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Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions

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Abstract

Genetic diversity was estimated among 42 U.S. Plant Introduction (PI) accessions of the genus *Citrullus* (of these, 34 PIs are reported to have disease resistance), and 5 watermelon cultivars, using 30 RAPD primers. These primers produced 662 RAPD markers that could be rated with high confidence. Based on these markers, genetic similarity coefficients were calculated and a dendrogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA). The analysis delineated three major clusters. The first cluster consisted of a group of five watermelon cultivars, a group of *C. lanatus* var. *lanatus* accessions, and a group of *C. lanatus* var. *lanatus* accessions that contained some *C. lanatus* var. *citroides* genes. The second cluster consisted of the *C. lanatus* var. *citroides* accessions, while the third cluster consisted of the *C. colocynthis* accessions. The two *C. lanatus* clusters differentiated from each other and from the *C. colocynthis* cluster at the level of 58.8% and 38.9% genetic similarity, respectively. Assessment of genetic diversity among accessions that have been reported to have disease resistance indicated that resistance to either anthracnose, downy mildew, powdery mildew, or watermelon mosaic virus is found among all major groups of *Citrullus* PIs. Additionally, resistance to gummy stem blight or Fusarium wilt may exist among *C. lanatus* var. *citroides* PIs. This study demonstrates that molecular markers can be useful in assessing genetic diversity, and in sorting *Citrullus* PIs into phylogenetic groups prior to their evaluation for disease or pest resistance.

Introduction

The genus *Citrullus* Schrad. ex Eckl & Zeyh. contains four diploid species: *Citrullus lanatus* (Thunb.) Matsum. & Nakai, found in tropical and subtropical climates worldwide, includes the cultivated watermelon (*C. lanatus* var. *lanatus*) that thrives in West Africa and is also called 'egusi' melon, and the preserving melon (*C. lanatus* var. *citroides*) that is grown in Southern Africa, and is called 'tsamma' melon (Whitaker and Bemis 1976). *C. colocynthis* (L.) Schrad ('bitter apple'), is a perennial species grown in sandy areas throughout northern Africa, southwestern Asia, and the Mediterranean (Zamir et al. 1984; Burkill 1985; Jarret et al. 1997). *C. ecirrhosus* Cogn. is a perennial, wild species (Meeuse 1962). *C. rehmii* De Winter is an annual, wild species (De Winter 1990). The last two species are endemic to the desert regions of Namibia (Meeuse 1962).

During the last century, watermelon production has increased steadily. Today, watermelon accounts for 2% of the world area devoted to vegetable production. Although many watermelon cultivars were developed throughout the world during the last century, there is an ongoing need for watermelon improvement, especially for increased disease and pest resistance.

In our evaluation of watermelon germplasm for gummy stem blight resistance, we used the Germplasm Resources Information Network (GRIN, Online Database, National Germplasm Resources Lab-

oratory, Beltsville, MD; www.ars-grin.gov). We identified 34 PIs that have been reported to contain resistance to one or more diseases in watermelon, and used them for analysis in the present study. Of these PIs, seventeen are designated *C. lanatus* var. *lanatus*, twelve are designated *C. lanatus* var. *citroides*, and five are designated *C. colocynthis* (GRIN database). Eight *C. colocynthis* PIs, collected from various parts of the world, were also included in the present study. Estimation of genetic relatedness among these PIs may be useful in determining whether closely related watermelon accessions have resistance to the same diseases. Furthermore, randomly amplified polymorphic DNA (RAPD) analysis can be useful in establishing core germplasm collections with disease resistances useful in watermelon breeding programs.

