Sequencing and Analysis of Leaf Transcriptomes of Impatiens Cultivars Differing in Downy Mildew Resistance

Krishna Bhattarai, Weining Wang, and Zhanao Deng

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

Introduction

Garden impatiens (*Impatiens walleriana* Hook.f.) is one of the most popular and widely grown bedding plants, with flowers available in virtually all colors, all over the world (Uchneat, 2007). Impatiens downy mildew (IDM), caused by *Plasmopara obducens* (J. Schröt.) J. Schröt., is a devastating disease to garden impatiens, causing severe infection in leaves and defoliation. Garden impatiens have been in high demand to consumers due to their vibrant colors, shade tolerance as a bedding plant, adaptability to containers, and performance in the pack (Uchneat, 2007). While garden impatiens is highly susceptible to IDM, New Guinea Impatiens (NGI) (*Impatiens xhawkeri*) has shown high levels of resistance to IDM (Cunnington et al., 2008). Transferring IDM resistance from NGI to garden impatiens is highly desirable, but it is complicated by cross incompatibility between the two species. RNA-seq has been used commonly to identify the genes involved in disease resistance/defense and obtain sequences for developing molecular markers for disease resistance. In this study, we report the sequencing and analysis of the leaf transcriptomes of one garden impatiens cultivar and one NGI cultivar that differ in resistance to IDM.

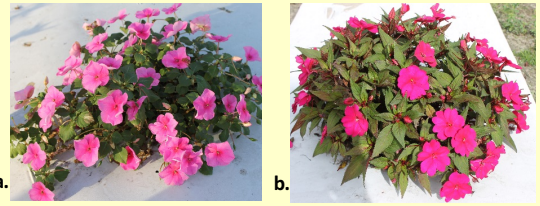


Figure 1. *Impatiens walleriana* Super Elfin® XP Pink (SEP) (a) and *Impatiens xhawkeri* SunPatiens® Compact Royal Magenta (SPR) (b).

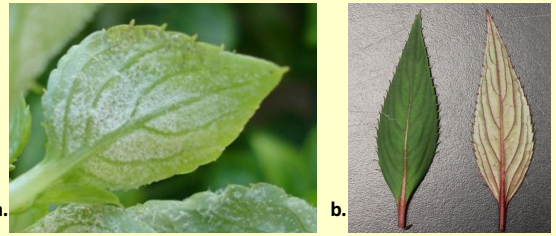


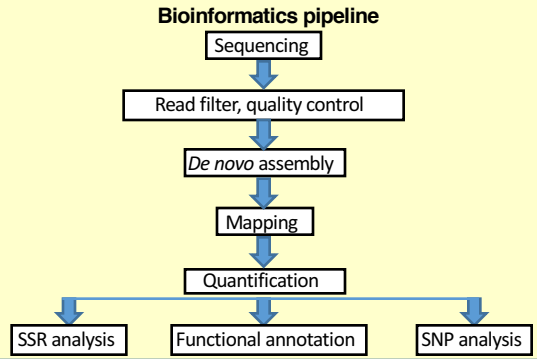
Figure 2. IDM-susceptible leaves of SEP covered with fungal spores (a) and IDM-resistant leaves of SPR showing no fungal spores (b).

Objectives

- To characterize the leaf transcriptomes of Sunpatiens® Compact Royal Magenta (SPR; IDM-resistant) and Super Elfin® XP Pink (SEP; IDM-susceptible).
- To identify resistance and defense-related genes in IDM-resistant SPR.
- To identify SSRs and SNPs for development of molecular markers.

