Journal of Plant Physiology 173 (2015) 28–32

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Short communication

Tobacco overexpressing β-ocimene induces direct and indirect responses against aphids in receiver tomato plants

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ARTICLE INFO

Article history:
Received 18 August 2014
Accepted 22 August 2014
Available online 2 September 2014

Keywords:
Macrosyphum euphorbiae
Aphidius ervi
Methyl salicylate
cis-3-Hexen-1-ol

ABSTRACT

In the last decade plant-to-plant communication has received an increasing attention, particularly for the role of Volatile Organic Compounds as possible elicitors of plant defense. The role of β-ocimene as an interspecific elicitor of plant defense has been recently assessed in multitrophic systems including different plant species (Solanaceae, Poaceae, legumes) and different pest species including chewer insects and phytophagous mites. Both chewer insects and phytophagous mites are known to elicit specific plant defensive pathways which are different (at least in part) from those elicited by sap feeders. The aim of this research was to fill this gap of knowledge and to assess the role of β-ocimene as an elicitor of plant defense against aphid pests, which are sap feeders.

For this purpose we used as transgenic tobacco plant releasing an odour plume enriched in this compound as emitter and a tomato plant as receiver. We selected the aphid *Macrosiphum euphorbiae* and its natural enemy, the parasitoid *Aphidius ervi*, as the targets of plant induced defense.

Tomato plant defense induced by β-ocimene was assessed by characterizing the aphid performance in terms of fixing behaviour, development and reproduction (direct plant defense) and the parasitoid performance in terms of attraction towards tomato plants (indirect plant defense). The characterization of tomato response to β-ocimene was completed by the identification of Volatile Organic Compounds as released by conditioned tomato plants.

Tomato plants that were exposed to the volatiles of transgenic tobacco enriched in β-ocimene resulted in less suitable for the aphids in respect to control ones (direct defense). On tomato plants "elicited" by β-ocimene we recorded: a significant lower number of aphids settled; a significant lower number newborn nymphs; a significant lower weight of aphids feeding.

In addition, tomato plants "elicited" by β-ocimene resulted became more attractive towards the parasitoid *A. ervi* than control ones.

These results could be explained at least in part by examining the composition of the Volatile Organic Compounds released by tomato plants "elicited" by β-ocimene. Indeed, we found a significantly higher release of several compounds including methyl salicylate and *cis*-3-hexen-1-ol. These two compounds have been demonstrated to impair aphid development and reproduction and to be involved in the attraction of the aphid parasitoid *A. ervi*.

By considering the ubiquity of β-ocimene and its ability to regulate the communication of plants belonging 30 to different species (if not families), we concluded that this compound is an ideal candidate for new 31 strategies of sustainable control of agricultural pests.

Introduction

Plant volatiles play a crucial role in plant communication with the surrounding environment performing a variety of functions from attraction of pollinators and seed dispersers to protection from biotic stresses such as phytopathogens and insect herbivore (Maffei et al., 2011). Among these functions, the ability to

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http://dx.doi.org/10.1016/j.jplph.2014.08.011
0176-1617/

promote plant defence has received increasing attention in the last decades, leading to the characterization of Volatile Organic Compounds (VOC) that elicit a physiological response in the emitter and in neighbouring individuals (Arimura et al., 2000; Frost et al., 2007; Glinwood et al., 2009). Heil and Karban (2010) reviewed papers retracing the main steps that strengthened the hypothesis of plant-to-plant communication. Field and laboratory studies have provided compelling evidence that receiver plants are able to respond to volatile cues from conspecific or interspecific emitter plants, by activating signalling pathways eventually leading to gene expression and the synthesis of defence metabolites. In most cases, the emitter plant was induced by mechanical or caterpillar damage, while the receiver one was tested for attractiveness towards predators and parasitoids and/or characterizing the "induced" VOC emission (Zebelo and Maffei, 2012 and references cited therein). Conversely, a few studies have focused on sap-sucking arthropods, demonstrating the existence of airborne plant-to-plant signalling that alters acceptance of the receiver plant by aphids, spider mites and attractiveness towards their natural enemies (Glinwood et al., 2004, 2009; Muroi et al., 2011). Nevertheless, there is a scarcity of information about the identity of chemical compounds that regulate these complex interactions. One functional approach to characterize the effect of single volatile compounds is the use of transgenic or mutant plants that are genetically modified in their potential to emit or receive VOC signals. Some evidence has highlighted the role of monoterpenoids (e.g., ocimene and myrcene) that regulate gene transcription and prime indirect defences in response to herbivore attack (Godard et al., 2008; Muroi et al., 2011). Such ubiquitously released VOCs are often present in the blends of volatiles emitted by damaged or induced plants. Broad bean plants that had been exposed to (*Z*)-jasmone in the vapor phase subsequently released significantly enhanced levels of β-ocimene compared with control plants, resulting more attractive for the aphid parasitoid *Aphidius ervi* (Birkett et al., 2000). The enhanced attractiveness could be partially explained by the high level of response of female parasitoids to purified β-ocimene in wind tunnel bioassay (Birkett et al., 2000).

