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Genetic diversity among *Lagenaria siceraria* accessions containing resistance to root-knot nematodes, whiteflies, ZYMV or powdery mildew

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Abstract

In recent years, there has been an increased interest in Europe and in the USA in grafting watermelon onto bottle gourd, *Lagenaria siceraria* (Mol.) Standl. In this study, genetic diversity and relationships were examined [using 236 sequence-related amplified polymorphism markers] among 56 United States plant introductions (PIs) of *L. siceraria* and PIs of important cucurbit crops [including *Cucurbita maxima* Duchesne (winter squash), *Cucurbita pepo* L. (squash and pumpkin), *Citrullus* spp. (watermelon), *Cucumis melo* L. (melon) and *Cucumis sativus* L. (cucumber)]. The analysis showed that *L. siceraria* is distinct and has similar genetic distances to the cucurbit species examined herein. The *L. siceraria* PIs were assembled into two major clusters. One cluster includes groups of PIs collected mostly in South Asia (India) and a few PIs collected in the Mediterranean region and in Northeast Africa. The second cluster includes groups of PIs collected mainly in Southern Africa and in North, Central and South America, and PIs collected in China, Indonesia and Cyprus. All *L. siceraria* PIs in this study were susceptible to the southern root-knot nematode (RKN) [*Meloidogyne incognita* (Kofoid and White) Sandground]. However, several PIs, among them a group of closely related PIs collected in Mexico and Florida, were less infected with southern RKNs. All *L. siceraria* PIs were infested with whiteflies [*Bemisia tabaci* (Gennadius)], while several PIs were less infested than others and need further evaluation and selection for developing breeding lines that may be less appealing to this pest. Most of the PIs that showed resistance to zucchini yellow mosaic virus and tolerance to powdery mildew were collected in India and belong to the same phylogenetic groups (PGs). Experiments with *L. siceraria* PIs representing different PGs showed similar grafting compatibility with watermelon. Findings from this study should be useful for the development of superior *L. siceraria* rootstock lines with enhanced resistance to diseases and insect pests of cucurbit crops.

Keywords: bottle gourd; cucurbits; grafting; nematodes; resistance; rootstocks; SRAP; whitefly; zucchini yellow mosaic virus

Introduction

Lagenaria is a genus in the Cucurbitaceae family with vine growth habit. There are seven known species:

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the wild *Lagenaria breviflora* (Benth.) G. Roberty, *Lagenaria abyssinica* (Hook. F.) C. Jeffrey, *Lagenaria rufa* (Gilg.) C. Jeffrey, *Lagenaria sphaerica* (Sonder) Naudin and *Lagenaria guineensis* (G. Don) C. Jeffrey, and the domesticated *Lagenaria siceraria* (Mol.) Standl. and *L. vulgaris* Ser. which was previously combined with *L. siceraria*. The *L. siceraria* is thought to be among the earliest domesticated plant species (Cutler and Whitaker, 1967). The name *Lagenaria* is derived from *'lagena'*, the Latin name for the Florence flask, referring to the shape of the *L. siceraria* fruit. The species name *siceraria* refers to its dry (*siccus*) mature fruit used by people throughout the world for making jars, utensils, tubes and musical instruments. For this reason, it is commonly known as the 'bottle gourd' (Decker-Walters *et al*., 2001; 2004; Erickson *et al*., 2005). It is also known as the white-flowered gourd 'Calabash' (Jeffrey, 1967; 1980). *L. siceraria* is indigenous to Africa (Richardson, 1972; Morimoto *et al*., 2006); however, remains of *L. siceraria* in archaeological digs point to the possibility that it reached temperate and tropical areas in Asia and the Americas over 10,000 years ago, perhaps with human migration (Erickson *et al*., 2005) or dispersed through natural ways. *L. siceraria* fruits are known to have the capacity to float on seas for many months without losing seed viability (Decker-Walters *et al*., 2004).

L. siceraria thrives in a wide range of soil types, including alluvial sandy soils along river banks, red silt, clay loam soils and rocky soils. It is more tolerant of a high water table (Yetisir *et al*., 2006) and to salt than watermelon (Colla *et al*., 2005). The ability of *Lagenaria* to thrive in different soils and its natural resistance to soil-borne diseases makes it desirable for grafting different cucurbit crops susceptible to soil pathogens. Grafting cucurbits, particularly watermelon, on *Lagenaria* rootstocks has been practiced in Eastern Asia for many years, and in recent years there has been an increased interest in Europe and in the USA in adopting this practice as one of the alternatives for methyl bromide fumigation for the control of fusarium wilt (Miguel *et al*., 2004; Cohen *et al*., 2007). In a recent study (Ling and Levi, 2007), a number of United States plant introductions (PIs) of *L.* siceraria were found to be resistant to zucchini yellow mosaic virus (ZYMV), and were moderately resistant to powdery mildew (Kousik *et al*., 2008). Edelstein *et al*. (2000) reported that *Lagenaria* rootstocks may confer resistance to the carmine spider mite (*Tetranychus cinnabarinus*) in the grafted scion of *Cucurbita* cv. Brava. In addition, *L.* siceraria rootstocks appeared to enhance lycopene content in watermelon fruits of grafted vines (Perkins-Veazie *et al*., 2007). For these reasons, there is an increased interest by researchers and breeders of watermelon in exploring the utility of *L. siceraria* germplasm for improving watermelon yield and quality.

