Expression of Defense Genes in Root Tissues of Two Soybean Cultivars with Different Levels of Partial Resistance to Phytophthora sojae

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Introduction

Partial resistance in the soybean-P. sojae interaction is race non-specific and quantitatively inherited [1]. Partially resistant plants restrict pathogen growth to the lower stem and tap root [5]. Race-specific resistance to P. sojae, controlled by individual Rps genes, is also present in soybeans and their deployment in commercial cultivars has been an effective method to control Phytophthora root and stem rot, with any single gene lasting from 8 to 15 years. However, recent surveys on pathogenic diversity in soybean fields have shown that the P. sojae population in Ohio has increased in number of pathotypes as well as complexity [2]. For this reason, relying on currently deployed resistance genes for disease control is not recommended. There is a need for more efficient incorporation of partial resistance to P. sojae in new or existing soybean cultivars as well as a better understanding of the molecular and/or biochemical mechanisms for this type of resistance [3]. To characterize the defense response in partially resistant soybean, we monitored mRNA levels for several plant defense-related genes following inoculation and subsequent infection of roots with P. sojae.

Objective

To determine which defense pathways are associated with partial resistance to P. sojae in soybean. Specifically, to test the hypothesis that transcription of defense-related genes during infection correlate with partial resistance to P. sojae in soybean.

Materials and Methods

- Soybean cultivars: Conrad (high level of partial resistance); OX20-8 (low level of partial resistance).
- P. sojae isolate: 34.S.5.1 (vir 1a, 1b, 1k, 2, 3a, 3c, 4, 5, 6, 7). Isolate was maintained on lima bean agar.

Fig. 1: A: Soybean plants affected by stem and root rot caused by Phytophthora sojae B: Initial symptoms of root rot in young soybean seedling caused by artificial inoculation of P. sojae C: Infected root section showing numerous oospores

Fig. 2: A: Plants grown in styrofoam cups with vermiculite B: Ten seedlings/tray over polyester cloth C: Root wounding D: Inoculation with a slurry of agar containing P. sojae mycelium E: Trays kept in buckets after inoculation F: 1.5cm root section removed from the lesion area

Slant board assay

- Samples were collected at 0, 6, 12, 24, 48 and 72 h.a.i. from test plants, non-inoculated, and mock inoculated control plants
- The experiment was repeated twice
- Root sections (1.5 cm) were taken from the interface between the expanding lesion and healthy tissue (Fig. 2F)

Northern analysis

- Total RNA was extracted from 20 root sections/ time point
- RNA (6 µg/ lane) was separated by electrophoresis under denaturing conditions
- RNA was transferred to nylon membrane
- Nine defense-related genes (Table 1) were selected to design probes for Northern blot analysis
- Signals were quantified with the ImageQuaNT software
- Signal values of the soybean actin gene were used for loading controls and to standardize the signal values of defense-related genes
Results and Discussion

Statistical analyses of mRNA signal levels for each individual gene indicated no significant differences between the two experiments. No signal was detected using F6H probe for inoculated and control samples at all time points. Both 4CL3 and D6aH signal accumulated at 48 h.a.i. at a significantly higher level in OX20-8 than Conrad (Fig. 4). In contrast, only the level of beta-1,3-endoglucanase (EGL) at 6 h.a.i. was significantly higher in Conrad than OX20-8. Interestingly, the expression levels of IPER and MMP at 72 h.a.i. were generally higher in Conrad than in OX20-8, but were never significantly different. A previous study from our lab found that the expression of 4CL1, 4CL-2, 4CL3 and D6aH, above the lesion site, was confounded by the wounding effect during the slant board test [6]. In the present work this effect was detected only during the first 24 h.a.i. and this difference could be attributed to the different sampling procedure. These results suggest a possible involvement of EGL, IPER and MMP as factors involved in the expression of partial resistance to *P. sojae* in soybean. MMP encodes an enzyme with in vitro anti microbial activity and it is hypothesized to be involved in a novel defense response of soybean against *P. sojae* infection [4].

![Image of a chart showing gene expression levels](image)

Fig. 4: Northern blot analysis of defense-related mRNA transcript accumulation during a time-course infection assay of root sections of soybean cultivars Conrad and OX 20-8 inoculated with *P. sojae*. The amounts of RNA loaded were visualized by ethidium bromide staining under UV light. Data point shown in the graphs are mean values of two independent experiments.

Conclusions

- *P. sojae* infection induced changes in defense-related gene expression over time in both cultivars
- mRNA levels of the majority of the defense-related genes tested were only effected by wounding during the first 24 h.a.i.
- Genes involved in the phenylpropanoid pathway together with PR1-a accumulated at a higher level in OX20-8 at 48 h.a.i.
- Defense-response pathways associated with expression of EGL, MMP and IPER may act as components of partial resistance of soybean to *P. sojae*
- Other physiological mechanisms, not explored in this study, might play additional roles in partial resistance to *P. sojae* in soybean

References


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