In previous studies, isozymes were used to examine genetic diversity and phylogenetic relationships in *Citrullus* spp. (Zamir et al. 1984; Navot and Zamir 1987; Biles et al. 1989). Isozymes were also used to determine genetic linkage between genes affecting bitterness and flesh color in watermelon (Navot et al. 1990). In these studies, most isozymes tested for watermelon were monomorphic. A higher number of informative markers was detected using the RAPD procedure (Zhang et al. 1994; Hashizume et al. 1993). RAPD markers were used to estimate genetic diversity among watermelon cultivars (Lee et al. 1996), and to construct an initial genetic linkage map for watermelon (Hashizume et al. 1996). Jarret et al. (1997) determined genetic variation among PI accessions of *C. lanatus* var. *lanatus*, *C. lanatus* var. *citroides* and *C. colocynthis* using the PCR-based molecular marker technique 'simple sequence repeat (SSR) length polymorphisms'. In a recent study, using RAPD analysis, we found a low genetic diversity among 46 heirloom cultivars of watermelon (unpublished) and concluded that cultivated watermelon has a narrow genetic base. In the present study, the objectives were: 1) to identify RAPD primers useful in distinguishing among *Citrullus* PIs and watermelon cultivars; 2) to assess genetic relatedness among PIs reported to have disease resistances and 3) to estimate genetic diversity among and within groups of PIs in *C. lanatus* var. *lanthus, C. lanatus* var. *citroides* and *C. colocynthis*.

Materials and methods

Plant material

Seeds of PIs were obtained from the USDA, ARS, Plant Genetic Resources Conservation Unit (Griffin, Georgia). Seeds of the cultivars Charleston Gray (NSL-5267), and Ironsides (NSL-7369) were obtained from the USDA, ARS, National Seed Storage Laboratory (NSSL, Fort Collins, Colorado). Seeds of the cultivars Allsweet, Mickylee and New Hampshire Midget were kindly provided by Novartis seeds (Napels, Florida). Seeds were germinated in the greenhouse, and 10 g of young leaves were collected from 4 to 5 plants (3 weeks old) of each PI or cultivar and stored at $-80\,^{\circ}C$.

Isolation of DNA

In order to avoid co-isolation of polysaccharides, polyphenols and other secondary compounds that damage DNA, a modified procedure was used with high concentrations of CTAB (2.5%) and SDS (0.5%) in the DNA extraction buffer (Levi and Thomas 1999).

DNA amplification conditions and gel electrophoresis: Ten decamer oligonucleotides manufactured by the University of British Columbia, Biotechnology Center, British Columbia, Canada or by Operon Technologies Inc., Alameda, California, were used for PCR amplification as described by Levi et al. (1993). Amplification products were separated by electrophoresis in 1.4% agarose gels in 0.5× Tris borate buffer (Sambrook et al. 1989). The gels were stained with 0.5 μg/ml ethidium-bromide solution for 30 min and destained for 15 min in distilled water. DNA fragments were visualized under UV light and photographed using a still video system (Gel Doc 2000, Bio-Rad, Hercules, CA). The molecular weights of the amplification products were calculated using 1-kb DNA ladder standards (Gibco BRL, Gaithersburg, MD).

Data analysis

A pairwise similarity matrix was generated using the Nei-Li similarity index (Nei and Li 1979) as follows: Similarity $= 2\ N_{ab}/(N_a + N_b)$, where N_{ab} is the number of RAPD fragments shared by two genotypes (a and b) and N_a and N_b are the total number of RAPD fragments analyzed in each genotype. A dendrogram was constructed based on the similarity matrix data by applying the UPGMA cluster analysis using the Numerical Taxonomic and Multi-Variant Analysis System for PC (NTSYS-PC version 2) (Rohlf 1993).

Results and discussion

Initially, 140 primers (60 to 80% GC content) were screened. Of these, 30 primers that produced polymorphic markers were selected for further analysis (Table 1). These 30 primers produced 662 RAPD markers that could be rated with high confidence and ranged in molecular weight from 100 to 2650 base pairs (bp) (Figure 1). The number of markers produced by each primer in this study is relatively high (an average of 22 marker bands per primer). This is mainly due to high GC content (60–80%) of primers, high polymorphism among the species *C. colocynthis* and the two subspecies *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*, in addition to polymorphism among accessions within each species (Figure 1).

