American Journal of Plant Sciences, 2015, 6, 1493-1500
Published Online June 2015 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2015.69148

Systemic Acquired Resistance of Cotton, Soybean and Common Bean to *Rhizoctonia solani* and *Sclerotium rolfsii* Induced by Shale Water Seed Treatment

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Received 16 May 2015; accepted 20 June 2015; published 23 June 2015

Abstract

Root rots of cotton, soybean and common bean caused by *Rhizoctonia solani* and *Sclerotium rolfsii* are basically soil-borne diseases and are difficult to control through the use of fungicides. One of the alternatives to control these diseases could be through the induction of Systemic Acquired Resistance (SAR). It is believed that shale water as a by-product obtained during the process of extraction of petroleum from fossil rock may act as an inducer of SAR to some pathogens of some crop plants. The objective of the present investigation was to verify the effect of seed treatment with shale water in inducing SAR to *R. solani* and *S. rofsii* root rots of cotton, soybean and common bean. Seed treatment experiments were conducted in the greenhouse on seedlings of these three crops using naturally or artificially infested soil with *R. solani* or with *S. rolfsii*. Treatments with seeds treated with shale water significantly reduced the average number of plants infected with the two pathogens. Consistent results were obtained in repeated experiments. SAR in cotton and common bean to *R. solani* varied between 86.16% and 91.13%, while for *S. rolfsii* in soybean and common bean varied between 84.0% and 57.54% and was long lasting. This is the first report giving strong indication of SAR of the three crops to *R. solani* and to *S. rolfsii*. Patent regarding this investigation is obtained with Petrobras, Brazil, under the number IVP 12/039.

Keywords

***Gossypium hirsutum, Glycine max*, *Phaseolus vulgaris*, Induced Resistance, Control**

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How to cite this paper: Mehta, Y.R., Marangoni, M.S., Bocatti, C.R., Rodrigues, H.P., Cunha, T.S. and Galbieri, R. (2015) Systemic Acquired Resistance of Cotton, Soybean and Common Bean to *Rhizoctonia solani* and *Sclerotium rolfsii* Induced by Shale Water Seed Treatment. *American Journal of Plant Sciences*, **6**, 1493-1500. http://dx.doi.org/10.4236/ajps.2015.69148

1. Introduction

Root rots of cotton (*Gossypium hirsutum*), soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) caused by *Rhizoctonia solani* (Telemorf *Thanetophorus cucumeris*), and *Sclerotium rolfsii* are economically important in several countries including Brazil. Most of the cultivars of these crops so far available for commercial cultivation are susceptible. Root rot symptoms caused by these pathogens on young seedlings are somewhat similar in all the three crops and can be easily verified as wilting, black necrosis and constriction of roots for *R. solani* and dark brown necrosis for *S. rolfsii* (**Figure 1** and **Figure 2**). Severity of root rot depends on the climatic conditions and the cultural practices. In Brazil, for example, some soybean farmers had to replant their crop because of the severe incidence of a root-rot complex caused by *R. solani* and *S. rolfsii*.

Figure 1. Seedlings infected with *R. solani*. Left—cotton seedlings; Right—common bean seedlings.

Figure 2. Root system of common bean seedlings 30 days after sowing. Left—healthy root system produced by shale water treated seeds; Right—root system infected with *S. rolfsii* produced by untreated seeds.

These are basically the soil-borne pathogens and are not controlled through the use of fungicides. They have wide host range attacking over 200 plant species [1] [2], and hence crop rotation practices alone may not help much to control these pathogens in a short period of time. Some information is available in the literature about the reduction of incidences and severities of soybean due to *R. solani* and *S. rolfsii* through the use of *Tricoderma* sp. [3]. However, so far there is no conclusive evidence in this respect to permit the generalized use of *Tricoderma* sp.

During the pyrolytic decomposition of organic material of fossil rock, petroleum and other by-products like retorted shale and shale water are obtained [4]. The shale water contains several macro and micro elements including phenol, pyridines, indol, phosphorus and salicylic acid (SA). It is believed that due to the presence of such elements the shale water may act as an inducer of Systemic Acquired Resistance (SAR) against the root rot pathogens and could be one of the alternatives to reduce crop losses caused by these pathogens.

