HortScience 50(8):1257–1259. 2015.

Fla. 8923: A Tomato Breeding Line with Begomovirus Resistance Gene *Ty-3* in a 70-kb *Solanum chilense* Introgression

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Additional index words. Solanum lycopersicum, *Tomato yellow leaf curl virus* (TYLCV) resistance, *Tomato mottle virus* (ToMoV) resistance

Begomoviruses vectored by the sweetpotato whitefly (*Bemisia tabaci*) are a major threat to tomato (*Solanum lycopersicum*) production in many regions around the world. Of the many begomoviruses, the strains that cause *Tomato yellow leaf curl virus* (TYLCV) are the most widespread and well known. TYLCV resistant cultivars are commercially available in production regions, and most of these use the dominant *Ty-1* gene that was introgressed from *S. chilense* accession LA1969. Producers in Florida often prefer to grow susceptible cultivars because of linkage drag effects that lower marketable yield and increase foliar disease infections. The *Ty-3* gene, introgressed from *S. chilense* accessions LA2779 and LA1932, was recently found to be allelic to *Ty-1* (Verlaan et al., 2013). Linkage drag in the form of increased foliar disease and reduced yield has also been a problem in lines possessing *Ty-3*. Fla. 8923 is a large-fruited breeding line with a much reduced introgression that is free of the negative effects of linkage drag based on observations of several promising hybrids derived from it over three seasons and three locations (Hutton and Scott, unpublished data). It is potentially suitable as a parent in commercial hybrids or as a *Ty-3* donor in tomato breeding programs where begomovirus resistant cultivars are the goal.

Origin

The pedigree of Fla. 8923 is shown in Fig. 1. Resistance was derived from *S. chilense* accession LA2779 that was crossed with Campbell 28 (C28) in 1990. Two interspecific F_1s were obtained from the 66 fruit that set from this cross. The F_1s were backcrossed to Fla. 7409 and a fair amount of seed was produced, but germination was less than 2%. The backcross to Fla. 7409 was advanced to the F_7 generation) in the field after inoculation with *Tomato mottle virus* (ToMoV) and selection for resistant plants each generation. The F_7 was highly resistant but wild in appearance. Seed was sent to Judith Milo at the Hebrew University in Rehovot, Israel, who crossed the F_7 with a begomovirus-susceptible greenhouse tomato inbred and returned F_2 seed from that cross. An F_2 ToMoV-resistant selection without wild species characteristics was made and advanced to the F_4 generation. The F_4 was fixed for ToMoV resistance and crossed to Fla. 7833; selections were thereafter tested in separate trials for both ToMoV and *Tomato yellow leaf curl virus* (TYLCV). In 2002 the F_8 Fla. 8680 was crossed to Fla. 7781 (Scott and Jones, 2000) and F_2 seed were screened for molecular markers flanking the *S. chilense* introgression (Ji et al., 2007). The following map positions are based on the Tomato-EXPEN 2000 map (http://www.sgn.cornell.edu). Of 719 F_2 plants, 30 recombinants were identified and genotyped for markers between 5 and 32 cM on chromosome 6, and recombinant inbred lines (RILs) were evaluated in the field for resistance to TYLCV. All RILs having introgressions that spanned 18–25 cM showed some resistance. Two of the resistant F_5 RILs, each containing this region, were selected and used to develop fine-mapping populations; the introgression in RIL 554 spanned from 5 to 25 cM, and the introgression in RIL 157 spanned from 18 to 32 cM. These RILs were crossed to Fla. 7776 (Scott et al., 2006), and F_2 populations were developed. Of nearly 11,000 plants genotyped with flanking markers, 299 recombinants were obtained, and 114 of these had crossovers between 18 and 25 cM. Phenotyping of cuttings from these recombinants in 2009 and genotyping with newly available single nucleotide polymorphism markers developed from sequences available through the tomato genome project mapped the *Ty-3* gene to a 2.5 cM region. Further testing of informative RILs in 2010 located the gene within a 0.3 cM region (Verlaan et al., 2013). Two resistant RILs (i.e., E942-482 and E948-725), each resulting from a crossover within this 0.3 cM interval and immediately on either side of *Ty-3*, were intercrossed to develop a *Ty-3* minimal introgression (Verlaan et al., 2013, Table S3). More than 2000 F_2 plants from this cross were grown in 2010 and tested with flanking markers to identify recombinants; three plants were found to have resulted from crossovers within this region and were heterozygous for a *Ty-3* introgression less than 70 kb in size. Progeny were grown and markers were used to select for homozygosity of the reduced region; one of these plants became Fla. 8879.