In order to estimate genetic diversity among *Citrullus* PIs and watermelon cultivars, genetic similarity coefficients were calculated, and a dendrogram was constructed (Figure 2). The dendrogram consisted of three major clusters. The first cluster contained a group of the five watermelon cultivars (Group I), a group of the *C. lanatus* var. *lanatus* accessions (Group II), and a branch of *C. lanatus* var. *lanatus* accessions (Group III) that appeared to be divergent and more closely related to *C. lanatus* var. *citroides* than the two other groups in the cluster (Figure 2). The second cluster contained a main group of *C. lanatus* var. *citroides* accessions (Group IV), and a small group of accessions that are more closely related to *C. lanatus* var. *lanatus* (group V). Among the accessions in this group is PI 296341 that was reported to contain resistance to Fusarium wilt (races 0, 1 and 2) (Netzer and Martyn 1989; Martyn and Netzer 1991), and is designated in the GRIN database as *C. lanatus* var. *lanatus* (Table 2). The third cluster consisted of closely related accessions (Group VI), and divergent accessions (Group VII) of *C. colocynthis*. This species is considered to have the broadest geographical distribution compared with

Table 1. The nucleotide sequences of RAPD primers used in the present study, and the number of polymorphic (PM), and monomorphic (MM) band markers produced by each primer. Primer names are according to manufacturer's identification system (Operon Technologies; OP, and University of British Columbia, UBC).

Primer	Nucleotide Sequence (5′ → 3′)	PM	MM
OPB-05	TGCGCCCTTC	11	2
OPB-11	GTAGACCCGT	24	
OPB-14	TCCGCTCTGG	14	1
OPC-05	GATGACCGCC	18	2
OPC-20	ACTTCGCCAC	15	1
OPD-02	GGACCCAACC	16	
OPD-07	TTGGCACGGG	17	
OPD-20	ACCCGGTCAC	25	
OPE-04	GTGACATGCC	19	
OPJ-06	TCGTTCCGCA	26	
OPJ-13	CCACACTACC	19	
OPK-14	CCCGCTACAC	21	
OPK-20	GTGTCGCGAG	17	
OPT-01	GGGCCACTCA	26	
OPT-05	GGGTTTGGCA	29	
UBC106	CGTCTGCCCG	32	3
UBC115	TTCCGCGGGC	25	1
UBC137	GGTCTCTCCC	21	
UBC147	GTGCGTCCTC	8	
UBC149	AGCAGCGTGG	25	
UBC152	CGCACCGCAC	22	4
UBC155	CTGGCGGCTG	16	1
UBC157	CGTGGGCAGG	24	1
UBC159	GAGCCCGTAG	10	
UBC186	GTGCGTCGCT	28	2
UBC199	GCTCCCCCAC	27	4
UBC212	GCTGCGTGAC	21	1
UBC218	CTCAGCCCAG	20	2
UBC222	AAGCCTCCCC	29	1
UBC228	GCTGGGCCGA	31	

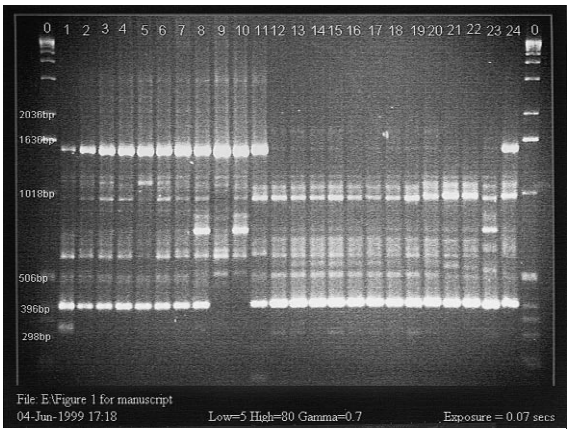

Figure 1. PCR-RAPD patterns (on 1.4% agarose gel) among eight *C. lanatus* var. *lanatus* (L), three *C. lanatus* var. *citroides* (C), and thirteen *C. colocynthis* (Y) PIs, produced by primer OPE-04. Lanes are: 0) DNA markers showing molecular size, 1) PI 189317 (L), 2) PI 192937 (L), 3) PI 203551 (L), 4) PI 248178 (L), 5) PI 249010 (L), 6) PI 270306 (L), 7) PI 270550 (L), 8) 271778 (L), 9) 296341 (C), 10) PI 482252 (C), 11) PI 271779 (C), 12) PI 220778 (Y), 13) PI 269365 (Y), 14) PI 386015 (Y), 15) PI 386016 (Y), 16) PI 386018 (Y), 17) PI 386019 (Y), 18) PI 386024 (Y), 19) PI 386025 (Y), 20) PI 386026 (Y), 21) PI 388770 (Y), 22) PI 432337 (Y), 23) PI 525082 (Y), 24) PI 525083 (Y).