Over 235 PIs of *L. siceraria* that were collected in different regions of the world are maintained by the USDA, ARS, Plant Genetic Resources and Conservation Unit (PGRCU) in Griffin, GA, USA (http://www.ars-grin.gov). These PIs can be valuable for the development of superior *L. siceraria* rootstock lines for grafting watermelon. However, information is insufficient with respect to genetic relationships among these *L. siceraria* PIs and their phylogenetic relations to important cucurbit crops [including *Cucurbita maxima* Duchesne (winter squash), *Cucurbita pepo* L. (squash and pumpkin), *Citrullus* spp. (watermelon), *Cucumis melo* L. (melon) and *Cucumis sativus* L. (cucumber)] that are being used as rootstocks for grafting watermelon (Miguel *et al*., 2004). Also, there is no information with respect to the resistance of *L. siceraria* PIs to southern root-knot nematode (RKN) [*Meloidogyne incognita* (Kofoid and White) Sandground], which can be a serious pest of cucurbit crops (Thies and Levi, 2003; 2007). In addition, information is lacking with respect to the resistance of these *L. siceraria* PIs to the sweetpotato whitefly [*Bemisia tabaci* (Gennadius)] which not only damages plants by its feeding, but it also transfers viruses in cucurbit crops. Furthermore, information is lacking with respect to grafting compatibility of *L. siceraria* PIs [representing different phylogenetic groups (PGs)] with cucurbit cultivars, particularly watermelon.

Decker-Walters *et al*. (2001; 2004) and Morimoto *et al*. (2006) examined genetic diversity among *L. siceraria* and related *Lagenaria* species employing random amplified polymorphic DNA marker analysis, and were able to differentiate among the *Lagenaria* species. Molecular markers can be useful in examining genetic diversity among the *L. siceraria* PIs, and in elucidating their genetic relations before setting up a breeding scheme for the development of superior rootstock lines with enhanced pest and disease resistance.

The objectives of this study were to: (1) examine genetic diversity among *L. siceraria* PIs collected in different parts of the world and examine their relatedness to PIs of important cucurbit species [including *C. maxima*, *C. pepo, Citrullus lanatus* and *Citrullus colocynthis* (L.) Schrader, *C. melo* and *C. sativus*], (2) examine resistance of the *Lagenaria* PIs to southern RKN (*M. incognita* race 3) and to the B-biotype sweetpotato (*B. tabaci*) whitefly, (3) examine the genetic relations among those *L. siceraria* PIs showing disease or pest resistances and (4) determine whether there are any significant differences in grafting compatibility of *Lagenaria* PIs with watermelon cultivars. The information found in this study can be useful for researchers and plant breeders interested in developing superior *L. siceraria* rootstock lines with enhanced disease and pest resistance.

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Materials and methods

Plant material

Seeds of *L. siceraria* PIs (Table 1) were obtained from USDA, ARS, PGRCU, Griffin, GA, USA. The *Cucurbita moschata* and *C. pepo* PIs (Table 1) were obtained from USDA, ARS, Plant Genetic Resources Unit, Northeast Regional PI Station, Geneva, NY, USA. The *Cucumis* sp. PIs (Table 1) were obtained from the USDA, ARS, North Central Regional PI Station, Ames, IA, USA (http://www.ars-grin.gov). The squash cultivar ESPN, the melon cultivar 'Ananas Yokneam' and the cucumber cultivar SMR58 were from seeds that have been maintained at the USDA, ARS, US Vegetable Laboratory, Charleston, SC, USA.

PIs of *C. moschata*, *C. pepo*, *C. sativus*, *C. melo*, *Citrullus* spp. and *L. siceraria* (Table 1) were grown in the greenhouse (day and night temperatures were ~27 and 22°C, respectively), and in the field during the summers of 2006 and 2007 in Charleston, SC, USA.

DNA isolation

Samples of young leaves (10 g) were collected from three to four plants (3-week old) of each PI, and stored in −80°C. DNA was isolated from the frozen leaves using an improved procedure [as described by Levi and Thomas (1999); Levi *et al.* (2001)].

Sequence-related amplified polymorphism (SRAP) analysis

The sequence-related amplified polymorphism (SRAP) procedure was based on polymerase chain reaction amplification of open reading frames, using forward and reverse primers (Table 2) designed to preferentially amplify exon (rich in C and G nucleotides) and intron regions (rich in A and T nucleotides), respectively. The forward primer was a 14 nucleotide sequence rich in C and G, and three selective bases at the 3′ end, while the reverse primer was a 15 nucleotide sequence rich in A and T and three selective bases at the 3′ end. The variation in exon, intron or promoter region sequences produced the polymorphism (Li and Quiros, 2001). We used 24 SRAP primer combinations (Tables 2 and 3). The SRAP markers were tested for polymorphism among the PIs using the same procedure recently described for the mapping of watermelon genome (Levi *et al.*, 2006). The oligonucleotides were synthesized by International DNA Technologies, Inc. (Coralville, IA, USA), and were tested for polymorphism among PIs. Those primer pairs that yielded sufficient polymorphism among PIs were selected for repeated tests.

