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Low Genetic Diversity Indicates the Need to Broaden the Genetic Base of Cultivated Watermelon

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Abstract. **Genetic diversity and relatedness were assessed among 46 American cultivars of watermelon (*Citrullus lanatus* var. *lanatus*), and 12 U.S. Plant Introduction accessions (PIs) of *Citrullus* sp. using 25 randomly amplified polymorphic DNA (RAPD) primers. These primers produced 288 distinct reproducible bands that could be scored with high confidence among cultivars and PIs. Based on the RAPD data, genetic similarity coefficients were calculated and a dendrogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA). The cultivars and *C. lanatus* var. *lanatus* PIs differentiated at the level of 92% to 99.6% and 88% to 95% genetic similarity, respectively. In contrast, the *C. lanatus* var. *citroides*, and *C. colocynthis* PIs were more divergent and differentiated at the level of 65% to 82.5% and 70.5% genetic similarity, respectively. The low genetic diversity among watermelon cultivars in this study emphasizes the need to expand the genetic base of cultivated watermelon.**

The xerophytic genus *Citrullus* Schrad. ex Eckl. & Zeyh. belongs to the Cucurbitaceae family. It comprises four known diploid (n = 11) species found in the temperate regions of Africa, Central Asia, and the Mediterranean (Jeffrey, 1975; Whitaker and Davis, 1962). Among these species is *C. lanatus* (Thunb.) Matsum & Nakai, from which the cultivated watermelon (*C. lanatus* var. *lanatus*) originated (Whitaker and Bemis, 1976; Whitaker and Davis, 1962). Watermelon has been cultivated in Central Africa for at least 5000 years, and in Egypt and in the Middle East for over 4000 years. By the 10th century it was introduced to China, which is the world's greatest producer and consumer of watermelon. By the 13th century, watermelon was grown in Europe, and the crop was introduced into North America during the 17th century (Jeffrey, 1975; Whitaker and Davis, 1962). Major U.S. production areas are in Florida, California, Texas, Georgia, and Arizona. U.S. watermelon production has increased from 1.2 million tons in 1980 to 3.9 million tons in 1999 with a farm value of $270 million [U.S. Dept. of Agriculture (USDA), Agricultural Statistics, 2000]. Over 500 diploid cultivars were developed in the United States during the last two centuries, but there is an ongoing need to improve watermelon, particularly with respect to development of disease and pest resistant cultivars.

There is little information regarding the ancestries of many American watermelon cultivars developed during the 19th and early 20th centuries (G.W. Elmstrom, personal communication). Identification of watermelon cultivars and determination of their genetic purity and relatedness relies mainly on fruit characteristics. Molecular markers can be an effective means to determine genetic relatedness among cultivars and among selections used in watermelon breeding programs. In previous studies designed to examine genetic diversity and phylogenetic relationships among watermelon cultivars using isozymes, most isozymes tested produced monomorphic patterns (Biles et al., 1989; Zamir et al., 1984). In contrast, RAPD procedure provided a sufficient number of informative markers that could distinguish among watermelon cultivars (Hashizume et al., 1993; Zhang et al., 1994). In a recent study (Levi et al., 2000), genetic diversity and relatedness were examined among U.S. Plant Introduction accessions (PIs) of *C. lanatus* var. *lanatus, C. lanatus var. citroides, and C. colocynthis* using RAPD analysis. RAPD markers were also used in the construction of an initial genetic linkage map for watermelon (Hashizume et al., 1996), and to determine genetic relatedness among Asian watermelon cultivars and breeding lines (Lee et al., 1996).

Three hundred and fourteen American cultivars are stored at the USDA, ARS, National Seed Storage Laboratory (NSSL, Fort Collins, Colo.), and are considered an essential germplasm resource for watermelon breeding programs. Among them are 'Allsweet', 'Au-Producer', 'Charleston Gray', 'Crimson Sweet', 'Jubilee', and 'Peacock', that are grown throughout the world and are widely used as parents for many hybrids. Currently, there are no molecular data assessing the genetic relatedness and diversity among these cultivars. The objectives of this study were to: 1) estimate genetic relatedness and diversity among American cultivars; and 2) compare their genetic diversity with that in PI accessions of *Citrullus*.