other *Citrullus* species (Zamir et al. 1984). Indeed, the genetic variation among *C. colocynthis* accessions coincides with their geographical distribution. *C. colocynthis* is found in the Mediterranean region (PI 432337 was collected in Cyprus), North Africa (PI 388770 was collected in Morocco and PI 525082 and PI 525083 were collected in Egypt), and in South-Central Asia (PI 386015, PI 386016, PI 386018, PI 386019, PI 386024, PI 386025 and PI 386026 were collected in Iran, while PI 220778 and PI 269365 were collected in Afghanistan). The closely related PIs (Group VI) were collected in South-Central Asia, and the divergent PIs (Group VII) were collected in South-Central Asia, the Mediterranean region and North Africa. PI 269365 (Afghanistan) and PI 386015 (Iran) are closely related, while PI 386026 (Iran) is closely related to PI 432337 (Mediterranean island of Cyprus) and to PI 388770 (Morocco) (Figure 2). PI 346082 (Afghanistan) is designated in the GRIN database as *C. lanatus* var. *citroides*. However, in the present study it appeared to be *C. colocynthis*(Figure 2). Our observations of plants of this PI confirmed that it has morphological characteristics of *C. colocynthis*, having deeply divided leaves, thick tendrils, and small globular bitter fruits.

The *C. lanatus* clusters differentiated from each

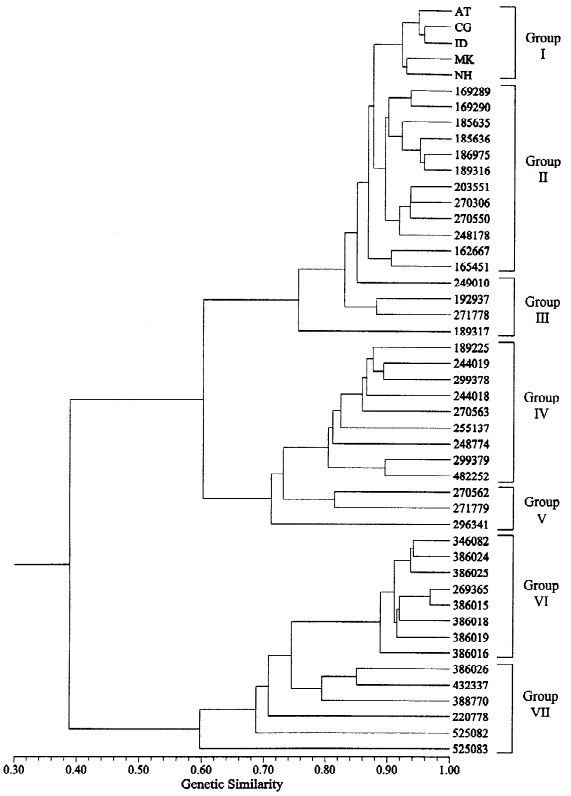

Figure 2. Dendrogram of *Citrullus* PIs and watermelon cultivars [Allsweet (AT), Charleston Gray (CG), Ironsides (ID), Mickylee (MK) and New Hampshire Midget (NH)] produced by UPGMA cluster analysis of similarity matrix.

other at the level of 58.8% genetic similarity, and from the *C. colocynthis* cluster at the level of 38.9% genetic similarity (Figure 2). These results are consistent with those reported for *Citrullus* by Navot and Zamir (1987) using isozymes, and by Jarret et al. (1997) using SSR length polymorphisms.

In the present study we used 17 PIs that are designated *C. lanatus* var. *lanatus*, 12 PIs that are designated *C. lanatus* var. *citroides*, and 13 PIs that are designated *C. colocynthis* (GRIN database). Most of these PIs, particularly those in the *C. lanatus* groups, were not chosen randomly but based on their resistance to diseases. Thus, these PIs may not accurately reflect the whole genetic diversity within each of the two *C. lanatus* subspecies. Nevertheless, the results in the present study are consistent with those in previous studies (Navot and Zamir 1987; Jarret et al. 1997), indicating higher levels of genetic diversity within *C. colocynthis* and *C. lanatus* var. *citroides* as compared with *C. lanatus var. lanatus*(Figure 2). The five watermelon cultivars in this study were chosen to

