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Note

Genetic Variation in Native and Introduced Populations of Tropical Soda Apple (*Solanum viarum*)[1]

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Abstract: Tropical soda apple samples were collected from 31 populations across the southeastern United States and from four populations in a portion of its native range in Brazil. The genetic relationships among these populations were examined by single primer amplification reactions (SPAR) and by sequencing a portion of the chloroplast genome. SPAR revealed no variation among the 132 individuals and only two chloroplast haplotypes were detected in the 50 individuals sequenced. The most common haplotype was present in all samples from the United States and most of the Brazilian samples, whereas the second haplotype was only found in one of the Brazilian populations. Within the limitations of these data, we conclude that Brazil is the best location to seek a potential biological control agent for tropical soda apple, and that, if identified, this agent should prove useful for populations throughout the United States.

Nomenclature: Tropical soda apple, *Solanum viarum* Dunal.

Additional index words: Chloroplast DNA, SPAR.

Abbreviations: cpDNA, chloroplast DNA; dNTPs, deoxynucleoside triphosphate; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; RAPDs, randomly amplified polymorphic DNA; SPAR, single primer amplification reactions.

INTRODUCTION

Molecular markers have proven to be useful tools in identifying the source(s) for an invading species and can broaden our understanding of invasion biology at a variety of levels. For example, molecular techniques were used to document potential dispersal vectors (Geller 1996) and the role of single vs. multiple dispersal events (Kreiser et al. 2000; Meekins et al. 2001) that characterize the history of the invasion process. Information on the geographic origins of nonnative species may provide insight into the likelihood that an invader will become established in an area. Species may vary across their range in the physiological tolerances that ultimately determine the success of an invasion (Bastrop et al. 1998).

For economically important pests, molecular tools may provide useful information in designing and implementing biological control programs. Given the highly variable success rates of biological control programs, Nissen et al. (1995) suggest that researchers should consider two issues. First, the genetic diversity present within a weed population may influence the success of a biological control agent because less genetically variable populations are more likely to be effectively controlled by a particular biological control agent (Burdon and Marshall 1981). Second, determining the geographic origin of a weed may help to focus the search for an appropriate biological control agent. Compatibility between a biological control agent and the host (weed) is likely the product of a long evolutionary history. Thus, selecting a potential control organism from a population that is adapted to the genotype(s) of a particular weed should improve the chances for success.

Tropical soda apple has become an important weed of pastures, vegetable crops, row crops, forested land, and urban and natural areas in the southeastern United States and elsewhere in the world (Hall et al. 1998; Mullahey et al. 1993). This weed belongs to the family Solanaceae, section *Acanthophora*, and subgenus *Leptostemonum* (Nee 1991). In tropical climates, tropical soda apple is a perennial shrub, but is a short-lived perennial or annual in subtropical and temperate regions. It is native to Argentina, Brazil, and Paraguay but has become a troublesome weed in many other areas of the world, including countries in Africa, Asia, the Indian subcontinent, the

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Caribbean Islands, and North and South America (Mullahey et al. 1996; Nee 1991). In its native range, tropical soda apple is found in scattered infestations (Bianco et al. 1997) but is not a major weed problem and rarely achieves population levels that have occurred elsewhere after its introduction, i.e., Florida (Mullahey et al. 1998).

In Florida, tropical soda apple was first found in Hendry County in 1987 and identified as a potential pest in 1988 (Mullahey et al. 1993). By 1994, tropical soda apple had spread throughout Florida and infested more than 200,000 ha of agricultural land and was estimated to cause losses of $11 million to cattle ranchers in Florida alone in 1993 (Mullahey and Cornell 1994). Since its introduction into Florida, tropical soda apple has continued to spread into other states and provinces in the United States including Alabama, Georgia, Louisiana, Mississippi, North Carolina, Pennsylvania, South Carolina, Tennessee, and Puerto Rico (Bryson and Byrd 1994; Bryson et al. 1995; Everest and Ball 1995; Mullahey et al. 1998). Tropical soda apple dispersal is attributed primarily to movement of livestock and to contaminated compost manure and grass seeds from infested areas (Coile 1993). Recently, tropical soda apple contaminated seed for wildlife food plots were detected in Mississippi (C. T. Bryson, unpublished data). Once tropical soda apple is established in an area, its continued spread is by domesticated animals and wildlife that spread seed through their feces (Mullahey 1996). Tropical soda apple was placed on the federal noxious weed list in 1996 (Hall et al. 1998). On the basis of ecological range studies, tropical soda apple has the potential to continue to spread into additional areas of the southeastern United States and adjacent regions (Patterson et al. 1997). On the basis of these ecological studies and the past history of tropical soda apple spread and if unchecked, it is possible that tropical soda apple–infested areas could double in the United States within the next decade (Bryson 1996).

