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Deciphering tomato defense after induction of resistance towards a biotrophic, a hemibiotrophic and a necrotrophic pathogen

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Abstract: In this paper we describe our strategy to unravel the connection between plant defenses and plant resistance to pests. With tomato (*Solanum lycopersicum*) as the reference plant, our ambition is to screen different plant defense stimulators (SPD) and measure (i) the resulting protection level towards *Phytophthora parasitica*, *Botrytis cinerea* and *Oidium neolycopersici* (ii) the genes involved in the corresponding defense mechanisms (iii) the proteins and secondary metabolites which could be effector of resistance. We already show that BABA induces a strong resistance to *P. parasitica* (100% protection, no symptoms). Conversely, SA does not protect tomato to *P. parasitica* infection even though some classical defense genes are highly up-regulated in the same manner after BABA and SA treatments. Preliminary results of RNA-Seq evidence that the expression of more than 1000 genes is modified by BABA treatment. The possible involvement of unreported functions is discussed.

Key words: *Solanum lycopersicum*, induced resistance, elicitors, *Phytophthora parasitica*, *Botrytis cinerea*, *Oidium neolycopersici*

Introduction

Current cropping systems have been developed since World War II to intensively produce food. A consequence is the use of high performance varieties that were not selected to resist efficiently to stresses (loss of rusticity). This was not so important as long as freely available water, fertilizers and pesticides could clearly minimize the effects of stress. However, the massive use of pesticides has become a major concern for health and environment. Modern agriculture urgently needs efficient alternatives and "old" strategies such as biocontrol or the stimulation of plant defences attract increasing interest. Plant innate immunity is very well known from a mechanistic standpoint, as illustrated by quite abundant literature mainly focused on two model plants, but there are still few examples of its effective use for crop protection. Implementing the induction of plant defences by elicitors (whatever their origin: PAMPs, MAMPs, DAMPs, non pathogenic microorganisms, hormones...) to obtain subsequent resistance to diseases of cultivated crops will clearly require important efforts in generating knowledge on cultivated non-model plants as well as the realization of "field assays" that were partly neglected in the past.

Tomato is an example of a partly studied crop. The interaction between tomato and *Cladosporium fulvum* was a pioneering model for understanding *R-Avr* and it allowed the

description of many genes involved in pathogen recognition, hypersensitive response and beyond (two decades of work from Van den Ackerveken *et al.*, 1992 to Xu *et al.*, 2012). Many Stimulators of Plant Defense (SPD) leading to tomato resistance to pathogens were described: the PGPR *Pseudomonas putida* (Akram *et al.*, 2008), hexanoic acid (Vicedo *et al.*, 2009), the elicitin oligandrin (Picard *et al.*, 2000) and the non-protein amino acid ß-amino-butyric acid BABA (Cohen *et al.*, 1993). BABA not only protects against the oomycete *Phytophthora infestans*, but also triggers resistance to root knot nematodes (Oka *et al.*, 1999). In the present paper, we describe our project to identify SPDs able to elicit tomato defenses that could be implicated in the resistance to a set of pathogens with different modes of infection and we present preliminary results obtained with BABA. The main question is: which defenses are induced against which pest?

Material and methods

Biological material

Solanum lycopersicum (var. Marmande) was grown in controlled conditions (24 °C, 16 h daylight). Five-week-old plants were sprayed with different SPDs including BABA and salicylic acid (SA), each in the form of an aqueous solution at a concentration of 1 mM. Two days after treatment, leaves were collected. Leaflets were individualized and further inoculated on the upper part with 20 µl of a zoospores suspension of *Phytophthora parasitica* (strain 149 from INRA Sophia collection, 40 000 z/ml). Inoculated leaflets were incubated in humid chambers for 5 days in the same conditions as mentioned above. Disease severity was estimated by the surface of the lesion after 4 days. Protection experiments against *Oidium neolycopersici* and *Botrytis cinerea* were carried out as previously described (Bardin *et al.*, 2008). Controls were water-treated plants.

Transcriptomics

24 h after treatments, total RNA was extracted from leaves with TRIzol® reagent according to the manufacturer's recommendations. RT-qPCR was carried out to follow the expression levels of a set of known defense-related genes coding for PR-proteins and key enzymes of the secondary metabolism. At the same time, RNA-Seq experiments with SOLID™ technology were also performed to evaluate the regulated genes without *a priori* assumptions. Mapping was achieved using the genomic database (http://solgenomics.net/).

Proteomics and metabolomics

48h after treatments, leaflets were ground in liquid N_2 then extracted in buffer (MES 20 mM, pH 6, 1 mM DTT) or in methanol to obtain the total soluble proteins and the metabolites, respectively. Proteins were fractionated using ion exchange chromatography, then fractions were reduced and alkylated prior to trypsin digestion. Proteins were identified after LC-MS/MS with Mascot search engine. Metabolites, after cleaning by Solid Phase Extraction to eliminate photosynthetic pigments and lipids, were analysed by HPLC/DAD.

Results and discussion

Protective effect of tested SPDs

In the different experiments, BABA consistently provided total protection of tomato towards the hemibiotrophic *Phytophthora parasitica* as previously described for the leaf pathogen

P. infestans (Cohen *et al.*, 1993). Additional protection experiments are in progress to estimate the protection level against the obligate parasite *O. neolycopersici* and the necrotrophic fungus *B. cinerea*. Interestingly, SA did not induce any significant resistance to *P. parasitica*.

Transcriptomic approaches

The results of q-RT-PCR showed, as expected, that the genes coding for PR proteins (PR1, PR2, PR5 and chitinases) are strongly up-regulated upon BABA and SA treatments. The profiles obtained with these two SPDs were almost comparable although SA triggered no resistance to *P. parasitica*.

This is the reason why we engaged a RNA-Seq strategy to better understand this apparent discrepancy. Preliminary runs were obtained after BABA treatment to evaluate the performance of the technique. Currently, data have only partially been processed. Among the 35,000 genes present in the tomato genome database (release 2.1), only 25,000 were retrieved from RNA-Seq mapping. It probably means that 10,000 genes are either never expressed in leaves or are pseudogenes. Among the 25,000 expressed genes, 1,300 are up-regulated and 190 are down-regulated with a robust level of significance (padj < 0.01). Some up-regulated genes were unexpected and the fine analysis of their expression is under way.

Preliminary proteomic analyses allowed the observation of major up-regulated PR1 proteins as well as Lipid Transfer Proteins (data not shown). HPLC profiles of secondary metabolites did not provide clear evidence for the biosynthesis of compounds. Nevertheless, some unknown phenolics belonging to the hydoxycinnamoyl derivatives class (minor HPLC peaks) seem to be good candidates as defense markers. However, their precise profiling requires a purification step prior to analysis. LC-MS should also help to determine both their structure and their link to defense responses.

We, now, have a clear "positive control" (BABA) that enables us to screen other SDPs. These elicitors should be from either known structure and/or composition or from microbial origin. The combination of different SDPs, their protective activity towards 3 highly different pathogens and their ability to trigger many different markers at gene, protein and metabolic levels will allow us to delineate profile specific resistance, thus to handle tools able to predict "which defenses against which pests".

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