Towards improvement of *Impatiens*©

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Abstract

Common impatiens, *Impatiens walleriana*, have traditionally been the most popular annual flower used for landscaping. However, impatiens downy mildew (*Plasmopara obducens*), a pathogen which has recently become virulent against this species, leaves plants defoliated and commercially unviable. Research was started to identify other species, from a genus of over one thousand, which were more resistant to the disease. Screening identified many species with significantly higher resistance, as well as trends in which species were susceptible. Using a range of breeding and propagation tools, we explored different ways to improve common impatiens and integrate the resistance we identified. These included making efforts to better characterize the available germplasm, ploidy manipulation, tissue culture, and interspecific hybridization. Here we describe existing techniques for impatiens improvement, as well as the modifications we have developed for them.

INTRODUCTION

Impatiens is one of the most popular annual bedding plants and have traditionally been an important source of income for many American greenhouse growers. Unfortunately, in 2004, commercial plants of the most common impatiens species, *Impatiens walleriana*, were reported as being completely defoliated by a new race of impatiens downy mildew, *Plasmopara obducens* (Wegulo et al., 2004). By 2011 the pathogen had spread worldwide and become a significant problem in the landscape. This disease results in wilting, leaf and flower drop and ultimately death of this important bedding plant. As older samples of the pathogen have been identified on the native North American jewelweeds (Saccardo, 1888), *I. capensis* (syn. *I. fulva*) and *I. pallida*, there is also some concern about whether the pathogen could impact native North American ecosystems. However, since the genus *Impatiens* contains more than one thousand described species, an investigation has begun into the general degree of susceptibility to this disease, and whether factors correlated to resistance can be identified. Reports on the disease have already recognized that New Guinea impatiens, *I. hawkeri*, exhibit a high degree of resistance (Cunnington et al., 2008). However previous research has also shown that *I. hawkeri* has a very low success rates in crosses with *I. walleriana* even after embryo rescue (Arisumi, 1985) and that the few hybrids produced and which make it to maturity are abnormal and weak (Arisumi, 1987), limiting its use in resistance introgression.

The original goal of this research was to identify sources of resistance to downy mildew that were also cross-compatible with *I. walleriana*. As we accumulated more species and cultivars, it became clear that the genus *Impatiens* has a lot to offer beyond its most well-known representative. Therefore we expanded our research goals to also incorporate other methods of working with this diversity, and hopefully producing something commercially viable. Many of the same techniques to increase and harness diversity are applicable to all of the germplasm in our collection. By diversifying the commercial genepool, we hope to broaden people's concept of what an “impatiens” is and help bring them out of the shade and into the limelight.

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AREAS OF INVESTIGATION

Germplasm acquisition

The majority of the roughly 200 species we have are donations from private collectors. Descriptions and observations of some of the species used in our research can be found in Table 1. Through funding from a USDA SARE grant (#GNE13-063) we also have purchased other species from a range of commercial sources. Unlisted cultivars or species were purchased from online retailers and small, hobbyist garden centers. The USDA's Ornamental Plant Germplasm Center has also recently acquired a range of accessions, mostly representing the native North American jewelweed species, but also with a few species from further afield.

Table 1. Primary *Impatiens* species investigated during this research. Cytological data compiled from Goldblatt and Johnson (1979) and Yuan et al. (2004).