Fla. 8879 was crossed to Fla 8111B (Scott, 2013), a globe-shaped tomato with large fruit size derived from Fla. 7777 (Scott et al., 2004). Fla. 8111B has a large vine and a number of good horticultural characteristics, but it is very susceptible to bacterial spot. The Fla. 8879 x Fla. 8111B F_1 was crossed to Fla. 8626, another large-fruited globe-shaped tomato inbred that also had Fla. 8111B in its

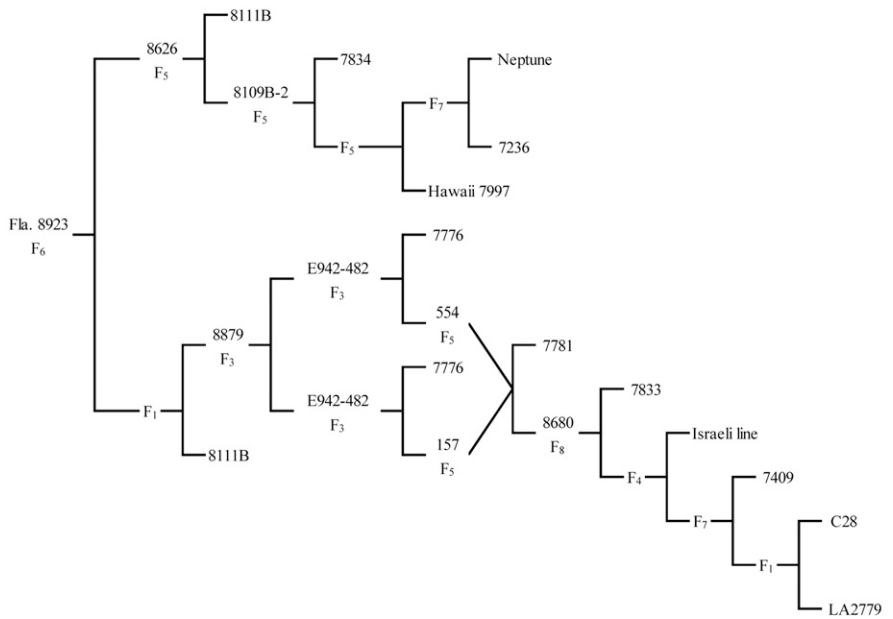

Fig. 1. Pedigree of Fla. 8923. All 7000 and 8000 numbered lines in the pedigree should have a Fla. prefix, which was left off for spacing considerations.

Received for publication 9 Apr. 2015. Accepted for publication 26 May 2015.
We thank Dolly Cummings, Tim Davis, Jose Diaz, and Rudy Jones for technical support.
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pedigree. Scott et al. (2009) reported that Fla. 8626 had resistance to bacterial wilt (*Ralstonia solanacearum*), but further testing showed this not to be the case (Scott, unpublished data). Marker assisted selection was used at the seedling stage in the F_1 and subsequent generations to identify and select plants containing *Ty-3*. In Spring 2012, an F_2 plant was selected that was heterozygous for *Ty-3*. A homozygous F_3 line was obtained with a good yield of very large fruit. Yield data were taken on more advanced selections in 2013. Seed was increased in the F_6 generation in Spring 2014 for release.

