

Registration of Three Germplasm Lines of Cotton Derived from *Gossypium barbadense* L. Accession GB713 with Resistance to the Reniform Nematode

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ABSTRACT

Three upland cotton (*Gossypium hirsutum* L.) germplasm lines, M713 Ren1 (Reg. No. GP-958, PI 665928), M713 Ren2 (Reg. No. GP-959, PI 665929), and M713 Ren5 (Reg. No. GP-960, PI 665930) resistant to the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira were developed and jointly released by the USDA-ARS and the Mississippi Agricultural and Forestry Experiment Station in 2012. The day-neutral, reniform-resistant germplasm lines originated from the photoperiodic *G. barbadense* L. accession GB713 and were selected using the tightly linked simple sequence repeat (SSR) markers GH 132, BNL 3279, and BNL 569. Egg production of the reniform nematode was suppressed to approximately 90% below that of the susceptible check, 'Deltapine 61' in greenhouse tests. The lines exhibit considerable differences for agronomic and fiber traits such as length, strength and micronaire, and with their high level of resistance they should be valuable to cotton breeding programs. The successful use of marker assisted selection for these specific SSR markers further validates their use in the selection of resistant plants in segregating generations.

Three upland cotton (*Gossypium hirsutum* L.) germplasm lines, M713 Ren1 (Reg. No. GP-958, PI 665928), M713 Ren2 (Reg. No. GP-959, PI 665929), and M713 Ren5 (Reg.

No. GP-960, PI 665930) resistant to the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira were developed and jointly released by USDA-ARS and the Mississippi Agricultural and Forestry Experiment Station in 2012. The three germplasm lines were derived from a cross between 'Sure-Grow 747' and GB713, a photoperiodic *G. barbadense* accession with subsequent backcrossing to Sure-Grow 747. After screening several hundred *G. barbadense* accessions, Robinson et al. (2004) reported that GB713 was resistant to the reniform nematode. Gutierrez et al. (2011) reported that reniform nematode resistance in GB713 is significantly associated with three QTLs: *Ren^{barb1}* and *Ren^{barb2}*, located on chromosome 21, and *Ren^{barb3}*, located on chromosome 18. The flanking simple sequence repeat (SSR)-marker loci associated with these QTLs are BNL1551_162 and GH 132_199 (*Ren^{barb1}*), BNL4011_155 and BNL3279_106 (*Ren^{barb2}*), and BNL1721_178 and BNL569_131 (*Ren^{barb3}*). Gutierrez et al. (2011) suggested that these markers could be used for the selection of resistance in segregating populations when GB713 was used in crosses.

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Abbreviations: HVI, high-volume instrument; MAS, marker-assisted selection; SSR, simple sequence repeat; UHM upper-half mean; UI, uniformity index.

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The reniform nematode has been an emerging problem in U.S. cotton and is now considered a serious pest in the southern United States (Robinson, 2007). Losses have been estimated to exceed \$100 million annually, with the greatest losses occurring in Georgia, Alabama, Mississippi, Louisiana, Arkansas, and Texas (Koenning et al., 2004; Blasingame, 2006; Blasingame and Patel, 2011). Resistant

cultivars are needed to help manage the reniform nematode; however, none of the currently available upland cultivars has appreciable levels of resistance. These germplasm lines should serve as a useful source of resistance to this nematode.

Methods

The genes for reniform nematode resistance in these three M713 lines were derived from a photoperiodic accession GB713 (PI 608139). Progeny of GB713 were screened for reniform nematode resistance in a growth chamber during the spring of 2007, and two highly resistant plants were potted and transferred to the greenhouse. When the day length was short during the winter of 2007–2008, one resistant flowering plant was crossed to Sure-Grow 747 (PI 656375 PVPO). The pedigree for Sure-Grow 747 can be found in Bowman et al., 2006. The F_1 generation was grown at the winter nursery, Tecomán, Colima, Mexico during 2008–2009 and self-pollinated to produce F_2 seeds. The F_2 population (800 plants) was grown in the field at Mississippi State, MS during the summer of 2009, and 204 day-neutral plants were selected.

