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Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection

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ABSTRACT

Grapevine (*Vitis* spp) is susceptible to serious fungal diseases usually controlled by chemical treatments. Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts which can stimulate plant defences. We investigated the effect of mycorrhization on grapevine stilbenoid defences. *Vitis vinifera* cvs Chasselas, Pinot noir and the interspecific hybrid Divico, on the rootstock 41B, were mycorrhized with *Rhizophagus irregularis* before leaf infection by *Plasmopara viticola* or *Botrytis cinerea*. Gene expression analysis showed an up-regulation of *PAL*, *STS*, and *ROMT*, involved in the stilbenoid biosynthesis pathway, in plant leaves, 48 h after pathogen inoculation. This defense response could be potentiated under AMF colonization, with an intensity level depending on the gene, the plant cultivar and/or the pathogen. We also showed that higher amounts of active forms of stilbenoids (*i.e* trans-form of resveratrol, ε- and δ-viniferins and pterostilbene) were produced in mycorrhized plants of the three genotypes in comparison with non-mycorrhized ones, 10 days post-inoculation with either pathogen. These results support the hypothesis that AMF root colonization enhances defence reactions against a biotrophic and a necrotrophic pathogen, in the aerial parts of grapevine.

1. Introduction

Main grapevine cultivars are very susceptible to aerial fungal pathogens, such as *Plasmopara viticola* or *Botrytis cinerea*, the causal agents of downy mildew and grey mould, respectively. Conventional treatments with fungicides contribute to diffuse environmental pollution and are suspected to have serious consequences for human health. Thus, there is a need for alternative biocontrol methods which include the use of beneficial bacteria and fungi (Berendsen et al., 2012). Among them, the widespread endosymbionts Arbuscular Mycorrhizal Fungi (AMF) are obligate biotrophs belonging to the phylum *Glomeromycota*, which naturally colonise the roots of most land plant species in a relatively non-specific way (Corradi and Bonfante, 2012). The fungus receives carbohydrates from its host, while the plant benefits from multiple positive effects of the symbiotic relationship. Plant growth is improved by increased uptake of mineral nutrients and water, especially under poor soil conditions (Bonfante and Genre, 2010). Another important effect of mycorrhizal symbiosis is the stimulation of the plant response to pathogen and pest attacks (Pozo and Azcón-Aguilar, 2007).

Plant inoculation with AMF has long proved efficient in conferring tolerance to various diseases caused by soil-borne agents (Jung et al., 2012). In addition, a few studies have recently shown that mycorrhization reduces symptoms due to aerial fungal pathogens, such as *Alternaria spp* (Fritz et al., 2006; Nair et al., 2015) or *Botrytis cinerea* (Fiorilli et al., 2011) in tomato, *Colletotrichum spp.* in cucumber (Jeun et al., 2008) and cyclamen (Maya and Matsubara, 2013), *Phytophtora spp* in pepper (Alejo-Iturvide et al., 2008) and potato (Gallou et al., 2011), or *Magnaporthe oryzae* in rice (Campos-Soriano et al., 2012). The bio-protective effect of mycorrhization

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could be attributed to the potentiation of plant defences, as higher amounts of defence-related molecules were produced in AMF colonized plants, compared to non-colonized ones, upon pathogen challenge, such as antioxidant enzymes and phenolic compounds (Alejo-Iturvide et al., 2008; Maya and Matsubara, 2013; Vos et al., 2013) or pathogenesis-related (PR) proteins (Campos-Soriano et al., 2012; Cordier et al., 1998; Gallou et al., 2011; Pozo et al., 1999).

In grapevine, AMF symbiosis has been shown to improve the tolerance to the root fungal pathogens *Cylindrocarpon macrodidynum* (Petit and Gubler, 2006) and *Armillaria mellea* (Nogales et al., 2010) and to the ectoparasitic soil nematodes *Meloidogyne incognita* (Li et al., 2006) and *Xiphinema index* (Hao et al., 2012). However, beneficial effects of grapevine mycorrhization on the tolerance to major aerial diseases have not yet been reported. The aim of our study was to test if AMF root colonization can stimulate the defence response to *P. viticola* and *B. cinerea*. Grapevine can produce stilbenoid compounds potentially involved in the tolerance to these pathogens, *i.e.* resveratrol and glycosylated or methylated derivatives, as well as resveratrol dimers (Jeandet et al., 2010). We measured the expression of several genes involved in stilbenoid biosynthesis in leaves of mycorrhized plants of Pinot Noir, Chasselas and Divico after inoculation by *P. viticola* and *B. cinerea*: i) a *phenylalanine ammonia-lyase* (*PAL*) gene involved in the conversion of phenylalanine into cinnamate (first committed step of phenylpropanoid pathway), ii) a group of *stilbene synthase* (*STS*) genes implied in the biosynthesis of resveratrol, and iii) a *ROMT* gene encoding a resveratrol O-methyltransferase which methylates resveratrol into pterostilbene. In addition, we quantified the trans- form of piceid, resveratrol, ε- and δ-viniferin and pterostilbene in leaves challenged with either pathogen.

