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Effect of dsRNA on growth rate and reproductive potential of *Monosporascus cannonballus*

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ABSTRACT

The effect of double stranded RNA (dsRNA) infection on growth rate and the reproductive potential of *Monosporascus cannonballus* was studied in 21 isolates collected in cucurbit growing areas of Spain and Tunisia. The isolates were incubated on potato dextrose agar (PDA) under different conditions of temperature, pH, and water potential (Ψ_s). They showed optimal growth temperatures over the range of 27–34 °C and perithecia formation was obtained mainly at 25 and 30 °C, although some isolates were able to produce perithecia at 35 °C. All isolates were able to produce perithecia in a broad range of pHs (4–8). Regarding the effect of Ψ_s, the isolates were more tolerant to grow on KCl than on NaCl. For each solute, radial growth decreased progressively as Ψ_s decreased and was severely limited at −5.0 to −6.0 MPa. Perithecia formation was highest at −0.5 MPa, decreased at −1.0 MPa and occurred just in some isolates at −2.0 MPa. Nine of the *M. cannonballus* isolates harboured dsRNA with 2–6 bands each and a size range of 1.9–18.0 Kb. Phenotypical data were subjected to multivariate factorial analysis. Most of the isolates clustered in two groups corresponding with the presence/absence of dsRNA elements. Isolates without detectable dsRNA produced more perithecia. However, isolates with dsRNA produced lower number of perithecia depending on the pH, Ψ_s, or solute used. These results improve our understanding of the behaviour and growth of this pathogen in soil, and can be useful to implement effective disease control.

Introduction

Monosporascus root rot and vine decline of cucurbits caused by the soilborne ascomycete *Monosporascus cannonballus* (Pollack & Uecker 1974) has become one of the most important cucurbit yield-limiting diseases worldwide, causing serious economic losses mainly in muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) crops

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(Martyn & Miller 1996; Bruton 1998; Cluck *et al*. 2009). Since the mid-1980s, this disease has been prevalent in cucurbit-production areas located in hot semi-arid to arid regions, as well as subtropical environments (Martyn & Miller 1996). In recent years, a re-emergence of *Monosporascus* root rot and vine decline has been noticed. Severe outbreaks of this disease have been reported in new areas such as Egypt (El-Desouky & El-Wakil 2003), Brazil, (Sales *et al*. 2004) and Italy (Chilosi *et al*. 2008), and expanded its geographical and host ranges in regions where it was already present (Sarpeleh 2008; Boughalleb *et al*. 2010; Sales *et al*. 2010).

Symptoms of the disease include the yellowing and death of the crown leaves that gradually radiate out and kill the vine as the fruit approach maturity (Martyn & Miller 1996; Cluck *et al*. 2009). *Monosporascus cannonballus* produces ascospores in perithecia formed on affected roots at the end of the cropping season (Martyn & Miller 1996). Ascospores probably function as the primary survival structure, as well as the primary inoculum of the fungus for root infection (Stanghellini *et al*. 1996, 2000).

Little information is available on the adaptability of *M. cannonballus* to different environmental variables such as soil temperature, water potential or pH, and how they may influence the phenotypic variability and the reproductive potential of this fungus. *Monosporascus cannonballus* appears to be adapted to hot, semi-arid climates with saline and alkaline soils (Martyn & Miller 1996). Information about the environmental requirements of *M. cannonballus* has been inferred from areas where the fungus has been found and by *in vitro* studies. *Monosporascus cannonballus* grows optimally at pH 6–7, can tolerate relatively high levels of sodium and calcium chloride salts (8–10 %) and maximum mycelial growth occurs at osmotic water potential (Ψ_s) of −0.6 to −0.8 MPa (Martyn & Miller 1996; Ferrin & Stanghellini 2006). Perithecial production is also affected by soil temperature, reaching its optimum between 25 and 30 °C (Waugh *et al*. 2003). Recently, an evaluation of soil factors associated with ascospore density conducted in watermelon fields in Tunisia, revealed that the pH of the soil had a strongly significant negative linear relationship with ascospore density indicating that pHs closer to the optimal one *in vitro* (pH 6–7) are more conducive for ascospore production (Boughalleb *et al*. 2010).