***Impatiens* species**	**2N**	**Description**	**Observations**
arguta	18, 20	Generally recumbent plants; flowers tubular, ranging dark to light purple, a white form is also available.	Reported hardy to USDA Zone 7.
balfourii	14	Plants generally 2-3 ft tall; flowers profusely, petals are purple and white; forms self seed readily.	1 month stratification; cuttings unsuccessful
balsamina	14	Plants 3-4 ft tall; many color forms; generally cleistogamous.	Fragrant, germinates within 1 week.
campanulata	20	Large, flat leaves; small white flowers with red spots, borne profusely.	Flowers year-round
capensis	20	Flowers generally orange with varying degrees of red spotting, unspotted forms are also common.	Native to North America; asexual propagation unsuccessful; 4-5 month stratification
cinnabarina	16	Plant 2-3 ft tall; leaves heart-shaped	Attractive form; flowers continuously
glandulifera	18	5 ft+ tall; tubular purple flowers.	1 month stratification; fragrant (melon).
grandis	20,36,40	Attractive flat leaves; flowers large and flat, generally white with red spots.	Difficult to flower; buds are very sensitive.
hamata	14	Small pink and white flowers.	Leaves always curled; a very weak plant.
hawker	16, 32	Many color forms available.	Slow to germinate.
hochstetteri	16	Small pinkish flowers borne in profusion; plant forms a trailing mound.	Might be a good filler plant; very pretty.
irvingii	14	Trailing; pubescent; flat; mauve flowers.	Seedpods resemble *I. balsamina*
laurentii		Plants forming sparse mounds 2-3 ft; flowers violet and flat.	Self-seed prolifically; seed may have a short dormancy requirement.
niamniamensis	32	Flower petals green with an enlarged spur.	Roots readily; free from most pests and diseases.
omeiana		Stoloniferous plant with short bouts of dormancy; many foliage forms; flowers yellow and tubular.	Flowering appears photoperiodic; reported hardy to USDA Zone 4.
pallida	20, 30	Different shades available in different forms, flowers yellow.	Native North America. 4-5 month stratification; asexual propagation unsuccessful.
pritzellii		Similar to *I. omeiana*, but taller. flowers yellow.	Likely hardy to USDA Zone 7. flowers more reliably than *I. omeiana*
repens	14	Prostrate, vining, red stems with small, rounded leaves; yellow flowers borne irregularly.	Very attractive foliage plant; good in green walls.
sodenii	16	5-7 ft tall; leaves whorled; flowers white or pink; several forms available.	Variability in self-seed set and floriferousness between forms.
usambarensis	16	Recumbent to slightly mounding; flowers red.	Leaves prone to thrip damage.
walleriana	16	Most common commercial species; many forms available.	Many forms not commercialized with different foliage and growth habits; rarely gets mite damage.

Interspecific hybridization

This approach, more than any other, has formed the basis for our improvement program. Toru Arisumi, a USDA scientist tasked with improving the genus *Impatiens* back in the 1970s, published extensively on his ovule-rescue techniques and the resulting hybrids (e.g. Arisumi 1973, 1977, 1980, 1985, 1987). We have modified our approach from his based on subsequent studies by other researchers. Han (1991) found that changing the carbohydrate source from sucrose to glucose resulted in better germination of rescued embryos. Later, Han (1994) demonstrated that the addition of glutamine to the medium resulted in higher survival rates of embryos. As browning persisted in our experiments, we also looked into using vitamin C as an antioxidant source. However, vitamin C as ascorbic acid tends to change the medium pH and can also deteriorate into an oxidant over time; something we have countered by using calcium ascorbate instead. These findings were incorporated into our medium recipe, although the substitution of glucose produced a softer medium that had to be amended with high concentrations of the gelling agent.

In addition to changes in medium, we have also explored changes to the actual technique of pollination. Initial crosses were done without emasculation, but resulted in self-pollinations in quite a few species. *I. balsamina* is particularly difficult, as it begins shedding pollen before the buds have developed color. The dissection required for bud emasculation also causes *I. balsamina* to release a range of browning chemicals; presumably phenolics. We now emasculate for almost all crosses, unless there is good evidence of sterility or self-incompatibility in the female parent. Pollinated flowers dropping before maturity has been a big problem, and we hoped to improve the retention rate by applying an anti-abscisic hormone to the base of the peduncle. We eventually decided on a commercial preparation for inducing tomato fruit set, "Blossom Set Spray," which contains kinetin. After comparing a few identical crosses done with or without hormones, the drop rate was roughly the same. However, the ovules from the treated flowers did appear quite a bit larger than those from the untreated ones, which may be an avenue worth pursuing in future breeding work.

The days-after-pollination (DAP) that a cross is rescued also appears to play a strong role in its chances of survival. While crosses rescued too early may not be developed enough to grow, crosses rescued too late may have already spontaneously aborted. Arisumi (1980) rescued ovules from a range of DAPs, but found variation in which of these ovules actually developed into seedlings. However, previous work by an esteemed colleague demonstrated that many crosses were not viable prior to 7 DAP (Kendra Hutchins, pers. commun.). We found that in crosses between two Himalayan species, ovules rescued at 10+ DAP showed a conspicuous brown, failed embryo within the ovule while crosses rescued at 7 DAP were uniformly white and appeared viable. In other crosses though, waiting for up to 14 DAP seemed to allow the ovule to develop further without mortality; the species combination in the cross is likely an important factor to gaging the appropriate age DAP.

