SCOPOLIN, A GLYCOSIDE FORM OF THE PHYTOALEXIN SCOPOLETIN, IS LIKELY INVOLVED IN THE RESISTANCE OF NICOTIANA ATTENUATA AGAINST ALTERNARIA ALTERNATA

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SUMMARY

Phenolic coumarins are secondary metabolites playing an important role in plant-environment interaction. Previously, we have shown that wild tobacco Nicotiana attenuata accumulates high amounts of the jasmonate (JA)-dependent phenolic coumarin, scopoletin, as a phytoalexin to defend itself against a necrotic fungal pathogen, Alternaria alternata (tobacco pathotype). Scopolin, a β-glycoside form of scopoletin, was strongly elicited and to a level even higher than that of scopoletin in host plants after A. alternata infection. However, it was not known whether scopolin functioned as a phytoalexin playing a role in the resistance or simply as a storage form of scopoletin. When supplied in PDA medium, scopolin exhibited a moderate but slightly weaker anti-fungal activity against A. alternata than that of scopoletin. In addition, A. alternata-elicited scopolin level was dramatically decreased in JA deficient plants. Thus, all the data suggest that scopolin is also a JA-dependent phytoalexin likely involved in the resistance of N. attenuata against A. alternata.

Keywords: Alternaria, fungal pathogen, Nicotiana, phytoalexin, plant resistance, scopoletin; scopolin.

Phytoalexins are antimicrobial chemicals of low molecular weight biosynthesized by host plants after pathogen attack (Ahuja et al., 2012). When Nicotiana attenuata, a wild tobacco, was attacked by Alternaria alternata (tobacco pathotype), a notorious necrotrophic fungal pathogen causing brown spot disease in Nicotiana species (LaMondia, 2001; Schuck et al., 2014; Sun et al., 2014a, 2016), strong blue fluorescence was observed around the infection zone under UV light. Silencing of Feruloyl-CoA 6'-hydroxylase 1 (F6'H1), the key gene for scopoletin biosynthesis, revealed that the blue fluorescence was mainly emitted by scopoletin and its β-glycoside form, scopolin (Sun et al., 2014b).

Scopoletin is a phenolic coumarin deriving from phenylpropanoid pathway (Fig. 1), and can be isolated from many plant species (Gnonlonfin et al., 2012). In N. attenuata, scopoletin has been considered as a phytoalexin, as it exhibits fungal toxicity to A. alternata in planta and in vitro, and scopoletin/scopolin-depleted plants generated by silencing NaF6'H1 are more susceptible to A. alternata (Sun et al., 2014b).

Fig. 1. Scheme of the proposed pathway of scopoletin and scopolin biosynthesis pathway in N. attenuata. Scopoletin and scopolin are phenolic coumarins derived from phenylpropanoid pathway, feruloyl-CoA 6'-hydroxylase 1 (F6'H1) and UDP-Glc:phenylpropanoid glucosyltransferases (UGT) are key enzymes for their biosynthesis.
Glycosylation is a common modification of plant secondary metabolites, and has been proposed to be involved in the detoxification and storage of secondary metabolites (Gachon et al., 2005). Scopolin is a β-glycoside form of scopoletin (Fig. 1), catalyzed possibly by scopoletin glucosyltransferase in tobacco (Gachon et al., 2004; Chong et al., 2002). It is currently not known whether scopolin is simply a storage form of scopoletin, or functions as a phytoalexin like scopoletin.

The levels of scopolin were dramatically increased in *N. attenuata* after *A. alternata* infection, and reached to 70 µg/g fresh leaf weight in plants transformed with an empty vector (EV) at 5 days post inoculation (dpi), but were not elicited in plants transformed with NaF6'H1-silencing construct (VIGS NaF6'H1), confirming both scopoletin and scopolin are NaF6'H1 dependent as shown in Sun et al. (2014b). Importantly, VIGS NaF6'H1 plants were highly susceptible to *A. alternata* (Sun et al., 2014b), suggesting that scopoletin and/or scopolin play an important role in the resistance. Scopoletin has been proved to function as a phytoalexin (Sun et al., 2014b), but it is not known how much scopolin contributes to the resistance.

When scopolin (BioBioPha http://www.biobiopharma.com) was supplied in PDA medium at a final concentration of 50 or 100 µg/ml, the growth of *A. alternata* mycelium was significantly inhibited (by 23% or 46%, respectively; Fig. 2). The inhibition rate was a little lower than that of scopoletin (Fig. 2), but was meaningful since the supplied concentrations were in the range of *A. alternata*-elicited levels.
dropping to 43.1 µg/ml after two days, and to 24.4 µg/ml mycelium, decreased during the growth of A. alternata. The results showed that scopolin levels significantly malized to the volume of the medium before solidification. In the PDA plates which were supplied with 50 µg/ml scopoletin against A. alternata, we measured both scopolin and scopoletin by LC-MS/MS according to Sun et al. Scopolin and scopoletin levels were further determined of the inoculated PDA medium with 70% of methanol. Both chemicals were extracted by homogenization for 0, 2, and 4 days. The levels of scopoletin elicited in WT were arbitrary set as 100%. Asterisks indicate the significance level of differences between WT and transgenic plants (Student’s t-test: *** p < 0.005).

To rule out the possibility that the inhibition of scopolin against A. alternata was due to its deglycosylated form, scoepoletin, we measured both scopolin and scopoletin levels in the PDA plates which were supplied with 50 µg/ml scopolin and inoculated with A. alternata for 3 days. The levels of scopoletin elicited in WT were arbitrary set as 100%. Asterisks indicate the significance level of differences between WT and transgenic plants (Student’s t-test: *** p < 0.005).

Fig. 4. A. alternata-elicited scopoletin and scopolin are strongly decreased in plants impaired in JA production (irAOC plants) or JA perception (irCOI1 plants). Mean (± SE) scopolin or scopolin levels were determined by LC-MS/MS in 5 replicated source-sink transition leaves of WT, irAOC, and irCOI1 plants infected with A. alternata for 3 days. The levels of scopoletin elicited in WT were arbitrary set as 100%. Asterisks indicate the significance level of differences between WT and transgenic plants (Student’s t-test: *** p < 0.005).

JA signalling was vital for A. alternata-elicited blue fluorescence under UV light, and scopoletin production (Sun et al., 2014b). Scopolin levels were also regulated by JA signalling, as very low levels were detected in plants strongly impaired in JA biosynthesis (irAOC, plants silenced in the JA biosynthetic gene NaAOC) and perception (irCOI1, plants silenced in NaCOI1, the gene for the JA-Ile receptor; Fig. 4). These low levels of scopolpin in irAOC and irCOI1 plants were very likely due to the impaired A. alternata-elicited transcripts of NaF6'H1 which encodes the key enzyme gene of scopoletin biosynthesis (Sun et al., 2014b).

Taken all together, scopolin is highly induced after A. alternata infection, and exhibits fungal toxicity in vitro. These results suggest that scopolin is also involved in the resistance of N. attenuata against A. alternata as a phytoalexin dependent on JA signalling, like scopoletin. However, it needs further efforts to quantify the contribution of scopolin to the resistance by generation of plants only impaired in scopoletin biosynthesis without affecting scopoletin production.

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REFERENCES

