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Modulation of the phenylacetic acid metabolic complex by quinic acid alters the disease-causing activity of *Rhizoctonia solani* on tomato

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ABSTRACT

The metabolic control of plant growth regulator production by the plant pathogenic fungus *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) and consequences associated with the parasitic and saprobic activity of the fungus were investigated. Fourteen genetically distinct isolates of the fungus belonging to anastomosis groups (AG) AG-3, AG-4, and AG-1-IA were grown on Vogel's minimal medium N with and without the addition of a 25 mM quinic acid (QA) source of carbon. The effect of QA on fungal biomass was determined by measuring the dry wt of mycelia produced under each growth condition. QA stimulated growth of 13 of 14 isolates of *R. solani* examined. The production of phenylacetic acid (PAA) and the chemically related derivatives 2-hydroxy-PAA, 3-hydroxy-PAA, 4-hydroxy-PAA, and 3-methoxy-PAA on the two different media was compared by gas chromatography coupled with mass spectrometry (GC–MS). The presence of QA in the growth medium of *R. solani* altered the PAA production profile, limiting the conversion of PAA to derivative forms. The effect of QA on the ability of *R. solani* to cause disease was examined by inoculating tomato (*Solanum lycopersicum* L.) plants with 11 isolates of *R. solani* AG-3 grown on media with and without the addition of 25 mM QA. Mean percent survival of tomato plants inoculated with *R. solani* was significantly higher when the fungal inoculum was generated on growth medium containing QA. The results of this study support the hypotheses that utilization of QA by *R. solani* leads to reduced production of the plant growth regulators belonging to the PAA metabolic complex which can suppress plant disease development.

1. Introduction

The soil fungus *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) possesses a unique combination of characteristics that enable it to function as both a pathogen of plants and a competitive saprobe in soil (Papavizas, 1970; Cubeta and Vilgalys, 2000). The production of the plant growth regulator phenylacetic acid (PAA, **1**) and hydroxy (OH, **2**–**4**) and methoxy (MeO, **5**) derivatives of PAA (Fig. 1) have been implicated as an important component of the *R. solani* infection processes on plants (Aoki et al., 1963; Frank and Francis, 1976; Mandava et al., 1980). The primary objective of this study was thus to critically examine the production of plant growth regulators in the PAA (**1**) metabolic complex by *R. solani* and to understand the role of these compounds (**1**–**5**) in disease-causing activity.

PAA (**1**) and its OH/MeO derivatives (**2**–**5**) are synthesized by *R. solani* from phenylalanine via the shikimate pathway (Lakshman et al., 2006; Kohmoto and Nishimura, 1975; Kohmoto et al., 1973, 1975). This pathway shares two metabolic intermediates with the quinic acid (QA, **6**, Fig. 1) carbon metabolic pathway, which is induced by its presence in the growth substrate as it is released from lignin in decaying plant material (Giles et al., 1967; Hawkins et al., 1982; Herrmann and Weaver, 1999). The switch between parasitic and saprobic phases of the life history of this fungus may be determined by interplay between these metabolic pathways. It has been recently demonstrated that induction of the QA (**6**) pathway leads to sequestration of intermediates from the shikimate pathway, and reduced production of the PAA (**1**) precursor phenylalanine in *R. solani* and in other filamentous fungi (Lamb et al., 1992; Liu et al., 2003a). Induction of the QA (**6**) pathway in the inoculum of one isolate of *R. solani* AG-3 has also been shown to reduce symptom development on potato (Liu et al., 2003a,b). It has been suggested that the manipulation of metabolic pathways within the fungus by the application of substrates containing QA (**6**) could potentially reduce the disease causing activity of *R. solani* in cultivated crops (Liu et al., 2003b). This *R. solani*

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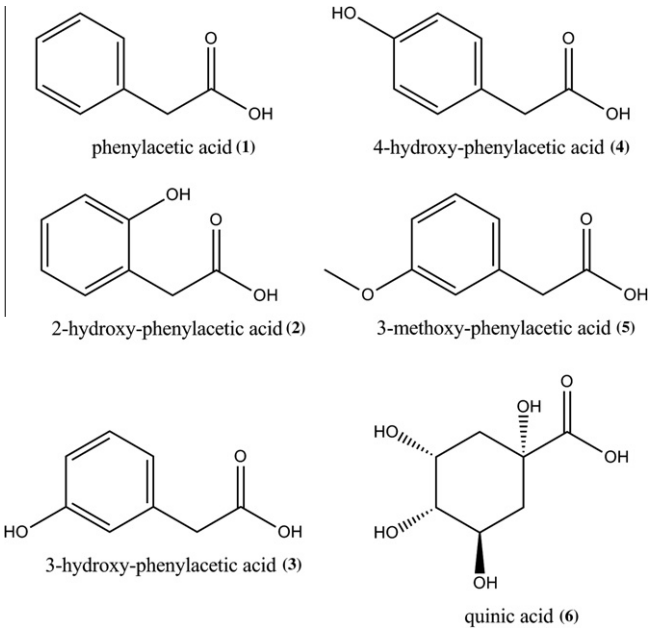

Fig. 1. Structures of phenylacetic acid (**1**), 2-hydroxy-phenylacetic acid (**2**), 3-hydroxy-phenylacetic acid (**3**), 4-hydroxy-phenylacetic acid (**4**), 3-methoxy-phenylacetic acid (**5**), and quinic acid (**6**).

disease management strategy based upon manipulation of fungal metabolism represents a novel approach, in contrast to current practices such as fungicide use that are primarily focused on reducing fungal population size (Leach and Garber, 1970; Sweetingham, 1996).

