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Aconitate and methyl aconitate are modulated by silicon in powdery mildew-infected wheat plants

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Abstract

The accumulation of 5,6-*O*-methyl *trans*-aconitate in wheat was previously found to be linked with the presence of powdery mildew (*Blumeria graminis*) and silicon (Si) feeding. In this work, we sought to determine if *trans*-aconitate (TA) could act as a precursor of methylated forms of TA in wheat and if a relationship existed between Si treatment, disease development, TA and methyl TA concentration within wheat leaves. In absence of infection, TA concentration increased over time regardless of Si feeding. By contrast, TA concentration remained fairly constant over time in both Si^- and Si^+-infected plants but Si^+ plants had a significantly lower level than Si^- plants. Conversely, methyl TA concentration increased in wheat leaves in response to infection and was linked to wheat's increased resistance induced by Si. The effect of Si feeding was only noticeable on methyl TA concentration in presence of the fungus. This suggests that Si does not act directly on TA concentration in leaves but somehow accentuate the production of methyl TA in stressed plants. Based on the concurrent increase in methyl TA and leveling off of TA concentration, it appears that the latter, instead of accumulating, is used by diseased plants to produce antifungal methylated forms of TA that would act as phytoalexins to limit disease development, a phenomenon more pronounced in plants treated with Si.

Introduction

Disease resistance in plants is usually associated with the activation of a wide variety of defense responses preventing pathogen ingress, infection, growth, invasion, reproduction or colonization. Induced resistance is the phenomenon by which a

Abbreviations: Bgt, *Blumeria graminis* f. sp. *tritici*; CA, *cis*-aconitate; HPLC, high-performance liquid chromatography; Si, silicon; TA, *trans*-aconitate.

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plant, once appropriately stimulated, exhibits an enhanced resistance upon elicitation or 'challenge' inoculation with a pathogen (Van Loon, 1997). Although silicon (Si) feeding has been associated with induced resistance in plants, its role in plant disease resistance has been elusive and debated from the initial hypothesis of a mechanical barrier to that of a potential activator of defense reactions (Carver et al., 1987; Menzies et al., 1991; Fawe et al., 2001; Bélanger et al., 2003). In support of the latter hypothesis, Si feeding has been shown to protect cucumber plants against *Pythium* root rot even in absence of Si accumulation in root tissues (Chérif et al., 1992). Further studies with cucumber have highlighted an accumulation of flavonoid phytoalexins in Si-treated plants challenged by powdery mildew (Fawe et al., 1998). Since those studies in cucumber, soluble Si has been linked with induced resistance in other plant species like rice, wheat and Arabidopsis (Bélanger et al., 2003; Rodrigues et al., 2003, 2004; Fauteux et al., 2006).

In wheat, Si-mediated resistance was linked with the production of neo-synthesized compounds in response to infection with *Blumeria graminis* (Bgt) (Bélanger et al., 2003; Rémus-Borel et al., 2005). These compounds were shown to have strong antifungal activity when assayed against *Cladosporium cucumerinum* and one of them was purified and identified as a methylated form of *trans*-aconitate (TA), a putative wheat phytoalexin (Rémus-Borel et al., 2006).

Chemical methylation of TA can lead to three mono-, three di- and one trimethylated TA (Rémus-Borel et al., 2006). Methylation of TA can be catalyzed by TA methyltransferase (Cai et al., 2001a). While this activity has yet to be reported in plants, it has been described in *Escherichia coli* and *Saccharomyces cerevisiae* (Cai and Clarke, 1999; Cai et al., 2001b).

Plants can accumulate relatively high levels (1–2% dry weight) of TA (Orioli and Thompson, 1990). Two pathways for biosynthesis of TA have been proposed (Figure 1): one involving aconitate isomerase (Thompson et al., 1990), which mediates the interconversion between *cis*-aconitate (CA) and TA, and one with citrate dehydratase (Brauer and Teel, 1981, 1982), which converts citric acid to TA through dehydration. However, the mechanisms involved in the production of methylated forms of TA in wheat remain unknown.

The concentration of CA in plants is lower than that of TA because the equilibrium for isomerization via aconitate isomerase is in favor of TA (Ambler and Roberts, 1948). For instance MacLennan and Beevers (1964) observed 65 times more TA than CA in maize. Thompson et al. (1990) showed that the quantity of aconitate isomerase was around 60-fold higher in wheat than in dicots and the quantity of aconitase 20-fold higher in wheat

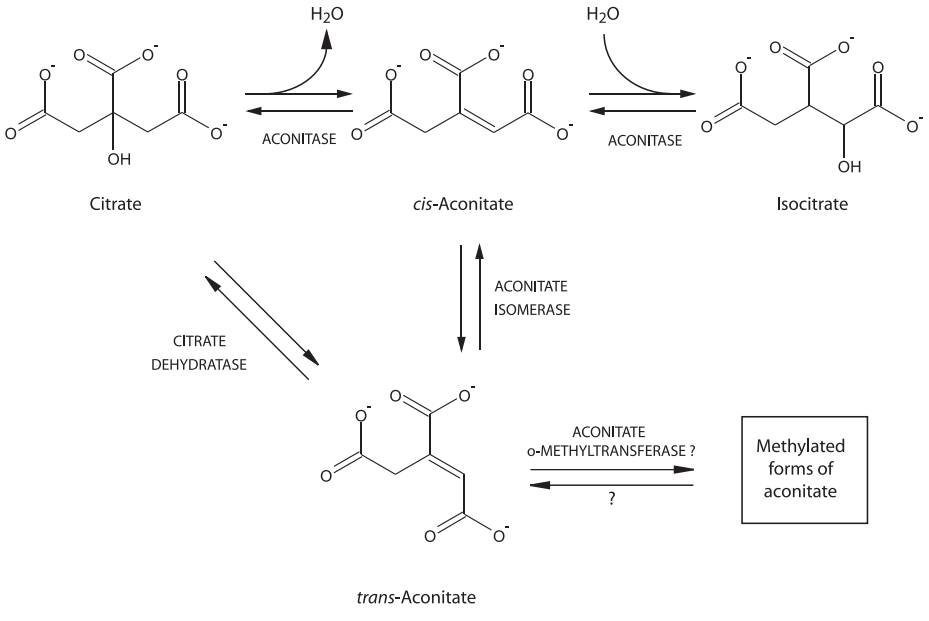

Figure 1. Krebs cycle schematic representation showing different ways to produce *trans*-aconitate, either by aconitate isomerase or by citrate dehydratase. Aconitase catalyzes citrate conversion into isocitrate and produces *cis*-aconitate, an enzyme-bounded intermediary. Citrate dehydratase, aconitase and aconitate isomerase are reversible. The presence of an aconitate *O*-methyltransferase has not been described yet.