Day-neutral F_2 plants were screened with the SSR markers GH 132_201, BNL3279_106, and BNL569_131. Based on marker-assisted selection (MAS), 15 F_2 plants homozygous for the three markers were selected and crossed back to Sure-Grow 747. The BC_1F_1 was grown in the greenhouse during the winter of 2009–10 and allowed to self pollinate. The 15 BC_1F_2 populations were grown in field plots during the summer of 2010. Each population, composed of at least 100 plants, was subjected to MAS with the above SSR markers linked to resistance. A total of 32 plants were selected from 10 populations (5 plants each from 4 populations, 3 plants each from 2 populations, 2 plants each from 2 populations, and 1 plant each from 2 populations) and crossed back to Sure-Grow 747. The BC_2F_1 was grown at the winter nursery during 2010–11 and self-pollinated to produce BC_2F_2 seeds. The 32 BC_2F_2 populations were grown in field plots during the summer of 2011, and 16 were selected based on early fruit production and screened with SSR markers. Three plants were selected from three populations based on MAS and on upland plant type, number of fruit present, and boll size and shape. The three plants were self-pollinated, and progeny (BC_2F_3) from these plants were increased at the winter nursery during 2011–12. Seed (BC_2F_4) of these three reniform-resistant lines are being released.

M713 Ren1 and M713 Ren2 are homozygous (SSR markers GH 132_201, BNL3279_106, BNL4011_152, and BNL569_131) for the three QTLs (*Ren barb1*, *Ren barb2*, and *Ren barb3*) associated with resistance. The line M713 Ren5 is homozygous (markers GH 132_201, BNL3279_106, and BNL4011_152) for the chromosome 21 QTLs (*Ren barb1* and *Ren barb2*) but is missing the QTL found on chromosome 18 (*Ren barb3*).

Nematode Evaluations

The three M713 germplasm lines—GB713, Deltapine 61, Sure-Grow 747—and line 713S were evaluated in two greenhouse experiments during the winter of 2011–12. Line 713S was a BC_1F_2 selection that did not have markers (GH 132,

BNL 3279, and BNL 569) associated with resistance present. Plants were grown in individual pots for 30 d in highly infested soil (~35,000 reniform nematodes 500 cm⁻³ soil) and scored on abundance of egg masses on roots using a scale of 1 (none) to 10 (high) (McCarty et al., 2012). Egg masses on roots were stained with 0.25% trypan blue as described by Sharma and Ashokkumar (1991) to facilitate scoring. Eggs from reniform nematodes were collected after root scoring and counted following the same procedure used by Gutierrez et al. (2010) to collect and count root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood] eggs. Each experiment consisted of two replications with five plants per entry per replication arranged as a randomized complete block. Scoring was conducted by two people on each plant; however, eggs were collected from all five plants per replication as one sample.

The open-bud mutant was observed following the cross of Sure-Grow 747 with GB713 in the F_2 generation. Kohel (1973) described this mutant as having flower buds open at the tip due to a shortened corolla resulting in the exposure of the stigma and upper anthers. Open-bud (*ob1ob1 ob2ob2*) results from duplicate recessive genes *ob1ob1* located on chromosome 18 in *G. barbadense* and *ob2ob2* located on chromosome 13 in *G. hirsutum* (Kohel, 1973; Endrizzi, 1975; Rhyne, 1979, Qian et al., 2009). We visually selected against open-bud and also used molecular marker BNL1721 (Qian et al., 2009) to select against *ob1ob1* on chromosome 18. Because of population size and number of plants tested, we may not have completely eliminated open-bud.

Characteristics

Nematode Resistance

The results averaged across tests showed a significantly lower mean egg-mass rating of 2.82, 2.70, and 4.00 for M713 Ren1, M713 Ren2, and M713 Ren5, respectively, compared with 9.14 for Deltapine 61 and 9.28 for Sure-Grow 747 (LSD 0.05 = 0.76). M713 Ren5 had an egg-mass rating significantly greater than M713 Ren1, M713 Ren2 and GB713. The egg-mass rating for line 713S was similar to that of the susceptible cultivars (Deltapine 61, Sure-Grow 747). Eggs per gram of root ranged from 4491 to 6625 for the three M713 lines compared to 2093 for GB713; whereas, the susceptible lines ranged from 40,312 to 52,131 (Table 1).

Agronomic Evaluations

High-volume instrument (HVI) fiber properties of the three resistant BC_2F_2 individual plants ranged as follows: upper half mean (UHM) length, 29.46–29.72 mm; uniformity index (UI), 83.4–84.8%; fiber strength, 300–367 kN m kg⁻¹; elongation, 5.9–6.4; and micronaire, 3.2–3.6. Values for Sure-Grow 747 were UHM, 28.44 mm; UI, 83.7%; strength, 297 kN m kg⁻¹; elongation, 7.0%; and micronaire, 4.6 (Table 2). A bulk sample of lint was collected from self-pollinated bolls from BC_2F_3 plants being grown for seed increase for the M713 lines at the winter nursery in Tecomán, Mexico. HVI data for these samples ranged as follows: UHM, 29.21–30.99 mm; UI, 83.4–84.5%; strength, 270–286 kN m kg⁻¹; elongation, 6.3–6.8%; and micronaire, 3.9–4.5 (Table 3).

