Report for 2005-2006 FNGLA Award on

Genetic Sterilization of Lantana

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Abstract

The goal of this project was to sterilize *Lantana camara* varieties through ploidy manipulation for invasiveness control. The specific objectives of this project were to identify additional diploids and tetraploids, to perform large-scale controlled pollination, to generate new triploids, and to improve seed germination. Four additional diploid and eight tetraploid varieties were identified out of 21 varieties analyzed. Three diploid and seven tetraploid varieties were chosen as parents. More than 12,000 flowers in 48 crosses were hand-pollinated and 27 new triploids were generated for eight varieties. Three treatments improved germination of seeds from open pollinations. The new triploids are being evaluated for pollen sterility, seed production, and horticultural values. Our previous studies demonstrated that triploid lantanas were highly sterile. These new triploids are expected to be developed into new sterile, non-invasive varieties for the nursery industry.

Objectives

Lantana camara can hybridize with Florida's native lantanta species (*L. depressa*), and produce numerous seeds that may be dispersed to neighboring areas where they produce new plants. This habit has led to its being classified as a Category I species by the State's Department of Environmental Protection (DEP), and the Florida Exotic Pest Plant Council (FEPPC). It was assigned "Caution" in North Florida and received "Do not use" in central and south Florida. It was indicated that sterile varieties of *L. camara* would likely receive more favorable acceptance by the State's DEP and the FEPPC (Fox et al., 2003).

Previously, we demonstrated that highly fertile varieties could be effectively sterilized through ploidy manipulation. The technique was tested on three highly fertile, very invasive commercial varieties, 'Cream', 'Pink Caprice', and 'Radiation'. We produced highly sterile triploids with pollen viability <5%. Several factors restrict efficient generation of new triploid sterile lantanas. There are few diploids and non-patented tetraploids available as breeding parents, controlled pollinations produce few berries, and collected seeds germinate poorly. These factors make it difficult to produce a sufficient number of new triploids with the desirable characteristics.

The specific objectives of this project were to identify additional diploids and tetraploids as breeding parents, to perform large-scale controlled pollination, to generate new triploids from invasive varieties, and to improve seed germination for efficient production of new triploids.

Materials and Methods

Twenty-one additional commercial varieties were analyzed for their ploidy levels. Three diploid and seven tetraploid varieties were selected as parents to make 48 controlled crosses. Flowers were emasculated before opening and pollinated immediately. Mature berries were collected; seeds were extracted, soaked in water overnight, and germinated in 5-inch pots containing a commercial container mix, under light. One and two months after sowing, seedling emergence was recorded. When plants were several months old, their ploidy level were analyzed, and new triploids were identified, as described below.

Tender leaves were collected from potted plants grown in the greenhouse at the GCREC, Wimauma, Fla. and kept fresh in plastic bags with moist paper towels. Leaf samples (~1/4 square inch) were chopped and stained in the UV Staining Ploidy, nuclei suspension was collected in plastic tubes and analyzed on a PA-I ploidy analyzer. Parameters including gain values, speed, etc were optimized for lantana ploidy analysis.

Berries were collected from open pollinated flowers of 'Pink Caprice' grown in a greenhouse. Seeds were extracted, and then subjected to different treatments before sowing. Seedling emergences were recorded one and two months after sowing.

Results and Discussion

1. **Identification of additional diploids and tetraploids**

   In the previous years (2003-2005), we found four diploids and fourteen tetraploids out of 29 commercial lantana varieties. To find additional diploids and tetraploids that can be used as parents for triploid production, 21 additional varieties were collected and analyzed for their ploidy levels. This effort resulted in identification of four more diploids and eight more tetraploids. Some of the identified diploids and tetraploids are not covered by plant patents and they can serve as parents for production of new triploids in the future.

2. **Generation of new triploids**

   We selected three diploids and seven tetraploid and made 48 crosses among them to produce new triploids (Table 1). A total of 12,993 flowers were pollinated, which resulted in 389 seeds. The seed set varied between 0 to 24.4% from cross to cross, but many crosses didn't produce any seed and the average seed set was only 3.0%, which is extremely low. Seed germination took two months or a longer time and seedling emergence was extremely low, an average of 8.2% one month after sowing and 11.6% two months after sowing.

Fig. 1. Pollinating lantana flowers

   Twenty-seven triploids have been produced (Table 1). These triploids are being evaluated for pollen sterility, seed production, flower color, floriferousness, and other horticultural value. Sterile and promising lines will be propagated in 2007 and trialed in 2008.

Table 1. **Summary on pollination, seed set, seedling emergences, and triploid production in lantana**												
No. crosses	No. flowers pollin-ated	Seed set		No. seeds sown	Seedling emergence				Triploid production			
					1 month		2 months					
		No.	%		No.	%	No.	%	No.	% of seed-lings	% of seeds	% of pollinated flowers
48	12,993	389	3.0	389	32	8.2	45	11.6	27	60.0	6.9	0.2

3. Improving seed germination

Poor fruit set and poor seed germination have been the greatest hurdles in generating new triploids. Several treatments are being evaluated to improve fruit set. To improve seed germination, we tested several treatments using seeds collected from open-pollinated flowers of 'Pink Caprice'. Data in Table 2 shows that treatments 2 to 4 could increase seed germination percentage by 62 to 74% (Table 2). These treatments are being applied to seeds from diploid by tetraploid crosses. Their effects are being tested.

Table 2. Seed emergence after eight treatments		
Seed treatment	Seedlings emergence (% of seeds sown)	
	One month	Two months
1	21.3	42.5
2	31.3	68.8
3	35.0	73.8
4	57.5	77.5
5	5.0	20.0
6	5.0	21.3
7	2.5	8.8
8	25.0	40.0

Conclusions

1. Four additional diploid and eight additional tetraploid lantana varieties were identified. Several of these varieties were not covered by plant patents, and they could be used as parents for production of new triploids, thus enriching our germplasm pool for genetic sterilization of lantana varieties.

2. Twenty-seven new triploids were produced for seven varieties that are highly fertile in pollen and seed production. These triploids are being evaluated for pollen viability, seed

production, flower color, floriferousness, and other horticultural values. Some of them are expected to be promising as new sterile varieties.

3. The percentage of triploids in the progeny populations from interploid crosses was pretty high (27/45 = 60.0%). However, the seed set from these crosses was extremely low. Artificial pollination of 12,993 flowers in 48 crosses produced only 389 seeds (3.0%).

4. Seeds produced from interploid crosses germinated very poorly. Several treatments increased germination of tetraploid seeds from open pollinated flowers of tetraploid varieties. It is anticipated that these treatments may improve the germination of triploid seeds.

Recommendations

1. Based on our studies so far, ploidy manipulation and triploid production provides an effective approach to sterilize lantana for invasiveness control. This approach should be useful for sterilizing other ornamental plants if it is necessary.

2. We have identified quite a number of diploid and tetraploid lantana varieties and found several treatments that could improve lantana seed germination. The most limiting factor in the production of large populations of triploids for development of new sterile, non-invasive lantanas is the extremely low percentage of seed set in diploid by tetraploid crosses. Therefore, improvement of seed set after artificial pollination should be the focus for future research.

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