Materials and Methods

Plant material. Forty-six watermelon cultivars (Table 1) and twelve PIs representing the three major *Citrullus* groups (*C. lanatus* var. *lanatus, C. lanatus* var. *citroides*, and *C. colocynthis*) (Table 2) were evaluated. Seeds of cultivars were obtained from commercial seeds companies, and the USDA NSSL in Fort Collins, Colo. (Table 1). Seeds of all PIs were obtained from the USDA Plant Genetic Resources Conservation Unit in Griffin, Ga. All plants were grown in the greenhouse. Young leaves were collected from four to five, 3-week-old-plants of each watermelon accession and stored at –80 °C.

Marker data collection. DNA was isolated from young watermelon leaves as described by Levi and Thomas (1999). One hundred and thirty eight primers (60% to 80% GC content) were screened for polymorphism using the cultivars Blackstone and Stone Mountain #5. These two cultivars were chosen for the initial screening of primers because of their difference in parentage (Table 1). RAPD-PCR reactions were performed 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 $mg \cdot mL^{-1}$ 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, Calif.). The molecular weights of amplification products were calculated using the "1-Kb Plus 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) according to the equation: Similarity = $2 N_{ab} / (N_a + N_b)$, where

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Table 1. Watermelon cultivars evaluated in this study, including source of seeds, parental background, year of introduction, and fruit characteristics.

Cultivar	Source of seeds	Breeding parentage	Year introduced	Fruit shape	Fruit wt	Flesh color	Flesh firmness	Rind color	Rind firmness	Maturity
Allsweet	Sunseeds[z]	(Miles x Peacock) x Charleston Gray	1972	Long	25	Pink	Firm	Green w/deep green wide stripes	Firm	Late
Astrakanski	Seed Savers[y]	---[x]	---	---	---	---	---	---	---	---
AU-Golden Producer	Hollar[w]	Selection from AU-Producer	1993	Globe	20	Light red	Firm	Light green w/green wide stripes	Firm	Mid-early
AU-Jubilant	Hollar	Jubilee x PI 271778	1985	Long	25	Light red	Firm	Light green w/green narrow stripes	Firm	Midseason
AU-Producer	Hollar	Crimson Sweet x PI 189225	1985	Globe	20	Light red	Firm	Light green w/green wide stripes	Firm	Mid-early
Black Diamond	Sunseeds	---	---	Globe	15	Light red	Soft	Dark green	Medium	Early
Blackstone	Hollar	Florida Giant, Fairfax	---	Globe	15	Light red	Soft	Dark green	Medium	Mid-early
Calhoun Gray	Sunseeds	---	---	Long	20	Light red	Firm	Light green/gray	Firm	Late
CalSweet	Sunseeds	---	---	Oblong	20	Deep red	Firm	Green w/deep green wide stripes	Firm	Late
Charleston Gray	NSL-5267[v] and NKL&G[u]	Africa 8, Iowa Belle,Garrison, Hawkesbury, Leesburg	1954	Long	20	Light red	Firm	Light green/gray	Firm	Late
Coles Early	NSL-5270	---	1892	---	---	---	---	---	---	---
Congo	Syngenta[t]	(African x Iow Belle) x Garrison	1949	Long	25	Light red	Firm	Deep green w/dark green narrow stripes	Firm	Late
Crimson Sweet	Hollar	(Miles x Peacock) x Charleston Gray	1963	Globe	20	Light red	Firm	Light green w/green wide stripes	Firm	Mid-early
Dixielee	Hollar	Texas W5, Wilt resistant Peacock, Fairfax, Summit	1979	Globe	20	Deep red	Firm	Light green w/green narrow stripes	Firm	Late
Dixie Queen	Sunseeds	---	1890	Globe	20	Deep red	Firm	Light green w/green narrow stripes	Firm	Late
Dunbarton	NSL-6637	(African x Iowa Belle) x Garrison	1953	---	---	---	---	---	---	---
Fairfax	Sunseeds	Garrison, African, Iowa Belle, Leesburg, Hawkesbury	1952	Long	20	Light red	Firm	Light green w/green narrow stripes	Firm	Late
Family Fun	Syngenta	---	1973	---	---	---	---	---	---	---
Garrison	NSL-2053	---	---	Long	20	Light red	Firm	Light green w/green narrow stripes	Firm	Late
Garrisonian	Willhite[s]	Africa 8, Iowa Belle, Garrison, Hawkesbury, Leesburg	1957	Long	20	Light red	Firm	Light green w/green narrow stripes	Firm	Late
Georgia Rattlesnake	Seed Savers	---	---	---	---	---	---	---	---	---
Golden Honey	Hollar	---	1954	Globe	12	---	Soft	---	Explosive	Early
Golden Midget	NSL-5288	Mixed strain from Japan Seed Co.	1959	Globe	12	Yellow	Soft	---	Tender	Early
Hawkesbury	Syngenta	New Hampshire Midget x Pumpkin Rind	1936	---	---	---	---	---	---	---
Iopride	Syngenta	---	---	Globe	20	Light red	Firm	Deep green w/dark green narrow stripes	Firm	Midseason