The aim of our study was to assess the role of β-ocimene as a trans-specific elicitor of plant defences against aphids. For this purpose we used transgenic tobacco plants releasing a plume enriched by β-ocimene as emitters (Muroi et al., 2011), and tomato plants cv San Marzano as receivers. We aimed for the biological, chemical and behavioural characterization of direct and indirect defensive responses against aphids in tomato elicited by transgenic tobacco volatiles.

Materials and methods

Plant material

The emitter plants used in all assays were wild-type tobacco, *Nicotiana tabacum* L. (Solanaceae) cv SR1 and the relative transgenic plants overexpressing a lima bean β-ocimene synthase that emits an odour plume enriched by β-ocimene (Muroi et al., 2011). The receiver plants used in all assays were tomato plants *Solanum lycopersicum* L. (Solanaceae) cv San Marzano (Digilio et al., 2012). All plants were grown in sterilized soil in three different greenhouses (one to rear the plants and the others for each treatment, see below) under the following conditions: $24 \pm 2\,^{\circ}C$ and $70 \pm 10\%$ RH, and a 16:8 light/dark photoperiod.

Plant treatments

Three week-old tomato plants were conditioned by placing them downwind (flow rate $30\,cm\,s^{-1}$) of transgenic (tomato transgenic tobacco system = TTT) and wild-type genotypes of tobacco (tomato wild-type tobacco system = TWT) for 72 h as described in Muroi et al. (2011).

Insects

The tomato and potato aphid *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) is permanently reared in an environmental cabinet at the Institute for Sustainable Plant Protection, National Research Council, on tomato plants, cv San Marzano under the same conditions as described above. The original strain was field-collected in 2003 in Scafati (Campania, Italy) from tomato cv San Marzano. The parasitoid wasp *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) is continuously reared in an environmental chamber at $20 \pm 1\,^{\circ}C$, with a photoperiod of 18/6 h of light/darkness and $60 \pm 5\%$ RH, on its natural host, the pea aphid *Acyrthosiphon pisum* (Harris), maintained on potted broad bean plants (*Vicia faba* L. cv Aquadulce), as previously described (Guerrieri et al., 2002).

Induction of direct defence

The induction of direct defence against aphids was assessed in terms of behaviour and performance of *M. euphorbiae* on conditioned tomato plants (TWT and TTT). Immediately after conditioning, receiver plants were sealed in a Perspex cage ($4.7\,dm^3$) together with a 2 ml Eppendorf tube, containing twenty aphids (apterous fourth instars or newly born adults), weighed as a pool. The tube was placed with its base in the soil, and its opening next to the tomato stem, to facilitate the aphids in walking/climbing on the plant to choose a feeding site. Each experiment was repeated 6 times. The Perspex cage was opened after 48 h and aphids, dead or alive, were counted indicating the number remaining in the Eppendorf tube, wandering on cage walls, pot or tube, or feeding/standing on the plant. For living aphids, the pooled weights, the nymphs produced and the aphids settled after 48 h on TTT and TWT were compared by ANOVA.

Induction of indirect defence (A. ervi attraction)

Attraction assay for the aphid parasitoid *A. ervi* was conducted in a single-choice wind tunnel bioassay. Behavioural observations were carried out as described previously (Sasso et al., 2009). In brief, ninety parasitoid females were tested for each target by releasing them individually in the odour plume 35 cm downwind from the target; each female was only used once and observed for a maximum time of 5 min. Experimental conditions were: $20 \pm 1\,^{\circ}C$, $65 \pm 5\%$ RH, $70\,\mu mol\,m^2\,s^{-1}$ PPF, $30\,cm\,s^{-1}$ wind speed. Flight behaviour data were recorded by calculating the percentage of oriented flights towards the target plant and landings on the target plant. Behavioural experiments were conducted on several days and the target plants offered in a random order to reduce any daily or hourly bias. For each target (TTT or TWT), five conditioned plants were tested. The percentage of response (oriented flights and landings on the target) to each target was evaluated. The number of parasitoids responding to each target in all experiments was compared by a *G*-test for independence with William's correction (Sokal and Rohlf, 1995). The resulting values of *G* were compared with the critical values of χ^2 (Sokal and Rohlf, 1995).

Identification of plant volatiles

Plant volatiles were collected from all target plants (TTT, TWT) immediately after the wind tunnel bioassay. All glassware, silicon, and Teflon connections were scrupulously cleaned and heated at $100\,^{\circ}C$ overnight before use. Plant volatiles were collected by an