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Research article

An altered tocopherol composition in chloroplasts reduces plant resistance to *Botrytis cinerea*

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ABSTRACT

Tocopherols are lipid-soluble antioxidants that contribute to plant resistance to abiotic stresses. However, it is still unknown to what extent alterations in tocopherol composition can affect the plant response to biotic stresses. The response to bacterial and fungal attack of the *vte1* mutant of *Arabidopsis thaliana*, which lacks both α- and γ-tocopherol, was compared to that of the *vte4* mutant (which lacks α- but accumulates γ-tocopherol) and the wild type (with accumulates α-tocopherol in leaves). Both mutants exhibited similar kinetics of cell death and resistance in response to *Pseudomonas syringae*. In contrast, both mutants exhibited delayed resistance when infected with *Botrytis cinerea*. Lipid and hormonal profiling was employed with the aim of assessing the underlying cause of this differential phenotype. Although an altered tocopherol composition in both mutants strongly influenced fatty acid composition, and strongly altered jasmonic acid and cytokinin contents upon infection with *B. cinerea*, differences between genotypes in these phytohormones were observed during late stages of infection only. By contrast, genotype-related effects on lipid peroxidation, as indicated by malondialdehyde accumulation, were observed early upon infection with *B. cinerea*. We conclude that an altered tocopherol composition in chloroplasts may negatively influence the plant response to biotic stress in *Arabidopsis thaliana* through changes in the membrane fatty acid composition, enhanced lipid peroxidation and delayed defence activation when challenged with *B. cinerea*.

1. Introduction

Plants are continuously subjected to biotic stresses by a range of microbes, some of which are pathogens. Depending on the pathovar *Pseudomonas syringae* can infect several plant species where disease symptoms can range from leaf spots to stem cankers (Hirano and Upper, 2000). *P. syringae* pv. *tomato* strain (*Pst*) DC3000 is a well-characterized pathogen that causes hemibiotrophic symptoms in susceptible tomato (*Solanum lycopersicum*) and *Arabidopsis thaliana* plants (Preston, 2000). *Botrytis cinerea* is a necrotrophic fungus with a wide range of host plant species (Staats et al., 2005), infecting important crops such as tomato, grapevine (*Vitis* spp.) and strawberry (*Fragaria* × *ananassa*) (Jarvis, 1977), as well as the model plant *A. thaliana*.

In both plants and animals, glycerol-based membrane lipids such as phospho- and galactolipids play an important role in stress resistance. In animals, eicosanoids may be derived from the oxidation of arachidonic acid (C20:4) derived from the acyl chains of phospholipids. Subsequent differential processing leads to the generation of prostaglandins, leukotrienes and thromboxanes (Funk, 2001). These eicosanoid-derivatives have roles in inflammation, vasoconstriction or vasodilatation, coagulation, pain and fever. Similarly in plants, glycerol-based membrane lipid (predominantly galactolipid) processing, mostly through lipoxygenase activity on α-linolenic acid (C18:3) acyl chains, produce a range of oxylipin signals. These include jasmonates, which are potent mediators of plant defense against certain pathogens and insects (Koo and Howe, 2009). Jasmonates are thought to act with ethylene primarily against necrotrophic pathogens such as *B. cinerea* whilst salicylic acid is thought to influence defense against *Pst* (Pieterse and Van Loon, 2004).

Central to jasmonates formation is the regulation of the initial release of polyunsaturated fatty acid (PUFA) precursors (e.g. C18:3) as free fatty acids from the glycerol-based membrane galactolipid. By

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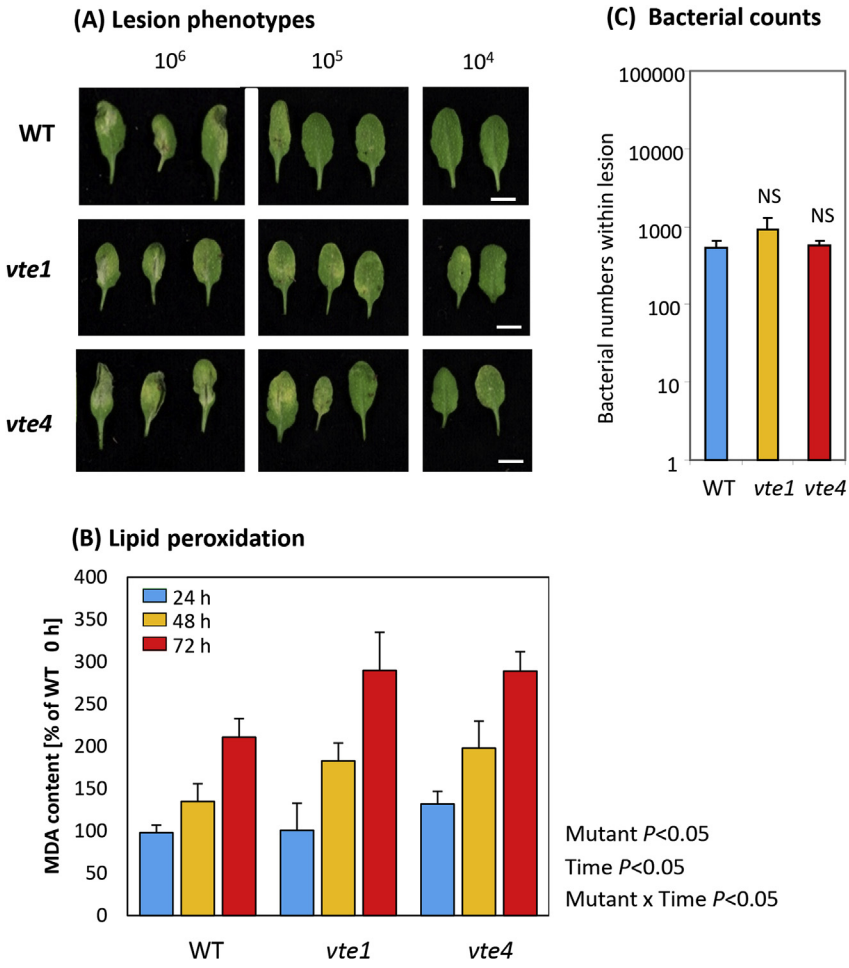