Table 1 continued on next page

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 unweighted pair-group method with arithmetic average (UPGMA) cluster analysis using the Numerical Taxonomic and Multi-Variant Analysis System for PC (NTSYS-PC version 2) (Rohlf, 1993).

Results and Discussion

Of the 138 primers that were initially screened, only 35 produced polymorphic RAPD patterns. Of these, 25 primers that produced distinct polymorphic bands were used for further analysis with all cultivars and PIs. The 25 primers produced 288 reproducible RAPD bands that ranged in molecular size from 100 to 3000 base pairs (bp) (Fig. 1). Of these bands, 26 were monomorphic for all cultivars and PIs, 9 were polymorphic among cultivars, but were monomorphic for all PIs. One hundred and sixty eight bands were polymorphic among PIs, but were monomorphic for all cultivars, while 85 bands were polymorphic among all cultivars and PIs (Table 3).

The RAPD marker data were used to construct a genetic similarity matrix among cultivars and PIs (data not shown) based on the Nei-Li estimate of similarity (Nei and Li, 1979). The similarity matrix was used in a UPGMA cluster analysis to produce a genetic similarity dendrogram (Fig. 2). High genetic similarity values (92% to 99.6%) were detected among watermelon cultivars and among PIs of *C. lanatus* var. *lanatus* (88% to 95%) which is considered the progenitor of the cultivated watermelon. Lower similarity values were found among the *C. lanatus* var. *citroides* and among the wild species *C. colocynthis* PIs (65% to 82.5%, and 70.5%, respectively) (Fig. 2). In an additional study designed to elucidate genetic diversity in *Citrullus* sp. (Levi et al., 2000), genetic diversity and relatedness were estimated among 17 *C. lanatus* var. *lanatus* PIs, 12 *C. lanatus* var. *citroides* PIs, 13 *C. colocynthis* PIs, and five cultivars ('Allsweet', 'Charleston Gray', 'Ironsides', 'Mickylee', and 'New Hampshire Midget') using RAPD analysis. In that study, the five watermelon cultivars grouped in a distinct cluster, indicating that they were derived from common parents. However, the low genetic diversity among *C. lanatus* var. *lanatus* PIs in the previous study (Levi et al., 2000), and in the present study (Fig. 2) indicates that the lack of genetic diversity among cultivars is due to a narrow genetic base in *C. lanatus* var. *lanatus*. Navot and Zamir (1987) found little isozyme variation among watermelon accessions, and suggest that this is due to the domestication of watermelon outside of its center of origin. They base this assumption on the probability that only a small fraction of plants of the progenitor species (a few plants that have desired qualities) were selected and used at the early stages of domestication (Ladizinsky, 1985). Katzir et al. (1996) could not detect any polymorphism among the watermelon cultivars Sugar Baby and Malali (diploids), and Tri-X-313 (triploid) using seven simple sequence repeat (SSR) markers. However, Jarret et al. (1997) could detect genetic diversity among *Citrullus* PIs using the same SSR markers. In that study, PIs of *C. lanatus* var. *citroides* were slightly more divergent than PIs of *C. lanatus* var. *lanatus*. Navot and Zamir (1987) considered *C. lanatus* var. *citroides* as the wild progenitor of *C. lanatus* var. *lanatus*.

