Developing Genetic and Molecular Tools for Assessing and Controlling the Invasive Potential of *Lantana camara* and Protecting Native Lantana

Zhanao Deng, David M. Czarnecki II, and Li Gong

University of Florida /IFAS, Gulf Coast Research and Education Center, 14625 County Road 672, Wimauma, FL 33598

BACKGROUND

- *Lantana depressa* is a rare and endangered species in south Florida. It has been under the threat of genetic contamination and displacement by its invasive relative, *L. camara*.
- Commercial *L. camara* cultivars provide a multitude of flower colors. With its ability to flower year-round and attract butterflies, *L. camara* has been among the most popular garden and landscape plants in south Florida and many other areas and has been a crop widely grown by many nurseries.
- The invasiveness of *L. camara* stems mainly from its seed and pollen production. Through seed dispersal, *L. camara* can invade natural and agricultural land. Through pollination, *L. camara* can hybridize with *L. depressa*. Male and female sterility is necessary for genetic control of *L. camara's* invasiveness.

Lantana depressa

Lantana camara

- Questions have been raised regarding the identity of some *L. depressa* stock plants used in commercial production: Are they true natives or hybrids from cross pollination?
- Can new sterile *L. camara* cultivars be developed that can protect the native species while provide materials for nursery production and landscape use?

MATERIALS AND METHODS

- Molecular marker development: DNA fragments containing simple sequence repeats (SSRs) were enriched, cloned, and sequenced. Unique sequences flanking SSR loci were selected for primer designing. Primers were tested in PCR for their capacity to amplify discrete products in lantana.
- To understand the genetic relationships among *L. camara* cultivars and *L. depressa* accessions, SSR alleles were amplified by PCR using Lantana-specific primers and detected on a LI-COR DNA analyzer. The presence or absence of alleles was scored and analyzed in NTSYSpc.
- To assess pollen viability and pollinating capacity of *L. camara*, anthers were collected from newly opened flowers and stained with cotton blue. Fully stained grains were counted under a microscope as viable ones. Collected pollen was applied by hand onto emasculated flowers of *L. depressa*, followed by seed count about four weeks after pollination

Controlled pollination

- Seed set of *L. camara* cultivars was assessed after natural pollination, or after hand-pollination with *L. depressa* pollen.
- Triploids were generated through cross pollination between diploids and tetraploids. Obtained triploids were evaluated for male and female sterility.

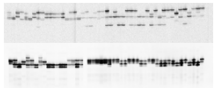
RESULTS

SSR marker development

A partial genomic library was constructed from the genomic DNA of *L. camara* cv Lola. Out of 384 sequences surveyed, 225 (58.6%) contain SSRs. One hundred and nine pairs of primers were designed from unique SSR-containing sequences, and 91 (83.5%) amplified discrete PCR products. The transferability rate was the highest in *L. depressa* (59.2%), but it rapidly declined to 30.6% in *L. montevidensis*, 22.4% in *L. involucrata*, and 10.2% in *L. canescens*.

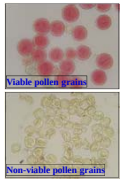
DNA-fingerprinting *L. camara* and *L. depressa*

Thirty-three polymorphic markers detected 160 alleles among *L. camara* and *L. depressa* accessions. Based on Jaccard similarity coefficients, 13 *L. camara* cultivars formed a large cluster, while four of five *L. depressa* accessions formed another cluster. One "*L. depressa*" accession was clustered with two cultivars that are known hybrids between *L. camara* and *L. depressa*, thus representing a cryptic interspecific hybrid.



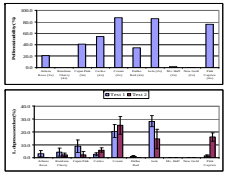
DNA fingerprinting patterns of 48 lantana cultivars and species accessions at two SSR loci

Clustering analysis shows that one *L. depressa* accession is a hybrid between *L. depressa* and *L. camara*.



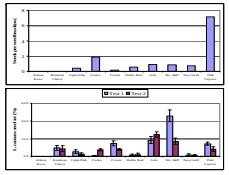
Invasive potential of *L. camara* as a pollen source

Pollen stainability ranged from 0.8% to 85.6%. Two cultivars had pollen stainability below 10%, another two cultivars had it between 20% and 40%, and the rest five cultivars had it above 40%. Based on hand pollination results, diploid cultivars (Cream and Lola) were the most effective pollinators to the native species and resulted in 14.5% to 28.3% seed set, followed by Pink Caprice (4x) and Cajun Pink (5x). Pollination of the native species with Ms. Huff and New Gold didn't result any seed set.



Invasive potential of *L. camara* as a seed producer

A range of variation in seed production capacity was observed among *L. camara* cultivars: The number of seeds per cluster under open pollination varied from 0.01 (Athens Rose) to 7.17 (Pink Caprice). When hand-pollinated with *L. depressa* pollen, Ms. Huff produced the most seeds, followed by Lola, Cream, and Pink Caprice. Athens Rose, Dallas Red and New Gold produced few seeds.

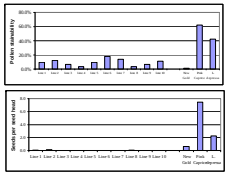


Ploidy level & reproductive biology

The ploidy level of *L. camara* cultivars ranges from diploid (2x) to hexaploid (6x) and it directly affected pollen viability and seed set: 2x > 4x > 5x and 6x > 3x. Some cultivars have evolved multiple reproduction pathways, including unreduced female gamete formation and apomixis. Some polyploids, even triploids, can produce large numbers of seeds through these pathways.

New sterile triploid lines

Several hundreds of triploids have been generated in *L. camara*. Many of them are highly male sterile, and some of them are highly female sterile. Some triploids can produce seeds through unreduced female gamete formation or apomixis. These triploids can be eliminated through sterility assessment tests.



CONCLUSION

- SSR markers are very powerful in revealing the genetic relationships among lantana species and cultivars and identifying cryptic interspecific hybrids.
- Some stock plants have been incorrectly identified as *L. depressa*. They pose an even more severe threat to the native species.
- *L. camara* cultivars vary greatly in pollen viability and seed production capacity. Some can pollinate *L. depressa* well and/or produce great amounts of seeds, while some others appear to be male and female sterile and not invasive.
- New sterile *L. camara* lines have been generated, and they may be used to replace the existing invasive types.

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