Fungal viruses or mycoviruses are present in many phytopathogenic species and, in general, most of them cause little or no obvious symptoms in their fungal hosts. However, in some cases infection by mycoviruses may lead to attenuation (hypovirulence) or enhancement of fungal virulence (hypervirulence) (Ghabrial & Suzuki 2009). In *M. cannonballus*, some isolates have been reported to harbour double stranded RNA (dsRNA) components, which cause different effects on this pathogen (Martyn & Miller 1996; Cluck *et al*. 2009). The presence of dsRNA in *M. cannonballus* isolates was first reported by Lovic *et al*. (1995). These authors, in a survey of two commercial muskmelon fields in Texas, found that approximately 65 % of the *M. cannonballus* isolates recovered from diseased plants harboured dsRNAs. A diverse assortment of different sizes and number of dsRNAs, ranging in size from ca. 1.7–15 Kb, based upon agarose gel electrophoresis, were associated with the isolates. Some isolates harboured only a single dsRNA, while others harboured as many as 13 dsRNAs. After laboratory maintenance, most dsRNA$^+$ isolates developed a degenerate culture phenotype characterized by slow growth, yellow to orange pigment accumulation, and reduced ascospore production in culture (Lovic *et al*. 1995; Martyn & Miller 1996). In addition, these dsRNA$^+$ isolates were hypovirulent on muskmelon in greenhouse pathogenicity tests. Cluck *et al*. (2009) conducted experiments to determine pigmentation, perithecial formation, and the presence of cellular dsRNA among a collection of *M. cannonballus* isolates obtained from different Spanish muskmelon growing areas. Thirty-one isolates were grouped based on dsRNA fragment sizes using cluster analysis and Euclidean distances. Three distinct dsRNA groupings were observed. Group 2 isolates containing 2, 3, and 3.5 Kb dsRNA appeared to exhibit a decrease in perithecial production compared to the other groups. Group 1 isolates exhibited yellow pigmentation only, while Group 3 isolates expressed grey (wild-type) and yellow (degenerate) pigmentation. Isolates that did not contain dsRNA (Group 4) exhibited wild-type pigmentation. In addition, Wheeler *et al*. (2004) reported that degenerate isolates from California, Texas, Honduras, Israel, and Spain did not produce melanin as opposed to wild-type isolates. They further postulated that the loss of fungal melanization in *M. cannonballus* may be associated with loss of virulence in the pigmented isolates.

Examining the influence of dsRNA infection, temperature, pH, water potential, and their interactions on mycelial growth and the reproductive potential of *M. cannonballus* would contribute to a better knowledge of the biology of this economically important pathogen. Thus, the objectives of this study were to (i) determine the presence of dsRNA in 21 isolates of *M. cannonballus* collected in cucurbit growing areas of Spain and Tunisia (ii) evaluate the effect of temperature, pH, and water potential on mycelial growth and perithecial production of these isolates and (iii) study the relationship among the presence or absence of dsRNA, growth rate, and perithecial production.

Material and methods

Fungal isolates

Twenty-one *Monosporascus cannonballus* isolates obtained from roots of watermelon and muskmelon crops exhibiting symptoms of *Monosporascus* root rot and vine decline in different cucurbit growing regions in Tunisia and Spain were arbitrarily selected and used in the present study (Table 1). All isolates were hyphal-tipped and stored at 25 °C in darkness in plastic vials containing sterilized peat (Gramoflor GmbH & Co., Vechta, Germany). Prior to use, a small portion of the colonized peat from each plastic vial was transferred to potato dextrose agar (PDA) (Biokar-Diagnostics, Zac de Ther, France) plates and allowed to grow at 25 °C in darkness for 10 d. After this period of incubation, the presence of yellow pigmentation of each isolate on agar was recorded.

dsRNA extraction and separation

To determine the presence of dsRNA, all *Monosporascus cannonballus* isolates were grown on top of sterile cellophane disks placed over PDA medium in Petri dishes (9 cm in