



Presence of both mating types of *Ascochyta rabiei* in Argentina suggests potential for sexual reproduction

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Abstract

The presence of both mating types was tested for in twelve isolates of *Ascochyta rabiei* in Argentina. A *MAT*-specific PCR assay was performed. Nine of these isolates were identified as *MAT1-1* and three of them *MAT1-2*. This is the first research regarding the mating types of *Ascochyta rabiei* in the country and suggests that there is a potential for sexual reproduction in Argentina.

Keywords *Didymella rabiei* · Sexual stage · *Ascochyta* blight

Ascochyta rabiei is a heterothallic ascomycete fungus which causes *Ascochyta* Blight (AB) of chickpea (Trapero-Casas and Kaiser 1992), one of the most globally devastating diseases for the crop. Since it was first reported in Argentina in 2012 (Viotti et al. 2012) it has caused yield losses of up to 100% (De Rossi et al. 2018). Until now, only the anamorph has been reported in Argentina and no research has been conducted on the sexual stages of this fungus in that country.

Sexual reproduction of *A. rabiei* is controlled by a single regulatory locus, the mating type or *MAT* locus, which has two idiomorphs, either the *MAT1-1* or *MAT1-2* forms (Turgeon and Yoder 2000; Barve et al. 2003; Ali et al. 2012). For development of teleomorphic state, both mating types must be present. The presence of a single mating type has been reported in several parts of the world (Barve et al. 2003).

It is well known that sexual reproduction contributes to an increase in pathogenic diversity, allowing fungicides resistance or breakdown of host resistance (Peever

et al. 2004; Pande et al. 2005; Rhaïem et al. 2007; Ali et al. 2012; Sharma and Ghosh 2016; Manjunatha et al. 2018). Moreover, pseudothecia formed on chickpea debris contribute to long-term survival of the pathogen. Its ascospores have an important role as the primary inoculum for the infection and long-distance spread of the fungus (Bayraktar et al. 2007). For these reasons, the aim of this work was to determine if both mating types *MAT1-1* and *MAT1-2* were present to establish whether sexual reproduction could occur in Argentina.

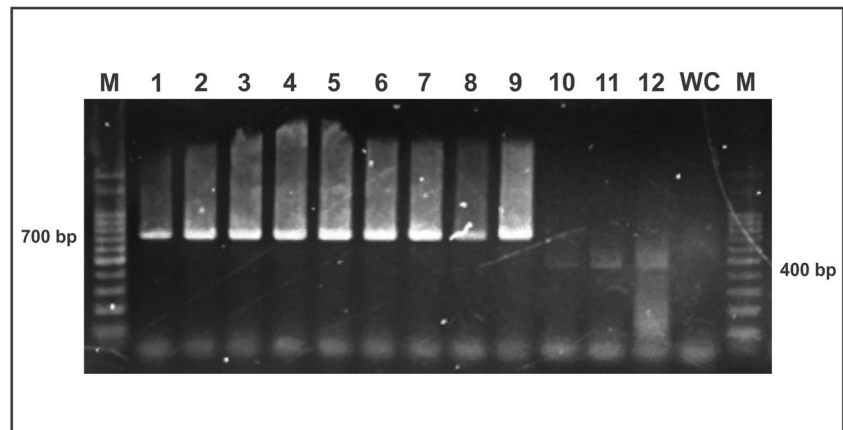
Twelve single spore isolates of *A. rabiei* were obtained from symptomatic chickpea plants, collected in fields of Córdoba province, Argentina. Molecular identification was carried out through phylogenetic inference based on the internal transcribed spacer (ITS) using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990); the partial sequence of β -tubulin gene using TUB2Fd (5'-GTBCACCTYCARACCGG YCARTG-3') and T2 (5'-TAGTGACCCTTGCC CCAGTTG 3') primers (Groenewald et al. 2013; O'Donnell and Cigelnik 1997) and large ribosomal subunit rDNA D1-D2 regions using D1/D2-NL4 (5'-GGTC CGTGTTC AAGACGG-3') and D1/D2-NL1 (5'-GCAT ATCAATAAGCGGAGGAAAAG-3') primers (O'Donnell 1993). The pathogenicity of the isolates was confirmed by Koch's Postulates. The isolates tested were deposited into the EEA La Consulta INTA (WDCM 904) collection. To verify the presence of both mating types a multiplex PCR assay was performed according to Barve et al. (2003), using the primers Tail5

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Fig. 1 Amplification of MAT-specific Multiplex PCR products from 12 random isolates of *Ascochyta rabiei*. Lanes 1–9: MAT1–1 isolates. Lanes 10–12: MAT1–2 isolates. M: 100-bp molecular weight marker. WC: Water Control



(5'-CGCTATTTTATCCAAGACACACC-3'), SP21 (5'-ACAGTGAGCCTGCACAGTTC-3') and Com1 (5'-GCATGCCATATCGCCAGT-3').

The results of this assay showed that nine of the analyzed isolates amplified a PCR product of 700 bp, which identifies them as being *MAT1-1* mating type, and three of them amplified a product of 400 bp, which shows that they include the *MAT1-2* idiomorph (Fig. 1).

This is the first research on mating types of *A. rabiei* in Argentina and confirms the presence of both idiomorphs. Mating type distribution is one of the important factors that contributes to variation in any pathogen population (Sharma and Ghosh 2016). This fact is a pre-requisite for the teleomorphic state with implications for spread, survival, and control of AB (Manjunatha et al. 2018).

Resistance to fungicides is one of the adaptations that may happen in recombinant populations (Sharma and Ghosh 2016). If this occurred, it would be particularly damaging as few fungicides are legally registered for AB control in Argentina and AB-resistant commercial varieties are not currently available to the farmers. The presence of a teleomorph in the life cycle of the pathogen contributes to variability within the population, and could lead to development of new pathotypes of *A. rabiei* (Sharma and Ghosh 2016). Pande et al. (2005) noted that more than 3000 AB-resistant lines had been obtained by ICARDA but most released cultivars succumbed to new pathotypes of *A. rabiei*. Thus, the possibility of the emergence of recombinant populations is essential information for breeders.

The work described here is a first step towards the study of the sexual stage in Argentina. Further research is needed to monitor the distribution and incidence of both idiomorphs and the teleomorph's occurrence in the Argentinian fields.

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