Table 2. Plant introduction accessions (PIs) used in the present study, the species (as designated in GRIN) and phylogenetic group (as classified in Figure 2) to which they belong, and the country from where they were collected. Among these are PIs that were reported (GRIN database) to have resistance to one of the following diseases: anthracnose races 1 and 3 (AC), anthracnose race 2 (AC2), downy mildew (DM), powdery mildew (PM), seed blight (SB) gummy stem blight (GSB), or watermelon mosaic virus (WMV), Fusarium wilt race 2 (FW). The level of resistance is reported at the range of 1–9, whereas 1 is fully resistant, and 9 is fully diseased. PIs that are not reported to be tested for disease resistance were designated (−).

				Disease Resistance							
PI#	Species	Group	Country	AC	AC2	DM	PM	SB	GSB	FW	WMV
162667	lanatus	II	Argentina	1	9	4	9	—	9	—	9
165451	lanatus	II	Mexico	1	9	4	9	9	9	—	9
169289	lanatus	II	Turkey	—	9	2	—	—	9	—	9
169290	lanatus	II	Turkey	9	9	2	1	9	9	—	9
185635	lanatus	II	Ghana	1	9	4	—	—	9	9	1
185636	lanatus	II	Ghana	1	9	4	—	—	9	—	9
186975	lanatus	II	Ghana	—	9	9	—	9	9	9	1
189316	lanatus	II	Nigeria	—	9	—	—	9	9	9	1
203551	lanatus	II	U.S.A.	1	1	—	—	9	9	9	9
248178	lanatus	II	Zaire	9	9	—	—	—	—	9	1
270306	lanatus	II	Philippines	1	9	—	—	9	9	—	9
270550	lanatus	II	Ghana	9	1	—	—	9	9	9	9
189317	lanatus	III	Zaire	—	9	—	—	9	9	—	1
192937	lanatus	III	China	—	9	4	—	9	9	—	1
249010	lanatus	III	Nigeria	1	9	9	—	9	—	—	9
271778	lanatus	III	S. Africa	—	1	—	—	9	1	7	9
189225	citroides	IV	Zaire	—	1	—	—	—	1	—	9
244018	citroides	IV	S. Africa	—	9	—	—	9	9	9	1
244019	citroides	IV	S. Africa	—	9	9	—	9	9	7	1
248774	citroides	IV	Namibia	1	9	—	—	9	—	—	9
255137	citroides	IV	S. Africa	—	9	9	—	9	9	9	1
270563	citroides	IV	S. Africa	1	9	—	—	9	9	—	9
299378	citroides	IV	S. Africa	—	9	—	—	1	9	7	9
299379	citroides	IV	S. Africa	1	1	—	—	9	9	7	9
482252	citroides	IV	Zimbabwe	—	—	—	—	—	—	9	1
270562	citroides	V	S. Africa	1	9	—	—	9	9	—	9
271779	citroides	V	S. Africa	1	1	—	—	9	9	5	9
296341	lanatus	V	S. Africa	—	—	—	—	9	9	1	—
269365	colocynthis	VI	Afghanistan	—	—	—	—	—	9*	9	—
346082	citroides	VI	Afghanistan	—	9	—	—	9	9*	9	1
386015	colocynthis	VI	Iran	—	—	—	—	—	9*	9	9
386016	colocynthis	VI	Iran	—	—	—	—	—	9*	9	1
386018	colocynthis	VI	Iran	—	—	—	—	—	9*	9	1
386019	colocynthis	VI	Iran	—	—	—	—	—	9*	9	9
386024	colocynthis	VI	Iran	—	—	—	—	—	9*	9	1
386025	colocynthis	VI	Iran	—	—	—	—	—	9*	9	1
220778	colocynthis	VII	Afghanistan	—	—	—	—	—	9*	—	—
386026	colocynthis	VII	Iran	—	—	—	—	—	9*	—	1
388770	colocynthis	VII	Morocco	—	—	—	—	—	9*	7	9
432337	colocynthis	VII	Cyprus	—	—	—	—	—	9*	9	9
525082	colocynthis	VII	Egypt	—	—	—	—	—	9*	—	—
525083	colocynthis	VII	Egypt	—	—	—	—	—	9*	—	—

* Our observations

represent a wide range of horticultural traits (fruit size and shape, flesh texture and color, rind color and firmness). Nevertheless, the RAPD-based genetic variation among these cultivars was relatively low (Figure 2). In an additional study that we conducted with 46 heirloom cultivars, RAPD analysis indicated that the cultivated watermelon *C. lanatus* var. *lanatus* has a narrow genetic base (unpublished).

