the critical third rating. FC220 had a significantly higher score (more susceptible) than the resistant checks and was not significantly different from the susceptible checks. FC221 had some resistance to curly top.

Sugarbeet Root Aphid

The germplasm were screened by Betaseed, Inc. (Shakopee, MN) in a greenhouse test for resistance to sugarbeet root aphid (*Pemphigus* sp.) at Shakopee during and after selection for resistance for rhizomania (Table 4). Seed was treated with Allegiance and Thiram and sown into germination medium. At the cotyledon stage, seedlings were transplanted to nursery flats with 6.4- by 5.6-cm celled inserts containing soilless growing medium. The transplants were grown in the greenhouse with a temperature range of 17 to 35°C and 16 h daylength. At 4 wk, the plants were transferred to 8.9-cm round pots, infested with approximately five aphids per pot, and grown for an additional 3 wk. Plants were evaluated individually on a 1 to 4 scale, where 1 designates a plant with no aphids, 2 indicates the presence of immature aphids, 3 has adult aphids or a small colony, and 4 indicates the presence of several small colonies or a larger colony with a diameter greater than 2.5 cm.

These tests were not randomized, and statistical analyses are not appropriate, but mean comparisons give some insight into the performance of these germplasm when challenged by sugarbeet root aphid (M. Rekoske and J. Miller, personal communication, 2007). In 2004 the population that became FC220 performed almost as well as the resistant commercial hybrid 'Monohikari', which has excellent field resistance to root aphid (Table 4). Again, in 2006

Table 2. Rhizoctonia root and crown rot resistance evaluations in Fort Collins, CO. FC220 (03-FC1030-15 and 05-FC1030-15), FC221 (03-FC1030-16 and 05-FC1030-16), and test-cross hybrids were tested. Given here are the disease index (DI), percentage healthy plants, and percentage harvestable plants. Experiments were planted in a completely randomized blocked design with five replications of 4-m single-row plots.

	2003			2004			2005		
Description	DI†	Healthy‡	Harvestable§	DI	Healthy	Harvestable	DI	Healthy	Harvestable
03-FC1030-15	4.2	8	29	2.0	44.6	72.8	3.2	28.0	50.9
03-FC1030-16	4.8	6	18	1.7	46.2	83.5	3.3	36.1	47.0
Susceptible check¶	5.2	4	10	2.5	33.2	66.1	5.3	4.4	10.0
Resistant check#	3.5	18	40	1.6	52.0	86.1	4.0	21.3	37.7
Highly Res. check††	3.5	13	43	1.6	44.6	90.0			
Experiment mean	5.1	4.5	14.6	2.7	29.3	63.6	4.5	17.0	28.7
$LSD_{0.05}$	1.17	13.7	20.3	0.77	14.36	17.45	1.19	17.7	21.4
CV	18.1	243.6	111.1	22.7	38.8	21.7	20.8	81.4	60.2

	2006 (5R)			2006 (11R)		
Description	DI	Healthy	Harvestable	DI	Healthy	Harvestable
05-FC1030-15	1.8	44.7	79.8	2.3	31	73
05-FC1030-15(Iso)				2.2	40	74
05-FC1030-15H50	3.7	11.8	46.5	2.8	35	59
05-FC1030-15H5				2.7	29	60
05-FC1030-16	2.4	34.1	64.8	2.6	31	68
05-FC1030-16(Iso)				2.5	32	67
05-FC1030-16H50	3.3	15.5	51.2	3.7	21	44
05-FC1030-16H5				3.5	18	51
Susceptible check¶	4.2	11.7	31.9	3.4	20	50
Resistant check#	2.2	32.8	72.4	1.8	40	82
Highly resistant check††	1.8	30.8	85.9	1.9	33	86
Experiment mean	3.5	19.5	48.3	2.5	32	69
$LSD_{0.05}$	1.18	19.5	20.1	0.98	17.8	19.0
CV				32.0	44.2	21.9

†DI is based on a scale of 0 (healthy) to 7 (plant dead).

‡Percentage of healthy roots (DI classes 0 and 1 combined). Percentages were transformed to arcsin-square roots to normalize the data for analyses.

§Percentage of diseased roots likely to be taken for processing (DI classes 0–3 combined). Percentages were transformed to arcsin-square roots to normalize the data for analyses.

¶FC901/C817.

#FC703.

††FC705/1.

FC220 performed comparable to Monohikari and better than all of the susceptible checks. The FC221 results were similar to the susceptible checks.