In contrast with the low DNA polymorphism, extensive variation in morphological characteristics existed among watermelon cul-

Table 1. Continued.

Cultivar	Source of seeds	Breeding parentage	Year introduced	Fruit shape	Fruit wt	Flesh color	Flesh firmness	Rind color	Rind firmness	Maturity
Ironsides	NSL-7369	(Leesburg x Hawkesbury) x Garrison	1950	---	---	---	---	---	---	---
Jubilee	Hollar	Africa 8, Iowa Belle, Garrison, Hawkesbury, Leesburg	1963	Long	25	Light red	Firm	Light green w/green narrow stripes	Firm	Mid-season
King and Queen	Hollar	---	---	Globe	12	Light red	Soft	Light green	Medium	Early
Kleckely's Sweet	Seed Savers	---	---	Globe	12	Light red	Soft	Deep green	Medium	Early
Klondike	Syngenta	---	1959	Long	20	Light red	Firm	Dark green	Firm	Late
Klondike Striped RS 57	Sunseeds	---	---	Long	20	Light red	Firm	Light green w/green narrow stripes	Firm	Late
Klondike Striped-Blue Ribbon	Hollar	---	1939	Long	20	Light red	Firm	Light green w/green narrow stripes	Firm	Late
Leesburg	NSL-7368	Selection from Kleckley Sweet	1936	---	---	---	---	---	---	---
Melitoplisky	Seed Savers	---	---	---	---	---	---	---	---	---
Mickylee	Hollar	Texas W5, Fairfax, Summit, Graybelle	1986	Globe	15	Deep red	Firm	Light green/gray	Firm	Midseason
Miles	NSL-6688	Dixie Queen x Klondike R-7	1948	---	---	---	---	---	---	---
Minilee	Hollar	Texas W5, Fairfax, Summit, Graybelle	1986	Globe	8	Deep red	Firm	Light green/gray	Firm	Early
New Hampshire Midget	Syngenta	Favorite Honey x Dakota Sweet	1951	Globe	6	Red	Soft	Light green/gray	Explosive	Early
Northern Sweet	Syngenta	---	1932	Globe	12	Red	Medium	Green w/lines	Medium	Early
Parker	Willhite	F_1	---	---	---	---	---	---	---	---
Peacock	Hollar	---	1939	Oblong	20	Red	Firm	Dark green	Firm	Late
Prince Charles	Syngenta	F1	1978	Long	20	Red	Firm	Light green/gray	Firm	Midseason
Sangria	Syngenta	F_1	1985	Long	25	Deep red	Firm	Green w/deep green wide stripes	Firm	Late
Sugar Baby	Sunseeds	Selection from Tough Sweets	1955	Globe	15	Orange red	Soft	Dark green w/dark stripes	Medium	Early
Stone Mountain	Hollar	---	1924	Oval	20	Light red	Firm	Light green w/green narrow stripes	Firm	Midseason
Stone Mountain #5	Syngenta	Stone Mountain x Iowa Belle	1936	---	---	---	---	---	---	---

[z]Sunseeds Co. (Acampo, Calif.).
[y]Seed Savers Exchange (Decorah, Iowa).
[x]Information unavailable.
[w]Hollar Seeds (Rocky Ford, Colo.).
[v]Accession number provided for each of the heirloom cultivars kept at the USDA National Seed Storage Laboratory (Fort Collins, Colo.).
[u]NK Lawn & Garden (Chattanooga,Tenn.).
[t]Syngenta Seeds (Naples, Fla.).
[s]Willhite Seed (Poolville, Texas).