Marker data collection and analysis

The markers were scored based on their presence or absence using the built-in fragment analysis software [provided with the Beckman CEQ-8800 system (Fullerton, CA, USA)] for DNA markers. Polymorphic markers were scored for the presence and absence of the corresponding bands among the genotypes. The scores '1' and '0' indicate the presence and absence of bands, respectively. A pairwise similarity matrix was generated using the Nei–Li similarity index (Nei and Li, 1979) as follows: similarity $= 2N_{ab}/(N_a + N_b)$, where N_{ab} is the number of SRAP fragments shared by two genotypes (a and b) and N_a and N_b are the total number of SRAP fragments analysed in each genotype. A cluster analysis was performed based on the SRAP marker data using the NTSYS-PC Version 2.02 (Rohlf, 1993) with the unweighted pair group method on arithmetic averages method.

Root-knot nematode experiment and analyses

M. incognita race 3 were cultured and prepared for inoculation of the 57 *L. siceraria* PIs. Commercial *L. siceraria* 'Emphasis' and *C. maxima* × *C. moschata* 'Strong Tosa' were included as susceptible reference controls. All PIs and cultivars were evaluated for resistance to *M. incognita* race 3 in replicated greenhouse tests, as described by Thies and Levi (2003; 2007). The experimental design was a randomized complete block for all genotypes with three replicates and five plants per replicate ($n = 15$) in each test. The seeds were sown in plastic trays containing 50 individual 0.2-l cells filled with Metro-Mix 360 (The Scotts Company, Marysville, OH, USA) and placed in a greenhouse maintained between 26 and 31°C. When seedlings were at the first true leaf stage, 3 ml distilled water containing approximately 2500 eggs of *M. incognita* race 3 were pipetted into the rhizosphere soil of each plant at a 1-cm depth. Plants were fertilized 2 and 5 weeks after sowing with one-half strength 20N–20P–16K water-soluble fertilizer (Peter's Fertilizer; United Industries Corporation, St Louis, MO, USA). Eight weeks later, the shoots of all plants were clipped at the crown, and the roots were removed from each cell and carefully washed. The root system of each plant was then submerged in a 15% solution of McCormick's (McCormick & Company, Inc., Sparks, MD, USA) red food colour (Thies *et al.*, 2002) for 15–20 min to stain the *M. incognita* egg masses. The root systems were carefully rinsed

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Table 1. *Lagenaria siceraria* plant introductions (PIs), the phylogenetic group (PG) they belong to (as shown in Fig. 1), the country they were collected from, their fruit shape, time-to-fruit maturity, gall indices of root-knot nematodes (RKN; *Meloidogyne incognita* race 3) and the average number of adult B-biotype sweetpotato whitefly (*Bemisia tabaci*) found on each PI

Lagenaria							
PIs	PG	Country of collection	Fruit shape	Maturity	RKN	Whitefly	Grafting survival (%)
PI 270456	(I)	Mexico	Oblate	L	3.75 a–g	2.88 cd (1.4 c)[a]	62.5
PI 271351	(II)	India	Elongated	M	4.37 c–l	6.60 cd	–
PI 271352	(II)	India	Elongated	M	4.61 g–l	20.24 a–c	–
PI 271354	(II)	India	Oblong	M	4.30 a–l	7.92 cd	75
PI 271357	(II)	India	Oblong	L	4.88 kl	4.81 cd	75
PI 271477	(I)	India	Elongated	L	4.58 g–l	9.88 a–d	–
PI 273662	(II)	Ethiopia	Round/Pear	L	4.83 j–l	7.35 cd	62.5
PI 273663	(III)	Ethiopia	Round	L	5.00 l	(59.46 a)	75
PI 280632	(II)	South Africa	Ovale	L	4.75 h–l	7.30 cd	75
PI 280636	(IV)	South Africa	Ovale	M	3.93 a–i	8.17 bc	–
PI 358056	(I)	Yugoslavia	Variable	M	4.38 c–l	25.88 a (4.63 bc)	–
PI 358059	(I)	Yugoslavia	Variable	M	4.45 e–l	10.54 a–d	–
PI 368365	(I)	Yugoslavia	Variable	M	–	–	–
PI 381832	(II)	India	Variable	M	4.66 h–l	9.30 a–d	–
PI 381844	(II)	India	Variable	M	4.55 g–l	9.29 b–d	75
PI 381845	(II)	India	Variable	M	4.45 d–l	7.29 cd	100
PI 381846	(II)	India	Elongated	M	4.23 a–l	10.08 a–d	75
PI 381847	(II)	India	Elongated	M	4.40 e–l	5.96 cd	–
PI 381848	(II)	India	Variable	M	–	5.13 cd	–
PI 381849	(II)	India	Elongated	M	4.10 a–k	10.83 a–d	–
PI 381850	(II)	India	Oblate	M	4.22 a–l	10.29 a–d	–
PI 381851	(II)	India	Elongated	M	4.38 d–l	6.50 d cd	75
PI 381854	(II)	India	Elongated	M	3.90 a–i	4.54 cd	100
PI 406857	(III)	Honduras	Elongated	L	4.33 a–l	3.92 cd (1.83 c)	–
PI 419089	(VII)	China	Oblong	M	4.75 h–l	–	–
PI 419090	(VII)	China	Oblong	M	4.46 e–l	11.54 a–d	62.5
PI 432340	(VII)	Cyprus	Elongated	L	4.75 i–l	5.70 cd	–
PI 432342	(VII)	Cyprus	Elongated	L	3.45 a–e	12.00 a–d	–
PI 438844	(VI)	Mexico	Oblate	L	3.30 a–d	6.29 cd	100
PI 438846	(–)	Mexico	Oblate	L	3.94 a–k	5.04 cd	–
PI 438847	(VI)	Mexico	Elongated	M	3.36 a–c	5.63 cd	–
PI 442368	(VI)	Florida	Elongated	E	3.24 ab	5.67 cd	87.5
PI 442369	(VII)	Mexico	Elongated	M	3.53 a–f	3.68 cd (3.46 bc)	–
PI 451856	(V)	Guatemala	Small-pear	M	3.79 a–i	6.08 cd	–
PI 451857	(V)	Guatemala	Large-pear	M	4.16 a–l	4.09 cd	–
PI 458736	(V)	Argentina	Oblong	M	3.20 a	7.33 cd	75
PI 470260	(VIII)	Indonesia	Oblong	M	4.03 a–k	10.04 a–d	–
PI 487482	(I)	Israel	Elongated	L	4.58 f–l	3.08 cd	–
PI 491252	(5)	Greece	Ovale	M	4.00 a–k	8.83 bc	–
PI 491266	(5)	Zimbabwe	Ovale	M	4.00 a–j	5.83 cd	62.5
PI 491267	(V)	Zimbabwe	Variable	M	5.00 l	10.00 a–d	75
PI 491268	(VI)	Zimbabwe	Variable	M	4.35 b–l	5.68 cd	–
PI 491269	(V)	Zimbabwe	Ovale	M	4.38 c–l	24.54 ab (9.39 bc)	–
PI 491270	(V)	Zimbabwe	Variable	M	4.17 a–l	5.00 cd (7.06 bc)	–
PI 491271	(V)	Zimbabwe	Round	M	4.44 e–l	2.13 cd (3.17 bc)	–
PI 491278	(V)	Zimbabwe	Variable	M	4.16 a–k	11.75 a–d	–
PI 491281	(V)	Zimbabwe	Variable	M	4.15 a–k	6.92 cd	–
PI 491287	(V)	Zimbabwe	Variable	M	4.28 c–l	8.38 b–d	–
PI 491294	(V)	Zimbabwe	Round	M	4.06 a–k	3.04 cd	87.5
PI 491295	(VI)	Zimbabwe	Variable	M	4.32 b–l	25.79 a (3.06 bc)	–
PI 534553	(IV)	Syria	Elongated	M	4.25 a–l	7.14 cd	–
PI 534555	(IV)	Syria	Variable	M	4.12 a–k	7.83 cd	–
PI 534556	(IV)	Syria	Elongated	M	3.80 a–h	5.75 cd	75
PI 636137	(II)	India	Oblong	M	4.53 f–l	8.69 bc	–
PI 639723	(III)	USA	Elongated	E	3.90 a–i	2.29 cd (8.77 bc)	75

