

POLYMORPHIC SSR MARKERS FOR *PLASMOPARA OBDUCCENS* (PERONOSPORACEAE), THE NEWLY EMERGENT DOWNY MILDEW PATHOGEN OF *IMPATIENS* (BALSAMINACEAE)¹

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- **Premise of the study:** Simple sequence repeat (SSR) markers were developed for *Plasmopara obducens*, the causal agent of the newly emergent downy mildew disease of *Impatiens walleriana*.
- **Methods and Results:** A 202-Mb draft genome assembly was generated from *P. obducens* using Illumina technology and mined to identify 13,483 SSR motifs. Primers were synthesized for 62 marker candidates, of which 37 generated reliable PCR products. Testing of the 37 markers using 96 *P. obducens* samples showed 96% of the markers were polymorphic, with 2–6 alleles observed. Observed and expected heterozygosity ranged from 0.000–0.892 and 0.023–0.746, respectively. Just 17 markers were sufficient to identify all multilocus genotypes.
- **Conclusions:** These are the first SSR markers available for this pathogen, and one of the first molecular resources. These markers will be useful in assessing variation in pathogen populations and determining the factors contributing to the emergence of destructive impatiens downy mildew disease.

Key words: de novo assembly; high-throughput marker identification; ornamental impatiens; *Plasmopara obducens*; population genetics; simple sequence repeats.

Downy mildew is a newly emergent disease of *Impatiens walleriana* Hook. f. (impatiens; Balsaminaceae), a high-value, flowering annual plant contributing \$105 million annually to the horticulture industry in the United States alone. This destructive disease threatens the cultivation of impatiens worldwide (Brasier, 2008). In 2011, widespread outbreaks of impatiens downy mildew (IDM) disease were observed for the first time in the United States, affecting plants grown in greenhouses, nurseries, and landscapes (e.g., Wegulo et al., 2004; Baysal-Gurel et al., 2012; Palmateer et al., 2013; Crouch et al., 2014). Similar disease outbreaks have been reported through the United Kingdom, continental Europe, and Australia (e.g., Lane

et al., 2005; Cunnington et al., 2008; Toppe et al., 2010). The causal agent of IDM, *Plasmopara obducens* (J. Schröt.) J. Schröt., is one of many obligate biotrophic plant pathogens in the Oomycota (Chromalveolata, Heterokontophyta) afflicting numerous economically important plants around the world (Kamoun et al., 2015). Impatiens infected with *P. obducens* are quickly defoliated, and death occurs within weeks of disease onset. Infected plants cannot be cured, and the pathogen might be capable of persisting in the soil from one season to the next.

Despite the global impact of IDM on cultivated impatiens, there is currently no information about pathogen population structure or the factors that led to the epidemics, delaying the development of effective mitigation strategies (Plantegenest et al., 2007). Downy mildew pathogens engage in classic gene-for-gene interactions with their hosts during the infection process, producing fast-evolving elicitor molecules that in turn give rise to diverse physiological races varying in their ability to infect a given plant (e.g., Lebeda and Cohen, 2011). As such, knowledge of pathogen variability provides key information required to develop durable host disease resistance. In this study, we developed 37 simple sequence repeat (SSR) markers from the genome of *P. obducens* to support investigations of population diversity, and demonstrate the utility of these markers in a sample of 96 *P. obducens* collected throughout the United States.

METHODS AND RESULTS

Genomic DNA from *P. obducens* sample H12.14-11 (Appendix 1) was extracted from a sporangial mass using the OmniPrep DNA Kit (G-Biosciences, St. Louis, Missouri, USA) following manufacturer's instructions, then purified

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