

## Registration of SR98 Sugarbeet Germplasm with Resistances to Rhizoctonia Seedling and Crown and Root Rot Diseases

J. Mitchell McGrath,\* Linda E. Hanson, and Lee Panella

### ABSTRACT

Sugarbeet (*Beta vulgaris* L.) germplasm SR98 (Reg. No. GP-287; PI 655951) was developed and released by the USDA–ARS at East Lansing, MI, in cooperation with the Beet Sugar Development Foundation, Denver, CO, and Michigan State University AgBioResearch, East Lansing, MI, to provide improved resistance to seedling disease and crown and root rot caused by *Rhizoctonia solani* Kühn in a smooth-root genetic background that contributes to low soil tare. Previous smooth-root releases have been highly susceptible to diseases caused by *R. solani*. SR98 was derived from Rhizoctonia-resistant germplasm released from the USDA–ARS sugarbeet germplasm enhancement programs at Ft. Collins, CO, and East Lansing, MI, and has shown good yield potential in agronomic trials and moderate resistance to *Aphanomyces* blackleg (caused by *Aphanomyces cochlodes* Drechs.) and *Cercospora* leaf spot (caused by *Cercospora beticola*, Sacc.). SR98 can be used as a pollinator for hybrid production or a population from which breeders can select pollinators for developing Rhizoctonia-resistant hybrids adapted to the Great Lakes growing region.

SIX SMOOTH-ROOT (SR) germplasm of sugarbeet (*Beta vulgaris* L.) have been released since 1990, each with improvements in sugar yield and/or disease resistance (Theurer, 1993; Saunders et al., 2000; McGrath, 2003; McGrath and Lewellen, 2004). The smooth-root trait reduces soil tare by approximately 50% relative to traditional sugarbeet germplasm, that is, soil adhered to the root at harvest, transported to the factory, whose disposal is often considered an industrial waste. However, they are susceptible to Rhizoctonia seedling disease and to crown and root rot (CRR) disease, both caused by *Rhizoctonia solani* Kühn.

Rhizoctonia CRR is problematic in many growing regions, and germplasm enhancement efforts over the past 40 yr have resulted in USDA–ARS germplasm releases with improved resistance (e.g., Panella, 1999). Poor emergence and stand establishment is also a perennial problem for sugarbeet growers, especially in the Great Lakes growing area. On average, 60% of planted seeds emerge, with wide variations in emergence depending on planting time, moisture availability, cultivar, and disease pressure. Fungal pathogens that are isolated frequently from diseased seedlings include *Rhizoctonia solani* Anastomosis Groups (AG) 2-2 and AG 4, *Aphanomyces* spp., and *Fusarium* spp., with *Rhizoctonia solani* AG 2-2 being the predominant pathogen isolated from diseased seedlings in Michigan over the past 5 yr (82% of isolates), either alone or in combination with other seedling pathogens (Kikkert et al., 2010; Hanson and McGrath, 2011; Llamas et al., 2012).

The same organism, *Rhizoctonia solani* AG 2-2, causes both Rhizoctonia seedling and CRR diseases. Seedling resistance to Rhizoctonia seedling disease (i.e., damping-off) was discovered in the germplasm release EL51 (PI 598074) (Nagendran et al., 2009), which was initially intensively selected for resistance to Rhizoctonia CRR (Halloin et al., 2000). The major difference in the etiology of these diseases is that the seedling phase is acute,

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Journal of Plant Registrations  
doi:10.3198/jpr2013.08.0052crg

Received 25 Aug. 2013. Accepted 6 Nov. 2014.

Registration by CSSA.