Aphanomyces Root Rot (Aphanomyces Black Root)

The germplasm were screened by Betaseed in a field nursery for resistance to aphanomyces root rot (caused by *Aphanomyces cochlioides* Drechs.) at Shakopee during and after selection for resistance for rhizomania (Table 5). All plots were two rows, 3 m long, with 56-cm row spacing. The seed was treated with standard rates of Allegiance and Thiram. Trials were planted into warm soils between late May and early June to facilitate pathogen development. Plots were thinned to a uniform stand of 8 to 9 cm between plants. Irrigation was used as needed to provide adequate moisture for initial stand establishment and to maintain conditions favorable for *A. cochlioides*. Fungicides were applied as needed to control rhizoctonia root rot and CLS. A visual 1 to 9 rating scale based on stand persistence and plant health was used to evaluate aphanomyces root rot damage. A rating of 1 is a complete stand of healthy plants, and a rating of 9 has no surviving plants. Ratings were taken one to three times during the growing season. Experimental design was a randomized complete block with three replications (M. Rekoske and J. Miller, personal communication, 2007).

FC 220 and FC 221 performed similarly when challenged by *A. cochlioides*, the causal agent of aphanomyces root rot (also called aphanomyces black root). In 2004, when the populations were under development, they had significantly less resistance than the tolerant checks but performed significantly better than the susceptible and moderately susceptible checks (Table 5). In 2006 both germplasm were comparable to Monohikari, a tolerant hybrid, and again had significantly less resistance than the tolerant checks (second evaluation 2006; Table 5) but performed significantly better than the susceptible and one of the two moderately susceptible checks. Their performance indicates a moderate resistance to *A. cochlioides*.

Table 3. Evaluation for resistance to beet curly top in the Beet Sugar Development Foundation's nursery near Kimberly, ID. FC220 (03/05-FC1030-15) and FC221 (03/05-FC1030-16) were tested during and after development. The entries were not randomized in 2004 and 2005, and statistical tests are not appropriate. In 2006 plots were planted in a completely randomized block design, replicated three times in 3-m, one-row plots, and analyzed using Proc GLM (SAS Institute, Cary, NC).

Variety	Description	2006				2005			2004			
		No.†	Rating 1‡ 7 Aug.	Rating 2 28 Aug.	Rating 3§ 11 Sept.	No.	Rating 1 23 Aug.	Rating 2 13 Sept.	No.	Rating 1 16 Aug.	Rating 2 30 Aug.	Rating 3 13 Sept.
03-FC1030-15	Inc. 01-FC1030-15 (*A*,*aa*)						4.0	5.0		3.3	3.7	4.0
03-FC1030-16	Inc. 01-FC1030-16 (*A*,*aa*)						4.0	5.0		3.3	3.7	4.0
05-FC1030-15(Sp)¶	03-FC1030-15*aa* × *A*		4.7	5.7	6.3							
05-FC1030-15(Iso)#	RZM 03-FC1030-15(*A*,*aa*)		5.0	6.0	7.0							
05-FC1030-16(Sp)	03-FC1030-16*aa* × *A*		4.0	5.0	5.0††							
05-FC1030-16(Iso)	RZM 03-FC1030-16(*A*,*aa*)		4.0	4.3	5.0††							
HM-PM21	Resistant check	6	3.6	4.0	4.2	3	3.8	4.6	1	2.3	3.0	3.0
US H11	Resistant check	3	3.5	3.9	4.2	2	3.9	4.6	6	3.0	3.2	3.5
03-, 04, 05-C37	Resistant check, Inc. C37	7	3.7	4.0	4.4	5	3.7	4.5	6	3.2	3.3	3.6
HM-E17	Susceptible check	1	4.5	6.5	7.5	1	4.7	7.0				
Monohikari	Susceptible check	5	4.9	6.7	7.6	4	5.4	7.5	4	4.4	4.9	5.9

†Number of times this check line was planted in the nursery.

‡Plots were visually evaluated and rated on a disease index scale of 0 to 9 (no symptoms to dead).

§Differences among lines at all three dates were significant ($P < 0.0001$).

¶Sp, increase using spatial isolation where genetic ms (*aa*) plants are tagged and pollinated randomly by male fertile plants (*A*_).

#Iso, increase in greenhouse isolation chambers where both *aa* and *A*_ plants are harvested in bulk.

††In a Duncan's Multiple Range Test, FC221 was significantly better than the susceptible checks and not significantly different from the resistant checks ($p = 0.05$).

Table 4. Sugar beet root aphid resistance evaluation by Betaseed, Inc., in greenhouse testing at Shakopee, MN (FC220 = 03-FC1030-15; FC221 = 03-FC1030-16).

