



Molecular Biology

Protein profiling and *tps23* induction in different maize lines in response to methyl jasmonate treatment and *Diabrotica virgifera* infestation



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ABSTRACT

Plant responses to herbivore insects involve direct and indirect defense with the production of signal molecules including jasmonic acid (JA) and its derivatives (e.g. methyl jasmonate, MeJA). In maize (*Zea mays*), root feeding by *Diabrotica virgifera* larvae activates an indirect defense mechanism, through entomopathogenic nematodes that are recruited after *Terpene Synthase 23* (*tps23*) upregulation and (*E*)- β -caryophyllene root emission.

In order to gain insight into the correlation between JA signaling and response to *Diabrotica* attack, we analyzed *tps23* expression and protein profiles in maize roots in response to MeJA treatment and insect infestation. Similar to herbivore feeding, MeJA treatment was found to increase *tps23* transcript accumulation, with consistent variations for both treatments in maize lines differing in (*E*)- β -caryophyllene production. Analysis of root protein profiles showed specific alterations leading to the identification of three proteins that were induced by MeJA treatment. We focused on a peroxidase-like protein (Px-like) showing that the corresponding transcripts accumulated in all tested lines.

Results show that exogenous application of MeJA upregulates *tps23* expression and specifically alters protein patterns in maize roots. Parallel effects on *tps23* transcript accumulation were observed upon hormone exposure and insect infestation in different maize lines. In contrast, Px-like transcript profiling showed differences between treatments. These results support the possible involvement of MeJA in mediating the upregulation of *tps23* in response to *Diabrotica* attack.

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Introduction

Plant survival depends on plants' ability to adapt to environmental changes through different mechanisms, including transcriptional reprogramming. Exposure to different stimuli triggers the activation of signaling pathways that interplay to produce specific responses. The oxylipin-derived hormone jasmonic acid (JA) and its derivative methyl jasmonate (MeJA) have been implicated

in many developmental processes including root growth and pollen production and are also thought to act as major mediators of responses to biotic and abiotic stresses such as pest attack, wounding and water deficit (Creelman and Mullet, 1997; McConn et al., 1997). Jasmonates are central signaling components in the regulatory cascades of plant inducible responses, accumulating both locally and systemically upon wounding (Glaser et al., 2008). Despite progress in understanding their function as long-distance signals mediating aboveground responses (Koo et al., 2009), the role of jasmonates in the activation of belowground defense against insect herbivores has been less investigated (Erb et al., 2012). Recently, exogenous application of JA in shoot and root in *Brassica oleracea* indicated that the two organs have a

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different JA signaling cascade, and JA response is not only tissue-specific but also dependent on the organ treated (Tytgat et al., 2013).

Measurements of jasmonate levels in roots after herbivore attack showed contrasting results: in Erbet et al. (2009) *Diabrotica virgifera* root infestation was shown to result in significant increases in JA accumulation, while in experiments from Erb et al. (2012) only a slight increase was registered. Moreover, in the maize genotype Mp708, constitutively elevated JA levels result in potent and prolonged defense against western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Gill et al., 2011). Mp708 has been shown to constitutively release (*E*)- β -caryophyllene, a terpenoid compound that was proposed to be associated with resistance (Smith et al., 2012), even if its expression was exclusively monitored in leaves.

Maize has become an established model system for studying herbivore-induced plant responses, and the interaction between caterpillars and the root system is well characterized. In response to feeding by *D. v. virgifera* larvae, maize roots activate an indirect defense mechanism by releasing the sesquiterpene (*E*)- β -caryophyllene that recruits natural entomopathogenic nematodes (Rasmann et al., 2005). (*E*)- β -Caryophyllene was shown to accumulate after herbivore damage in maize lines from European breeding programs and the wild ancestor teosinte, but was absent in maize lines originating from North American breeding programs (Degen et al., 2004). The production of (*E*)- β -caryophyllene is related to *Terpene Synthase 23* (*tps23*) gene expression: transcript levels are upregulated in response to *D. virgifera* root feeding and vary among maize lines exhibiting different accumulation of (*E*)- β -caryophyllene (Köllner et al., 2008).

Different clues indicate a possible correlation between herbivore damage, activation of JA signaling and *tps* gene induction. In Douglas-fir seedlings, (*E*)-caryophyllene was seen to significant increase following application of MeJA to the soil (Huber et al., 2005).