Table 2. Twelve U.S. plant introduction accessions (PIs) examined in the present study, the *Citrullus* group to which they belong, the country from which they were collected, and their fruit characteristics as described by the Germplasm Resources Information Network (www.ars-grin.gov).

PI #	*Citrullus* type	Country of origin	Rind color background	Rind color pattern	Flesh color	Fruit diameter	Fruit shape	Frit maturity
162667	*lanatus*	Argentina	Medium green	Solid	Red	15/30	Oblong	Midseason
165451	*lanatus*	Mexico	Medium green	Solid	Pink	18/25	Oblong	Midseason
169289	*lanatus*	Turkey	Dark green	Solid	Red	36/36	Round	Early
185636	*lanatus*	Ghana	Medium green	Solid	White	10/10	Round	Late
189316	*lanatus*	Nigeria	Dark green	Striped	White	12/12	Round	Late
203551	*lanatus*	U.S.A.	Light green	Striped	White	12/12	Round	Midseason
271778	*lanatus*	S. Africa	Medium green	Striped	Yellow	22/27	Oblong	Midseason
270564	*citroides*	S. Africa	Light green	Solid	Yellow	20/25	Oblong	Early
482251	*citroides*	Zimbabwe	Medium green	Striped	Yellow	12/12	Round	Midseason
271779	*citroides*	S. Africa	yellow	Striped	Yellow	30/45	Oblong	Midseason
386014	*colocynthis*	Iran	Light green	Striped	White	9/9	Round	Early
388770	*colocynthis*	Morocco	Light green	Striped	White	12/12	Round	Early

tivars used in this study (Table 1). These characteristics included rind color and thickness, fruit shape and size, flesh texture and color, sugar content, seed shape and color, days to fruit maturity, and disease resistance. Most of these characteristics are qualitative traits affected by a single or a few gene mutations (Rhodes and Dane, 1999; Rhodes and Zhang, 1995) that could not be readily detected by the RAPD markers in this study. However, some of these mutations may be detected through a bulked segregant analysis procedure (Michelmore et al., 1991) using a large number of RAPD primers.

The watermelon cultivars in the present study are open-pollinated or F_1 hybrid diploid types (n = 11). The parentage records for most of these cultivars are incomplete (Table 1). However, records available for some of the cultivars are consistent with the RAPD-based results (Fig. 2). For instance, the gray-green rind and round fruit type cultivars Mickylee and Minilee were developed from sister plants that had the cultivars Fairfax, Summit, and Texas-W5 in their genetic background (Table 1) (Crall, 1986). 'Mickylee' and 'Minilee' appeared closely related in the present analysis (Fig. 2). The cultivar Garrison contributed to the genetic background of the oblong fruited cultivars Congo, Charleston Gray, and Garrisonian (Table 1). In the present analysis, 'Congo', 'Garrison', and 'Garrisonian' are in the same group, while 'Charleston Gray' is in a closely related group (Fig. 2). The cultivar Allsweet has a green-striped rind and oblong fruit (Table 1), and according to the records it

