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Soil Biology & Biochemistry 37 (2005) 2180–2184

Soil Biology & Biochemistry

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Variation in oxalic acid production and mycelial compatibility within field populations of *Sclerotinia sclerotiorum*

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Received 2 July 2004; received in revised form 17 March 2005; accepted 24 March 2005

Abstract

This study was aimed at detecting mycelial compatibility groups and variations in oxalic acid production in *Sclerotinia sclerotiorum*. For this purpose, 121 isolates of this plant pathogen recovered from lettuce, soybean and sunflower field crops, and grouped in 46 MCGs were tested for their ability to release oxalic acid and other organic acids to the medium. Oxalic acid production on liquid media was measured spectrophotometrically and release of organic acids was estimated by isolate abilities to discolour solid media amended with bromophenol blue. There were significant differences among MCGs in both oxalic acid and organic acids releasing, ranging the mean production of oxalic acid between 18 and 110 μg oxalic acid mg^{-1} dry wt. When isolates were grouped by their hosts, those obtained from soybean presented the highest release of oxalic acid (71 μg oxalic acid mg^{-1} dry wt), while those from sunflower showed the highest release of other acids to the medium. Solid medium discoloration was not correlated with oxalic acid concentration in liquid medium (Spearman $R=-0.085$; $P=0.126$).
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Keywords: *Sclerotinia sclerotiorum*; Oxalic acid; Organic acids; Mycelial compatibility groups

1. Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a widespread fungal pathogen that causes disease on many economically important vegetables and field crops (Boland and Hall, 1994). Approximately 90% of its life cycle is spent in soil as sclerotia and their high persistence make of *S. sclerotiorum* a very successful pathogen (Adams and Ayers, 1979).

This fungus produces and secretes millimolar concentrations of oxalic acid into their surroundings (Cessna et al., 2000). The observation that mutants unable to synthesize oxalate were non-pathogenic, whereas revertant strains displayed normal virulence confirms oxalate as a pathogenicity determinant in *S. sclerotiorum* (Godoy et al., 1990). Besides oxalic acid, *S. sclerotiorum* may release other dicarboxylic organic acids such as succinic, malic, fumaric and glycolic (Vega et al., 1970).

Considerable research interest has been focused on oxalic acid production by this pathogen and the following mechanisms of action have been proposed to explain its involvement in pathogenesis: (1) lowering infected tissues pH, what enhances the activity of extracellular enzymes produced by the pathogen (Bateman and Beer, 1965), (2) chelation of cell wall Ca^{2+} by the oxalate anion, what softens plant cell wall and compromises the function of Ca^{2+}-dependent defence responses (Bateman and Beer, 1965), (3) direct toxicity to host plants, what weakens the plant and facilitates invasion (Noyes and Hancock, 1981), and (4) suppression of the host plant oxidative burst (Cessna et al., 2000).

It has been established that field crops populations of *S. sclerotiorum* are clonal and that several clones may infect each field (Kohn, 1994). One of the criterions for detecting clonality is the mycelial compatibility grouping. When paired in culture, all members of an MCG can anastomose to form one confluent colony with no reaction line. These members also share a unique complex DNA fingerprint (Kohn et al., 1991; Carbone et al., 1999).

Identifying an association between MCGs and oxalic acid, one of the main determinants of *Sclerotinia*

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0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.03.017

pathogenicity, is important for developing strategies to combat its spread among agronomically important crops, particularly because some MCGs have shown to persist from year to year specially as resistant sclerotia in soil, covering a very wide geographical area being some of them responsible for a large proportion of infections (Hambleton et al., 2002; Durman et al., 2003).

Therefore, the determination of oxalic acid in *S. sclerotiorum* is of importance, and numerous methods have been used including $KMnO_4$ tritation (Bateman and Beer, 1965), enzyme-based colorimetric assay (Yriberri and Possen, 1980), spectrophotometry (Allan et al., 1986; Zhou and Boland, 1999), high pressure liquid chromatography (Jarosz-Wilkolazka and Gad, 2003), for this and other fungi.