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Table 1. *Continued*

Lagenaria PIs	PG	Country of collection	Fruit shape	Maturity	RKN	Whitefly	Grafting survival (%)
PI 641946	(III)	India	Oval	M	3.80 a–i	3.25 cd (3.91 bc)	–
PI 642039	(VI)	USA	Elongated	M	4.39 b–l	3.77 cd	83.4

E, early season; M, mid season; L, late season.
[a] Data in parenthesis are from the second experiment for whiteflies.

under running tap water and evaluated for galling severity and egg mass production using a 1–5 scale in which 1 = 0–3%, 2 = 4–25%, 3 = 26–50%, 4 = 51–79% and 5 > 80% of root system galled (Thies and Fery, 1998). Gall index data were converted to the midpoint of the percentage range designated for each gall index score. Then, percentage of root system galled was arcsine transformed before analysis. Data were analysed using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS) for Windows, Version 8.0 (SAS Institute, 2002), and means were separated using Fisher's protected least significant difference test. Non-transformed data are shown in Table 1.

Whitefly experiments

Trials for infestation of the B-biotype *B. tabaci* on *L. siceraria* PIs were conducted in the greenhouse. Each PI entry was established in a seedling tray (10.2 cm wide × 10.2 cm long × 6.4 cm deep) in Fafard Heavyweight Mix #52 (Conrad Fafard, Inc., Agawam, MA, USA) potting soil. There was a 10-seedling set for each of the PI entries, which were replicated thrice. Each replicate of each set was randomly placed on separate tables in an otherwise empty greenhouse. During the experiment, temperature in the greenhouse averaged 25.2°C (range 19–32°C) and relative humidity (RH) averaged 65% (range 46–77% RH). The seedlings were maintained free of pests until the first true leaf stage. Collard seedlings that were infested with whiteflies were then taken from a whitefly colony and placed on empty tables, about 1.2 m from the test plants. Leaf growth was variable among the test plants and was 1–3 true leaf stage during the start of the evaluation. The upper leaf was sampled from one random plant in the 10-plant set of each replicate. The number of adult whiteflies was counted from the top and lower leaf surfaces of each sampled plant. Sampling was conducted seven times over a 3-week period, and mean whitefly counts were averaged across sample dates. Whitefly data were analysed using SAS (SAS Institute, 2002) computations, and significance was determined at $P < 0.05$. Significantly different means for whitefly counts among PIs were separated using the Student–Newman–Keuls test.