Fig. 1. Characterization of *Pseudomonas syringae* pathovar tomato (*Pst*) *avrRpm1* interactions with the *vte1* and *vte4 A. thaliana* mutants. **(A)** Lesion phenotypes in wild type plants and the *vte1* and *vte4* mutants at 72 h following inoculation with 10^6, 10^5 or 10^4 cell/mL of *PstavrRpm1*(bar = 1 cm). **(B)** Lipid peroxidation at 24 h, 48 h and 72 h following inoculation with *Pst avrRpm1* as estimated by MDA accumulation. **(C)** Bacterial numbers within lesion, obtained in dissected tissue, after 24 h of infection in wild type plants and *vte1* and *vte4* mutants. Data are the mean ± SE of $n = 4$. Significant differences between genotypes, time of infection and its interaction are given in the panels (ANOVA, $P \leq 0.05$). NS, not significant.

analogy with the animal systems it is assumed that α-linolenic acid release is mediated by lipases. However, it is clear that lipid peroxidation occurring as a result of oxidative stress additionally plays a key role in activating free fatty acid release from membrane bound glycerol-based lipids. Lipid peroxidation is an inevitable consequence of the generation of reactive oxygen species – the oxidative burst - that are characteristic of plant responses to pathogens. This is particularly the case with the hypersensitive response elicited by avirulent pathogens (Mur et al., 2008). Hydroxyl radicals generated as part of oxidative burst will readily abstract a proton from, for example, phospholipids to initiate a lipid radical – lipid hydroperoxide chain reaction. The propagative nature of lipid radical generation necessitates its careful regulation to limit cell death to the site of infection and regulate the production of any derived signals.

Lipid peroxidation can be suppressed by non-polar antioxidants such as tocopherols. Tocopherols consists of four homologues (termed α, β, γ, and δ) that differ only in the number and position of methyl groups in the chromanol ring. In plants, the most important forms are α- and to a lesser extent γ-tocopherol in photosynthetic tissues. α-Tocopherol protects the photosynthetic membranes from the propagation of lipid peroxidation, helps to maintain the membrane stability and in a coordinated action with other antioxidants such as glutathione and ascorbic acid enables the correct functioning of the photosynthetic machinery under stress conditions (Munné-Bosch, 2005; Munné-Bosch et al., 2013). γ-Tocopherol is the precursor of α-tocopherol and accumulates in small quantities in young or senescing tissues only (Munné-Bosch and Alegre, 2002; Szymanska and Kruk, 2008). There is some controversy about whether or not tocopherols can have more functions beyond their antioxidant role (Falk and Munné-Bosch, 2010). Some studies suggest that tocopherols can act in cell signaling which affect processes outside chloroplasts (Cela et al., 2011). Mutants with an altered tocopherol biosynthetic pathway have suggested that altered antioxidant capacities led to an increase in anthocyanin accumulation in senescing leaves which may be linked to alterations in jasmonic acid contents (Munné-Bosch et al., 2007). Also, tocopherol-deficient mutants of *A. thaliana* show alterations in photoassimilate transport at low temperatures through alterations in callose deposition in the phloem (Maeda et al., 2006) and endoplasmic reticulum PUFA metabolism (Maeda et al., 2008).

Given the centrality of lipid peroxidation in plant defense we hypothesized that lack of tocopherols or an altered tocopherol composition would influence interactions with pathogens. With the aim of evaluating the role of tocopherols in plant responses to biotic stress, we examined here the response of *vte1* and *vte4* mutants to *P. syringae* and *B. cinerea*. While the avirulent *P. syringae* used in this study is a well-characterized pathogen that causes hemibiotrophic symptoms and increases salicylic acid contents, *B. cinerea* is a necrotrophic fungus that elicits jasmonic acid-related resistance. Emphasis was put on evaluating the lipid and hormonal profiling of leaves to unravel possible mechanistic links between tocopherols and defence signal production.

2. Materials and methods

2.1. Plant material and sampling

Seeds of *A. thaliana* Columbia ecotype (Col 0) and *vte1* and *vte4* mutants, which were provided by Kathleen Brückner (University of Kiel, Germany), were used in this study. The *vte1* mutant lacks both α-