Source	Variety	Avg. DI†	No. of plants‡	SD§
	2006			
06BOB30	05-FC1030-15(Sp)	2.7	13	1.38
06BOB31	05-FC1030-16(Sp)	4.0	16	0.00
06 BOB37	Resistant check Monohikari	2.6	13	1.26
Betaseed	Susceptible check	3.8	16	0.40
Betaseed	Susceptible check	3.2	13	1.30
Betaseed	Susceptible check	3.7	12	0.49
Betaseed	Susceptible check	3.4	12	1.00
Betaseed	Susceptible check	3.3	13	1.18
	2004			
BOB08	03-FC1030-15	2.0	13	
BOB09	03-FC1030-16	3.0	13	
BOB01	Resistant check Monohikari	1.8	16	
BOB02	Susceptible check Beta 4430R	3.4	15	
Betaseed	Susceptible check	3.7	16	
Betaseed	Susceptible check	3.6	16	
Betaseed	Susceptible check	3.4	16	

†DI, disease index: 1–4 scale, where 1 = plant with no aphids, 2 = presence of immature aphids, 3 = adult aphids or a small colony, and 4 = presence of several small colonies or a single larger colony with a diameter > 2.5 cm.

‡Tests are not randomized and statistical analysis is not appropriate.

§Standard deviation of a sample.

Cercospora Leaf Spot

The germplasm were screened by Betaseed, Inc., in a field nursery for resistance to CLS at Rosemount, MN, during and after selection for resistance for rhizomania (Table 6). All plots were two rows, 3 m long, with 56-cm row spacing. The seed was treated with Allegiance (metalaxyl; Gustafson LLC, Plano, TX), Thiram (Gustafson LLC, Plano, TX), and Tachigaren (hymexazol; Sanyko Co., Tokyo). Trials were planted in early May and thinned to a uniform stand of 17 cm between plants. The nursery was inoculated during the first 2 wk of July with a 2:1 mixture of talc to dry *C. beticola*–infected leaves at a rate of 16.8 kg ha^{-1}. Solid set irrigation was used to provide adequate moisture for initial infection and as needed to maintain conditions favorable for CLS development. The KWS rating scale (Kleinwanzlebener Saatzucht, 1970) was used to evaluate leaf spot infection (1 = an absence of leaf spot spots; 9 = leaves that are entirely necrotic). Ratings were taken each week during the period of infection. Individual ratings and an average of all ratings were provided. Experimental design was a randomized complete block with three replications (M. Rekoske and J. Miller, personal communication, 2007).

FC220 and FC221 perform similarly when challenged by *C. beticola*. Both in 2004 when the populations were under development and in 2006 when the released germplasm were tested, they had significantly less resistance than the tolerant checks (Table 6). Although significantly more resistant than the susceptible checks, they were significantly better than some but not all of the moderately susceptible checks (Table 6). These germplasm are moderately susceptible to CLS.

Field Performance and Sugar Yield

FC220 and FC221 have shown favorable yield characteristics when evaluated as lines and pollinators for experimental (test-cross) hybrids in five trials grown under rhizomania conditions at Salinas in 2006 (Table 7). Because the frequency of the *Rz1* allele that conditions resistance to BNYVV was less than 100% and different between these two lines, the relative performance of these lines may have been confounded in these trials. Under these conditions, FC220, both as a line and in two experimental hybrid combinations, always had a slight to significantly higher sucrose concentration than FC221. Compared with SP7322-0 (increased from SP 6322-0, PI 615525), a widely known and used breeding line without resistance to rhizomania, both sugar yield and sucrose concentration of FC220 and FC221 were significantly higher (Table 7) (Coe and Hogaboam, 1971). In the experimental hybrid combination with rhizomania-resistant C833-5CMS (*Rz1Rz1*), the sugar yield of both hybrids was not significantly different from the mean of two widely grown rhizomania-resistant commercial hybrids, but the sucrose concentrations were significantly higher. In experimental hybrid combinations with rhizomania susceptible C790-15CMS *(rzrz*), in which not all hybrid plants carry *Rz1_*, the sugar yield was not significantly lower than the mean of four commercial hybrids with resistance to rhizomania and equal for sugar content (Table 7).

Availability

Breeder seed of FC220 and FC221 is maintained by USDA–ARS and will be provided in quantities sufficient for reproduction on written request to Sugarbeet Research, USDA–ARS, Crops Research Laboratory, 1701 Centre Ave., Fort Collins, CO 80526-2083. Seed of these releases will be deposited in the National Plant Germplasm System, where it will be available for research purposes, including development and commercialization of new cultivars. We request that appropriate recognition be made of the source when this germplasm contributes to a new cultivar. U.S. Plant Variety Protection will not be requested for FC220 and FC221.

