Diagnostic Chloroplast DNA Haplotypes to Distinguish Cultivated from Citron Type Watermelon

Fenny Dane and Rasima Bakhtiyarova
Department of Horticulture, Auburn University, AL 36849

Plant molecular evolution has been dominated by studies of the chloroplast genome (cpDNA). There are several reasons for this focus on a single organelle which accounts for less than 0.1% of the genetic complement of plants. cpDNA is an abundant component of total cellular DNA, which has facilitated the early molecular characterization of its genome. The cp genome is small, being 155 kb in *Citrullus* (2) and most genes are essentially single copy. cpDNA has a conservative mode of nucleotide substitution and slow rate of molecular evolution which are ideal for the study of plant phylogenetic relationships. The high degree of sequence conservation has facilitated the use of PCR primers in unrelated species (7). Universal primers have been constructed on the basis of conserved sequences of cpgenes and used to amplify the DNA located between the primer binding sites (6, 7). This study was undertaken to detect phylogeographic patterns among a phenotypically and geographically diverse array of cultivated and wild types of *Citrullus lanatus*.

Methods: Seeds of more than 20 different Plant Introduction (PI) accessions of cultivated watermelon, *C. lanatus* var. *lanatus*, and more than 45 citron types, *C. lanatus* var. *citroides* PIs, originating from many different countries, were obtained from the S-9 PI collection in Griffin, GA or from The Cucurbit Network (Table 1). Seeds from the following watermelon cultivars :'AU-Producer', 'Blackstone', 'Crimson Sweet', 'Dixielee', 'Ferrari', 'Jubilee', 'Klondike', 'Mardi Gras', 'Mickylee', 'Peacock', 'Regency', 'Royal Sweet', 'Sangria', 'Starbrite', and 'Stone Mountain' were obtained from commercial breeding companies. Cultivars and accessions were selected based on dendrograms using SSR polymorphism (4) or RAPD marker polymorphisms (5). DNA was extracted from young leaves or seeds using the Qiagen plant DNeasy kit (Qiagen, Valencia, CA). Chloroplast specific primer pairs were used to amplify the corresponding regions (1). PCR products were digested with several different restriction enzymes and informative regions were sequenced. DNA sequences of informative primer pairs and amplification reaction conditions are given in Table 2.

Results: Even though variability within *Citrullus lanatus* was very low, unique cultivar-specific patterns were detected using the following primer / restriction enzyme combination: *ndh*F 1955-607R and *Alu*I for *C. lanatus* var. *lanatus; ycf*6-*psb*M and *Taq*I for *C. lanatus* var. *citroides*. When these cpDNA regions were sequenced, the differences were due to single nucleotide substitutions at restriction enzyme recognition sites. No polymorphism was detected between phenotypically diverse *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides* sequences. *C. lanatus* var. *citroides* PI 179881, and *C. lanatus* var. *lanatus* PI 482251 (Zimbabwe), PI 494529 (Nigeria), and 'AU-Producer' showed identical sequences at *ycf*6-*psb*M and *ndh*F regions. Similarly, *C. lanatus* var. *citroides* PI 271769 (S. Africa) and PI 482252 (Zimbabwe) had homologous sequences at these regions.

Morphological characteristics of many of the citron types described in the GRIN database indicate high phenotypic variability. Fruit size varies from small (10x10 cm for PI 244018) to medium (30x30 cm for PI 270563), fruit shape from round to oblong, flesh color white to yellow, and seed size from 5 x 8 mm to 8 x 15 mm. Citron seeds generally lack the flatness of watermelon cultivar seeds. Several of the var. *citroides* PI accessions showed the *C. lanatus* var. *lanatus* haplotype and have medium-sized fruit with white, yellow or red flesh. Cultivated watermelons similarly have variable fruit types ranging from small fruit (10 x 10 cm for PI 494527) with white flesh and Egusi type edible seeds (5 x 27 mm), to the large round or oblong watermelon fruit with red or yellow flesh presently available on the U.S. market. Levi et al. (5) detected higher levels of genetic variation using RAPDs among *C. lanatus* var. *citroides* accessions as compared to *C. lanatus* var. *lanatus*. However, in this study no association between

Table 1. List of investigated *C. lanatus* Plant Introductions and their geographical origin (see GRIN database for morphological characteristics of most accessions).

C. lanatus var. lanatus	Origin	*C. lanatus var. citroides*	Origin
165451	Mexico	179881, 288316	India
176492	Turkey	189225, 532738	Zaire
185636, 271751	Ghana	244018, 255136, 255137, 270563, 271769, 271779, 295850	S. Africa
211011	Afghanistan	296334, 296335, 295842, 296341, 482293, 596665, 596667	S. Africa
241689	Chile	525081	Egypt
254742	Senegal	254744	Senegal
273481	Ethiopia	248774, TCN 1126	Namibia
295845, 271778	S. Africa	346082	Afghanistan
385964	Kenya	379243	Yugoslavia
482251	Zimbabwe	482246, 482259, 482261, 482279, 482303, 482311, 482319,	Zimbabwe
494527, 494529, 494531	Nigeria	482324, 482334, 482361	Zimbabwe
500314, 500324, 500353	Zambia	532664, 532667	Swaziland
507858	Hungary	532819	China
536453	Maldives	TCN 1360, TCN 1337	U.S.
549160	Chad	542114, 532669, 542123, 483583	Botswana
15 different cultivars	U.S. (see text)	512385, 512854	Spain

Table 2. DNA sequences of *C. lanatus* informative chloroplast specific primer pairs and PCR amplification reaction conditions.

