# Towards Improvement of Impatiens

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## Introduction

The genus *Impatiens* contains over 1300 species, with centers of diversity in Africa and Southern Asia. *I. walleriana* is best known commercially, but has been threatened by the recent spread of a virulent race of Impatiens Downy Mildew (*Plasmopara obducens*). Through our other research, we have identified species which are resistant to this disease. However, many of these species have characteristics which make them commercially inviable in their current state. Other species have the potential to bring much-needed diversity into the *I. walleriana* gene pool, but require special techniques and efforts in order to obtain hybrids. To improve and incorporate these species, we reviewed some of the literature around impatiens breeding and biotechnology that has been published since impatiens were domesticated in the 1970's. We also made our own observations, based on experience and observations.

## Germplasm Acquisition

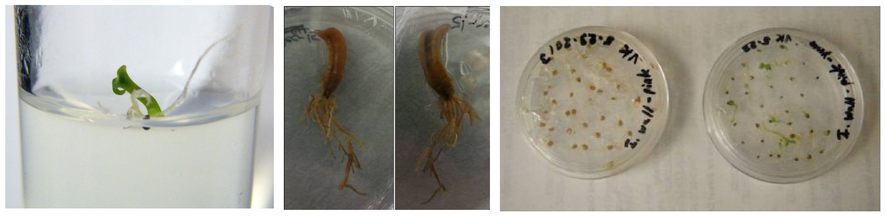
- Most accessions were generously donated by Derick Pitman
- Additional species were provided by Sean Hogan of Cistus Nurseries
- Seed of many unusual cultivars and species are available online
- Small commercial establishments are also good sources

## Interspecific Hybridization

- Arisumi (1985) techniques and crosses had the best success rates
- Phylogenetic data from Yuan et al. (2004), Janssens et al. (2006), and Janssens et al. (2009) identified species relationships for compatible crosses
- Many clades within the genus appear to contain intracompatible species
- 7-10 Days After Pollination (DAP) was best for embryo rescue *in vitro*
- Treatment with a commercial kinetin spray did not prevent flowers from dropping, but did appear to increase embryo size

## Embryo/Ovule Rescue

- Media as described by Nitsch & Nitsch (1969), with modifications by Arisumi (1985)
- Han (1991) suggested using glucose as a carbohydrate source
- Browning of the embryos was prevented with the addition of calcium ascorbate, a pH neutral form of Vitamin C
- Many interspecific hybrid embryos developed abnormally, but some differentiated into growing points



Left: A viable seedling obtained through ovule rescue.
Center: Top and bottom views of an inviable seedling obtained through ovule rescue. Note the lack of a growth point.
Right: Ovule rescues from self-pollination, showing the effect of more (left) versus fewer (right) Days After Pollination.

Plants of *I. balsamina* on media as described by (left-right): Chou (2000), Dan et al. (2010), Baxter (2005), and Xiang and Wang (2005)

Plants of *I. hawkeri* on media as described by (left-right): Chou (2000), Dan et al. (2010), and Baxter (2005).
Note the flower bud indicated by the arrow.

Control (top) and treated (bottom) plants of *I. balsamina*, after exposure to EMS. Inset: a treated seedling that did not survive to maturity.

Putative 2N (left) and 4N (right) flowers of *I. walleriana.*

Putative 2N (left) and 4N (right) flowers of *I. balsamina.*

## Tissue Culture

- Surface sterilization was the main difficulty when establishing plants *in vitro*. Some species were substantially more difficult than others
- Different hormone combinations had variable growth effects on different species
- Unmodified Murashige & Skoog (1962) media was effective for general maintenance of cultures

## Mutagenesis

- The methods for the mutagen Ethyl methanesulfonate (EMS) as described by Weigle & Butler (1983) were followed, with a buffer described by Kim et al. (2006)
- Treatment with sodium thiosulfate was an effective cleanup method for EMS (Arnason, 1974)
- Six out of 120 treated seeds survived. Many more originally survived, but were not vigorous enough to grow to maturity
- Dormancy mechanisms in some species prevented effective treatment and evaluation
- Results treating *in vitro* cultures were inconclusive

## Polyploid Induction

- Polyploid induction was used to bridge species with dissimilar chromosome counts or to restore fertility by creating amphiploids of interspecific hybrids
- The 'Bruno' series of *I. walleriana* was reported to be tetraploid, but is no longer commercially available
- 0.2% colchicine in 2% DMSO was used
- There was a high mortality rate, which may be partially from the DMSO
- Putative polyploids from *I. walleriana* and *I. balsamina* were recovered

## Conclusions

The incredible genetic diversity within the genus *Impatiens* can provide excellent resources for breeding and development of new cultivars. Embryo rescue of interspecific hybrids has allowed us to access some of this diversity, and we have had good success propagating these new variety using tissue culture. Our future breeding efforts will likely utilize ploidy manipulation to restore fertility in these crosses, as well as to permit crosses between species with disparate chromosome numbers. Species with a limited genetic base, either due to genetic isolation or eroded diversity through domestication, may show a wider range of phenotypes after mutagenesis. By leveraging all of these techniques, we hope to harness and shape some of the potential present in this genus. This paper is part of our project to breed in Downy Mildew resistance into *Impatiens walleriana* hybrids.

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