

## Development and validation of a multiplex qPCR assay for detection and relative quantification of *Diaporthe aspalathi*, *D.caulivora*, *D.miriciae* and *D.longicolla* on soybean

Eilyn Mena<sup>1\*</sup>, Pablo Grijalba<sup>2</sup> and Inés Ponce de León<sup>1</sup>

1 Departamento de Biología Molecular, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

2 Departamento de Producción de Plantas, Universidad de Buenos Aires, Argentina

\*emena@iibce.edu.uy

Soybean (*Glycine max* L.) is one of the most economically important crops in Uruguay. Soybean is affected by several pathogens, including fungal *Diaporthe* species that cause soybean stem canker (SSC), which reduces yield worldwide. The main pathogen causing SSC are *Diaporthe aspalathi*, *D. caulivora*, *D. masirevicii*, *D. miriciae* and *D. longicolla* (Mena *et al.* 2020; Mena *et al.* 2024). Disease symptoms are similar between *Diaporthe* species and consist in brown-to-reddish necrotic lesions on the stems. Similar morphological and disease characteristics of these pathogens constitutes a challenge for conventional disease diagnosis. The present study was carried out to detect and quantify *Diaporthe* species associated to SSC in Uruguay by multiplex qPCR. Four species-specific TaqMan primer-probe sets were designed based on translation elongation factor 1-alpha gene (tEF1a) sequences for the species: *D. aspalathi*, *D. caulivora*, *D. miriciae* and *D. longicolla*. The specificity and efficiency of the primer-probe sets were tested using PCR products and genomic DNA from pure cultures of *Diaporthe* species. In addition, multiplex qPCR assay was evaluated in soybean plants inoculated with one or more *Diaporthe* species. Our results indicate that these *Diaporthe* species can be detected and quantified alone and in parallel with a multiplex qPCR assay. Thus, our qPCR assay could be a useful tool for diagnosis of *D. aspalathi*, *D. caulivora*, *D. miriciae* and *D. longicolla*, as well as for designing strategies to manage SSC disease.

**Keywords:** molecular diagnostic of *Diaporthe*, real-time PCR, and soybean stem canker