5585 Guilford Rd., Madison, WI 53711 USA

\*Corresponding author (mitchmcg@msu.edu)

J.M. McGrath and L.E. Hanson, USDA–ARS, Sugarbeet and Bean Research Unit, 494 PSSB, Michigan State Univ., East Lansing, MI 48824; L. Panella, USDA–ARS, Crops Research Lab., 1701 Centre Ave., Fort Collins, CO 80526. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

**Abbreviations:** AG, Anastomosis Group; CRR, crown and root rot; EL, East Lansing; FC, Fort Collins; RCB, randomized complete block; SR, smooth root.

leading to loss of stand, while the adult phase is chronic, leading in most cases to a harvestable but lower-yielding crop. Until recently, few measures were available to control *Rhizoctonia* seedling disease. Strobilurin fungicides applied at planting or early in the season reduced seedling disease incidence by 25 to 95% relative to untreated checks in 2011 (Michigan Sugar Co., 2011). However, control is relatively short-lived and does not protect against the chronic, mature-root, crown and root rot phase of the disease. To date, germplasm exhibiting *Rhizoctonia* seedling disease resistance also exhibit resistant to *Rhizoctonia* CRR. Thus, deployment of seedling resistance is likely to confer season-long genetic protection, in addition to preserving stands that are essential for profitability of the crop.

An urgent need exists for *Rhizoctonia*-resistant germplasm with better sucrose yield and agronomic characteristics, particularly for the unique challenges of the Great Lakes growing region. The *Rhizoctonia* disease resistant EL51 is unacceptable for sucrose yield, and the genetic base of EL51 was derived from a few breeding lines that were intensively reselected for six generations in the East Lansing inoculated CRR nursery (Hallowin et al., 2000). Breeding of SR98 (Reg. No. GP-287; PI 655951) was initiated from EL51 to develop a smooth-rooted, agronomically acceptable sugarbeet germplasm with resistance to *Rhizoctonia* seedling disease and resistance to *Rhizoctonia* CRR.

## Methods

General breeding methods for sugarbeet germplasm enhancement, including general methods used for the East Lansing program, have been summarized (Panella et al., 2008; McGrath, 2011), and these methods were used in developing SR98. Initial breeding stock consisted of mother roots selected from the *Rhizoctonia* CRR nursery in East Lansing, MI (Table 1), and subsequent selections from the progeny of this population were from mother roots harvested from the *Rhizoctonia* seedling disease nursery in East Lansing. Weed control followed the micro-rate methods of Bollman and Sprague (2007) as amended (McGrath, 2011). Seed was produced in the East Lansing greenhouses as per Panella et al. (2008) with the minor modification in that only field nursery-harvested mother roots were used.

All Michigan nurseries were planted at 76-cm row spacing in plots 7.3 m long, either at the Saginaw Valley Bean and Beet Farm in Saginaw, MI, or on the campus of Michigan State University in East Lansing, following standard industry agronomic practices (Anonymous, 2011). Nurseries were planted between 25 April and 15 May of each year.

The Fort Collins *Rhizoctonia* CRR nursery methods followed those described by Panella et al. (2008), with a one-row, five-replication, randomized complete block (RCB) design. An established root disease index using a scale of 0 = healthy to 7 = dead was calculated from individual roots, summed across each class (0–7) per plot, and transformed (arcsin-square root) for calculating (i) overall disease reaction and (ii) machine-harvestable roots (Ruppel et al., 1979; Panella et al., 2008). Classes 0 to 3 are considered machine harvestable, and results here are expressed as a percentage of machine-harvestable roots (i.e., roots likely to be delivered to a processor for sucrose extraction).

*Rhizoctonia* seedling disease reaction was evaluated in East Lansing in one-row plots in a breeding nursery. Formal nursery methods for *Rhizoctonia* seedling disease reaction have not been developed, in part due to the recent description of *Rhizoctonia* seedling disease resistance in sugarbeet, as well as the lack of available resistant germplasm to use as check cultivars. Thus, results described herein reflect the developmental nature of this methodology. Logistically, the difference between seedling and CRR resistance testing was the timing of inoculation (for CRR, plants at the ~14- to 18-leaf growth stage are inoculated). Seedlings were inoculated at the two- to four-true leaf growth stage with *R. solani* AG 2-2 isolate R1-infested ground barley inoculum at a rate of 1.0 g m<sup>-1</sup> of row using a spreader as described by Hanson and McGrath (2011). Experimental units were blocked into similar soil types, and only entries with full stands before inoculation (defined as 20 or more plants per plot, across all plots of that entry) were evaluated. Disease was evaluated 30 d after inoculation as the number of surviving plants per plot, where wilted seedlings were excluded from the surviving plant counts. It should be noted that *Rhizoctonia* diseases are progressive and continue throughout the season, and such wilted seedlings do not survive until harvest.

