Comparison of Different Staining Methods for Coffee Proteomic Analysis

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SUMMARY

Proteomic methods, such as bidimensional electrophoresis (2-DE) and mass spectrometry, have been extensively used for the study of protein differential expression in several plants including *Arabidopsis thaliana*, rice and wheat. Specifically in the 2-DE method, deep attention must be given to the protein staining technique, which often involves silver nitrate or Coomassie Brilliant Blue (CBB). Silver staining is usually preferred over CBB due to the higher sensitivity obtained. Nevertheless, silver-staining resolution could significantly vary according to the studied organism and more specifically to the researched tissue. In *Coffea* spp., 2-DE analysis has been rarely employed. Some studies of protein expression have been reported in this culture mainly involving the biosynthesis of caffeine and metabolism during seed germination. The study of the global protein expression in coffee plants in response to biotic stress conditions had not been reported until now. Phytonematode infection can be considered one of the most important biotic stresses that affect coffee production and *Meloidogyne paranaensis* is one of the major nematode species that infects coffee plants. In this report, the protein expression of infected- and non-infected roots of coffee (*Coffea canephora*) were analyzed and the protein pattern determined by 2-DE. Gels were stained with silver nitrate or CBB, in order to obtain an optimized method for proteomic analysis of plant-nematode interaction. The 2-DE analysis revealed an enhanced number of protein spots, as well as differentially expressed proteins, when CBB was used. A total of approximately 70 and 100 spots were observed in silver and CBB stained gels, respectively. Moreover, 18 differentially expressed proteins were observed in CBB gels, and only 8 in the silver stained gels. This report showed that the staining method was crucial for an optimized protein analysis of coffee. Similar results were obtained for cotton roots and therefore these results may be extended to other plant species in order to better understand the host-pathogen interaction.

INTRODUCTION

Proteomic methods, such as bidimensional electrophoresis (2-DE) and mass spectrometry, have been extensively used for the study of protein differential expression in several plants. Specifically in the 2-DE method, deep attention must be given to the protein staining technique, which often involves silver nitrate or Coomassie Brilliant Blue (CBB). Silver staining is usually preferred over CBB due to the higher sensitivity obtained. Nevertheless, silver-staining resolution could significantly vary according to the studied organism and more specifically to the researched tissue.

In *Coffea* spp., 2-DE analysis has been rarely employed. Some studies of protein expression have been reported in this culture mainly involving the biosynthesis of caffeine and metabolism during seed germination. The study of the global protein expression in coffee plants in response to biotic stress conditions had not been reported until now.

Phytonematode infection can be considered one of the most important biotic stresses that affect coffee production and *Meloidogyne paranaensis* is one of the major nematode species that infects coffee plants. Several attempts have been made in order to control nematode infection, including the use of nematicides and crop rotation; however, the control of this pathogen using these methods is generally inefficient. The use of resistant genotypes and also genetically modified plants with enhanced resistance has been pointed as an alternative, which could contribute to a significant reduction in economic losses caused by *Meloidogyne* spp. Therefore, the knowledge of plant resistance genes and the elucidation of plant-pathogen interaction mechanisms are of great importance to control phytonematodes.

OBJECTIVE

The objective of this study was to analyze the protein expression of infected- and non-infected roots of coffee (*Coffea canephora*) by 2-DE and compare different staining methods in order to maximize the number of proteins being analyzed.

MATERIAL AND METHODS

Plant material

Coffee (*C. canephora*) plants were grown in sterile soil and maintained in green house. After six months, coffe roots were used for nematode infection. Non-infected plant roots were colected, washed and frozen in liquid nitrogen for further protein extraction and considered as the control condition.

Nematode culture and inoculation

Tomato roots were washed and homogenized in a blender with 0.5% (v / v) sodium hypochloride in order to obtain nematodes. The resulting material was sifted and the eggs were removed. After eclosion, juveniles were collected by centrifugation for 30 min at 2,500 x g and counted for plant infection. Each plant was infected with approximately 10,000 juveniles of *M. paranaensis*. Infected roots were collected 6 and 10 days after inoculation.

Protein extraction and bidimensional electrophoresis

Total proteins and isoelectric focusing were performed as described by Mot & Vanderleiden (1989). Approximately 150 ug of proteins were loaded onto the gel after a pre-run. Molecular mass separation was performed according to Laemmli (1970). After running, 2D gels were fixed overnight in a solution containing 30% ethanol and 10% acetic acid. Silver staining was carried out according to Blum et al. (1987). For CBB staining, a Coloidal Coomassie solution (0.1% Coomassie G250; 2% phosphoric acid; 10% ammonium sulphate and 20% methanol) was used.

RESULTS AND DISCUSSION

In the present report, coffee roots infected with *M. paranaensis*, were used for protein extraction and analyzed by 2-DE. Gels were stained with silver nitrate or CBB, in order to obtain an optimized method for proteomic analysis of plant-nematode interaction.