Previous studies using isozymes (Zamir et al. 1984; Biles et al. 1989) or RAPD markers (Hashizume et al.

1993; Zhang et al. 1994; Lee et al. 1996) also indicated low polymorphism within *C. lanatus* var. *lanatus* that is considered to be the wild progenitor of the cultivated watermelon. Katzir et al. (1996) could not detect any polymorphism among a tripolid (Tri-X-313) and two diploid cultivars (Sugar Baby and Malali) of watermelon, using seven SSR length polymorphism markers. In their study, Navot and Zamir (1987) relate the low level of isozyme polymorphism to the domestication of *C. lanatus* var. *lanatus* away from its center of origin. These same authors also suggest that *C. lanatus* var. *citroides* is likely to be the wild progenitor of *C. lanatus* var. *lanatus*. This is due to the fact that most of the alleles detected in *C. lanatus* var. *lanatus* are also common in *C. lanatus* var. *citroides*. Jarret et al. (1997) also reported higher genetic diversity among *C. lanatus* var. *citroides* as compared with *C. lanatus* var. *lanatus*.

Fifteen accessions in the present study have been tested for resistance to races 1 and 3 of anthracnose (GRIN database) that cause foliar disease in watermelon. Of these, twelve were reported to contain resistance (Table 2). A few of these resistant accessions appeared to be closely related. For example, PI 162667 and PI 165451 were reported to have resistance to races 1 and 3 of anthracnose (Table 2), and are closely related (Figure 2, group II). Similarly, PI 185635 and PI 185636, or PI 203551 and PI 270306 have resistance to these two races of anthracnose (Table 2), and are closely related (Figure 2, Group II). Five of the *C. lanatus* var. *citroides* PIs in this study have been tested, and are resistant to races 1 and 3 of anthracnose (Table 2).

Most watermelon PIs that were resistant to race 1 were also resistant to race 3, but not to race 2 of anthracnose (Jenkins et al. 1964). Twenty-seven accessions in this study have been tested for resistance to race 2 of anthracnose (GRIN database). Of these, six PIs [PI 203551 and PI 270550 (Group II), PI 271778 (Group III), PI 189225 and PI 299379 (Group IV), and PI 271779 (Group V)] were reported to be resistant to this race of anthracnose (Table 2). Among these, PI 203551 and PI 270550 appeared to be closely related (Figure 2, Group II). Three of these PIs (PI 203551, PI 299379, and PI 271779) were reported to be resistant to all three races (1, 2 and 3) of anthracnose (Table 2)(Sowell et al. 1980; Boyhan et al. 1994).

Thirty-eight accessions in this study have been tested for gummy stem blight (GSB) resistance (GRIN database). Of these, only PI 189225 (Group IV) and PI 271778 (Group III) were reported to contain resistance to this disease (Sowell and Pointer (1962), Sowell (1975); Table 2). GSB is considered a major watermelon disease in North America. Our preliminary observations for resistance to this disease indicate that a few *C. lanatus* var. *citroides* accessions contain higher resistance as compared with *C. lanatus* var. *lanatus* accessions (data not shown), while all *C. colocynthis* accessions are highly susceptible (Table 2). The preliminary ratings for GSB severity on the Horsfall-Barratt scale of 1–12 (symptomless-100% diseased) were in the ranges of 4–9, 6–10 and 7–12, for *C. lanatus* var. *citroides*, *C. lanatus* var. *lanatus* and *C. colocynthis*, respectively (data not shown). Based on these observations resistance to GSB may exist among *C. lanatus* var. *citroides* accessions. Currently, only a few of the *C. lanatus* var. *citroides* accessions are reported to have been tested for resistance to GSB (GRIN database). Therefore, additional efforts are needed to expedit the testing of this group of PIs for resistance to GSB.

