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Metabolic responses of *Thellungiella halophila/salsuginea* to biotic and abiotic stresses: Metabolite profiles and quantitative analyses

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ABSTRACT

The metabolite profiles of the model crucifer *Thellungiella salsuginea* (salt cress) ecotype Shandong subjected to various biotic and abiotic stresses were analyzed using HPLC-DAD-ESI-MS. Two different cruciferous microbial pathogens, *Albugo candida*, a biotrophic oomycete, and *Leptosphaeria maculans*, a necrotrophic fungus, elicited formation of the phytoalexins wasalexins A and B without causing visual damage on inoculated leaves. Analyses of non-polar and polar metabolites led to elucidation of the chemical structures of five metabolites: 4′-*O*-(*E*)-sinapoyl-7-methoxyisovitexin-2″-*O*-β-D-glucopyranoside, 4′-*O*-(*E*)-sinapoylisovitexin-2″-*O*-β-D-glucopyranoside, 4-*O*-β-D-glucopyranosyl-7-hydroxymatairesinol, 5′-*O*-β-D-glucopyranosyldihydroneoascorbigen and 3-*O*-β-D-glucopyranosylthiane. 3-*O*-β-D-glucopyranosylthiane, an unique metabolite for which we suggest the name glucosalsuginin, is proposed to derive from the glucosinolate glucoberteroin. In addition, the identification of a broad range of polar metabolites identical to those of other crucifers was carried out. Quantification of several metabolites over a period of eight days showed that concentrations of the polar phytoanticipin 4-methoxyglucobrassicin increased substantially in leaves irradiated with UV light (λ_{max} 254 nm) relative to control leaves, but not in leaves subjected to other stresses.

1. Introduction

Cruciferous extremophiles of the genus *Thellungiella* (Brassicaceae family) appear to show resistance to stress caused by salinity, cold and draught. *T. halophila* (C.A. Meyer) O.E. Schultz, *T. salsuginea* (Pallas) O.E. Schulz and *T. parvula* (Schrenk) Al-Shehbaz and O'Kane are among the species of *Thellungiella* described so far. The Shandong (from China) and Yukon (from Canada) ecotypes have been cited as *T. halophila* (salt cress), although both ecotypes appear to belong to the species *T. salsuginea* (Amtmann, 2009; hence, these ecotypes will be hereon referred as *T. salsuginea*). The Shandong (Inan et al., 2004) and Yukon ecotypes are annual crucifers and important model systems due to their small genomes (about twice that of *Arabidopsis thaliana*) and high resistance to salinity. *T. salsuginea* (Shandong and Yukon ecotypes) abiotically stressed with $CuCl_2$ produced the phytoalexins wasalexins A (**1**) and B (**2**), 1-methoxybrassenin B (**3**) and rapalexin A (**4**) (Pedras and Adio, 2008). The Shandong ecotype exposed to UV-radiation (λ_{max} 254 nm) produced the largest quantities of wasalexins A (**1**) and B (**2**), together with the unique wasalexin photoaddition products biswasalexins A1 (**5**) and A2 (**6**) (Pedras et al., 2009). By contrast, irrigation of *T. salsuginea* Shandong with a NaCl solution induced substantially smaller amounts of phytoalexins. The production of biswasalexins A1 (**5**) and A2 (**6**) in leaves of UV stressed Shandong appeared to result from a photochemical reaction that might protect plants from UV-radiation (Fig. 1). The phytoanticipins indolyl-3-acetonitrile (**7**), arvelexin (**8**), caulilexin C (**9**), neoascorbigen (**10**) and methylsulphanylpropylisothiocyanate (**11**) were also isolated from both elicited and control leaves. To date, polar metabolites from *Thellungiella* species subjected to any of those abiotic stresses or even in naturally healthy plants remain to be reported.

To continue with the evaluation of stress responses of cruciferous species, we have analyzed the metabolite profiles of *T. salsuginea* Shandong inoculated with two different cruciferous microbial pathogens, *Albugo candida* (Pers. ex Chev.) Kuntze, a biotrophic oomycete, and *Leptosphaeria maculans* (Desm.) Ces. et de Not. (asexual stage *Phoma lingam* (Tode ex Fr.) Desm.), a necrotrophic fungus. Races of *A. candida* show specific pathogenicity to different *Brassica* species and cultivars (Rimmer et al., 2000). Similarly, the fungus *L. maculans* comprises various subgroups virulent to *B. napus* and *B. rapa* or *B. juncea*. In this study, (i) *A. candida* races 7 V (virulent to *B. rapa* cv. Reward, rapeseed) and 2 V (virulent to *B. juncea* cv. Cutlass, brown mustard) and (ii) *L. maculans* isolates virulent to