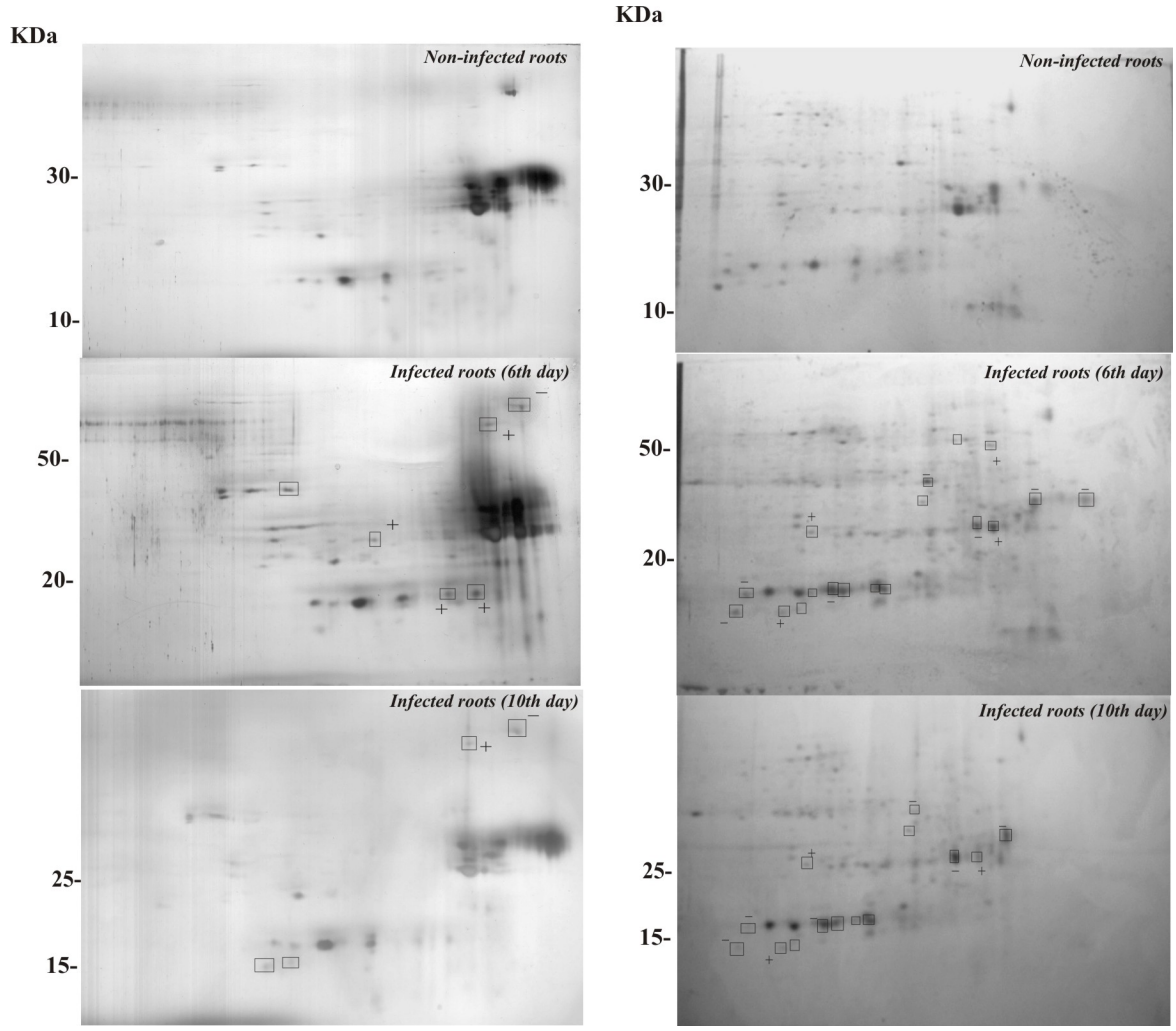

Figure 1. Bidimensional gels of coffee roots stained with silver nitrate (right) and CBB (left) of non-inoculated roots and infected roots 6 and 10 days after inoculation, as indicated. The pH of the gels ranged from 4-8. Squares indicate differentially expressed proteins in infected roots when compared to non-infected roots. The symbols + and – represent up- and down-regulated proteins, respectively, squares without symbols indicate new proteins and arrows point to differential spots observed in silver and CBB stained gels.

A total of approximately 70 and 100 spots were observed in silver and CBB stained gels, respectively. In order to identify differentially expressed proteins, the protein maps of the 6th and 10th day after nematode infection were determined and further compared to maps obtained from non-infected roots. CBB colored gels revealed at least 18 differentially expressed proteins at 6 days after inoculation including 4 up-, 7 down-regulated and 7 totally new and 14 differential spots at 10 days after inoculation, including 3 up-, 6 down- regulated and 5 new. A surprising result was obtained when gels were silver stained. By using this staining procedure, only 6 different proteins (4 up-, 1 down-regulated and 1 new) were visualized at the 6th day and 4 spots (1 up-, 1 down-regulated and 2 new) at the 10th day after inoculation (Figure 1). Another unexpected result showed that most differentially expressed

proteins identified in the silver stained gels were not observed in the CBB gels. Only 2 differential spots were common to the gels colored with both methods (Figure 1). Vediyappan et al. (2000) obtained similar results in the comparison of different staining methods for the analysis of *Candida albicans* proteins. It is possible that differences in the staining capacities of silver nitrate and CBB are responsible for the revelation of different proteins. In order to standardize this procedure, higher protein amounts were loaded onto the 2-D gel stained with silver nitrate. However, this modification lead to the presence of more pronounced stripes and background (data not shown), clearly reducing gel visibility and spot identification.

CONCLUSION

The results obtained herein showed that the staining method is crucial in order to maximize the number of proteins observed and that in the analysis of root proteins in coffee, CBB staining is more efficient. Similar results were obtained for cotton roots and therefore this conclusion may be extended to other plant species in order to better understand the host-pathogen interaction. We conclude that more than one coloring method should be employed when screening for proteins involved in specific biological processes.

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