Cercospora leaf spot (caused by *Cercospora beticola*, Sacc.) evaluation nurseries were at the Saginaw Valley Bean and Beet Farm in one-row, three-replication, RCB design, plant-to-stand trials with 125 seedballs per plot as described by Hanson et al. (2011). Aphanomyces disease testing was done at the Betaseed, Inc. nursery in Shakopee, MN, Fusarium disease testing was done at the Betaseed, Inc. nursery in Sabin, MN, and rhizomania (caused by *Beet necrotic yellow vein virus*) disease testing was done by R. Lewellen (USDA–ARS retired) in Salinas, CA, as described by Panella et al. (2008). Agronomic evaluations were conducted at the Saginaw Valley Bean and Beet Farm in two-row plots with four replications in a RCB design, which were manually thinned to approximately 20 cm between seedlings by 15 June of each year. Total root weights were obtained, and 15

**Table 1. Parental germplasm used and their numerical mother root contribution used in development of SR98.**

| Source germplasm        | PI         | Citation                                | No. of mother roots |
|-------------------------|------------|---|---------------------|
| EL51                    | 598074     | Hallowin et al., 2000                   | 14                  |
| EL0204                  | 632750     | McGrath and Lewellen, 2004              | 7                   |
| FC705/1                 | 590754     | Hecker and Ruppel, 1985                 | 2                   |
| FC709                   | 598641     | Hecker and Ruppel, 1988                 | 6                   |
| FC712                   | 590766     | Hecker and Ruppel, 1986                 | 2                   |
| FC724                   | 632251     | Panella and Hanson, 2004                | 2                   |
| SR donors (96N7, 95HS3) | unreleased | unpublished                             | 5                   |
| SP6322 × fodder         | unreleased | Coe and Hogaboam, 1971; and unpublished | 2                   |

roots were subsampled for sucrose and water content analyses (McGrath and Fugate, 2012).

SR98 was developed from diverse germplasm channeled through a continued population enhancement effort to increase genetic diversity in USDA–ARS sugarbeet germplasm releases and foster recombination among advanced public sugarbeet breeding materials. Parent materials and their donor proportion contributing to SR98 are listed in Table 1. Rhizoctonia resistance was contributed by germplasm releases EL51, FC705/1, FC709-2, FC712, and FC724, and smooth rootedness was contributed by germplasm release EL0204 and unreleased SR breeding materials (specifically, breeding lines 96N7 and 95HS3), which also contributed to the development of other released SR germplasm. A small percentage (5%) of SR98's parentage included a hybrid between SP6322 (PI 615525, Coe and Hogaboam, 1971) and an unspecified fodder beet variety grown in the same mother root selection nursery. SR98 is diploid, self-sterile, multigerm, and biennial.

Parental materials were selected as mother roots, that is, the first year's growth that is harvested, stored, and replanted for the second year of the biennial life cycle, under conditions favoring Rhizoctonia CRR development in the 2002 East Lansing Rhizoctonia nursery (i.e., late inoculation). Mother root selections, based on freedom from disease, were open pollinated as an isolated group in the 2003 East Lansing greenhouse. Seed was harvested from individual plants, and seed from plants derived from the same source was combined, with the exception that seed from the 12 Fort Collins-released mother roots (prefixed "FC" in Table 1) were combined and designated EL-A013703. The EL-A013703 population was planted in the 2007 East Lansing Rhizoctonia nursery, which was artificially inoculated at the seedling stage to incite Rhizoctonia seedling disease (i.e., early inoculation). Ten mother roots with freedom from disease were selected from a population of 60 surviving

EL-A013703 individuals at the end of the season. Seed was produced from interpollinating these 10 mother roots in isolation in the 2008 greenhouse, and this seed was designated and tested as EL-A023047 and released as SR98.

## Characteristics

SR98 showed high levels of resistance to Rhizoctonia CRR in both the 2007 and 2008 Fort Collins disease nursery. The ANOVA indicated significant variation among the experimental breeding lines and check entries (Table 2). SR98 was significantly more resistant than the susceptible check in both years, significantly more resistant than the resistant check in 2007 but not in 2008, and not significantly different than the highly resistant check in either year, as indicated by percentage harvestable roots (Table 2).