Description

Fla. 8923 has a determinate, open vine with intermediate vigor typical of most tomato vines with globe-shaped fruit. Fruit cover is fair. The deep round- (globe-) shaped fruit have jointed pedicels, shoulders are uniform green, and blossom scars are stellate and usually smooth. In two seasons of testing, total marketable yields of Fla. 8923 were similar to all the commercially available hybrids that were tested (Tables 1 and 2). Fla. 8923 has very large fruit. In Spring 2013 Fla. 8923 had extra-large fruit yield similar to 'Sanibel' but greater than that of 'Sebring', 'Florida 47', and 'Solar Fire', while overall fruit size was greater than that of all the commercial hybrids, except 'Sanibel' (Table 1). In Fall 2013 Fla. 8923 had total and extra-large yields statistically similar to those of commercial hybrids. Overall fruit size was greater than all three commercial hybrids (Table 2). Firmness was comparable to tomato cultivars grown in Florida in two seasons of testing (Table 3). Overall, the external and internal fruit color tended to be slightly pale red. In Spring 2013, the external L and hue angles of Fla. 8923 were similar to 'Sebring' but higher than those of Tasti-Lee® and 'Florida 47' suggesting a trend for the fruit to be lighter and paler red, respectively. Likewise, in Fall 2013 the external L and hue angles were significantly higher than those of the commercial hybrids. Internally, the L and hue angles for Fla. 8923 in Spring 2013 were similar to 'Florida 47' but higher than Tasti-Lee® and 'Sebring'. The latter two are crimson (*og*^{c} gene) and have a deeper red color than Fla. 8923 or 'Florida 47'. Likewise in Fall 2013, the internal L values and hue angles for Fla. 8923 and 'Florida 47' were similar and significantly higher than those of Tasti-Lee®, indicating the latter was a deeper red.

Disease Resistance

Fla. 8923 had intermediate resistance to both TYLCV and ToMoV under the heavy disease pressure that resulted when seedlings were inoculated for 2 weeks (Table 4). On grower farms in Florida, the resistance of the cultivar Tygress, heterozygous for *Ty-1,* would be rated very close to 0 on our severity scale. Observations for genotypes with *Ty-3* have shown that when young plants are

Table 1. Marketable yield, extra-large yield and fruit size for tomato inbreds and control hybrids at the Gulf Coast Research and Education Center, Balm, FL, Spring 2013.

	Marketable yield (11.4 kg box/ha)		
Genotype	Total	Extra large	Fruit size (g)
Fla. 8872B	2,132 a[z]	1,846 a	230 a
Fla. 7781	2,110 a	540 d	139 e
'Solar Fire'	1,836 ab	681 cd	150 e
Fla. 8923	1,653 ab	1,265 b	206 b
Fla. 8925	1,649 ab	1,250 bc	193 bc
'Sanibel'	1,530 ab	1,099 bc	184 b–d
'Sebring'	1,184 b	744 cd	176 c–e
'Florida 47'	1,151 b	609 d	162 de

[z]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$. The experiment was a completely randomized block design with two blocks and eight plant plots.

Table 2. Total and extra-large marketable yield and fruit size for tomato inbreds and control hybrids at the Gulf Coast Research and Education Center, Balm, FL, Fall 2013.

	Marketable yield (11.4 kg box/ha)		
Genotype	Total	Extra large	Fruit size (g)
Fla. 8925	2,174 a[z]	1,746 a	206 b
'Solar Fire'	1,947 ab	967 b	166 c
Tasti-Lee®	1,608 a–c	758 b	163 c
Fla. 8857	1,485 a–c	943 b	182 bc
'Florida 47'	1,151 b	1062 b	213 b
Fla. 8923	1,235 bc	1,150 ab	260 a
Fla. 8124C	1,148 c	754 b	188 bc

[z]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$. The experiment was a completely randomized block design with two blocks and 10 plant plots.

Table 3. External color, internal color, and firmness for fruit of Fla. 8923 and comparison of tomato cultivars over two seasons at the Gulf Coast Research and Education Center, Balm, FL 2013.

