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Implication of signaling pathways involving calcium, phosphorylation and active oxygen species in methyl jasmonate-induced defense responses in grapevine cell cultures

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Summary

Perception of elicitors triggers plant defense responses via various early signal transduction pathways. Methyl jasmonate (MeJA) stimulates defense responses in grapevine (*Vitis vinifera*). We investigated the involvement of various partners (calcium, ROS, reversible phosphorylation) in MeJA-induced responses by using a pharmacological approach. We used specific calcium channel effectors and inhibitors of serine/threonine phosphatases, superoxide dismutase and NAD(P)H oxidase and investigated production of stilbenes (resveratrol and its glucoside, piceid, the major form), which are the grapevine phytoalexins. RNA accumulation of two genes encoding enzymes involved in stilbene synthesis (*PAL* and *STS*), three genes encoding pathogenesis-related proteins (*CHIT4C*, *PIN* and *GLU*) and one gene encoding an enzyme producing jasmonates (*LOX*) were also assessed. Calcium and its origin seemed to play a major role in MeJA-induced grapevine defense responses. Phytoalexin production was strongly affected if calcium from the influx plasma

Abbreviations: CHIT4C, acidic class IV chitinase; DEPC, diethylpyrocarbonate; DETC, diethyldithiocarbamate; DPI, diphenylene iodonium; EGTA, ethylene glycol tetraacetic acid; GLU, β-1,3-glucanase; IP3R, inositol triphosphate receptor; LOX, lipoxygenase; NO, nitric oxide; OGA, oligogalacturonide; PAL, phenylalanine ammonia lyase; PGIP, polygalacturonase-inhibiting protein; PIN, inhibitors of serine proteases; PP1, phosphatase 1; PP2A, phosphatase 2A; PR proteins, pathogenesis-related proteins; PVPP, polyvinylpolypyrrolidone; ROS, reactive oxygen species; RR, ruthenium red; RyR, ryanonide receptor; SOD, superoxide dismutase; STS, stilbene synthase..

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membrane was inhibited, whereas calcium from the intracellular compartments did not seem to be involved. ROS production seemed to interfere with MeJA-stimulated defense responses, and protein phosphorylation/dephosphorylation events also played a direct role.

Introduction

Grapevine is a major fruit crop worldwide and is particularly sensitive to various diseases caused by pathogens, particularly fungi (*Botrytis cinerea, Erysiphe necator* for example). These diseases are currently controlled by the intensive use of phytochemicals. However, the economic cost and negative environmental impact associated with these applications has led to developing alternative strategies such as activation of natural plant defenses. Plant defenses include preformed barriers and constitutively expressed antimicrobials as well as inducible mechanisms. These inducible mechanisms have led to a broad spectrum of metabolic modifications, such as cell-wall reinforcement, production of antimicrobial metabolites (phytoalexins), pathogenesis-related (PR) proteins and enzymes involved in oxidative stress protection, lignification, and frequently the hypersensitive response (HR), a form of programmed host cell death at the infection site associated with limited pathogen development (Kombrink and Somssich, 1995).

Activation of these natural plant defenses can be induced upon contact with pathogen-derived compounds, plant-derived molecules or synthetic chemicals, thus providing protection against a wide spectrum of pathogens. Jasmonates such as methyl jasmonate (MeJA), the most active derivative of jasmonic acid, act as an elicitor. They are involved in plant development and defense and are overproduced during wounding, fruit ripening and drought stress (Creelman and Mullet, 1997; Wasternack, 2007). Jasmonates induce genes encoding pathogenesis-related proteins (PR) such as chitinases, β-1,3-glucanases (GLUs) and proteinase inhibitor in several plants like tobacco and tomato (Enkerli et al., 1993; Fukuda and Shinshi, 1994). Jasmonic acid (JA) induces the production of a wide range of secondary plant metabolites such as alkaloids, terpenoids, flavonoids, phenolic compounds and phytoalexins in various cell cultures (*Cupressus lusitanica, Taxus cuspidata, Phaseolus vulgaris* and *Rauwolfia serpentina* for example) and in *Arabidopsis thaliana* plants (Brader et al., 2001; Memelink et al., 2001; Mirjalili and Linden, 1996; Mueller et al., 1993; Zhao et al., 2001). In grapevine, MeJA has been shown to stimulate the accumulation of PR proteins in suspension-cultured cells (Repka et al., 2004) and in leaves, and also to trigger deposition of callose in leaves (Hamiduzzaman et al., 2005). We have recently shown in grapevine that MeJA stimulates phytoalexin production in cell suspension cultures and in grapevine foliar cuttings and induces the RNA accumulation of transcripts encoding PR proteins (Belhadj et al., 2006, 2008). A correlation has already been reported between the combined activities of chitinase and GLU (Giannakis et al., 1998), PGIP activities (De Lorenzo and Ferrari, 2002; D'Ovidio et al., 2004) and increase resistance of plants to fungi. Moreover, resveratrol has been shown to confer a tolerance to powdery mildew and downy mildew (Dai et al., 1995) and piceid could be a form of storage or resveratrol transport in the plant (Douillet-Breuil et al., 1999). Schnee et al. (2008) also showed that the synthesis of viniferins (resveratrol oligomers) at infection sites seems to be closely linked to the inhibition of grapevine powdery mildew. Correlated with the induction of all these molecules and other defense ones, MeJA triggers enhanced resistance to *Erysiphe necator*, the fungus responsible for powdery mildew (Belhadj et al., 2006).

Plant–microbe interactions and plant defense responses, as well as the signal transduction pathways involved, have been studied extensively (Scheel, 1998). Despite the intensive characterization of MeJA as a general elicitor, and particularly as an elicitor of secondary plant metabolites (Memelink et al., 2001), little is known about the signal transduction pathways involved in MeJA transmission to defense responses in grapevine. The earliest event in plant cells exposed to an elicitor is its recognition mediated by plasma membrane-localized receptors (Kaku et al., 2006; Zimmermann et al., 1997).

Elicitor perception activates receptor-coupled effectors such as GTP-binding proteins (G-proteins) or protein kinases and phosphatases which further mobilize or generate diverse signaling molecules directly or indirectly (such as free calcium, nitric oxide [NO] and reactive oxygen species [ROS]). These signaling events regulate many processes leading to an amplification of the defense response (Aharon et al., 1998; Lecourieux-Ouaked et al.