In this study, three hypotheses related to the addition of QA (**6**) to the fungal growth environment were tested: (1) QA (**6**) does not negatively alter mycelial growth of *R. solani*; (2) QA (**6**) reduces production of PAA (**1**) and the 2-OH (**2**), 3-OH (**3**), 4-OH (**4**) and 3-MeO (**5**) derivatives of PAA by *R. solani*; and (3) QA (**6**) reduces disease symptom development on tomato (*Solanum lycopersicum* L.) inoculated with *R. solani*. Fourteen isolates belonging to three different anastomosis groups (AG) of *R. solani* were examined. The results of preliminary experiments have been previously published (Bartz et al., 2012).

2. Results and discussion

2.1. QA stimulates the growth of R. solani

Fig. 2 summarizes the fungal biomass of 14 isolates of *R. solani* and a non-inoculated control in response to the addition of 25 mM QA (**6**) to minimal medium N (Vogel, 1956). The mycelial dry wt was significantly increased by amendment of the medium with QA (**6**) for of all isolates except CsKa ($p = 0.3819$ for CsKa, $\alpha = 0.05$). Although mean biomass of isolates Bs69, Rs113, Rs182, Rs183, and Rs88 differed significantly between experimental runs, the increase in mycelial dry wt upon QA (**6**) treatment was significant for both experimental runs of each isolate (Bs69 $p = 0.022$ and 0.0036, Rs113 $p = 0.0052$ and <0.0001, Rs182 $p = 0.0043$ and 0.0007, Rs183 $p = 0.0014$ and 0.0003, and Rs88 $p < 0.0001$ and <0.0001 for the first and second experimental run for each isolate respectively; $\alpha = 0.05$). There were no significant differences in biomass between experimental runs for the remaining isolates, all of which displayed a significant increase in mycelial dry wt upon QA (**6**) treatment ($p =$ <0.0001, 0.0008, 0.0014, 0.0005, 0.0127, <0.0001, <0.0001, and 0.0037, for Rhs1A1, Rhs1AP, Rs114, Rs138, Rs191, Rs29, T2, and Tom7b, respectively; $\alpha = 0.05$). Because the addition of QA (**6**) to the growth medium of *R. solani* stimulates growth, any inhibition of disease causing activity or PAA (**1**) production resulting from QA (**6**) treatment cannot be attributed to reduced biomass.

2.2. QA modulates the production of PAA (*1*) and OH and MeO derivatives (*2–5*) of PAA by R. solani

The production of PAA (**1**), 2-OH-PAA (**2**), 3-OH-PAA (**3**), 4-OH-PAA (**4**), and 3-MeO-PAA (**5**) by 14 isolates of *R. solani* grown in Vogel's minimal medium N with and without the addition of 25 mM QA (**6**) was quantified by gas chromatography coupled with mass spectrometry (GC–MS). The effect of QA (**6**) on production of PAA (**1**) differed from the effect on the production of derivatives of PAA (**2–5**) (Fig. 3). While more PAA (**1**) was produced in the medium containing QA (**6**), the mean production of each of the

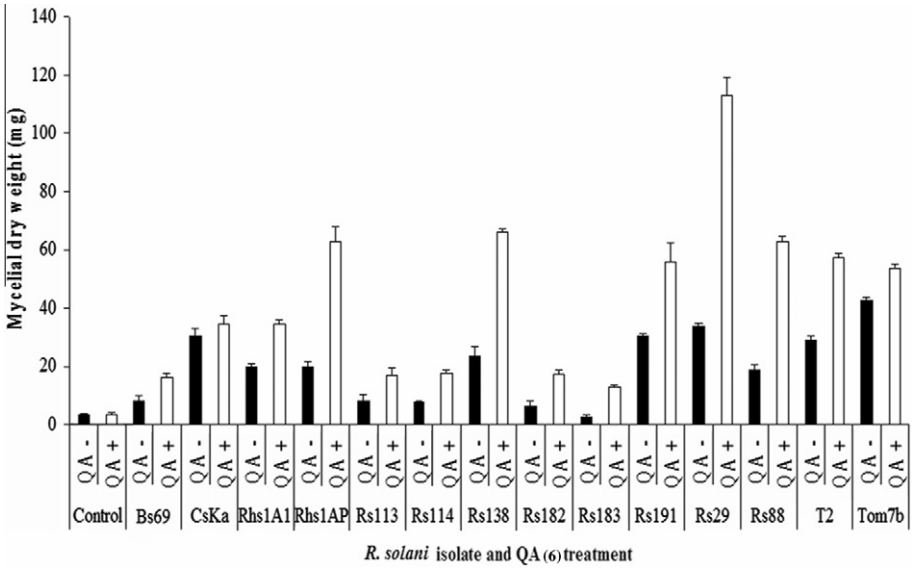

Fig. 2. *Rhizoctonia solani* mycelial dry wt after 3 weeks of growth in Vogel's medium N amended with quinic acid (**6**) (QA). Error bars indicate s.e. of the mean dry wt for each isolate (and non-inoculated control) and QA (**6**) treatment.