Table 5. Aphanomyces root rot resistance evaluation in the Betaseed, Inc., nurseries at Shakopee, MN, in 2004 and 2006 (FC220 = 03/05-FC1030-15 and FC221 = 03/05-FC1030-16). Experimental design was a randomized complete block with three replications of two-row plots, 3 m long.

Variety	Source	2006			2004
		First evaluation†	Second evaluation	Mean	Evaluation
05-FC1030-15(Sp)	03-FC1030-15*aa* × *A*	3.5	3.7	3.6	
05-FC1030-16(Sp)	03-FC1030-16*aa* × *A*	3.8	3.5	3.7	
Beta 4430R	Betaseed, Inc.	3.5	3.2	3.3	
Moderately susceptible check	Betaseed, Inc.	4.3	5.3	4.8	
Susceptible check	Betaseed, Inc.	5.5	6.7	6.1	
Tolerant check	EL-SP7322-0	2.0	1.2	1.6	
Tolerant check	Monohikari	3.7	3.7	3.7	
03-FC1030-15	Increase 01-FC1030-15				4.0
03-FC1030-16	Increase 01-FC1030-16				3.3
Beta 4430R	Betaseed, Inc.				2.0
Moderately susceptible check 2	Betaseed, Inc.				5.8
Susceptible check	Betaseed, Inc.				8.5
Tolerant check	EL-SP7322-0				1.0
Tolerant check	Monohikari				2.8
$LSD_{0.05}$		1.31	1.47	1.31	1.06
CV (%)		23.41	25.72	23.16	19.4

†A visual 1 to 9 rating scale used to evaluate aphanomyces root rot damage was based on stand and plant health. A rating of 1 is a complete stand of healthy beets, and a rating of 9 has no surviving plants.

Table 6. Evaluation for resistance to cercospora leaf spot in the Betaseed, Inc., nursery at Rosemount, MN, in 2004 and 2006 (FC220 = 03/05-FC1030-15 and FC221 = 03/05-FC1030-16). Experimental design was a randomized complete block with three replications of two-row plots 3 m long.

Variety	Description	2006		2004	
		Last evaluation†‡	Mean	Last evaluation	Mean
05-FC1030-15(Sp)	Increase 03-FC1030-15*aa* × *A*	6.9	4.8		
05-FC1030-16(Sp)	Increase 03-FC1030-16*aa* × *A*	7.4	4.7		
HM-E17	(MI hybrid) tolerant check	5.9	4.0		
EL-SP22-0	(EL-SP7322-0) tolerant check	5.9	3.8		
Beta 4430R	Susceptible check	8.6	6.4		
Monohikari	Moderately susceptible check	7.3	5.0		
Betaseed	Susceptible check	9.1	6.6		
Betaseed	Tolerant check	4.5	3.0		
03-FC1030-15	Increase 01-FC1030-15			4.7	3.2
03-FC1030-16	Increase 01-FC1030-16			5.3	3.3
Beta 4430R	Susceptible check			7.0	4.5
EL-SP22-0	(EL-SP7322-0) tolerant check			4.3	2.8
Monohikari	Moderately susceptible check			5.3	3.5
Betaseed	Moderately susceptible check			4.3	3.1
Betaseed	Susceptible check			7.7	4.0
Betaseed	Tolerant check 1			2.7	1.6
	$LSD_{0.05}$	0.79	0.49	0.85	0.5
	CV (%)	7.97	7.45	10.3	9.6

†The last reading is usually most severe of the epiphytotic.

‡The visual score was based on the KWS rating system, where 1 = absence of leaf spot spots and 9 = leaves entirely necrotic.

Table 7. Sugar yield and sucrose content of FC220 (05-FC1030-15), FC221 (05-FC1030-16), their test-cross hybrids, and checks when grown under rhizomania conditions in five yield trials at Salinas, CA, in 2006.