Currently, there are no data available to determine whether tropical soda apple populations in the southeastern United States originated from a single introduction or multiple introductions. Determining the origin and genetic makeup of tropical soda apple populations may help determine whether introduction of tropical soda apple was a single event or multiple events. Also, determining the genetic makeup may aid in the search for biological control agents. The objective of this study was to compare patterns of genetic variation in populations of tropical soda apple from its native land and in the southeastern United States, where leaf material could be acquired.

MATERIALS AND METHODS

Plant material (leaves) was collected from populations of tropical soda apple or from undistributed herbarium specimens with a goal of sampling individual leaves from three to five plants in each population. For many populations, acquiring ample sample material was easily performed; however, sampling of tropical soda apple populations was not adequate for several locations in the United States and South America. Overall, we were able to obtain collections from 31 populations across the southeastern United States and from four populations in Brazil for a total of 146 individuals. Sample size per site in the United States ranged from 1 to 10 individuals (average = 4.0) and two to nine for the Brazilian populations (average = 5.75). We also collected comparative material from two other *Solanum* species, the nipplefruit nightshade (*Solanum mammosum* L.) and red soda apple (*Solanum capsacoides* All.). Herbarium vouchers for populations and species sampled were placed in the Southern Weed Science Research Unit herbarium (SWSL), Stoneville, MS.

DNA Isolation. DNA was extracted from dried, recently pressed leaf tissue or herbarium specimens following a modification of the cetyltrimethylammonium bromide procedure presented by J. J. Doyle and J. L. Doyle (1987). Briefly, a single leaf was ground in liquid nitrogen and then approximately 50 to 100 mg of tissue was incubated in 800 μl of extraction buffer at 50 C for 1 h. The supernatant was twice extracted with 24:1 chloroform–isoamyl alcohol, and then the DNA was precipitated by the addition of two-thirds volume of isopropanol and storage at −20 C overnight. The DNA was pelleted by centrifugation, twice washed with 70% ethanol, air dried, and then redissolved in about 300 μl of Tris–ethylenediaminetetraacetic acid (EDTA) (10 mM Tris, 1 mM EDTA, pH 8.0) depending on the size of the pellet. The ribonuclease treatment in the original protocol was not performed. The quality of the extracted DNA was assessed by agarose gel electrophoresis, and the concentration was estimated by visual comparison with samples previously quantified by a fluorescent spectrophotometer. Before use, each DNA sample was further diluted 1:24 with Tris–EDTA.

Single Primer Amplification Reactions. Simple sequence repeats are loci comprised of tandem repeats of various nucleotide motifs in which each repeat unit is typically between two and four bases in length. These loci are ubiquitous and often highly polymorphic within the genomes of eukaryotes. One method of detecting

variation in these loci is to design primers flanking the locus of interest. However, this approach requires knowledge of the DNA sequence in the flanking regions. An alternative method to use these simple sequence repeat loci was described by Gupta et al. (1994). A primer was designed on the basis of the repeat motif and then used in a single primer amplification reaction (SPAR). This approach is similar to randomly amplified polymorphic DNA (RAPDs; Williams et al. 1990), which is a commonly used technique in genetic studies.