Methodology

- Total RNA was extracted from leaves of SEP and SPR cultivars (Figs. 1 and 2) and converted into cDNA
- Libraries were created from cDNA; quality control was done by Agilent 2100 Bioanalyzer and quantified by ABI StepOnePlus Real-Time PCR
- Sequencing was performed on Illumina HiSeq™2000 to generate 100-bp paired-end reads



Results

- *De novo* assembly generated 121,497 unigenes with an average length of 1,156 nucleotides and the N50 of 1,778 nucleotides
- The total number of contigs and unigenes in SPR were 122,166 and 87,415 and in SEP 104,752 and 69,369 respectively
- In total 91,187 (75%) unigenes and 89,490 (73.66%), 59,403 (48.89%), 54,521 (44.87%), 37,576 (30.93%), and 70,190 (57.77%) unigenes were annotated to NR, NT, Swiss-Prot, KEGG, COG, GO databases
- Fifteen resistance and defense-related genes were expressed highly in SPR but not or rarely in SEP (Table 1)
- There were 22,484 SSRs identified, among which trinucleotides (10501; 46.7%) were the most common. There were 245,936 and 120,073 SNPs discovered in SPR and SEP cultivars, respectively; the dominant type was transition 139,794 (56.84%) and 71,181 (59.28%) for SPR and SEP respectively (Figure 3)

Table 1. Resistance and defense-related genes expressed in impatiens

Gene ID	Subject description
CL10.Contig1	Putative disease resistance protein At5g05400
CL12505.Contig5	Putative disease resistance RPP13-like protein 1
CL12796.Contig1	LETM1-like protein
CL14017.Contig1	Cation efflux family protein
CL14259.Contig1	Disease resistance protein (CC-NBS-LRR class) family
CL1855.Contig4	Putative disease resistance RPP13-like protein 1
CL2138.Contig3	Uncharacterized mitochondrial protein AtMg00860
CL3381.Contig3	Probable disease resistance protein At5g45510
CL8803.Contig4	Leucine-rich repeat (LRR) family protein
CL8803Contig5	Leucine-rich repeat (LRR) family protein
Unigene2713	Receptor like protein 35
Unigene13240	Putative disease resistance protein At1g59780
Unigene2747	Putative disease resistance protein At3g14460
Unigene3612	DNA-binding storekeeper protein-related transcriptional regulator
Unigene6688	Disease resistance protein (TIR-NBS-LRR class)

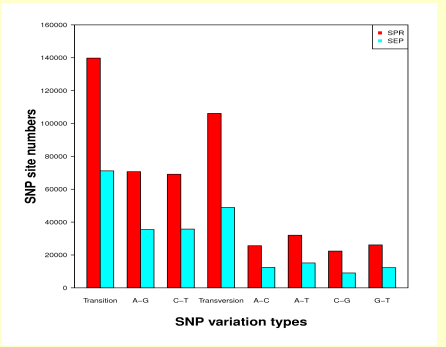


Figure 3. Types of single nucleotide polymorphic sites (SNPs) (a) and simple sequence repeats (SSRs) (b) in impatiens.

Conclusions

- Characterization of impatiens leaf transcriptomes has laid a foundation for future genomic and molecular studies in impatiens
- Sequences of the identified resistance and defense-related genes provide templates for gene cloning, structural analysis, and function confirmation
- SSRs and SNPs identified will be useful for development of molecular markers, marker-assisted selection, and discovery of quantitative trait loci

References

- Cunington, J. H., R. Aldaoud, M. Loh, W. S. Washington, and G. Irvine, 2008. First record of *Plasmopara obducens* (downy mildew) on impatiens in Australia. Plant Pathology 57:371.
- Uchneat, M. S. 2007. Impatiens In: N.O. Anderson (ed.). Flower breeding and genetics: Issues, challenges and opportunities for 21st century. Springer, London. Pp. 277-299.

Acknowledgements

- The American Floral Endowment and the Fred C. Gloeckner Foundation for partial financial support.
- Beijing Genomics Institute, China for transcriptome sequencing and bioinformatics analysis. Zhe Cao for tissue sampling, Gail Bowman and Joyce Jones for managing plants in the greenhouse.

Contact: krishnabhattarai@ufl.edu