In maize, release of sesquiterpene volatile compounds including (*E*)- β -caryophyllene was stimulated by direct application of JA and volicitin (*N*-(17-hydroxilinolenoyl)-L-glutamine), a compound present in beet armyworm (*Spodoptera exigua*) oral secretions (Schmelz et al., 2001). In addition, the *tps1* gene was over-expressed in leaves and roots upon JA treatment (Feng et al., 2010). Recently, co-localization of volatile sesquiterpenes and JA were also observed in terpene-emitting leaf tissues upon herbivore damage suggesting a role for jasmonates in propagating the damage signal (Köllner et al., 2013). However, dissection of root-herbivore responses remains complicated due to the concomitant action of many interacting factors (Erb et al., 2009). Transcriptomic profiling of maize roots after herbivore attack by *D. u. howardi* or in response to JA and salicylic acid (SA) application indicated that herbivore-insect responses are complex and involve both JA- and SA-dependent and independent signals, resulting in differential expression of genes associated to direct defense such as cystatin and pathogenesis-related protein 10 (PR10) (Lawrence et al., 2012). Beyond transcriptomic profiling, no direct studies using proteomic approaches were reported for maize root responses to herbivore attack.

Here, we report protein signature changes occurring in roots of different maize lines after exogenous application of MeJA or direct feeding by herbivore larvae of *D. v. virgifera*.

To explore the possible links between MeJA and indirect defense against herbivore damage, we also monitored expression of the *tps23* gene. By integrating different proteomic approaches and gene expression analysis we also provide novel information supporting the possible link between JA activation and response to herbivore attack in roots.

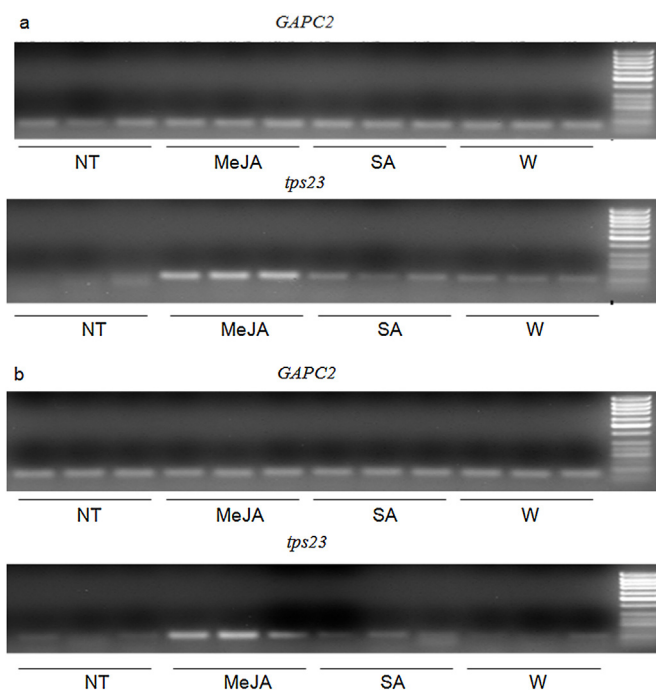


Fig. 1. Time course of (*E*)-beta-caryophyllene synthase *tps23* gene (GenBank accession no. EU259633) expression in roots of B97 maize plants grown under hydroponic conditions. (A) 4 h time point; (B) 8 h time point. MeJA = methyl jasmonate, SA = salicylic acid, W = wounding, NT = not treated. The glyceraldehyde-3-phosphate dehydrogenase *GAPC2* (GenBank accession no. NM.001112230) was used as a reference. For each time-point and treatment three biological replicates were tested.

Results

Specific alterations in *tps23* expression and SELDI-ToF protein profiles in roots of the B97 maize line upon different treatments

In order to analyze and compare responses of maize roots to different treatments related to herbivore attack, we analyzed the expression of the *tps23* gene, considered as a marker of the indirect defense response mediated by (*E*)- β -caryophyllene. Plants were subjected to mechanical root damage or treated with MeJA or SA, and Reverse Transcription PCR (RT-PCR) was used to follow the timing of transcriptional responses. The B97 inbred line was used because it is known to specifically respond to *D. v. virgifera* infestation by expressing *tps23* (Köllner et al., 2008). The *tps23* gene was scarcely expressed in standard hydroponic conditions, while expression was evident following mechanical injury and SA treatment. A marked increase in transcript abundance was detected at both 4 and 8 h of MeJA exposure (Fig. 1), supporting the existence of a possible link between MeJA and the indirect defense response of maize roots to feeding.

In order to further characterize root responses to different treatments, protein profiling variations were evaluated using Surface-Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-ToF-MS).

Specific and distinct variations in root protein profiles were evident upon treatment with MeJA or SA, while no significant alterations were observed after wounding (Fig. 2) as supported by statistical analysis. Protein profiling alterations were appreciable from 4 h of treatment, with differentially expressed peptide/protein peaks (*P*-value <0.05) specific for each compound used: peaks at 7141 Da and 7412 Da were detected for MeJA and SA treatment, respectively. Over time, the number of differentially expressed peaks observed increased for both treatments (Supplementary

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