Eleven accessions in this study have been tested for downy mildew resistance (GRIN database). Of these, PI 169289 and PI 169290 were reported to be resistant to this disease (Table 2). These two PIs also appear to be closely related (Figure 2, Group II). PI 169290 was also reported to contain resistance to powdery mildew (Table 2). The incidences of this disease in watermelon fields are increasing in North America in recent years, and there is a great need to find new sources of resistance to powdery mildew among the *Citrullus* PIs (B. D. Bruton, personal communication).

Twenty-six accessions in the present study have been tested for Fusarium wilt resistance; *Fusarium oxysporum* f. sp. *niveum* race 2 (Table 2). Of these, PI 296341 (Group V) was reported to be resistant (Netzer and Martyn 1989; Martyn and Netzer 1991), while PI 271779 (Group V) and PI 189317 (Group III) were reported to be moderately resistant to this disease (Dane et al. 1998). These researchers also report that a *C. lanatus* var. *citroides*, PI 271769 (not included in the present study), contains high resistance to Fusarium wilt race 2.

Thirty-seven accessions in this study have been tested for watermelon mosaic virus (WMV) resistance (GRIN database). Of these, sixteen were reported to contain resistance (Table 2). These resistant accessions are found in all major groups (Groups II, III, IV, VI and VII, Table 2). Among these, PI 186975

and PI 189316 are closely related (Figure 2, Group II). PI 346082 that is designated (GRIN database) *C. lanatus* var. *citroides*, but appeared to be *C. colocynthis* in the present study (Figure 2), has been reported to be WMV resistant (Table 2). Five other *C. colocynthis* accessions (PI 386016, PI 386018, PI 386024, PI 386025 and PI 386026) tested were also reported to be resistant to WMV (Table 2)(Gillaspie and Wright 1993).

The data in the present study indicate that resistance to either anthracnose (races 1, 2 and 3), downy mildew, powdery mildew, or WMV exist in most of the major groups (Table 2). This includes accessions of *C. lanatus* var. *lanatus* (Group II) that are closely related to the cultivated watermelon (Figure 2), and can be readily used in breeding programs. On the other hand, resistance to the major diseases, gummy stem blight and Fusarium wilt, may exist among accessions that are closely related or belong to *C. lanatus* var. *citroides* (Groups III, IV and V).

Conclusion

Of the 1600 *Citrullus* PIs collected at the USDA, ARS, Plant Genetic Resources Conservation Unit (Griffin, Georgia), 1480 are *C. lanatus* var. *lanatus*, 102 are *C. lanatus* var. *citroides*, and 18 are *C. colocynthis* (GRIN). The *C. lanatus* var. *lanatus* PIs are preferred in watermelon breeding programs because of their close genetic proximity to the cultivated watermelon. However, the two other *Citrullus* types might also be an essential source of genes that would confer disease and pest resistance in the cultivated watermelon, although they do not have the desirable horticultural traits of *C. lanatus* var. *lanatus*. For example, in a recent study (Simmons and Levi 2000) *C. colocynthis* PIs had high resistance, while all *C. lanatus* PIs were highly susceptible to whitefly (*Bemisia*). Thus, additional efforts are needed to expand and test the *C. colocynthis* and *C. lanatus* var. *citroides* PIs for resistance to important diseases and pests of watermelon. The present study demonstrates that molecular markers can be useful in evaluating genetic diversity, and in classifying *Citrullus* PIs to phylogenetic groups based on their genetic similarity values. Such classification will enable the selection of representatives from each group of closely related PIs, that can be tested for disease or pest resistance. This approach may facilitate the development of representative core collections of valuable *Citrullus* PIs.

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References

Biles C.L., Martyn R.D. and Wilson H.D. 1989. Isozymes and general proteins from various watermelon cultivars and tissue types. HortScience 24: 810–812.

Boyhan G.E., Norton J.D., Abrahams B.R. and Wen N.H. 1994. A new source of resistance to anthracnose (Race 2) in watermelon. HortScience 29: 111–112.

Burkill H.M. 1985. The Useful Plants of West Tropical Africa. Royal Botanic Gardens, Kew, 2nd edn. 1.

Dane F., Hawkins L.K. and Norton J.D. 1998. New resistance to race 2 of *Fusarium oxysporum* f. sp. *niveum* in watermelon. Cucurbit Genet. Coop. Report 21: 37–39.