Gupta et al. (1994) tested 23 primers on nine different eukaryotes. We selected five primers that they found produced multiple, polymorphic bands in tomato (*Solanum lycopersicon* L.), the closest related species to tropical soda apple. These primers included (GTG)6, (ACTG)4, (GACA)4, (GATA)4, and (GACAC)4. Although anchor nucleotides are sometimes used on SPAR primers, we used the primers described by Gupta et al. (1994) without modification. The primer (GACAC)4 was selected for use in this study because it was the only one that produced consistent and repeatable results. Similarly, the annealing temperature used was selected after several optimization trials. Amplifications using this primer were conducted in a total volume of 25 μl using 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.01% gelatin, 2 mM $MgCl_2$, 200 μM deoxynucleoside triphosphate (dNTPs), 0.75 units *Taq* polymerase,[3] 1 μM of the primer, approximately 100 ng of template DNA, and water to the final volume. A reaction without template DNA was included as a negative control for each set of reactions. The polymerase chain reaction (PCR) was performed using a Perkin–Elmer GeneAmp® 9700[4] with cycling conditions consisting of an initial denaturing step of 95 C for 3 min followed by 35 cycles of 1 min at 95 C, 1 min at 35 C, and 5 min at 70 C. A final elongation step of 3 min at 70 C ended the cycle. Amplified fragments were separated on 1.5% agarose gels in 1× Tris–borate–EDTA buffer, stained with ethidium bromide (0.5 μg/ml) and then observed under ultraviolet light. Each band was scored for size by comparison with known size standards (Promega 1 kb and 100-bp DNA ladders).

Chloroplast DNA. The development of universal primers for noncoding regions of the chloroplast genome (Taberlet et al. 1991) has facilitated its use in population studies of native and invasive plants. Many of these studies have reported intraspecific variation in various portions of the chloroplast genome (Aoki et al. 2003; Petit et al. 1993). We screened selected individuals for variation in one noncoding region by sequencing the intron between the 5′ and 3′ exons of the *trn*L (UAA) gene. This region was amplified using the B49317 ("c") and A49855 ("d") primers of Taberlet et al. (1991). Amplifications were conducted in a total volume of 50 μl using 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.01% gelatin, 2 mM $MgCl_2$, 200 μM dNTPs, 0.75 units *Taq* polymerase, 0.3 μM of each primer, approximately 100 ng template DNA, and water to the final volume. PCR was performed using a Perkin–Elmer GeneAmp® 9700 with cycling conditions consisting of an initial denaturing step of 95 C for 1 min followed by 30 cycles of 1 min at 95 C, 1 min at 50 C, and 1 min at 72 C. A final elongation step of 7 min at 72 C ended the cycle. PCR products were checked by gel electrophoresis (1% Tris–borate–EDTA gel), and successful amplifications were cleaned using the QIAquick PCR purification kit.[5] This fragment was then sequenced using a BigDye Terminator cycle sequencing kit.[6] Most individuals were only sequenced in one direction using the c primer, but selected individuals were also sequenced in the reverse direction with the d primer. Excess dye terminators were removed from the sequencing reactions with Centri-Sep columns.[7] Reactions were air dried and then mailed to Iowa State University DNA Sequencing and Synthesis Facility for gel runs. The sequence data were aligned and edited with Sequencher 4.1.[8]

RESULTS AND DISCUSSION

High-quality, large molecular weight DNA appeared to be obtained from all dry, freshly pressed plant and herbarium specimens. However, a few individuals, mainly herbarium samples, failed to amplify perhaps because of the presence of PCR inhibitors. The SPAR appeared to be most sensitive to the quality of the DNA, although it was robust with respect to the quantity of DNA in the reaction.

Single Primer Amplification Reactions. SPAR with the (GACAC)4 primer produced five bands in tropical soda apple of approximate sizes 275, 350, 450, 600, and 775

[3] *Taq* DNA Polymerase in storage buffer B, Promega Co., 2800 Woods Hollow Road, Madison, WI 53707.

[4] GeneAmp® 9700 PCR system, Perkin–Elmer, 850 Lincoln Drive, Foster City, CA 94404.

[5] QIAquick PCR Purification Kit, Qiagen Inc., 28159 Avenue Stanford, Valencia, CA 91355.

[6] ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Perkin–Elmer/Applied Biosystems, 850 Lincoln Drive, Foster City, CA 94404.

[7] Centri-Sep columns, Princeton Separations, Inc., P.O. Box 300, Adelphia, NJ 07710.

[8] Sequencher 4.1, Gene Codes Co., 2901 Hubbard Street, Ann Arbor, MI 48105.