Variety	Sugar yield[†]	% Sugar[‡]	Apparent purity[§]	Rhizomania	
				Canopy[¶]	DI[#]
	kg ha^{-1}				
Yield trial 2006 (Test 1906)[††]					
05-FC1030-15(Sp)	9,740	16.2	78.1	2.4	
05-FC1030-16(Sp)	10,080	15.5	79.5	2.9	
SP7322-0[‡‡]	6,270	13.0	78.1	3.9	
$LSD_{0.05}$	940	0.6	2.0	0.5	
CV	9	3.8	2.6	24.2	
Yield trial 2006 (Test 3106)[§§]					
05-FC1030-15(Sp)	11,870	17.9	83.1	2.7	3.9
05-FC1030-16(Sp)	11,650	16.8	84.5	2.8	4.7
05-FC1030-15H5O[¶¶]	11,820	17.9	84.9	2.7	3.9
05-FC1030-16H5O[¶¶]	11,610	16.8	85.8	2.8	4.9
SP7322-0	7,170	14.9	83.3	4.6	5.2
Commercial hybrids[##]	14,590	17.4	87.5	2.0	3.0
Commercial hybrids[†††]	8,660	16.6	85.6	3.7	4.9
$LSD_{0.05}$	2,130	0.8	3.2	0.6	0.5
CV	16	4.3	3.4	25.2	11.2

Variety	Sugar yield[†]	% Sugar[‡]	Apparent purity[§]	Rhizomania	
				Canopy[¶]	DI[#]
	kg ha^{-1}				
Yield trial 2006 (Test 3206)[‡‡‡]					
05-FC1030-15(Sp)	10,720	18.1	84.2	2.0	3.9
05-FC1030-16(Sp)	10,090	16.7	84.0	3.3	5.2
SP7322-0	6,690	14.8	84.8	4.8	5.4
$LSD_{0.05}$	2,430	1.1	2.6	0.8	0.5
CV	14	4.6	3.1	31.0	9.6
Yield trial 2006 (Test 1806)[§§§]					
05-FC1030-15H5[¶¶¶]	13,770	16.6	79.8	1.6	
05-FC1030-16H5[¶¶¶]	13,280	16.3	79.9	1.9	
Commercial hybrids[###]	12,600	15.2	81.1	1.5	
Test mean[††††]	12,530	15.6	78.7	1.6	
$LSD_{0.05}$	1,370	0.7	3.1	1.5	
CV	11	5.0	4.1	0.5	
Yield trial 2006 (Test 2006)[‡‡‡‡]					
05-FC1030-15H50	10,780	16.1	79.4	2.1	
05-FC1030-16H50	11,050	16.0	80.7	2.5	
Commercial hybrids[§§§§]	11,640	15.9	81.5	1.4	
Test mean[¶¶¶¶]	11,790	16.3	79.7	1.7	
$LSD_{0.05}$	950	0.5	1.5	0.5	
CV	8	3.2	2.0	26.9	

[†]Sugar yield = root yield × % sugar.

[‡]Percentage of fresh weight.

[§]Apparent purity = raw juice apparent purity [(% sugar/% total soluble solids)100].

[¶]Rhizomania canopy scores (associated with rhizomania severity) were taken just before harvest. 1–5 scale, where 1 = dark green, 2 = green, 3 = light green, 4 = mostly yellow, and 5 = 100% uniformly yellow.

[#]DI, rhizomania disease index, where individual roots were scored for rhizomania on a 0–9 scale, where 0 = no visual evidence of disease, 5 = classical symptoms of rhizomania, and 9 = dead.

[††]One-row plots, 6.7 m, randomized complete block experimental design (RCB), 8 replications, planted 5 May 2006, , harvested 3 Oct. 2006.

[‡‡]SP7322–0 is the rhizomania-susceptible check.

[§§]One-row plots, 3.3 m, RCB, 6 replications planted 5 May 2006, harvested 20 Nov. 2006.

[¶¶]Experimental hybrids of FC220 [05-FC1030-15(Sp)] and FC221 [05-FC1030-16(Sp)] test crossed to monogerm, *rzrz* tester C790-15CMS.

[##]Mean of rhizomania-resistant hybrids, Beta 4430R and HH142.

[†††]Mean of rhizomania-susceptible hybrids, ACH555, MH-E17, Monohikari, and Roberta.

[‡‡‡]One-row plots, 3.3 m, RCB, 4 replications, planted 5 May 2006, harvested 22 Nov. 2006.

[§§§]One-row plots, 6.7 m, RCB, 8 replications, planted 4 May 2006, harvested 28 Sept. 2006.

[¶¶¶]Experimental hybrids of FC220 and FC221 test crossed to monogerm, *Rz1Rz1* tester, C833-5CMS.

[###]Mean of commercial hybrids Beta 4430R and Phoenix.

[††††]Mean of the 24 hybrid entries in this test.

[‡‡‡‡]One-row plots, 6.7 m, RCB, 8 replications, planted 4 May 2006, harvested 28 Sept. 2006.

[§§§§]Means of commercial hybrids Beta 4430R, Phoenix, HH142, and Beta 4309R.

[¶¶¶¶]Mean of 48 experimental hybrid entries.

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