A previous report from Durman et al. (2003) based on more than 140 *S. sclerotiorum* isolates collected from soybean, sunflower and lettuce crops in the Buenos Aires province, demonstrated that these populations presented a clonal structure and that each one was made up by several MCGs. The objectives of this study were: (1) to evaluate the ability to release oxalic acid by different *S. sclerotiorum* isolates from economically important field crops and (2) to establish the presence of an association between oxalic acid release and specific genotypes (or MCGs) or host.

2. Materials and methods

2.1. Isolates

The study was performed on 121 *S. sclerotiorum* isolates confined, from a previous study (Durman et al., 2003), to 46 different MCGs. Nineteen isolates were obtained from sunflower, 49 from lettuce and 53 from soybean field crops in the Buenos Aires province, Argentina (Fig. 1).

2.2. Oxalic acid determination

For oxalic acid determination isolates were grown in 60 ml flasks containing 15 ml of potato dextrose broth (PDB, 2% glucose and 0.4% fresh potato extract in distil water). Flasks were statically incubated for 3 days at room temperature. Cultures were vacuum filtered and the supernatant was used as oxalic acid sample. Mycelial fractions dry weights were determined after drying at 80 °C, 72 h. Oxalic acid was determined in the PDB culture following Xu and Zhang (2000) with few modifications. Reaction mix contained 0.2 ml of sample (or standard oxalic acid solution), 0.11 ml of bromophenol blue (BPB, 1 mM), 0.198 ml of sulfuric acid (1 M), 0.176 ml of potassium dichromate (100 mM) and 4.8 ml of distilled water. The reaction mix was placed in a water bath at 60 °C and quenched by adding 0.5 ml sodium hydroxide solution (0.75 M) after 10 min. The absorbance was measured at 600 nm by means of a spectrophotometer (Spectronic 20D+, Spectronic Instruments, Inc. USA) and PDB was used as the blank control. Oxalic acid concentration was calculated comparing with a standard curve and was expressed as μg oxalic acid mg^{-1} dry wt mycelium. Assay was run in triplicate and repeated once.

2.3. Estimation of total acids production

Plugs from 3-days old *S. sclerotiorum* colonies were inoculated onto Petri plates filled with potato dextrose agar and BPB (50 mg ml^{-1}). Plates were incubated for 3 days in darkness and 25 °C. Since bromophenol blue is a pH-indicator that turns from blue to yellow when pH is 3–4.6, the presence of a yellow halo was considered an evidenced of acid releasing by the fungus. Radii of yellow halo and colony were both

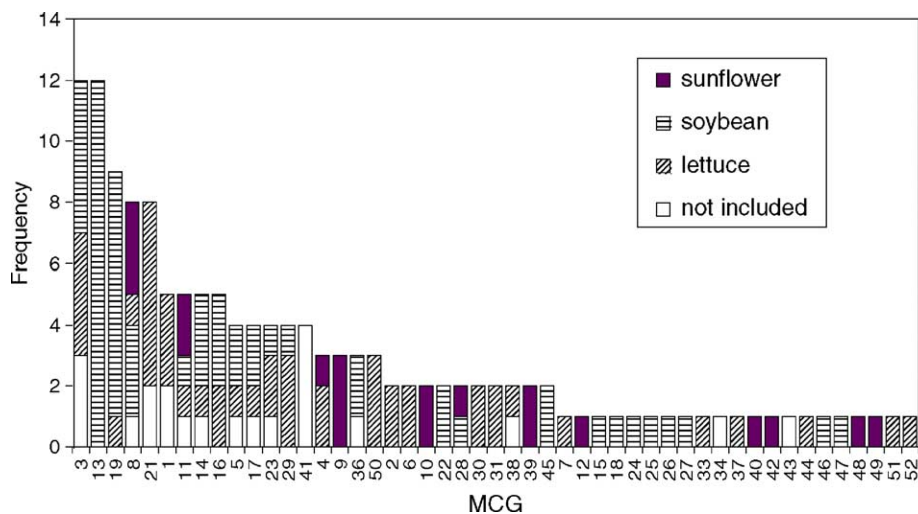

Fig. 1. *S. sclerotiorum* MCG frequencies. Shaded area in each column indicates number of isolates included in this study, discriminated by the host from which they were collected.

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