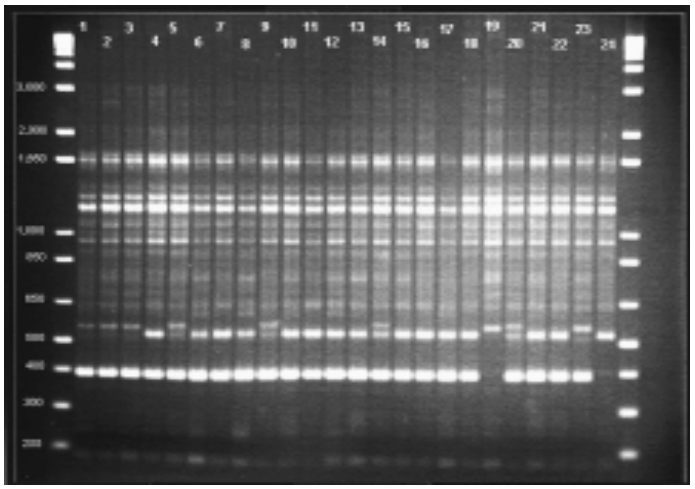

Fig. 1. PCR-RAPD patterns (on 1.4% agarose-gel) of watermelon cultivars produced by primer UBC-222. Lanes are: 0) 1-Kb Plus DNA ladder markers (Gibco BRL, Rockville, Md.), 1) 'Allsweet', 2) 'Astrakanski', 3) 'AU-Golden Producer', 4) 'AU-Jubilant', 5) 'AU-Producer', 6) 'Blackstone', 8) 'Calhoun Gray', 9) 'Calsweet', 10) 'Charleston Gray' (NSL-5267), 11) 'Charleston Gray' (NK Lawn & Garden Co), 12) 'Coles Early', 13) 'Congo', 14) 'Crimson Sweet', 15) 'Dixielee', 16) 'Dixie Queen', 17) 'Dunbarton', 18) 'Fairfax', 19) 'Family Fun', 20) 'Garrison', 21) 'Garrisonian', 22) 'Georgia Rattlesnake', 23) 'Golden Honey', 24) 'Golden Midget'.

Table 3. 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	

is comprised of 'Charleston Gray' (50%), 'Miles' (25%), and 'Peacock' (25%) (Table 1). The present analysis shows that 'Allsweet' is closer to 'Charleston Gray' than to the two other cultivars (Fig. 2). The cultivar AU-Golden Producer (globular fruit with green, wide-striped rind) is reported to be an orange fleshed mutation of 'AU-Producer' (Table 1). Predictably, these two cultivars are closely related (Fig. 2). 'AU-Producer' is derived from a cross of 'Crimson Sweet' (globular fruit with green-striped rind) with PI 189225 that is reported to have resistance to gummy stem blight (Sowell and Pointer, 1962). In the present study, 'Crimson Sweet' is indeed closely related to 'AU-Producer'. Like 'Allsweet', 'Crimson Sweet' also is comprised of 'Charleston-Gray' (50%), 'Miles' (25%), and 'Peacock' (25%) (Table 1). 'Allsweet' is indeed in the same group with 'Crimson-Sweet' and its descendents 'AU-Producer' and 'AU-Golden Producer' (Fig. 2). The cultivar Blackstone (globular fruit with dark green rind) contains 'Black-Diamond' (also known as Florida-Giant) and 'Fairfax' in its genome (Table 1). Indeed, 'Black-Diamond' and 'Blackstone' have similar characteristics (Table 1) and are in the same group (Fig. 2). 'Dixilee' is in the same group with 'Charleston-Gray' (Fig. 2). A detailed review (data not shown) reveals that these two cultivars are derived from common parents ('Peacock', 'Garrison', 'Leesburg', and 'Hawkesbury', Table 1).

Not all the results are fully consistent with parental records or with morphological characteristics. For instance, the cultivar Leesburg is a selection from 'Kleckely-Sweet' (Table 1). The present analysis shows that these two cultivars are in closely related groups, but are not clustered together (Fig. 2). Also, 'Stone-Mountain #5' is relatively distant from 'Stone-Mountain' (Fig. 2), which is considered one of its original parents, the other being 'Iowa Bell' (Table 1). The cultivar Jubilee is distant from 'Charleston Gray' and 'Garrisonian' (Fig. 2), which supposedly have similar parental backgrounds (Table 1). 'Jubilee' is also distant from its descendent 'Au-Jubilant' (Fig. 2).