Table 1. Summary of the number of tropical soda apple collection localities, the total number of samples per site, the number of samples scored for the SPAR locus, the number of samples sequenced, and the types of cpDNA haplotypes detected.[a,b]

Country	State or province	Locations sampled	Total samples	SPAR-scored samples	cpDNA samples sequenced	Haplotype	
						#1	#2
United States	Alabama	5	17	17	5	5	
	Florida	3	17	16	3	3	
	Georgia	15	60	50	13	13	
	Mississippi	5	20	18	5	5	
	South Carolina	1	3	3	1	1	
	Tennessee	2	6	6	2	2	
Brazil	Rio Grande Do Sol	4	23	22	21	17	4

[a] A complete list of collection sites is available from the first author.

[b] Abbreviations: SPAR, single primer amplification reaction; cpDNA, chloroplast DNA.

bp. This amplification pattern was the same for each of the 110 individuals from the United States and the 22 individuals from Brazil that were successfully amplified. For comparison, we also amplified an individual from the congeneric species red soda apple and nipplefruit nightshade. The red soda apple individual only produced one reliable band of approximately 500 bp, whereas the nipplefruit nightshade individual produced fragments of approximate sizes 450, 600, and 775 bp.

Chloroplast DNA. Amplifications with c and d primers produced a 571-bp fragment in tropical soda apple, of which we obtained sequence for 528 bp. We only detected two haplotypes among the 50 individuals sequenced (Table 1). These haplotypes differed by two bases for a total of 0.38% sequence divergence. The most common haplotype (#1) was present in the 29 samples from the United States and most of the individuals from Brazil (17 of 21). The second haplotype (#2) was present only in the population at Igre Jinha, where it was found in four of seven individuals sequenced. Sequencher 4.1 was used to determine the location of 96 restriction enzymes. None of these restriction sites varied between the two haplotypes. The lack of a diagnostic restriction site between the two haplotypes meant that we did not have a cost-effective means of screening every individual, such as using restriction fragment length polymorphisms, from the United States. Thus, we were forced to rely on sequencing a selected individual from most of the representative sites. The sequences that we obtained for red soda apple and nipplefruit nightshade were unique, and they differed from the tropical soda apple haplotypes by two bases and six bases, respectively. The sequences for each haplotype have been deposited in GenBank (AY4159358–AY4159361).

Tropical soda apple from across the southeastern United States were identical at the two markers used in this study. Furthermore, the SPAR pattern and chloroplast DNA (cpDNA) haplotype in these populations match the one most commonly observed in the portion of the native range that we sampled in Brazil. Although we used molecular markers that have successfully revealed intraspecific genetic variation in other studies (Aoki et al. 2003; Gupta et al. 1994), we failed to detect substantial amounts of genetic variation among or within the populations we examined. These results were consistent with a lack of noticeable phenotypic variation in the populations sampled. This paucity of genetic variation imposes some constraints on the conclusions we can draw from these data because the limited amount of variation observed may be a real pattern or may be attributed to the portions of the genome we examined. Furthermore, although we were able to obtain samples from much of the native and introduced portions of the range, there were still some gaps in our sampling and that too limits our ability to interpret the data. With these caveats in mind, we interpret our data in the following manner. The United States populations may be the product of a single or multiple introductions. In either case, the introduction(s) appear to be from a common source, and the close relationship between the United States and Brazilian populations suggests that Brazil may be this source. The fact that only one of the two cpDNA haplotypes was detected in the United States suggests that the colonizing individuals either lacked the second haplotype or that it was lost at some point during its introduction and spread across the United States (e.g., via a founder effect). However, without additional sampling or additional genetic markers (or both), this conclusion is still very much tentative.

Samples from other portions of the native range (Argentina) would be required to better characterize the extent of genetic variation within native populations. In addition, this study would benefit from the inclusion of additional markers such as RAPDs, amplified fragment

length polymorphisms, or intersimple sequence repeats, which may or may not reveal additional genetic variation. Only the inclusion of variable markers would allow us to strengthen our interpretations of the invasion history of tropical soda apple in the United States. Regardless of what additional insight more data might provide, our data do provide useful information for potential biological control programs. First, the degree of genetic similarity found among the United States populations suggests that these populations should be susceptible to the same biological control agent. Second, the close relationship between the United States and Brazilian populations indicates that Brazil would be an appropriate region to search for a potential biological control agent.

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