Proteomic analysis of soybean accessions resistant and susceptible to soybean rust (Phakopsora pachyrhizi)

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ABSTRACT

Screening accessions to find resistance to soybean rust infection.

- PI51789A and PI567104B supported fewer lesions compared to other accessions. Lesion of these accessions didn't produce any protein spots.
- PI208492 and PI662332 showed red-brown reaction without protein spots.
- The rest of the accessions produced high number of tan lesions with protein spots (Fig. 5 and Table 2).
- Significant soybean rust DNA accumulation was detected at 4 DAI in susceptible lines (PI548631 and 53M640, respectively) compared to resistant lines (PI51789A and PI567104B). A.
- No soybean rust DNA accumulation was detected in other resistant lines (PI468631 and PI567104B).

Soybean rust, caused by Phakopsora pachyrhizi, is a menace to worldwide soybean growers. This disease is studied on soybean in the continental U.S. In late 2004 and has the potential to cause severe yield reduction and billions of dollars in economic losses due to that all U.S. commercial soybean varieties are susceptible to this disease. In an effort to understand compatible and incompatible host-pathogen interactions at the molecular level, fourteen accessions were evaluated with rust spores collected in Louisiana. Two accessions showed consistent immune response in both detached leaf assay and greenhouse inoculation. qRT-PCR was conducted to compare the progress of fungal proliferation in resistant and susceptible lines. Fungal biomass increased significantly four days after infection in susceptible lines whereas no or little increase was detected in resistant lines. Comparison of protein profiles between two resistant and one susceptible line with or without rust infection has found differently expressed proteins in both resistant and susceptible lines. Some of the differentially expressed proteins that have been previously identified in plants, such as pathogenesis related protein 10 (PR10), S-adenosylmethionine synthase I and chalcone flavonone isomerase 1 (CHI). The identities of other differentially expressed proteins are being determined through peptide sequencing. The transcript levels of these differentially expressed proteins are analyzed using qRT-PCR. The potential importance of these differentially expressed proteins in soybean-P. pachyrhizi interaction is also discussed.

INTRODUCTION

Asian soybean rust (ASR) disease caused by P. pachyrhizi was first reported in Japan in 1982 and it is now an emerging disease in the continental United States since its discovery in late 2004 in Louisiana (LA). P. pachyrhizi infection can cause quick defoliation and severe yield losses up to 30% (Yang et al., 1991). According to studies of soybean rust spor viability under southern U.S. winter conditions (Park et al., 2008; Jurick et al., 2008), soybean rust is predicted to establish in the south and spread gradually to the north, which will pose a serious threat to U.S. soybean growers in the future. All U.S. commercial soybean cultivars are susceptible to the fungus and the only method to control this disease is timely and costly application of fungicides. To identify resistant lines, gel-based screening studies were conducted and soybean accessions resistant to P. pachyrhizi isolates from Nigeria, Paraguay and Vietnam were identified (Mills et al., 2008; Pham et al., 2006). However, the effectiveness of resistance can be overcome by virulent ASR isolates (Hartman et al., 2005). In order to develop approaches to effectively control this disease, it is necessary to understand how soybean rust infects the host and how host responses to pathogen attack at the molecular level. A proteomic approach has been successfully used to examine host-pathogen interactions in earlier studies between wheat and Puccinia triticina (Rampitsch et al., 2006), rice and Magnaporthe grisea (Kim et al., 2004), maize and Aspergillus flavus (Chen et al., 2008), and Arabidopsis and Alternaria brassicae (Grunwald et al., 2003). In this study, we determined the resistance levels of over a dozen soybean accessions against LA isolate. These lines had been previously identified as resistant to susceptible to rust isolates from other regions. A proteomic approach was adopted to compare leaf protein profile differences between resistant and susceptible soybean lines with or without rust inoculation to identify differentially expressed proteins.

OBJECTIVES

1. Identify soybean accessions with resistance to soybean rust spores collected in Louisiana.
2. Identify proteins differentially expressed between resistant and susceptible lines through protein profile comparisons.
3. Characterize the differentially expressed spots to identify proteins with important roles in host plant defense against rust infection.

CONCLUSION

- PI51789A and PI567104B showed immune response and PI567104B produced high levels of lesion and tan reaction after ASR infection.
- PI567104B completely stopped ASR proliferation whereas PI51789A allowed some extent of ASR proliferation, but not enough to cause the disease.
- Proteins were differentially expressed in resistant and susceptible lines after ASR infection.
- Some spots were commonly induced in both resistant lines but others were induced uniquely in each accession, indicating that they might have different defense mechanisms against ASR infection.
- PR10 gene expression was detected from all accessions at 100h, but its expression level was significantly higher in resistant lines.

MATERIALS & METHODS

1. Soybean leaf rust inoculation: 14 accessions, PI20680, PI20700, PI20702, PI41709, PI41710, PI567104, PI567108, PI567109, PI567114, PI567115, PI567116, PI567117, PI567118, PI567119. These were kindly provided by the Plant Genetic Resource Unit, USDA-ARS, Urbana, IL.

2. Inoculation of detached leaves. Inoculation of a detached leaf is similar to the method described in the protocol for soybean rust inoculation. Inoculated leaves were observed and sampled at 10 and 30 days after infection for each accession.

3. Proteomic analysis: The fourth and sixth trifoliate leaves were collected at R1 and R2 stages for detached leaf assay for each accession. For the proteomic analysis, PI41709A and PI567104B were selected for resistant and susceptible accessions, respectively. Leaves were collected from the fourth and sixth trifoliate leaves at 10 and 30 days after infection for each accession.

4. Protein extraction: Protein was released from detached soybean leaves that had been washed three times with distilled water and then air-dried. The leaves were homogenized with liquid nitrogen and ground to a fine powder. The homogenate was then extracted with 200 mL of cold 8 M urea. The supernatant was then centrifuged at 10,000 rpm for 10 min at 4°C.

5. Protein staining: Protein samples were mixed with silver stain reagents and stained according to the manufacturer's instructions. The gels were scanned and analyzed using ImageMaster 2D software.

6. Protein quantification was performed using ImageMaster 2D software. The spots were excised from the gels and digested with trypsin. The digested peptides were analyzed by MALDI-TOF mass spectrometry.

7. Protein identification: Protein spots were identified using MALDI-TOF mass spectrometry. The identified proteins were confirmed using Western blotting.

8. Real-time PCR: RNA was extracted from detached leaf tissues at each growth stage using TRIzol sample collection kit (Invitrogen). To prepare cDNA, 1 μg of total RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol. Real-time PCR was performed using 2X SYBR Green PCR Master Mix (Applied Biosystems) in 25 μl reaction volume with 25 ng of cDNA, 15 μl of SYBR green, and 1 μl of each primer. The same protocol was used for 18S rRNA internal control primer. ABI 7500 Fast Real-Time Detection System (Applied Biosystems) was used for real-time PCR under standard conditions.

9. Draw conclusions: Significant differences in the expression of genes were found between resistant and susceptible lines.

10. Future work: Further studies are required to understand the expression of genes involved in the defense mechanisms against soybean rust.

ACKNOWLEDGMENTS

This study was funded by the Louisiana Agricultural Experiment Station and the Southeastern Regional Soybean Promotion Board and the Louisiana Board of Regents Research Competitive Grant LEQSF(2008-10)-RD-01.

REFERENCES

[1] reference list