Most of the crosses we have attempted have been informed by either previous publications on successful crosses (Arisumi, 1987), base chromosome number (conveniently listed for many species on http://www.tropicos.org/Project/IPCN (Goldblatt and Johnson, 1979), or phylogenetic proximity of the species (Janssens et al., 2009; Yuan et al., 2004). Publications list some crosses between rather distantly related species (e.g. *I. uguenensis* [syn. *I. sodenii*] × *I. campanulata* in Arisumi, 1987), but phylogeny and chromosome numbers suggest some potential combinations of closely-related species that have not been attempted yet. One of the groups in which we see great potential, and have had some measure of success with, are the Himalayan species. These are mostly re-seeding annuals, and we have also found that many of them have excellent downy mildew resistance. Little has been documented in attempted interspecific hybrids among these species, but they contain a great diversity of colors and forms. One concern with this group is the potential for invasiveness. Already, *I. glandulifera* is considered a noxious weed across much of Europe (Global Invasive Species Database, 2009), and *I. balfourii* has been suggested to have some potential in that area as well (Schmitz and Dericks, 2010). However, this is one instance where higher sterility levels in interspecific hybrids might be an advantage. Another group great potential are the species from Madagascar and the surrounding islands. Several

commercial series have already been produced from interspecific hybrids among these species, such as the African Orchid series popular among collectors and the recently released Downtown series from Fry Road Nursery. They have also been combined with *I. walleriana* to form the Seashell and Fusion series, created by Burpee and Ball Horticultural respectively (Pitman, 2004). These are popular for combining an *I. walleriana*-like plant with yellow flowers not normally found in the species. While we have found the Madagascar/*I. walleriana* hybrids to be very susceptible to downy mildew, Madagascar hybrids and species on their own seem to possess decent resistance. Many of the interspecifics not involving *I. walleriana* also have at least partial fertility, allowing more complex breeding projects. The major drawback to many of these species is their more cupped flower shape, which resonates with collectors but does not seem to appeal as much to consumers expecting the typical "impatiens shape" (read: *I. walleriana*). This is where *I. laurentii* and the closely related *I. lyallii*, with their flat flowers, might be very useful. Our crosses between these and other Madagascar species have produced hybrids with a flatter flower and wider range of colors. More advanced breeding, such as backcrossing, might improve appearances even more.

Interspecific hybridization has the potential to improve a wide range of traits beyond those explored in our breeding work. One often unexpected side effect of producing interspecific hybrids is the presence of double flowers in the progeny. Arisumi (1987) noted this in hybrids between *I. flaccida* and *I. repens* as well as *I. flaccida* and *I. walleriana*, and we have observed this phenomenon ourselves even between some of the closely related Madagascar species. Fragrance is something not typically associated with impatiens, but in surveys has been reported as the number one most consumer-desired trait in ornamentals (Clark et al., 2013). Quite a few fragrant species of impatiens are commercially available, perhaps *I. tinctoria* most famously, and crosses to bring this trait into a more manageable plant could be very interesting. Perenniality is another trait possessed by a range of impatiens species, with the best hardiness known being in *I. omeiana*, and which could be transferred to showier species. Hybridization with other species to expand the growing range of a species, such as *I. platypetala* has given to *I. hawkeri* for the appropriately named 'Sunpatiens' series or *I. flaccida* has contributed to *I. hawkeri* in the 'Fanfare' series (Guillen, 2002; Pitman, 2004), is another admirable goal. Several impatiens species are native to areas with drought, heat, and high sun, and would be great candidates for pushing the boundaries of impatiens cultivation.

Mutagenesis

Another technique which has great promise for improving impatiens is mutagenesis. While mutagenesis has a bad reputation in the edibles world, due to incremental changes in difficult-to-measure phenotypes (e.g., yield) and concerns about affecting consumers, these are non-issues in the ornamental world: our phenotypes are primarily visual and consumers generally do not actually consume our plants (Schum, 2003). Instead, mutagenesis has great potential to broaden the genetic base of a species without having to collect new populations of the plant from the wild; something that is increasingly challenging in the modern world. Also, mutagenesis allows retention of a given phenotype with modification of only a small handful of traits, rather than the sometimes larger-scale changes brought about by traditional breeding, and without the stigma associated with genetic engineering. This is not to say that exploration and introduction of new germplasm or traditional breeding are any less useful, just that mutagenesis adds another tool to the plant improvement toolbox.