	Fruit color[z]				
	External		Internal		Firmness[y]
Genotype	L	Hue angle	L	Hue angle	(mm deformation)
Spring 2013					
Fla. 8923	47.2 a[x]	69.7 a	34.8 a	55.6 a	4.2
Tasti-Lee®	42.4 b	53.1 b	30.6 b	44.5 b	4.1
'Florida 47'	44.4 b	54.8 b	35.1 a	55.8 a	3.9
'Sebring'	47.6 a	61.0 a	31.0 b	44.5 b	3.6
					NS
Fall 2013					
'Solar Fire'	40.9 b	47.7 b	36.3 ab	40.5 b	3.9
Fla. 8923	44.6 a	52.1 a	38.3 a	44.7 a	3.7
'Florida 47'	40.4 b	45.0 b	38.1 ab	45.3 a	3.4
Tasti-Lee®	39.6 b	45.6 b	36.2 b	37.7 c	3.3
					NS

[z]Data taken with a Minolta CR-300 chromameter; higher "L" numbers indicate lighter color (value), lower hue angles indicate more red color (hue).
[y]Determined with an Institute of Food and Agricultural Sciences pressure tester using a 1-kg weight for 5 s with a fruit contact plate 1.5 cm in diameter. Pressure applied over a locule in equatorial plane. Lower values indicate greater firmness.
[x]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$. The experiments were completely randomized block designs with two blocks and 8 and 10 plant plots for spring and fall, respectively. Ten fruit per plot were harvested at the breaker stage and ripened in a laboratory at 24 °C until table ripe for color and firmness measurements.

Table 4. *Tomato yellow leaf curl virus* (TYLCV) and *Tomato mottle virus* (ToMoV) disease severity[z] for tomato genotypes inoculated at the seedling stage at the Gulf Coast Research and Education Center, Balm, FL, Spring 2014.

Genotype	Begomovirus resistance gene	TYLCV	ToMoV
Horizon	—	3.9 a[y]	3.3 b
HMX 1823	*Ty-3*/+	2.5 b	3.0 bc
'Tygress'	*Ty-1*/+	1.8 c	3.0 bc
(Fla. 8624 × Fla. 8923)	*Ty-3*/+, *Ty-6*/+	1.7 c	—
Fla. 8923	*Ty-3*	2.0 c	2.7 c
Fla. 8680	*Ty-3, Ty-6*	0.3 d	0.1 d
TAMU 5	*Ty-2*	0.0 e	3.9 a

[z]Rated at 66 d after inoculation began on a 0–4 scale where lower value indicated less disease; for scale and inoculation information see Hutton et al. (2012).
[y]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$. The TYLCV and ToMoV experiments were each a completely randomized block design with two blocks and six plant plots.

inoculated they show some disease symptoms, but often gradually improve as the plants continue to grow in the field. The inheritance is additive, so plants heterozygous for *Ty-3* alone show more disease symptoms than plants homozygous for *Ty-3* alone (Verlaan et al., 2013). When *Ty-3* is combined heterozygously with other TYLCV resistance genes, a good level of resistance is often obtained which is evidenced in the (Fla. 8624 × Fla. 8923) hybrid (Table 4). Fla. 8923 is resistant to *Fusarium* wilt races 1 and 2 (*Fusarium oxysporum* f. sp. *lycopersici*), gray leafspot (*Stemphyllium* sp.), and *Verticillium* wilt race 1 (*Verticillium dahliae*). It is more sensitive to bacterial spot race T4 (*Xanthomonas perforans*) than many susceptible genotypes and it is similar to Fla. 8111B in that regard. However, hybrids of Fla. 8923 crossed with other bacterial spot susceptible parents do not show extreme susceptibility as is the case for crosses among many other very susceptible inbreds (Hutton and Scott, unpublished observations). The primary disorder has been zippering. It has a high level of resistance to graywall.

Availability

Fla. 8923 is an inbred breeding line release. Seed distribution is controlled by Florida Foundation Seed Producers, P.O. Box 309, Greenwood, FL 32443. Initial seed requests should be made to S.F. Hutton.

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