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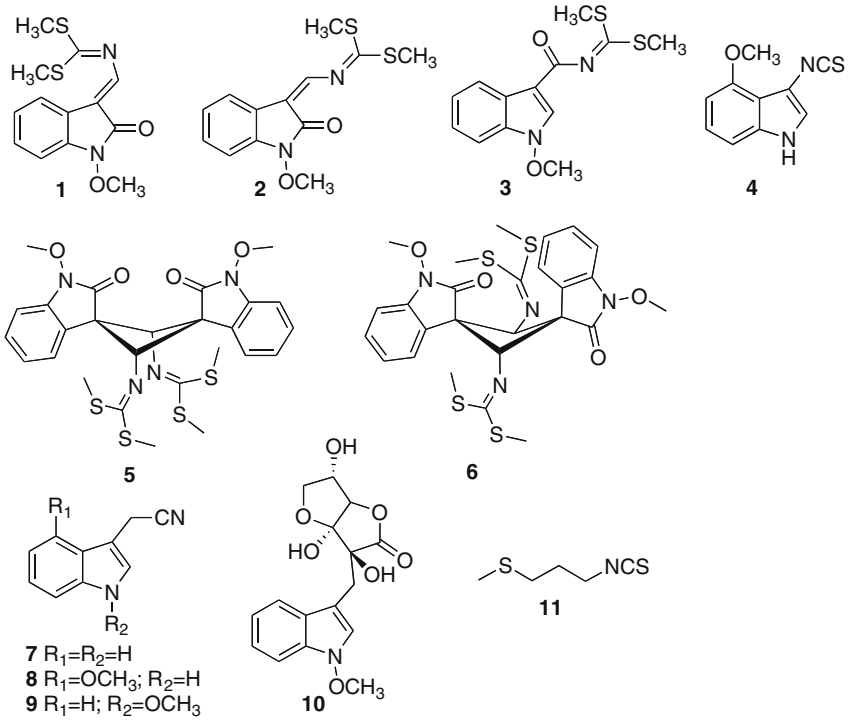

Fig. 1. Phytoalexins **1**–**6** and phytoanticipins **7**–**11** produced in leaves of *Thellungiella salsuginea* Shandong ecotype irradiated with UV light (Pedras et al., 2009).

B. napus (cv. Westar, canola) and *B. juncea* (cv. Cutlass) were used to inoculate *T. salsuginea* Shandong. To the best of our knowledge, no evaluation of potential interactions between *T. salsuginea* and any of these cruciferous pathogens has been reported to date. In addition, the profiles of polar metabolites of *T. salsuginea* Shandong subjected to stress caused by UV-radiation, NaCl irrigation and $CuCl_2$ spray were analyzed.

In this work, while no visual damage on leaves of *T. salsuginea* Shandong was caused by any of the pathogens, formation of the phytoalexins wasalexins A (**1**) and B (**2**) were induced in every plant–microbe interaction. In addition, elucidation of the chemical structures of five new glucosylated metabolites and identification of a very broad range of polar metabolites identical to those of other cruciferous species was achieved. Among the new metabolites, 3-*O*-β-D-glucopyranosylthiane (**21**) is a particularly interesting structure, as no naturally occurring thianes appear to have been reported to date.

2. Results and discussion

2.1. Microbial elicitation and analysis of non-polar metabolites

Leaves of *T. salsuginea* were inoculated with *A. candida* races 2 V and 7 V and *L. maculans* isolates BJ-125 and Laird-2, as described in the Experimental. Plants were harvested 2, 4, 6 and 8 days after elicitation, the aerial parts were frozen in liquid nitrogen, ground, and extracted with MeOH. Control plants were extracted similarly. After concentration to dryness, the residues were rinsed with CH_2Cl_2 to yield non-polar fractions (soluble in CH_2Cl_2), and polar fractions (remaining residues soluble in MeOH–H_2O). Compounds detected in each extract were identified either by direct comparison with authentic samples available in our metabolite libraries (HPLC-DAD and HPLC-ESI-MS), or by isolation and structure determination using spectroscopic data previously reported (Pedras et al., 2006, 2007, 2008b). Four known phytoalexins, wasalexin A (**1**), wasalexin B (**2**), 1-methoxybrassenin B (**3**), and rapalexin A (**4**), and two phytoanticipins, caulilexin C (**9**, 1-methoxyindolyl-3-acetonitrile) and 3-methylsulfanylpropylisothiocyanate (**11**) were detected in extracts of leaves inoculated with the four

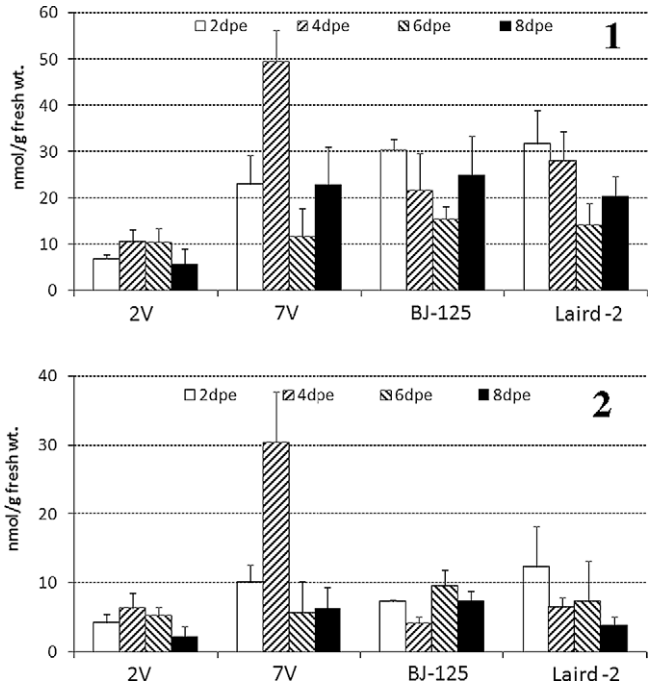

Fig. 2. Production of wasalexins A (**1**) and B (**2**) in leaves of *Thellungiella salsuginea* Shandong ecotype incubated with microbial pathogens *Albugo candida* races 2 V and 7 V, *Leptosphaeria maculans* isolates BJ-125 and Laird-2 up to eight days post-elicitation (dpe).