When evaluated for 30-d post-inoculation stand in the East Lansing Rhizoctonia seedling disease inoculated nursery in 2007 and 2008, where ANOVA showed significant variation existed, SR98's mean post-inoculation stand was not significantly different than the resistant check EL51 in either year and was significantly different from the susceptible checks in both years (Table 3). Germplasm EL51 is the only germplasm to date to have demonstrated Rhizoctonia seedling disease resistance (Nagendran et al., 2009). Field performance of SR98, as measured by the persistence of good stands 30 d after inoculation at the two- to four-true leaf growth stage, was not significantly different than EL51, while the stands of the susceptible SR donor lines were reduced by approximately 90% (Table 3).

Agronomic performance of SR98 was tested in trials at the Saginaw Valley Bean and Beet Farm in Saginaw, MI, in 2007 and 2008, which included 48 and 35 entries in total, respectively. SR98 was significantly lower yielding than the commercial check B5833R, in 2007 but not significantly

**Table 2. Reaction of SR98 in the Ft. Collins, CO, Rhizoctonia crown and root rot nursery. Means were extracted from a larger dataset that contained 44 experimental entries in 2007 and 60 experimental entries in 2008.**

| Year | Entry                            | % Harvestable roots |           |            |            |                |
|------|----------------------------------|---------------------|-----------|------------|------------|----------------|
| 2007 | SR98                             | 81.1                |           |            |            |                |
|      | Highly resistant check (FC705/1) | 81.5                |           |            |            |                |
|      | Resistant check (FC709-2)        | 56.4                |           |            |            |                |
|      | Susceptible check (FC901/C817)   | 45.8                |           |            |            |                |
|      | Trial mean                       | 47.9                |           |            |            |                |
|      | LSD (0.05)                       | 20.0                |           |            |            |                |
|      |                                  | <b>ANOVA source</b> | <b>df</b> | <b>SS†</b> | <b>MS†</b> | <b>F ratio</b> |
|      | Entry                            | 47                  | 69,729.8  | 1483.6     | 5.78       | <0.001         |
|      | Error                            | 172                 | 44,149.9  | 256.7      |            |                |
|      | Corrected total                  | 219                 | 113,879.8 |            |            |                |
| 2008 | SR98                             | 76.7                |           |            |            |                |
|      | Highly resistant check (FC705/1) | 73.7                |           |            |            |                |
|      | Resistant check (FC709-2)        | 63.5                |           |            |            |                |
|      | Susceptible check (FC901/C817)   | 36.1                |           |            |            |                |
|      | Trial mean                       | 38.6                |           |            |            |                |
|      | LSD (0.05)                       | 21.3                |           |            |            |                |
|      |                                  | <b>ANOVA source</b> | <b>df</b> | <b>SS</b>  | <b>MS</b>  | <b>F ratio</b> |
|      | Entry                            | 63                  | 54,519.4  | 865.4      | 2.96       | <0.001         |
|      | Error                            | 236                 | 69,012.9  | 292.4      |            |                |
|      | Corrected total                  | 299                 | 123,532.3 |            |            |                |

† SS, sum of squares; MS, mean square.

