Morphological and molecular characterization of European species of the *Diaporthe/Phomopsis* complex associated with Soybean Seed Decay

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I. Introduction

- The *Diaporthe/Phomopsis* Complex (DPC) has been reported to be involved in several soybean diseases, including *Phomopsis* seed decay (*P. longicolla*), pod and stem blight (*D. phaseolorum* var. *sojae*) and stem canker (*D. phaseolorum* var. *meridionalis* and *D. phaseolorum* var. *caulivora*), resulting in significant yield and quality losses (Figure 1). Accurate species identification of DPC is critical in understanding disease epidemiology and for developing effective control measures.

Figure 1: (A) Seed decay (B) Pod and stem blight (C) Stem canker

The objectives of this study were to isolate and identify DPC species of European soybean seeds based on morphological and molecular criteria and also to classify the isolated DPC species according to their mating-type loci.

II. Materials and Methods

- Collection of infected seeds (Germany, France and Austria)
- Pre-treatment of seeds (NaOCl 1%)
- Plating on acidified potato dextrose agar (APDA)
- Incubation (30 d at 24±1 °C)
- Subculture of each putative isolate of DPC species
- Purification of the isolates by single-spore method
- Morphological identification
- Extraction of DNA
- PCR [ITS1/ITS4 and EF1-728F/EF1-986R primers]
- Sequencing
- Mating-type diagnosis [PCR with MAT1-1-1FW/RV and MAT1-2-1FW/RV primers]

III. Results

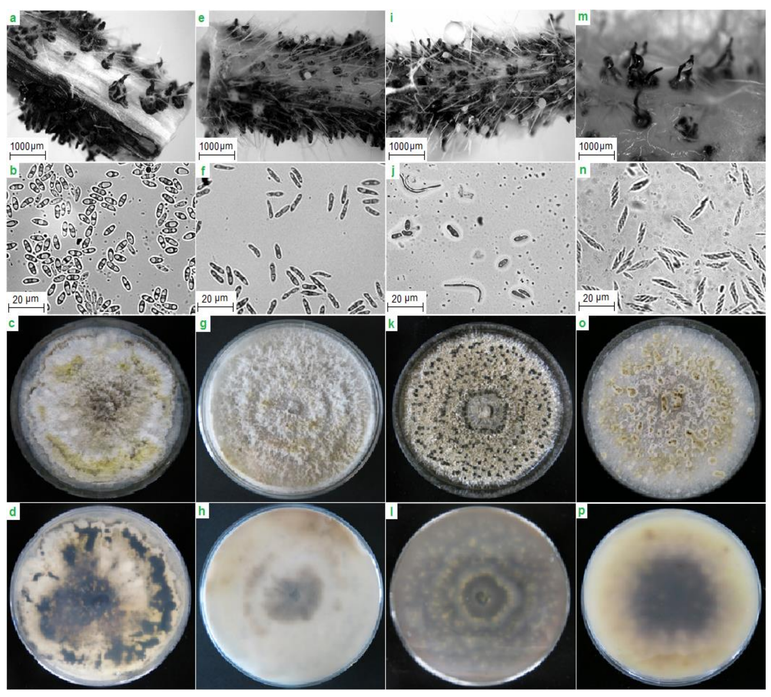

Figure 2: Morphology of *P. longicolla* (a-d), *D. novem* (e-h), *D. caulivora* (i-l) and *D. eres* (m-p) **a, e, i.** Pycnidial necks and **m.** Perithecia protruded on autoclaved soybean stem pieces placed on water agar. **b and f.** α-conidia **j.** α- and β-conidia **n.** Asci and ascospores **c, g, k and o.** upper view of the cultures on APDA **d, h, l and p.** Reverse view of the cultures.

