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MPSG ANNUAL EXTENSION REPORT

PROJECT TITLE: Alternative methods to reduce root rots in soybean and pulses

PROJECT START DATE: 1 December 2014 **PROJECT END DATE: 1 December 2017**

DATE SUBMITTED: 1 December 2016

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

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PART 2: EXECUTIVE SUMMARY

Outline the project objectives, their relevancy to pulse and soybean farmers, and a summary of the project to date, including methods and preliminary results.

Root rot causes serious reductions in plant stand and yield in soybeans. Several alternatives strategies have the potential to control root rot pathogens in soybeans. This research involves the evaluation of beneficial bacteria in combination with some natural compounds such as plant extracts, chitosan and phosphite in reducing the effect of root rot in soybeans. The main objective of this part of the study is to characterize the most effective bacteria and identify the mode of action of the successful treatments.

Our previous results showed that we were able to collect a total of 100 bacteria isolates from soil samples and healthy soybean plants from different soybeans fields in Manitoba. Among these collection, 5 isolates were able to strongly reduce the growth of P. sojae in vitro. These antagonistic bacteria used in combination with phosphite (Phi) revealed a beneficial effect in reducing the disease severity in planta. Significant differences in seedling growth parameters and root rot severity were observed among the two tested cultivars (suceptible and tolerant to P.sojae race 4, respectively).

The most effective bacteria isolates S10 and S11 identified as Streptomyces sp. were isolated from healthy soybeans roots. Previous studies emphasized on the beneficial properties of endophytes and their exploitation in agriculture since these microorganisms are the best adapted to the native conditions.
Studies of the mode of action of the successful treatments is ongoing through the determination of the amounts of secondary metabolites produced and through molecular investigation of the expression of some genes involved in hormones and phenols pathway.

PART 3: PROJECT ACTIVITIES AND PRELIMINARY RESULTS

Outline project activities, preliminary results, any deviations from the original project and communication activities. You may include graphs/tables/pictures in the Appendix.

The identification of bacteria isolates-based on 16S rRNA gene sequence showed that the most effective isolates S10 and S11, belonged to the genus of Streptomyces and were closely related to S. glebosus strain LMG 19950 (98% identity). Indeed, endophytic streptomyctes have been shown to be effective in reducing disease symptoms caused by several plant pathogens and to promote the growth of the host plants. The research in this phase of the project aimed to evaluate the beneficial effect of bacteria in combination with phosphites in reducing the impact of root rots and to understand the molecular mechanisms behind any protection resulting from induction with either treatment or the combination of both.

To understand the protective effect of the antagonistic bacteria and elucidate their mechanism of action against P. sojae, two isolates (S10 and S11) were selected on the basis of their inhibitory activities against P. sojae in vitro and in reducing the disease severity in planta. Roots were inoculated with the bacteria in combination with Phosphite and were subsequently inoculated with P. sojae zoospores. Seedling physiology, colonization of root tissue by the pathogen and mortality were monitored. Phosphite (Phi) compounds are salts derived from phosphorous acid that were shown to protect plants against different pathogens. In this stage of this research project, we aimed to evaluate the beneficial effect of bacteria in combination with phosphites in reducing the impact of root rot and to understand the mechanisms behind the protection with either or combined treatments.

Our previous results showed that application of the bacterial isolate S11 as a seed coating reduced the disease severity and enhanced plant growth, resulting in a greater protection against the infection than other beneficial bacteria. In addition, a significant positive correlation was recorded between the in vitro and in planta effects of this bacterial strain, suggesting both a direct and indirect mechanism in mitigating the disease effect.
The bacterial treatment in combination with phosphites was efficient in protecting soybeans against P. sojae and all bacteria+Phi-treated plants survived the infection. In addition, measurements of growth parameters, i.e., root and whole plant weight, indicated an improvement in comparison to the diseased plants.
In a series of experiments, HPLC analyses indicated an increase in isoflavones content in soybean plants in response to P. sojae. Pre-treatment with the beneficial bacterial strain S11 improved the accumulation of these compounds. The highest concentration was recorded in the susceptible soybean line. These results suggest that S11 applied to the plant induces defense responses in its tissues.

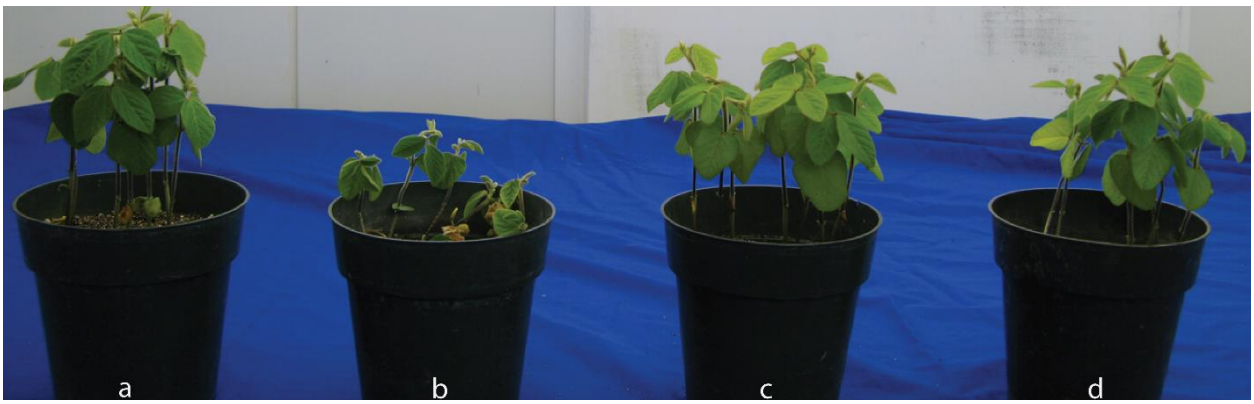
Examination of the expression of some defense-related genes involved in the isoflavonoids pathway showed that the S11 treatment primed the production of the phenylpropanoid key enzyme PAL in soybean roots. Examination of the expression pattern of others genes related to the Salicylic Acid pathway and Jasmonic Acid pathways is ongoing. The concentration of phosphite in infected roots is being quantified as well by ion chromatography, in order to assess the amounts used by the plant. The bacteria and P. sojae DNA contents in infected roots is going to be quantified by real time PCR in order to determine if there's any corresponding values between the reduction of disease severity and the amount of phosphites, bacterial and fungal DNA in treated versus infected plants.

In conclusion, our results suggest that the combination of native PGPR bacteria with chemical inducers, such as Phophite would contribute to the development of novel strategies for integrated control of root rot of soybean. However, further research is required to investigate possible interactions with environment or soybean genotype and application of chemical fungicides. These interactions should be investigated under both laboratory and field conditions in order to provide an efficient alternative.

APPENDIX

Scientific publications

Arfaoui A, L.R. Adam, F. Daayf. Isolation and identification of native bacteria associated with soybeans and investigation of their biocontrol activities against Phytophthora sojae (in preparation)



Picture showing the effects of different treatments on soybean plants:

a: Control plants, with no inoculation
b: Plants inoculated with *P. sojae*
c: Plants treated with bacterial strain S11
d: Plants treated with bacterial strain then inoculated with *P. sojae*