Quantitative detection methods for evaluating induction of disease resistance against Verticillium dahliae correlated with increased defense responses in tobacco plants treated with coactyl.

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Introduction

Verticillium wilt remains one of the most serious soil borne diseases worldwide. An array of different strategies to reduce the consequences of pathogen pressure is available. Among these methods, application of natural compounds, which stimulate locally and systemically plant defense reactions, is becoming an industrial alternative to the application of chemicals with deleterious side effects.

Our objective was to investigate, through the quantitative and very sensitive real-time RT-PCR technology, the potential of Coactyl™, composed of humic acid associated to a phenolic acid, to act as a plant defense inducer conferring resistance to Verticillium dahliae wilt in tobacco used as a model.

Material and Methods

We investigated by real time PCR how the organic amendment Coactyl delayed the appearance of disease caused by Verticillium dahliae. On one hand the effect of coactyl on defense mechanisms was determined by quantifying defense-related gene transcripts in a time course experiment (Fig 1), and on the other hand, the effectiveness of coactyl was quantified in presence of V.dahliae by molecular detection of pathogen before any symptom could be observed (Fig 2).

For RT-PCR experiments, RNA analysis, total RNA were extracted from 60 mg of crushed apical root using Nucleospin RNA plant kit (Macherey-Nagel, Germany) following manufacturer’s instructions. DNase-treated RNA were quantified by measuring the absorbance at A260nm and RNA quality was assessed by OD260/OD280 ratios and by electrophoresis on 1% formaldehyde agarose gels stained with Sybr Gold. First strand cDNA synthesis was carried out in 20 μl reactions containing 1 μg RNA with (IMV-derived) reverse transcriptase and mix of oligo(dt) and random primers from iscript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Real time RT-PCR was performed on 25 ng cDNA using IQ Syber green supermix in an iCycler iQ (Bio-Rad Laboratories, Hercules, CA). The reaction efficiency of PCR (1.99 - 2.02) was determined on the slope of serial dilution curves of pooled cDNAs with each specific primer pairs at harvested times.

For PCR diagnostic assay, plant/fungus genomic DNA were extracted using genomic DNA from plant kit (Macherey-Nagel, Germany) following manufacturer’s instructions. To evaluate the resistance of a plant to a pathogenic fungus it was necessary to quantify the degree (ng fungus DNA/ng plant DNA) to which it is colonized by that fungus. Specific primers, even more in greenhouse condition, were required. Sequencing amplicon identified only the internal transcribed spacer ITS of V. dahliae, target of V. dahliae sequence and no tobacco sequence demonstrated the specificity of the reaction. Calibration curve (CT/ng ADNg; 25ng-2.5 pg) were performed with 10ng/μl gDNA of V dahliae extracted form conidia cultivated in messiae medium and 100ng/μl gDNA N.tabacum from healthy plants. After each run, a dissociation curve was acquired to check for amplification specificity by heating the samples from 60°C to 95°C.

Results of PCR diagnostic assay

We investigated, through the quantitative and very sensitive real-time RT-PCR technology, the potential of Coactyl™, composed of humic acid associated to a phenolic acid, to act as a plant defense inducer conferring resistance to Verticillium dahliae wilt in tobacco used as a model.

Discussion

First, the use of the real-time RT-PCR technology allowed to quantify the level of plant defense genes, thus assessing the level of plant defense mechanisms. Second, PCR quantifying the level of fungus highlight the potential of Coactyl as a novel compound able to provide commercially significative resistance (93%) of tobacco against V. dahliae through the activation of molecular defense responses. In conclusion, these molecular techniques provide useful tools to evaluate both the action of “inducing agent” in plant defense mechanism”, and the effectiveness of the commercial product against pathogen infection.