Other researchers have previously published on induced mutagenesis in impatiens. Klozová (1962) used X-rays on *I. balsamina* and found changes in the type and quantity of anthocyanins produced. Bose and Basu (1967) applied diethyl sulfate to *I. balsamina* and reported plants without side-branches, as well as ones with fasciated stems. However, the experiment off of which we have based most of our work is Weigle and Butler (1983). They treated seeds of *I. platypetala* with a range of concentrations of ethyl methansulfonate (EMS) and found that a treatment of 80 mM for 24 h resulted in approximately 17.5% mortality. While this is lower than the 50% mortality they had hoped for in order to get saturated

mutagenesis, they still found several mutated plants including a dwarf form.

We tested a similar protocol on seeds of *I. balsamina.* However, we added a phosphate buffer solution to improve uptake (Kim et al., 2006) and a treatment with sodium thiosulfate afterwards to stop the mutagenesis reaction (Arnason, 1974). Pre-soaking the seeds in the buffer before treatment resulted in good germination but no leaf formation for any of the seedlings, suggesting a high mutation rate. Placing dry seeds into the buffer and treating immediately also lead to good germination rates, and 16 of the 120 treated actually produced leaves, in addition to all 8 of the control seeds. Of these 16, 6 matured into viable plants; although some of the loss here may have come from damping off during the slow maturation of the seedlings. These six showed distinctly different phenotypes from the controls (Figure 1). This method was also used to treat 240 seeds of *I. laurentii*, with 16 controls, but the germination rate of both the controls and the treated seeds was very low; leading us to believe that there may be some dormancy mechanisms in place that would need to be accommodated.

Figure 1. Plants of *Impatiens balsamina* grown from the same lot of seed, untreated (top) and treated (bottom) with EMS. The pink flower color is from segregation in the original population, other traits are presumed to be induced mutations.

Polyploid induction

Originally we started creating polyploid forms of different impatiens species as a way provide resources for our interspecific breeding work. Crossing two polyploid plants provides a complete set of chromosomes for each species, making meiosis smoother and sometimes increasing fertility of the hybrid. Arisumi (1973) documented that while most induced polyploid impatiens were less fertile than their diploid progenitors, inducing polyploidy in interspecific hybrids sometimes restored fertility. His best example of this was in crosses between a species from Java (possibly *I. platypetala*) and a species from New Guinea (likely *I. hawkeri*), where the diploid interspecific hybrids did not produce seeds but the induced tetraploids of the same plants set 11-23 seeds per capsule. While Arisumi (1987) also reported good results in getting interspecific hybrids between *I. walleriana* and *I. niamniamensis*, these hybrids were sterile, and there is no follow-up publication on whether inducing polyploidy might restore fertility. As *I. niamniamensis* is 2N=32 and *I. walleriana* is 2N=16, it would be interesting to recreate this cross with a 4N plant of *I. walleriana* and see if that hybrid had better fertility. If, on the other hand, sterility is desired in a cross, such as in the invasive species hybrids described previously, creation of odd-ploidy hybrids (e.g. triploids, etc.) that divide unevenly can be used to prevent proper

meiosis. Another advantage of polyploidy is larger and more rounded organs, such as flowers (Arisumi, 1973). The 'Bruno' series of *I. walleriana*, a tetraploid line released by Floranova (Uchneat, 2006), took advantage of this, with larger flowers and thicker leaves. Online reviews by gardeners praised the series' resiliency, claiming they grew in a wider range of conditions and tolerated abiotic stress better than diploid plants of *I. walleriana*. However, we have been unable to find a commercial or private source that still carries these.

As we were unable to find commercially available polyploid forms of common impatiens species, we endeavored to create our own. Arisumi (1973) described a technique of treating cuttings topically with a 0.2% colchicine solution, which we also employed. To improve penetration of the colchicine into the tissue we also diluted it with 2% DMSO, as we had previous experience with in other species. However, we found that the addition of DMSO seemed to increase phytotoxicity beyond the amount from the colchicine. Another modification was to use food-grade glycerin to dilute the colchicine in place of water, as it is thicker and less prone to running off the plant. It was unclear whether this actually improved transformation, but the treated surfaces did appear wet longer. One modification we have not extensively tested yet, but with excellent demonstrated potential in other species, is changing the anti-mitotic agent from colchicine to something less toxic and more potent, such as oryzalin.