Using a contingency test, we could not detect any significant association between RAPD markers and fruit shape. Therefore, it is likely that the RAPD markers here do not provide the resolution required to elucidate gene loci that control fruit characteristics, but elucidate random parts of the watermelon genome. Another indication for this assumption is that cultivars with different morphological characteristics appear closely related. For instance, the cultivar Calhoun-Gray, which has an elongated fruit with light green-gray rind, is in the same group with 'Blackstone' and 'Black Diamond' (Fig. 2), which have globular fruit and dark rind (Table 1).

Most cultivars in the present study have not been examined in previous studies of genetic relatedness using molecular markers. However, three cultivars ('Family Fun','Sugar Baby', and 'New Hampshire Midget') were examined together with watermelon cultivars and breeding lines developed in Asia (Lee et al., 1996). The genetic distance between 'Sugar Baby' and 'New Hampshire Midget' in the present study is comparable with that in the previous study. However, 'Family-Fun' appeared to be distant from most watermelon cultivars (Fig. 2), and not as closely related to 'Sugar Baby' as reported by Lee et al. (1996). This difference may be due to the use of different RAPD primers and RAPD procedures, or it may be due to use of seeds of cultivars that were produced by different sources. Due to the open-pollinated nature of watermelon, different genotypes may occur in a cultivar during seed increase, resulting in different RAPD patterns. To test this possibility, we examined 'Charleston Gray' plants from the original seed stock (NSSL) vs. 'Charleston Gray' plants from seeds provided by NK Lawn & Garden, Co. (Chattanooga, Tenn.). The RAPD patterns (as shown in Fig. 1) confirmed that these two types are alike, but not identical (Fig. 2). Two distinct RAPD markers (470 and 790 bp) produced by primer OPD-07 and UBC-155, respectively, were present in the original Charleston Gray cultivar (NSSL), but not in the type obtained commercially. The 790 bp marker was unique to the 'Charleston Gray' provided by NSSL and was not found in any other cultivar or PI in this study. These differences between two sources of the same cultivar indicate that changes in cultivar genotype may occur during seed increase. Thus, the source of the seeds for each cultivar tested should be taken into account in genetic studies and in DNA fingerprinting of watermelon cultivars.

The high genetic similarities among *C. lanatus* var. *lanatus* PIs, and among watermelon cultivars indicate that many cultivars developed in the United States over the last two centuries have a narrow genetic background. Therefore, it is essential to broaden the genetic base of the cultivated watermelon to reduce its vulnerability to diseases and insect pests. The recent study of Levi et al. (2000) indicated that the wild species *C. colocynthis*, which has the widest geographic