Based on the results from the initial trial, an additional replicated trail was conducted for PI 270456, PI 273663, PI 358056, PI 406857, PI 442369, PI 491269, PI 491270, PI 491271, PI 491295, PI 639723 and PI 641946. A total of 14 samples were collected during the 2 months.

Table 2. The sequence-related amplified polymorphism (SRAP) forward primers that were labeled with a DNA sequencing dye forward labeled primers (FLP) and the reverse unlabeled primers (RUP) used in different combinations to produce the SRAP markers (in Table 3) in watermelon

FLP	Primer sequence 5′–3′
Me1	TGAGTCCAAACCGGATA
Me2	TGAGTCCAAACCGGAGC
Me3	TGAGTCCAAACCGGAAT
RUP	Primer sequence 5′–3′
ba3	GTCGAGCTGCCAATTTGC
ba5	GTCGAGCTGCCAATTAAA
ba6	GTCGAGCTGCCAATTAAC
ba11	GTCGAGCTGCCAATTTGA
Em1	GACTGCGTACGAATTAAT
Em2	GACTGCGTACGAATTTGC
Em3	GACTGCGTACGAATTGAC

Grafting experiment

Pilot experiments for grafting compatibility with watermelon cultivars were conducted with 23 PIs in this study (Table 1). About 8 seeds out of 23 selected *L. siceraria* PIs (Table 1) were sown in trays containing Jiffy Mix soil and beach sand (mixed in ratio of 9:1). Four days later, seeds of the watermelon cultivars 'Crimson Sweet' and 'Charleston Gray' were sown in the same type of soil. The watermelon seedlings (2- to 3-d old) were grafted (as scions) on *L. siceraria* rootstocks (6- to 7-d old seedlings) using the 'hole insertion' grafting procedure optimized for cucurbits as described by Amaido (2004) and Hassell *et al.* (2008). Post-grafting plants were kept in humid conditions for 1 week as described by Hassell *et al.* (2008). The grafted plants were kept in plastic trays (1.75 in. deep) in the greenhouse and their development

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Table 3. The sequence-related amplified polymorphism primer pair combinations (PC), the number of polymorphic fragments (PF) produced in *Lagenaria* siceraria and/or in cucurbit species (Fig. 1) by each of these primer pairs

PC	PF	Fragments (size; bp)
Me2ba5	26	96, 104, 106–108, 110, 126, 143, 154–156, 163, 168, 185, 203, 254, 283, 286, 287, 291, 302, 316, 333, 347, 349, 356
Me1ba5	23	82, 91, 109, 111, 114, 115, 141, 151, 153, 158, 160, 177, 180, 184, 185, 220, 277, 287, 297, 332, 335, 368, 418
Me2em2	21	97, 110, 114, 116, 175, 186, 206, 213, 218, 243, 305, 307, 316, 317, 319, 356, 392, 399, 402, 419, 420
Me1em5	16	89, 158, 160, 178, 187, 211, 213, 214, 218, 230, 232, 233, 240, 251, 289, 291
Me1ba6	13	95, 123, 126, 129, 185, 186, 204, 209, 216, 248, 263, 282, 322
Me2ba11	12	157, 182, 216, 217, 245, 246, 247, 251, 261, 262, 263, 290, 291, 293, 306
Me2ba6	12	77, 92, 95, 100, 122, 128, 159, 216, 254, 266, 286, 317, 365, 366
Me3ba5	12	120, 122, 123, 135, 170, 172, 274, 276, 281, 313, 354, 363
Me2ba3	11	125, 127, 141, 143, 159, 161, 175, 177, 178, 207, 284
Me2ba3	10	96, 118, 148, 154, 162, 165–167, 169, 259
Me3ba11	9	116, 118, 121, 141, 142, 159, 172, 178, 245, 246
Me1ba11	8	103, 117, 120, 123, 154, 206, 227, 237
Me1em2	8	99, 103, 110, 112, 132, 218, 382, 407
Me3ba11	8	112, 120, 122, 123, 142, 183, 238, 317
Me3ba6	8	74, 96, 120, 121, 259, 270, 273, 316
Me1ba11	7	122, 181, 229, 288, 291, 295, 296
Me3em3	7	84, 97, 106, 131, 139, 152, 259
Me1em1	6	79, 99, 103, 116, 230, 267
Me3ba3	5	72, 147, 149, 201, 203, 245, 248
Me3em2	4	109, 116, 132, 205
Me2em1	2	123, 153

bp, base pair.

was observed during 4 weeks following grafting. Rootstocks that gave rise to healthy grafted watermelon plants with elongated stems and new healthy leaves and flower buds during 4 weeks post-grafting were considered to be compatible for grafting with watermelon.

Results

Genetic analysis based on SRAP markers

The SRAP markers in this study are polymorphic among the *L. siceraria* PIs and are reproducible from experiment to experiment. The 21 SRAP primer pair combinations (Tables 2 and 3) produced 236 markers polymorphic among the PIs of *L. siceraria* and cucurbit species (Table 1). About 2–26 polymorphic markers were produced by each of the SRAP primer pairs (Tables 2 and 3).

The *L. siceraria* group is distinct from the three other cucurbit genera examined in this study (*Cucurbita* spp., *Citrullus* spp. and *Cucumis* spp.; Fig. 1). The cluster analysis (based on 236 polymorphic SRAP markers) produced genetic similarity of 77–93% among the *L. siceraria* PIs, which is comparable with the genetic diversity that exists among the *Citrullus* species and subspecies (*C. lanatus* var. *lanatus*, and *C. lanatus* var. *citroides versus C. colocynthis* PIs; genetic similarity ranges from 79 to 94%) and among *Cucurbita spp.* examined here (*C. pepo versus C. maxima* PIs; genetic similarity ranges from 83 to 95%; Fig. 1).

