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Changes in *L*-phenylalanine ammonia-lyase activity and isoflavone phytoalexins accumulation in soybean seedlings infected with *Sclerotinia sclerotiorum*

Research Article

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Abstract: Soybean [*Glycine max* (L.) Merr.] cultivars (Meli, Alisa, Sava and 1511/99) were grown up to V1 phase (first trifoliate and one node above unifoliate) and then inoculated with *Sclerotinia sclerotiorum* (Lib.) de Bary under controlled conditions. Changes in *L*-phenylalanine ammonia-lyase (PAL) activity and isoflavone phytoalexins were recorded 12, 24, 48 and 72 h after the inoculation. Results showed an increase in PAL activity in all four examined soybean cultivars 48 h after the inoculation, being the highest in Alisa (2-fold higher). Different contents of total daidzein, genistein, glycitein and coumestrol were detected in all samples. Alisa and Sava increased their total isoflavone content (33.9% and 6.2% higher than control, respectively) as well as 1511/99, although 48 h after the inoculation its content decreased significantly. Meli exhibited the highest rate of coumestrol biosynthesis (72 h after the inoculation) and PAL activity (48 h after the inoculation). All investigated cultivars are invariably susceptible to this pathogen. Recorded changes could point to possible differences in mechanisms of tolerance among them.

Keywords: *Glycine max* • *Isoflavone phytoalexins* • *L-phenylalanine ammonia-lyase* • *Sclerotinia sclerotiorum*

1. Introduction

Stem rot caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is an important disease in soybean (*Glycine max* (L.) Merr.) crop zones of Europe, America and Australia. Numerous reports show that decrease in mineral uptake, sudden leaf wilt, reduction in seed yield and development of lesions on the tap root and basal portion of the stem precede the onset of wilting of plants infected with *S. sclerotiorum* and cause them to die within a few days [1].

Rapid and localized cell death (hypersensitive response, HR) and the accumulation of antimicrobial compounds, which play an important role in many plant-pathogen incompatible interactions, known as phytoalexins, represent a wide variety of protective mechanisms in plants designed to restrict pathogen growth [2,3]. After the infection, plants produce soluble phenolic compounds as a part of their defence. Accumulation of phenolic polymers, such as melanin or lignin, reinforce plant cell walls limiting fungal penetration [4]. In addition, impregnation of cell walls by esterified phenolic compounds increases resistance by modifying the cell wall properties, which are no longer recognized by the depolymerizing enzymes produced by the fungus [5].

Accumulation of the soybean phytoalexins correlated with rapid and large increases in the activities of enzymes of general phenylpropanoid and flavonoid metabolism, such as *L*-phenylalanine ammonia-lyase (PAL) and acetyl-CoA carboxylase and chalcone synthase [6-8]. Phenylalanine ammonia-

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