De Winter B. 1990. A new species of *Citrullus* (Benincaseae) from the Namib desert, Namibia. Bothalia 20: 209–211.

Gillaspie A.G. Jr and Wright J.M. 1993. Evaluation of *Citrullus* sp. germ plasm for resistance to watermelon Mosaic Virus 2. Plant Dis. 77: 352–354.

Hashizume T., Sato T. and Hirai M. 1993. Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) using random amplified polymorphic DNA (RAPD). Jpn. J. Breed. 43: 367–375.

Hashizume T., Skimamoto I., Harushima Y., Yui M., Sato T., Imai T. et al. 1996. Construction of a linkage map for watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] using random amplified polymorphic DNA (RAPD). Euphytica 90: 265–273.

Jarret R.L., Merrick L.C., Holms T., Evans J. and Aradhya M.K. 1997. Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Genome 40: 433–441.

Jenkins S.F., Winstead N.N. and McCombs C.L. 1964. Pathogenic comparison of three new and four previously described races of *Glomerella angulata* var. *orbiculare*. Plant. Dis. Rptr. 48: 619–623.

Katzir N., Danin-Poleg Y., Tzuri G., Karchi Z., Lavi U. and Creagan P.B. 1996. Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor. Appl. Genet. 93: 1282–1290.

Lee S.J., Shin J.S., Park K.W. and Hong Y.P. 1996. Detection of genetic diversity using RAPD-PCR and sugar analysis in watermelon [*Citrullus lanatus* (Thunb.) Mansf.] germplasm. Theor. Appl. Genet. 92: 719–725.

Levi A., Rowland L.J. and Hartung J.S. 1993. Production of reliable randomly amplified polymorphic DNA (RAPD) markers from DNA of woody plants. HortScience 28: 1188–1190.

Levi A. and Thomas C.E. 1999. An improved procedure for isolation of high quality DNA from watermelon and melon leaves. Cucurbit Genet. Coop. Report 22: 41–42.

Martyn R.D. and Netzer D. 1991. Resistance to races 0, 1 and 2 of Fusarium wilt of watermelon in *Citrullus* sp. PI-296341-FR. HortScience 26: 429–432.

Meeuse A.D. 1962. The *Cucurbitaceae* of Southern Africa. Bothalia 8: 1–111.

Navot N. and Zamir D. 1987. Isozyme and seed protein phylogeny of the genus*Citrullus* (Cucurbitaceae). Plant Syst. Evol. 156: 61–67.

Navot N., Sarfatti M. and Zamir D. 1990. Linkage relationships of genes affecting bitterness and flesh color in watermelon. J. Hered. 81: 162–165.

Nei M. and Li W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Netzer D. and Martyn R.D. 1989. PI 296341, a source of resistance in watermelon to race 2 of *Fusarium oxysporum* f. sp. *niveum*. Plant Disease 73: 518.

Rohlf F.J. 1993. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System. Exter Publishing, Ltd., Setauket, New York, Version 2.00.

Sambrook J., Fritsch E.F. and Maniatis T. 1989. Molecular Cloning. A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

Simmons A.M. and Levi A. 2000. Evaluation of watermelon germplasm for resistance to *Bemisia*. Entomological Society of America Annual Meeting. Abstract No. 15611.

Sowell G. Jr 1975. An additional source of resistance to gummy stem blight in watermelon. Plant Dis. Reptr. 59: 413–415.

Sowell G. Jr and Pointer G.R. 1962. Gummy stem blight resistance of introduced watermelons. Plant Dis. Reptr. 46: 883–885.

Sowell G. Jr, Rhodes B.B. and Norton J.D. 1980. New Sources of resistance to watermelon anthracnose. J. Amer. Soc. Hort. Sci. 105: 197–199.

Whitaker T.W. and Bemis W.B. 1976. Cucurbits. In: Simmonds N.W (ed.), Evolution of crop plants. Longman, London, pp. 64–69.

Zamir D., Navot N. and Rudich J. 1984. Enzyme polymorphism in *Citrullus lanatus* and*C. colocynthis* in Israel and Sinai. Plant Syst. Evol. 146: 163–137.

Zhang X.P., Rhodes B.B. and Skorupska H.S. 1994. RAPD molecular markers in watermelon. Cucurbit Genet. Coop. Report 17: 116–119.