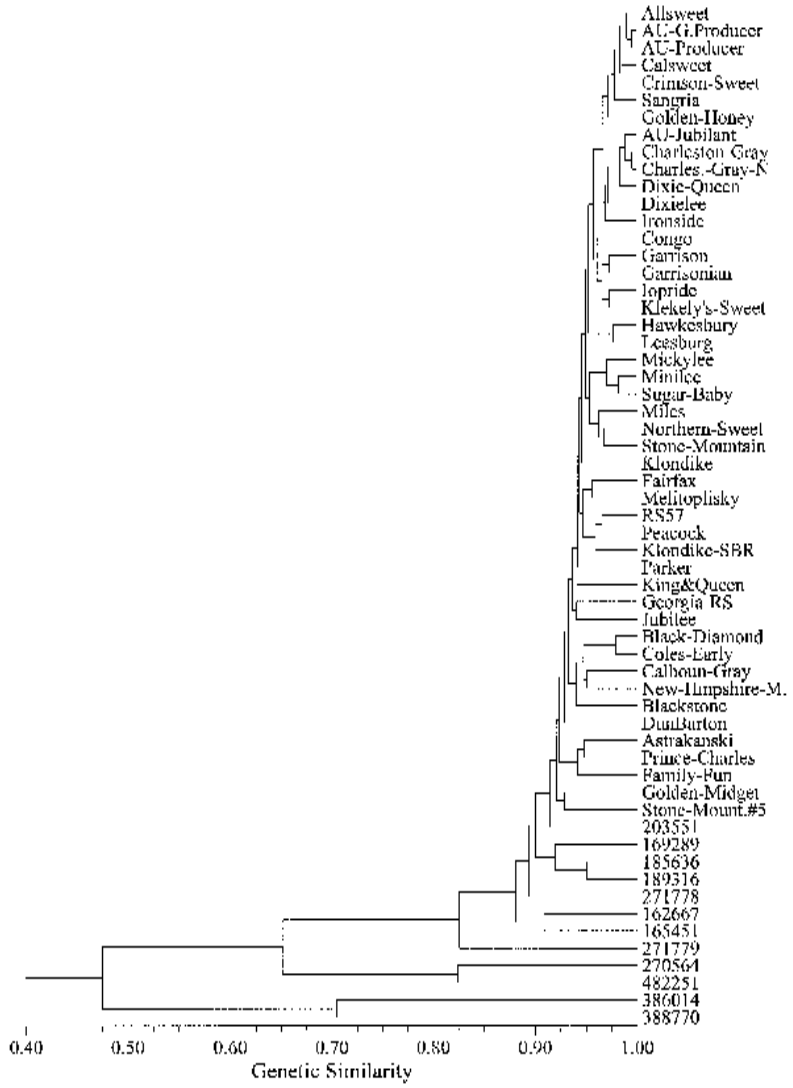

Fig. 2. Dendogram of watermelon cultivars and U.S. Plant Introduction accessions (PIs) produced by UPGMA cluster analysis of similarity matrix. 'AU-Golden Producer' = AU-G. Producer, 'Charleston Gray' (NSL-5267) = Charleston Gray, 'Charleston Gray' (NK Lawn & Garden Co.) = Chrles.-Gray-N, 'Georgia Rattlesnake'= Georgia-RS, 'Klondike Striped Blue Ribbon' = Klondike-SBR, 'New Hampshire Midget' = New-Hampshire-M., 'Klondike Striped RS 57' = RS57.

distribution, also has the highest genetic diversity among *Citrullus* species. That study also indicated higher genetic diversity within the wild subspecies *C. lanatus* var. *citroides*, than in *C. lanatus* var. *lanatus*.

Accessions of *C. lanatus* var. *lanatus* are preferred in watermelon breeding programs because of their horticultural qualities and their close genetic proximity to the cultivated watermelon. Previous studies indicate that resistance to anthracnose (Boyhan et al., 1994; Sowell et al., 1980), or watermelon mosaic virus (Gillaspie and Wright, 1993) exists among accessions of *C. lanatus* var. *lantus*. Although the two former *Citrullus* types are wild and do not have desirable fruit qualities, they might be an essential source of genes that would confer resistance to major diseases and pests. Resistance to gummy stem blight and Fusarium wilt, which are major diseases of watermelon, may exist among accessions of *C. lanatus* var. *citroides* (Dane et al., 1998; Martyn, and Netzer, 1991; Sowell, and Pointer, 1962). 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 tabaci*). The *Citrullus* germplasm collection at the USDA, Agricultural Research Service, Plant Genetic Resources Conservation Unit (Griffin, Ga.), contains 1480 *C. lanatus* var. *lanatus* PIs, but only 102 *C. lanatus* var. *citroides* PIs, and 21 *C. colocynthis* PIs (Germplasm Resources Information Network, www.ars-grin.gov). Thus, in order to broaden the genetic base of watermelon cultivars, further efforts are required to expand the collection of *C. lanatus* var. *citroides* and *C. colocynthis* PIs, and to evaluate additional accessions for resistance to important diseases and pests of watermelon.

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