In single-row plots at the winter nursery, plant height and over-all plant size were similar to those of the cultivar Sure-Grow 747. Morphologically, the lines resemble *G. hirsutum* in most respects even though they were derived from crosses with *G. barbadense*. All M713 lines produce white lint; however, M713 Ren5 has green seed fuzz whereas the other two lines have white seed fuzz.

Table 1. Reniform nematode resistance of three M713 germplasm lines, GB713 (source of resistance), two cultivars, and line 713S (susceptible line derived from GB713).

Entry	Resistance genotype [†]	Reniform nematode eggs	
		Egg-mass rating	Eggs per g root
		1–10 [‡]	no.
M713 Ren1	AAA	2.82 ± 0.22	4491 ± 1445
M713 Ren2	AAA	2.70 ± 0.14	5668 ± 1271
M713 Ren5	AAB	4.00 ± 0.72	6625 ± 2512
GB713	AAA	2.16 ± 0.15	2093 ± 566
Deltapine 61	BBB	9.14 ± 0.30	52131 ± 15886
Sure-Grow 747	BBB	9.28 ± 0.28	40312 ± 4290
Line 713S	BBB	8.30 ± 0.32	49936 ± 15026
LSD 0.05		0.76	19550

[†]AAA = *Ren barb1*, *Ren barb2*, and *Ren barb3*; AAB = *Ren barb1* and *Ren barb2*.

[‡]Reniform nematode egg-mass rating on roots for two tests, mean of two individual raters; 1 = none and 10 = high.

Table 2. High-volume-instrument fiber data for three reniform nematode lines and the cultivar Sure-Grow 747. Fiber from open-pollinated bolls from individual selected BC₂F₂ plants.

Entry	Upper-half mean length	Uniformity index	Strength	Elongation	Micronaire	Fiber reflectance	Fiber yellowness
	mm	%	kN m kg ⁻¹	%		Rd [†]	+b [‡]
M713 Ren1	29.46	83.4	342	6.3	3.6	—	—
M713 Ren2	29.72	83.7	300	6.4	3.2	73.9	8.8
M713 Ren5	29.46	84.8	367	5.9	3.5	76.2	7.9
Sure-Grow 747	28.44	83.7	297	7.0	4.6	73.2	7.5

[†]Rd is the percentage of the reflectance; the higher the value, the lighter the fiber color.

[‡]Hunter's +b value is a measure of increasing yellowness of fiber.

Table 3. High-volume-instrument fiber data for three reniform nematode lines and cultivars Stoneville 474 and FM958. Fiber sample from self pollinated bolls from a 15 hill unit, BC₂F₃, grown at the winter nursery in Tecomán, Mexico during 2011–12 (one single bulk sample of fiber per line).

Entry [†]	Upper-half mean length	Uniformity index	Strength	Elongation	Micronaire	Fiber reflectance	Fiber yellowness
	mm	%	kN m kg ⁻¹	%		Rd [‡]	+b [§]
M713 Ren1	30.99	84.5	286	6.8	3.9	76.4	7.9
M713 Ren2	29.71	83.5	247	6.8	4.5	74.5	7.1
M713 Ren5	29.21	83.4	270	6.3	4.2	76.4	7.3
Stoneville 474	28.70	84.5	267	7.3	5.0	73.5	4.7
Fibermax 958	30.73	85.3	300	6.5	4.0	75.2	6.5

[†]A fiber sample was not available from the winter nursery for Sure-Grow 747.

[‡]Rd is the percentage of the reflectance; the higher the value, the lighter the fiber color.

[§]Hunter's +b value is a measure of increasing yellowness of fiber.

Availability

Small amounts of seed of the three M713 germplasm lines are available for distribution to cotton breeders and other research workers until the present supply is exhausted. Written requests should be addressed to Jack C. McCarty, Crop Science Research Laboratory, P.O. Box 5367, Mississippi State, MS 39762–5367 or by email to the corresponding author. Seed of M713 Ren1, M713 Ren2, and M713 Ren5 has been deposited in the National Plant Germplasm System, where it will be available 5 yr after the date of publication for research purposes including development and commercialization of new varieties/cultivars. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new breeding line or variety.

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