**Table 3. Mean 30-d post-inoculation stand count of SR98 following inoculation of two- to four-leaf seedlings.**

| Year | Entry                      | No. of plots        | Mean post-inoculation stand |           |            |            |                |
|------|----------------------------|---------------------|-----------------------------|-----------|------------|------------|----------------|
| 2007 | SR98                       | 2                   | 30.0                        |           |            |            |                |
|      | EL51 (resistant check)     | 4                   | 25.5                        |           |            |            |                |
|      | Susceptible SR check       | 2                   | 2.0                         |           |            |            |                |
|      | Trial mean                 |                     | 20.8                        |           |            |            |                |
|      | LSD (0.05)                 |                     | 10.6                        |           |            |            |                |
|      |                            | <b>ANOVA source</b> |                             | <b>df</b> | <b>SS†</b> | <b>MS†</b> | <b>F ratio</b> |
|      | Entry                      |                     | 2                           | 964.5     | 482.3      | 28.37      | 0.002          |
|      | Error                      |                     | 5                           | 85.0      | 17.0       |            |                |
|      | Corrected total            |                     | 7                           | 1049.5    |            |            |                |
| 2008 | SR98                       | 7                   | 25.7                        |           |            |            |                |
|      | EL51 (resistant check)     | 4                   | 21.0                        |           |            |            |                |
|      | Susceptible check (SP7322) | 4                   | 2.5                         |           |            |            |                |
|      | Trial mean                 |                     | 18.3                        |           |            |            |                |
|      | LSD (0.05)                 |                     | 5.5                         |           |            |            |                |
|      |                            | <b>ANOVA source</b> |                             | <b>df</b> | <b>SS</b>  | <b>MS</b>  | <b>F ratio</b> |
|      | Entry                      |                     | 2                           | 1412.5    | 706.3      | 54.88      | <0.0001        |
|      | Error                      |                     | 12                          | 154.4     | 12.9       |            |                |
|      | Corrected total            |                     | 14                          | 1566.9    |            |            |                |

† SS, sum of squares; MS, mean square.

different in 2008 (Tables 4 and 5). The reverse was true when compared with the commercial check E17. When averaged over years, SR98 produced 73% of the yield of hybrid B5833R and 83% of hybrid E17. Dry matter content expressed as a percentage of fresh weight of SR98 was 93.7% of the check hybrids. In 2008, the only year in which sucrose content was measured, percentage sucrose of SR98 on fresh weight basis was 94.1% of checks, and dry matter expressed as a percentage of fresh weight was 91.1% of checks. Sucrose percentage of SR98 expressed as a percentage of dry matter was 103.3% of hybrid checks.

SR98 was evaluated for disease reaction in cooperator nurseries. Reaction to *Cercospora beticola*, the causal agent of Cercospora leaf spot, was moderate in the 2007 and 2008 Cercospora leaf spot nurseries at the Saginaw Valley Bean and Beet Farm in Saginaw, MI (2007: SR98 Disease Index [0 = no disease, 9 = dead] = 3.3, vs. highly resistant and susceptible checks of 1.7 and 5.3, respectively; 2008: SR98 Disease Index = 3.7 relative to the highly resistant and susceptible checks of 2.1 and 4.7, respectively). In 2008 only, SR98 was tested in the Betaseed, Inc. Aphanomyces nursery in Shakopee, MN, with

an average Disease Index of 3.3 relative to the resistant and susceptible checks of 1.5 and 4.3, respectively. Also in 2008, SR98 was tested in the Betaseed, Inc. Fusarium nursery in Sabin, MN, with Disease Index of 1.4 (0 = no disease, 9 = dead) compared with the resistant and susceptible checks of 2.1 and 6.9, respectively. SR98 had no appreciable resistance to rhizomania as tested in the 2008 Salinas, CA, rhizomania nursery.

SR98 is being released as a germplasm source for breeders to use in developing parental lines with resistance to Rhizoctonia crown and root rot that combines smooth rootedness with higher levels of Rhizoctonia CRR resistance than is currently available in current USDA-ARS smooth root germplasm releases, as well as a source of resistance to Rhizoctonia seedling disease. Genetic material of SR98 has been deposited in the National Plant Germplasm System, where it will be available for research purposes, including development and commercialization of new cultivars. It is requested that the authors be notified if this germplasm contributes to the development of a new breeding line or cultivar.

**Table 4. Agronomic performance of SR98 conducted at the Saginaw Valley Bean and Beet Farm, Saginaw, MI: yield component means. Means were extracted from a larger data set that contained 48 entries in 2007 and 35 entries in 2008.**