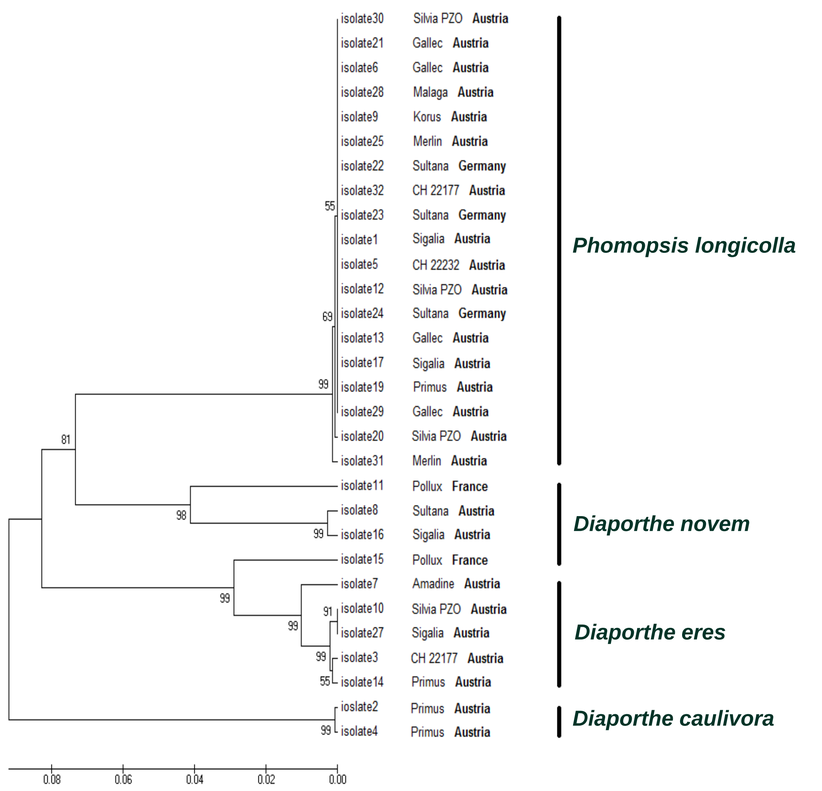

Figure 3: Phylogenetic tree resulting from MEGA6 analysis of the combined 2-genes sequence alignment (*TEF1* and *ITS*), included: Isolates, cultivars and their origin, respectively.

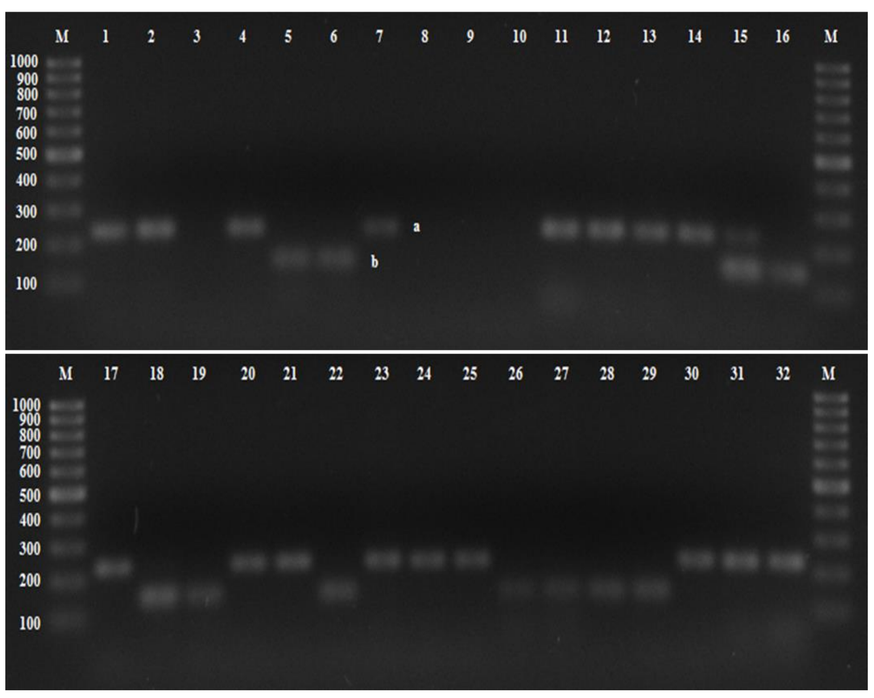

Figure 4: PCR analyses of isolates. *MAT1-1-1* (b, 150 bp), and *MAT1-2-1* (a, 230 bp). [1.5 % agarose gels, 100 V, 60 min]
M: DNA ladder. Numbers (1 to 32) represent DPC isolates
DPC isolates: [1, 5, 6, 9, 12, 13, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31 and 32 = *P. longicolla*]
[2 and 4 = *D. caulivora*] [3, 7, 10, 14 and 27 = *D. eres*]
[8, 11, 15 and 16 = *D.* novem]

IV. Conclusion

- 32 DPC strains isolated and identified based on the morphological characteristics and molecular analysis and they could be assigned to four different DPC species:
  - *Phomopsis longicolla*
  - *Diaporthe caulivora*
  - *Diaporthe eres*
  - *Diaporthe novem*
- Also, the results from Mating-type experiments revealed that MAT primers used in this study allowed Mating-type diagnosis of the 28 isolates.

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