2. Results

2.1. Influence of root mycorrhization on the expression of stilbenoid pathway genes after pathogen infection

The expression of *PAL*, *STS* and *ROMT* was quantified by q-RT PCR in infected leaves of mycorrhized and non-mycorrhized plants, relatively to non-infected leaves (Fig. 1).

In non-mycorrhized plants, the expression level of these genes was slightly enhanced upon pathogen infection, with a few exceptions, for instance *PAL* (unchanged) and *ROMT* (down-regulated) in Chasselas (Fig. 1A and B). In mycorrhized plants, however, infection by either pathogen induced the up-regulation of *PAL* and *ROMT* genes in the three grapevine genotypes, while *STS* genes showed a different response in relation to cultivar and fungal pathogen. In most cases, gene expression induction was significantly higher than in non-mycorrhized plants, suggesting that mycorrhization could prime *PAL*, *STS* and *ROMT*, for an enhanced expression after pathogen inoculation. This priming effect was more evident when plants were challenged with *P. viticola* (Fig. 1A) rather than *B. cinerea* (Fig. 1B) and its intensity depended on the plant genotype, as it was the lowest in Chasselas and the strongest in Pinot Noir, on average, after downy mildew infection.

2.2. Effect of root mycorrhization on the production of stilbenoid compounds after infection by P. viticola

The amount of piceid, resveratrol, δ-viniferin, ε-viniferin and pterostilbene was measured by HPLC in leaves of mycorrhized and non-mycorrhized plants, non-infected or infected with *P. viticola* spores. Results are shown in Fig. 2.

In leaves of non-infected control plants, mycorrhization didn't induce dramatic modifications of the stilbenoid content; δ-viniferin and pterostilbene were not detected, and resveratrol was present in low concentration (<1.5 μg g^{-1}FW). By contrast, infection by *P. viticola* induced important modifications of the stilbenoid composition. In non-mycorrhized plants, the concentration of piceid, resveratrol and viniferins increased significantly in the three varieties compared with uninfected leaves. In addition, the amount of pterostilbene was enhanced in the resistant genotype Divico. In mycorrhized plants, all stilbenoid compounds were produced in higher amounts after leaf infection in the three varieties and concentrations were higher than in non-mycorrhized plants, except for piceid and resveratrol in Chasselas. Interestingly, in Chasselas, pterostilbene was only detected in mycorrhized plants after infection. Genotypic response showed a role in phenylpropanoids biosynthesis: Pinot Noir and Divico seem to better exploit the effects of mycorrhization. On the whole, these results suggest the potentiation of stilbenoid synthesis by AMF symbiosis.

2.3. Effect of root mycorrhization on the production of stilbenoid compounds after infection by B. cinerea

Stilbenoids were quantified in leaves infected by *B. cinerea* using the same procedure as above (*P. viticola* infection experiment). However, before pathogen inoculation, leaves were wounded with the point of a needle to facilitate entry of the pathogen into the plant tissues. Consistently, non-infected control leaves were wounded in the same way. Results are shown in Fig. 3.

In non-infected conditions, we observed that preliminary wounding induced the production of most stilbenoid compounds (Fig. 3), when compared to intact control leaves used in *P. viticola* infection experiment (Fig. 2). However, infection by *B. cinerea* increased the production of most stilbenoids at a higher level than needle injury in mycorrhized and non-mycorrhized plants of the three genotypes. In non-mycorrhized plants, we observed the higher synthesis of resveratrol (three genotypes), piceid (Chasselas and Divico), as well as viniferins (Divico) and pterostilbene (Pinot Noir). In mycorrhized plants, the concentration of stilbenoids (except piceid in Divico) was also significantly enhanced after infection, particularly resveratrol in Chasselas and Divico, ε- and δ-viniferins in Pinot Noir and Divico, and pterostilbene in all three genotypes. Moreover, in most cases, the level of increase was significantly higher in mycorrhized than in non-mycorrhized plants. As in *P. viticola* infection experiment, AMF symbiosis per se (non-infected conditions), had rather few effects on the stilbenoid content of plant leaves, the concentration of piceid, resveratrol and pterostilbene being slightly enhanced in Divico and decreased in Chasselas (Fig 3.). Altogether, these results strongly suggest that AMF symbiosis can potentiate grapevine defence reactions against *B. cinerea*, a necrotrophic pathogen. However, stilbenoid molecules accumulated differentially depending on the cultivar: the amount of piceid was the highest in Chasselas, that of the two viniferins in Pinot Noir and that of pterostilbene in Divico.

3. Discussion

We observed that mycorrhization had little effect on the stilbenoid content of leaves of non-infected plants. Changes in the total phenolic composition of grapevine after mycorrhization has already been reported, but no modification of the stilbenoid content was mentioned (Eftekhari et al., 2012; Krishna et al., 2005; Torres et al., 2015). Although AMF root colonization can induce biological changes in the whole plant (Jung et al., 2012), contradictory observations have been made about the expression of defence related molecules in the absence of infection. Liu et al. (2007) reported the systemic induction of numerous genes involved in defence in shoots of non-infected mycorrhized *Medicago truncatula*, however, a reduced accumulation of several