Of the 15 species and cultivars we treated with colchicine, the best survival appeared to be in cuttings of the 'Xtreme' series of *I. walleriana*, seedlings of *I. balsamina*, and cuttings of *I. flaccida*. Flowers from treated plants showed a marked difference from those of untreated ones (Figures 2 and 3), except for in the case of *I. flaccida* where the two were indistinguishable and leading us to believe they escaped transformation. Some vegetative characteristics were also noticeable, such as thicker leaves in *I. walleriana.* Although the transformation of *I. balsamina* appeared to be uniform throughout the plant, likely due to the single growth point treated on the seedling, treated plants of *I. walleriana* appeared chimeric. Taking cuttings from visually distinct sections ameliorated this somewhat, but did not completely eliminate the variability. In one case, a cutting from a particularly thick-leafed section resulted in a plant which appeared incapable of setting flowers. The ploidy of this sport has not been tested, but we suspect it to be a higher-level polyploid (e.g. octoploid or above).

Figure 2. Flowers from plants of *Impatiens walleriana* treated (right) and untreated (left) with colchicine.

Figure 3. Flowers from plants of *Impatiens balsamina* treated (right) and untreated (left) with colchicine.

Tissue culture

Concurrent with our other work, we have kept an active tissue culture program of *Impatiens* species going. Originally we started this as a way to maintain large or difficult-to-grow species without having to allocate specialized greenhouse space. However, we also found that some species that normally deteriorate at the end of the growing season, such as *I. balfourii*, can also be grown beyond their normal senescence point through micropropagation. We are also hoping to use this as a way to efficiently apply mutagens to species that do not readily produce seeds, such as *I. repens*, but do not have conclusive results from this yet.

Fortunately, there have been several articles published on growing *Impatiens* in vitro. Many of these come from attempts to produce transgenic *I. walleriana* for a variety of reasons, such as resistance to impatiens necrotic spot virus (INSV). We contrasted four media recipes, based on the basal salts and vitamins published by Murashige and Skoog (1962) but with varying amendments (Baxter, 2005; Chou, 2000; Dan et al., 2010; Xiang and Wang, 2005). Each medium produced pronounced differences within each species, but these differences did not always follow from species to species. Routine preparation of media with co-autoclavable hormones, such as thidiazuron, was much easier than using partially heat-labile hormones, such as zeatin, that had to be sterile-filtered and added as the media cooled (Kyte et al., 2013). Based on the growth trends we have observed, we vary our media use to suit our current needs.

Another challenge we faced with tissue culture was that most of the articles published on impatiens use surface-sterilized seeds as the tissue source. This works well for species that readily produce seed and whose seed lack strong dormancy mechanisms, but did not fulfill our needs for other species that fell outside these criteria. Gunapala et al. (2008) tested several chemical formulations for surface-sterilizing plants of *I. repens* and found that mercury chloride yielded the lowest contamination rates. Unfortunately, mercury chloride is very toxic for humans. Instead, we tried a variety of other surface-sterilization techniques, using varying concentrations of several antiseptics. One discovery was that 70% ethanol, a standby in many surface-sterilization protocols, resulted in high phytotoxicity for the species we tested, especially when followed with a commercial bleach treatment. Instead, we began soaking the tissue in sterile water (amended with a few drops of 1N HCl to inhibit bacteria) on a rotary shaker for 2-3 h, following this with 10-15 min. in 3% hydrogen peroxide (to lift surface contaminants), a rinse with sterile water, 10-15 min. in 10-20% bleach, and 3 rinses

with sterile water. The rinse with sterile water between the hydrogen peroxide and bleach treatments was added after we observed vigorous bubbling when adding the bleach solution directly after the hydrogen peroxide, leading to be concerned about adverse chemical reactions. Following the above protocol produces plant material with low rates of contamination for most species. However, several species seem to be particularly prone to contamination even with the above protocol, possibly either due to coarse tissue surfaces or endophytic infection. Using all of these techniques, we have maintained up to 30 species in vitro.

CONCLUSIONS

There is a wide range of techniques available to both improve and utilize the diversity present in the genus *Impatiens*. We have outlined some of the ones that form the focus of our research, but this list is nowhere near exhaustive. Through our work, we hope to create germplasm that researchers with other skill-sets can build from. Interspecific hybrids provide a mechanism to transfer interesting traits between species, a process that can likely be aided by making crosses between individuals at different ploidy levels. Tissue culture allows us to preserve these hybrids, as well as other germplasm we have acquired, and multiply it so that we can create backups and share it more easily. Mutagenesis acts as a way to increase the diversity of species with narrow genetic bases, without the cost or environmental impact of seeking out wild populations. Drawing upon all of these, we have developed impatiens populations that are not only resistant to downy mildew, but also diverse and resilient enough to face new, unknown challenges from the environment, pests and pathogens, or even changing consumer trends. The genus *Impatiens* has a lot to offer already, and we hope that through our work we can make it more accessible.

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