Primer pair	Primer sequence	PCR condition
*ndh*F: 1955F-607R (6)	TAT ATG ATT GGT CAT ATA ATC G ACC AAG TTC AAT GTT AGC SAG ATT AGT C	4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min 55°C, 2 min at 65°C, 10 min at 65°C.
*ycf*6 F-*psb*M R (3)	CTT GGG CTG CTT TAA TGG GTA AAT ATT CTT GCA TTT ATT GC	4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min 50°C, 2 min at 65°C, 10 min at 65°C.

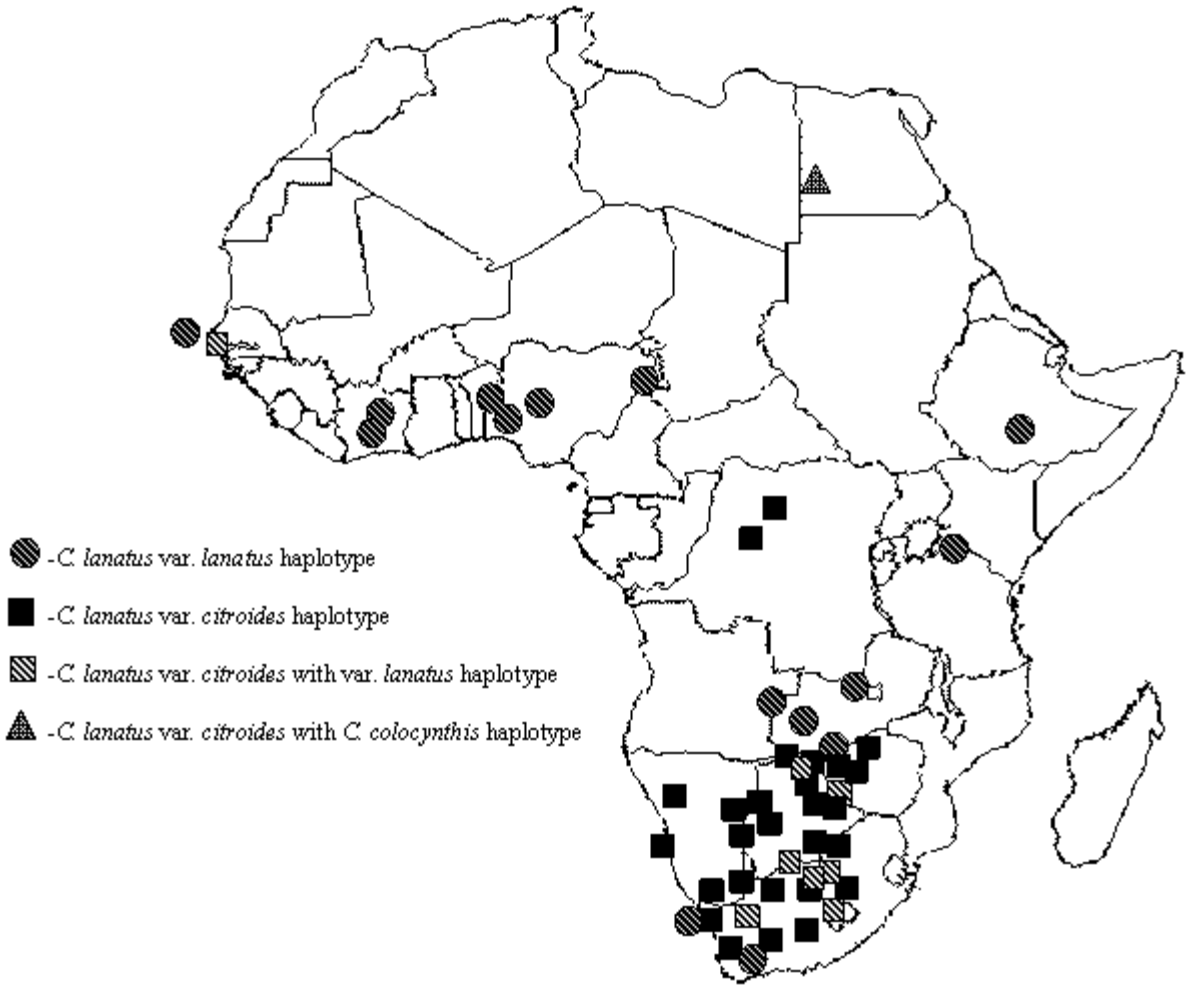

Figure 1. Geographical distribution of *Citrullus lanatus* haplotypes on the African continent.

phenotypic variability and chloroplast DNA patterns was detected.

It is interesting to note that several accessions classified as *C. lanatus* var. *citroides* showed the *C. lanatus* var. *lanatus* haplotype (PI 179881, PI 254744, PI 255136, PI 271779, PI 295850, PI 295842, PI 482319, PI 482293, and PI 482334), while two accessions (PI 386082 and PI 525081) showed the *C. colocynthis* haplotype. Since cpDNA is maternally inherited in *Citrullus* (2), only the maternal parent of these accessions can be identified. We are currently working on nuclear DNA markers (AFLPs) to detect the paternal origin.

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