| Year | Entry      | Tonne/hectare | Sucrose (% FW)† | DM (% FW) | Water (%) | Sucrose (% of DM)† |
|------|------------|---------------|-----------------|-----------|-----------|--------------------|
| 2007 | SR98       | 66.6          | –               | 19.9      | 80.1      | –                  |
|      | B5833R     | 93.9          | –               | 21.2      | 78.8      | –                  |
|      | E17        | 71.3          | –               | 21.3      | 78.7      | –                  |
|      | Trial mean | 68.3          | –               | 19.0      | 77.6      | –                  |
|      | LSD (0.05) | 14.1          | –               | 1.7       | 1.9       | –                  |
| 2008 | SR98       | 44.0          | 16.8            | 21.2      | 78.8      | 79.4               |
|      | B5833R     | 57.9          | 17.9            | 23.4      | 76.6      | 76.7               |
|      | E17        | 62.5          | 17.8            | 23.1      | 76.9      | 77.0               |
|      | Trial mean | 50.2          | 17.9            | 22.4      | 77.6      | 79.91              |
|      | LSD (0.05) | 14.8          | 1.7             | 1.9       | 1.9       | 4.24               |

† FW, fresh weight; DM, dry matter.



**Table 5. Agronomic performance of SR98 conducted at the Saginaw Valley Bean and Beet Farm, Saginaw, MI: ANOVA table. Means were extracted from a larger data set that contained 48 entries in 2007 and 35 entries in 2008.**

| Trait†            | ANOVA source    | 2007 |          |       |         |          | 2008 |        |      |         |          |
|-------------------|-----------------|------|----------|-------|---------|----------|------|--------|------|---------|----------|
|                   |                 | df   | SS‡      | MS‡   | F ratio | Prob > F | df   | SS     | MS   | F ratio | Prob > F |
| Tonne/hectare     | Entry           | 47   | 9,414.2  | 200.3 | 9.97    | <0.0001  | 34   | 710.1  | 20.9 | 0.938   | 0.57     |
|                   | Error           | 144  | 2,892.5  | 20.1  |         |          | 105  | 2337.0 | 22.3 |         |          |
|                   | Corrected total | 191  | 12,306.7 |       |         |          | 139  | 3047.1 |      |         |          |
| Sucrose (% FW)    | Entry           | –§   | –        | –     | –       | –        | 34   | 71.6   | 2.1  | 1.508   | 0.06     |
|                   | Error           | –    | –        | –     |         |          | 105  | 146.7  | 1.4  |         |          |
|                   | Corrected total | –    | –        |       |         |          | 139  | 218.3  |      |         |          |
| DM (% FW)         | Entry           | 47   | 130.0    | 2.8   | 1.85    | 0.003    | 34   | 135.9  | 4.0  | 2.192   | 0.00     |
|                   | Error           | 144  | 215.0    | 1.5   |         |          | 105  | 191.5  | 1.8  |         |          |
|                   | Corrected total | 191  | 345.0    |       |         |          | 139  | 327.4  |      |         |          |
| Water (%)         | Entry           | 47   | 130.0    | 2.8   | 1.85    | 0.003    | 34   | 135.9  | 4.0  | 2.192   | 0.00     |
|                   | Error           | 144  | 215.0    | 1.5   |         |          | 105  | 191.5  | 1.8  |         |          |
|                   | Corrected total | 191  | 345.0    |       |         |          | 139  | 327.4  |      |         |          |
| Sucrose (% of DM) | Entry           | –    | –        | –     | –       | –        | 34   | 502.5  | 14.8 | 1.618   | 0.03     |
|                   | Error           | –    | –        | –     |         |          | 105  | 959.2  | 9.1  |         |          |
|                   | Corrected total | –    | –        |       |         |          | 139  | 1461.7 |      |         |          |

† FW, fresh weight; DM, dry matter.

‡ SS, sum of squares; MS, mean square.

§ Data not obtained.

## Acknowledgments

Contributions of the Beet Sugar Development Foundation and the support of its member companies to the USDA–ARS East Lansing, MI, program, as well as support of the Michigan State University AgBioResearch (formerly Michigan Agricultural Experiment Station), is gratefully acknowledged. Thanks are also extended to Timothy M. Duckert for his expert technical assistance. Efforts of Dr. Robert Lewellen of the USDA–ARS, J. Miller and M. Rekoske of Betaseed, Inc., and S. Shaw, T. Goodwill, and R. Naegele at East Lansing in providing valuable disease nursery and agronomic testing assistance is gratefully acknowledged.

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