

Special Research Report #305: Plant Breeding & Genetic Engineering DNA Fingerprinting of Floral Crops

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BACKGROUND

Recently, economically significant intellectual and ethical issues have been raised in the floral crop industry with regard to plant patent protection and breeder's rights. Accurate and sensitive cultivar identification is vital to improving this situation. Thus, molecular methods are being tested in a wide array of floral crops. Amplified Fragment Length Polymorphism (AFLP) has been used in many floral crops, including geranium, Peruvian lily, rose, New Guinea impatiens, orchids, and poinsettia.



We have utilized AFLP to detect polymorphisms

which were assembled into DNA fingerprints for poinsettia and New Guinea impatiens for differentiating cultivars. A major goal of our efforts, not previously addressed by others, was validation of fingerprints and elimination of AFLP fragments that were not cultivar-linked polymorphisms. Reproducibility of the fingerprint was insured through testing of multiple plants from the same cultivar from many sources. We determined that cultivar series and breeder could be identified using fingerprints. In addition, we created a database of the fingerprints. They serve as a record for a large set of cultivars that can be used to identify cultivars and breeding material. This was shown to be effective for cultivar identification in poinsettia. While AFLP is highly reproducible, it can be technically difficult and time consuming. Polymorphisms useful in cultivar identification may also be identified

by repetitive sequences called SSRs, or microsatellites, and by retrotransposons. Microsatellites (SSR) are also highly reproducible, and can be performed in a single step. SSR is beginning to be used in floral crops such as geranium and carnation. Retrotransposon based analysis is a new method that has not yet been used in floral crops. The objectives of this project were: 1) To identify floral crops that could benefit from molecular tools; 2) Extend DNA fingerprinting capabilities using AFLP and assess SSR and retrotransposon markers; and 3) Test SSR and retrotransposon sequences in selected floral crops to develop protocols for fingerprinting and/or markers for simply inherited traits.

METHODOLOGY & RESULTS

To isolate microsatellites in New Guinea impatiens, we created a genomic library by cloning

fragments of New Guinea *impatiens* DNA. This allowed us to obtain approximately 442,000 bases of sequence. The sequence was screened for microsatellites using a computer algorithm. A total of 14 usable microsatellite motifs were isolated. Primers were designed to flank each of the motifs. Using these primers and a novel PCR product labeling technique, the motifs were amplified and the products analyzed on a LI-COR Fragment Analysis System. This technique allowed us to purchase a single labeled primer and use a single procedure to amplify all of the motifs. This resulted in significant time and cost savings. To test the microsatellites, 46 cultivars of New Guinea *impatiens* were selected representing 5 breeding programs and 11 cultivar series. The microsatellite analysis was able to differentiate all 46 cultivars of New Guinea *impatiens*, as

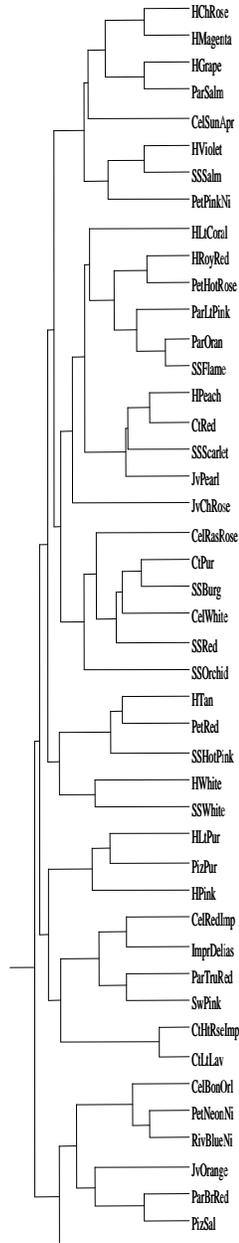


Fig 1. Example of DNA fingerprinting for New Guinea *impatiens*

NEXT QUESTIONS

The next phase of the project is to develop the retrotransposon into a straightforward method for fingerprinting that is widely applicable. The following questions will be addressed:

- (1)·Can retrotransposon-based fingerprints differentiate between cultivars?
- (2)·How can we make the methods and data accessible to the industry?

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