The *L. siceraria* PIs were assembled into two major clusters (Fig. 1). The first major cluster includes PIs collected mostly in South Asia (India) and a few PIs collected in the Mediterranean region (Israel, Syria and Yugoslavia) and Northeast Africa (Ethiopia; Fig. 1; groups I–IV). The second major cluster includes PIs collected mainly in Southern Africa (South Africa and Zimbabwe) and in North, Central and South America (Florida, Mexico, Guatemala, and Argentina), and a few PIs collected in China, Indonesia and Cyprus (Fig. 1; groups V–VIII). The cluster analysis (Fig. 1) indicated that the PIs collected in India, but not those collected in China, are distinct from the PIs collected in Southern Africa and in the Americas.

Root-knot nematode resistance

All *L. siceraria* PIs evaluated in this study were susceptible to the southern RKN (Table 1). The gall indices (GIs) were 4.7 and 5.0 for the control commercial cultivars 'Emphasis' (*L. siceraria*) and 'Strong Tosa' (*Cucurbita*), respectively. The GIs of the PIs examined

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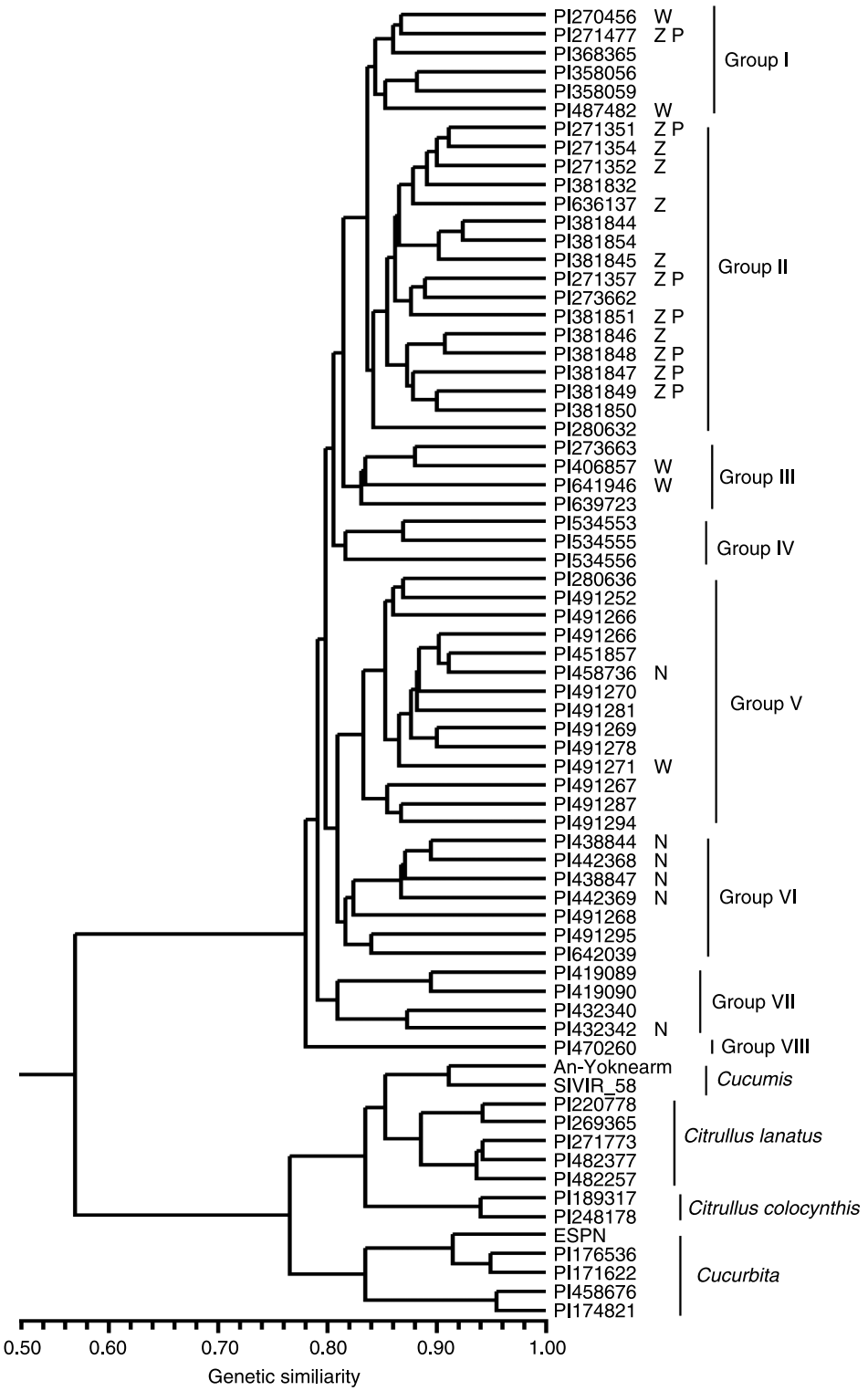

Fig. 1. Genetic diversity among *Lagenaria siceraria, Cucumis, Citrullus* and *Cucurbita* plant introduction (PIs) collected throughout the world, and the phylogenetic *L. siceraria* groups with PIs resistant to zucchini yellow mosaic virus (Z; Ling and Levi, 2007) and tolerant to powdery mildew (P; Kousik *et al.*, 2008), and moderate tolerance to the root-knot nematode (*Meloidogyne incognita* race 3; N), or less appealing to whiteflies (B-biotype *Bemisia tabaci*; W) (as indicated in Table 1).

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herein ranged from 3.20 to 5.00 ($P < 0.05$; Table 1). However, six PIs exhibited moderate galling response and may be classified as moderately susceptible (GI range: 3.2–3.5). Although these six PIs allowed nematode reproduction in their roots (egg mass data not shown), they had a moderate root galling response to southern RKN compared with the other PIs examined herein. Four out of the six PIs (PI 438844, PI 438847, PI 442369 collected in Mexico, and PI 442368 collected in Florida) are in the same PG (Fig. 1, group VI), indicating that their similar genetic background may play a role in reaction to southern RKNs. The two other PIs with moderate susceptibility were in the adjunct PG V (PI 458736 collected in Argentina) and group VII (PI 432342 collected in Cyprus; Fig. 1).

Resistance to whiteflies

In this study, all PIs examined were infested with the B-biotype sweetpotato whitefly (*B. tabaci*). Mean counts among all PIs evaluated ranged from 0.4 to 25.8 adult whiteflies per leaf in the first experiment, and from 1.4 to 59.5 adult whiteflies per leaf in the second experiment (Table 1). However, several PIs including PI 270456 and PI 442369 (collected in Mexico), PI 487482 (collected in Israel), PI 641946 (collected in India), PI 491271 and PI 491294 (collected in Zimbabwe), and PI 406857 (collected in Honduras) had the least whiteflies (>4 adult whiteflies per leaf in both the experiments). Although the whitefly counts were not significantly different from those on other PIs, these PIs consistently had low counts for whitefly adult, egg and nymphal counts.

Grafting experiments

The experiments resulted in high grafting compatibility (ranging from 62.5 to 100% success; Table 1) between rootstocks of *L. siceraria* PIs (representing the different PGs; Table 1; Fig. 1) and scions of the watermelon cultivar Crimson Sweet.

Discussion

Genetic analysis based on SRAP markers

The SRAP markers were polymorphic among the *L. siceraria* PIs, and are useful in determining intraspecific and interspecific genetic relations among the cucurbit species examined herein. The high reproducibility of SRAP markers is a result of amplification with two specific primers (each primer is 17–18 bp) that represent sequences in the proximity of coding regions (Li and Quiros, 2001). A large number of the SRAP markers in this study are polymorphic in one or a few base pairs (Table 3). These small differences could not be detected using standard gel electrophoresis system. However, they could be detected in this study using the advanced capillary electrophoresis technology employed in DNA sequencing and genotyping (as shown for DNA markers for watermelon genome by Levi *et al.* (2006)).

The *L. siceraria* PIs clustered into distinct groups and have overall genetic similarity of 57% from the PIs of three cucurbit genera examined in this study (*Cucurbita* spp., *Citrullus* spp. and *Cucumis* spp.; Fig. 1). Kocyan *et al.* (2007) evaluated evolutionary relationships among cucurbit species using chloroplast gene sequences. Their comprehensive cucurbit dendrogram based on chloroplast gene markers showed that the *Lagenaria*, *Citrullus* and *Cucumis* species belong to the *Benincaseae* clade which is adjunct to the *Cucurbitae* clade. The similar genetic diversity that exists within the *Lagenaria, Citrullus* and *Cucurbita* groups might be due to the fact that they belong to the same monophyletic group and are descended from common ancestors. Furthermore, the SRAP markers are related to gene sequences (Li and Quiros, 2001) that are know to be conserved within and among species compared with non-coding regions (Fulton *et al.*, 2002).

The assembly of *L. siceraria* PIs into two major clusters is in agreement with the supposition that African and American landraces *of L. siceraria* (subsp. *siceraria*) are distinctly different from Asian landraces (subsp. *asiatica*; Decker-Walters *et al.*, 2001; 2004). Whitaker (1972) suggested that the origin of *L. siceraria* is in Africa because all known wild *Lagenaria* species are also found in that continent. Richardson (1972) also suggested that *L. siceraria* may have originated in Africa and that it was dispersed from there to Asia and to the Americas, where it was domesticated (Decker-Walters *et al.*, 2001). Erickson *et al.* (2005) suggested that the *L. siceraria* that was domesticated over 10,000 years ago in America may have originated in Asia, and that it was introduced to the Americas by the Palaeo-Indians (the ancient inhabitants of the Americas) during their migration into the continent at the last ice age, over 10,000 years ago. The cluster analysis in this study (Fig. 1) indicated that the PIs collected in India, but not those collected in China, are distinct from the PIs collected in Southern Africa and in the Americas. It is possible that the PIs examined in this study are derived from *L. siceraria* genotypes that were introduced from Southern Africa to North and South America in recent times.

L. siceraria has been domesticated throughout the world and evolved into different types that have been classified into subspecies according to their fruit shape (Widjaja and Reyes, 1993). Additional studies

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using chloroplast and mitochondrial DNA sequence (as has been shown for *Cucumis* spp.; Renner and Schaefer, 2008) may be needed to determine the centre of origin of *Lagenaria* and the phylogenetic relations among *Lagenaria* species and among *L. siceraria* subspecies.

Root-knot nematode resistance

The results in this study indicate that the PIs in group VI (collected in Mexico and Florida) are the least susceptible to RKNs. Evaluating additional germplasm collected in Florida and Mexico may result in the identification of an accession that is tolerant or resistant to RKNs. Root-knot nematodes cause serious damages to cucurbit rootstocks (Thies and Levi, 2003; 2007) and are known to increase the incidence and severity of fusarium wilt in different crops (Mai and Abawi, 1987). *L. siceraria* proved to be useful in reducing soil-borne diseases in grafted watermelon scions (Lee and Oda, 2003; Taylor *et al.*, 2006; Cohen *et al.*, 2007; Yetisir *et al.* 2007), and is known to be resistant to *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *niveum* (E.F. Sm.; W.C. Snyder and H.N. Hans) that causes fusarium wilt in watermelon (Murata and Ohara, 1936; Miguel *et al.*, 2004; Cohen *et al.*, 2007). In Asia, *L. siceraria* is the preferred rootstock for grafting watermelon (Davis *et al.*, 2008). However, *Fusarium oxysporum* f. sp. *lagenariae* Matsuo and Yamamoto was identified in infected roots of *L. siceraria* (Sato and Ito, 1962; Sakata *et al.*, 2007) and has been related to its extensive use as a rootstock throughout Asia (Davis *et al.*, 2008). Identifying and selecting *Lagenaria* PIs with tolerance to RKNs should be useful in developing superior rootstock lines. Different *Lagenaria* germplasms, including the entire US *Lagenaria* PI collection, should be evaluated to identify potential sources with the lowest response to RKNs. Additional studies to evaluate plant growth performance in nematode-infested fields are being conducted (in Charleston, SC, USA) to determine the development of plants grafted on *Lagenaria* PIs with different root galling response.

Resistance to whiteflies

All PIs examined were infested with whiteflies, and the counts were not significantly different among PIs (Table 1). However, the few PIs that consistently had low whitefly adult, egg and nymph counts may be considered moderately susceptible [but not resistant to whiteflies, as indicated for the bitter watermelon *C. colocynthis* that thrives in desert regions (*C. lanatus* var. *lanatus*; Simmons and Levi, 2002)]. These PIs were selected for further evaluation and experiments to select and develop lines that are less appealing for whiteflies.

Whiteflies are serious pests that attack and transmit viruses into cucurbit crops (Simmons and Levi, 2002; Lecoq *et al.*, 2003). Edelstein *et al.* (2000) indicated that *L. siceraria* rootstocks reduced carmine spider mite infestation on grafted watermelon scions. Thus, identifying and developing *Lagenaria* rootstocks resistant to whiteflies and/or melon aphids might be useful in reducing their presence in grafted cucurbit vines. Further studies are needed to evaluate the US *Lagenaria* PI collection for whitefly and/or melon aphid infestation.

Grafting experiments

The high grafting compatibility between the *L. siceraria* rootstocks and the watermelon cultivar scions confirms the findings of high compatibility in grafting watermelon on *L. siceraria* rootstocks (Yetisir *et al.* 2007). The results herein indicate that *L. siceraria* of different genetic backgrounds are compatible with watermelon. In Asia, *L. siceraria* is considered a valuable rootstock for grafting watermelon (Davis *et al.*, 2008).

A number of the *L. siceraria* PIs in this study are likely to be derived from landraces that, over many years of domestication, developed tolerance to biotic and abiotic stress unique to their geographical region. Indeed, in this study, *L. siceraria* PIs that belong to certain geographical regions (South Asia *versus* Africa or South America) showed different disease or pest resistance (Table 1). A number of the PIs collected in India, including PI 271351, PI 271352, PI 271353, PI 271354, PI 271357, PI 271477, PI 381845, PI 381846, PI 381847, PI 381848, PI 381849, PI 381851 and PI 636137 (Fig. 1; group II) showed high resistance to ZYMV (Ling and Levi, 2007). A few of these PIs (including PI 271351, PI 271353, PI 271357 and PI 271477, PI 381847, PI 381848, PI 381849 and PI 381851; Fig. 1; group II) also have intermediate resistance to powdery mildew (Kousik *et al.*, 2008).

Edelstein *et al.* (2000) indicated the potential of *L. siceraria* rootstocks in reducing infestation of the carmine spider mite on grafted watermelon scions. However, not all disease or pest resistance modes are transferable through grafting (Edelstein *et al.*, 2000; Cohen *et al.*, 2007). Further studies are needed to evaluate the effect of virus-resistant *Lagenaria* rootstock on grafted watermelon vines.

Conclusions

The *L. siceraria* PIs examined in this study belong to different PGs and show different levels of resistance to

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diseases or whitefly pests. A significant number of the *L. siceraria* PIs that were collected in India (PGs I and II) contain resistance to ZYMV and/or tolerance to powdery mildew. Some of the *L. siceraria* PIs that were collected in South, Central or North America (PGs V–VII) showed lower response to RKNs. The phylogenetic data in this study should be useful in developing breeding schemes aiming to develop superior rootstock lines with enhanced disease and pest resistance, valuable for grafting watermelon and other important cucurbit crops.

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