SAR is associated with accumulation of pathogenesis-related genes (*PR* genes) in both local and systemic tissues. Durant and Dong, 2004 [5], Soares *et al*., 2004 [6] and Zhang *et al*., 2008 [7], have given a comprehensive account of the molecular basis of SAR. According to these authors the SAR induced genes include effector genes that confer resistance as well as regulatory genes such as transcription factors and the resistance conferred is long lasting in the plant. There are several reports in the literature to demonstrate this phenomenon. Benzothiadiazole as a novel class of inducers of SAR, for example, has been developed commercially as a plant activator, inducing SAR in wheat and conferring systemic protection against powdery mildew [8]. Francis *et al*., 2009 [9] have shown that soil application of an insecticide imidacloprid and related SAR-inducing compounds produce effective and persistent control of citrus canker. In case of common beans, Vigo *et al*., 2012 [10] reported that chemicals like pyraclostrobin and acibenzolar-S-methyl sprayed on snap bean induced systemic resistance against common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli*; however the latter did not induce systemic resistance of common bean against *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* [3].

In Brazil, a large percentage of seed of each one of the three crops is pirated which makes the use of resistant or less susceptible cultivars even more difficult.

Our earlier field and greenhouse studies have already indicated induction of SAR of some crop plants to some pathogens (unpublished data). The objective of the present investigation was to verify the efficiency of seed treatment by shale water in inducing systemic acquired resistance (SAR) of cotton, soybean and common bean to *R. solani* and to *S. rolfsii* under greenhouse conditions.

2. Material and Methods

2.1. Shale Water

Shale water is produced by Petrobras located at Irati (São Mateus do Sul), Pr, Brazil, by retorting bitumen shale and the process is known as PETROSIX. The shale water was brought to the laboratory, distilled at 60˚C and after distillation 4% of Dodecil Sodium Sulfate (Lauryl) was added as a fixing agent of the volatile substances. Later, soybean oil (1%) was added as a spreader and the shale water was stored at room temperature in dark for further use. The distillation of shale water was done especially to eliminate some elements with high molecular weight. This final composition of shale water is also referred as EAX (Extrato Aquoso de Xisto) in Brazil.

2.2. *In Vitro* Test with *R. solani*

The effect of shale water on the development of *R. solani* mycelium in Petri plates containing Potato Dextrose Agar (PDA) with or without shale water was studied under laboratory conditions. Four concentrations of shale water between 1% and 10% were used. For this purpose, mycelial discs of 0.5 cm were placed on the PDA plates. The plates were later incubated at room temperature and after one week the colony diameter of *R. solani* was measured.

In vivo* tests with *R. solani. For greenhouse experiments, seeds of the three crops were treated just before planting with 5.0 ml of 5% shale water per 100 g of seed. Un-treated seeds served as control. Soil was autoclaved for one hour at 121˚C and was used for greenhouse experiments unless otherwise mentioned. Seeds were sown in plastic trays (30 × 20 × 12 cm) containing infested or un-infested soil.

After emergence wilted plants of the three crops were uprooted every 48 h and were examined for the symptoms. Periodically, a few infected plants were used for the isolation of the pathogen to confirm the visual identi-

fication of the disease. The experimental design was randomized blocks with replications and the data were compared by analysis of variance.

Soybean. Greenhouse experiments were performed using cv. Embrapa 48. Two experiments were conducted on soybean. Four hundred seeds were sown in 10 replications in plastic trays containing mixture of soil, sand and compost in equal proportions. Soil of each tray was infested with 30 g of *R. solani* inoculum multiplied on autoclaved sorghum seeds for three weeks. For this purpose, inoculum of 30 g of sorghum seeds was blend in liquidizer for five min. with 100 ml of distilled water and later the inoculum was mixed in the soil of each tray. Trays were later maintained on the greenhouse bench where atmospheric conditions were not controlled. The treatments were: T1 = seeds not treated and the soil not infested; T2 = seeds not treated and the soil infested with *R. solani*; T3 = seeds treated and the soil infested with *R. solani.*

Common bean. Two experiments were conducted with common bean cv. Carioca. No artificial infestation of the soil was done because the soil was naturally infested. The first experiment was conducted using naturally infested soil, with 400 seeds treated with shale water and the other 400 seeds un-treated in eight replications of 50 seeds in each plastic trays.

In the second experiment we also used the same lot of soil naturally infested with *R. solani*, but used 2000 treated and another 2000 untreated seeds. Seeds were planted in plastic trays (50 seeds per tray) in 40 replications with un-sterilized soil. The treatments were: T1 = seeds not treated; T2 = seeds treated with shale water and sown soon after the treatment.

Cotton. Cotton seeds of cv. IPR Jatai were used. Four hundred seeds were treated and the other 400 seeds not treated and were sown in plastic trays containing the same lot of naturally infested soil as used for the common bean experiments.

2.3. *In Vivo* Tests with *S. rolfsii*

Seeds of the three crops were treated just before planting with 5.0 ml of 5% shale water per 100 g of seed. Soil sterilized in autoclave for one hour at 121˚C was used. Seeds were sown in plastic trays (30 × 20 × 12 cm) containing artificially infested soil.

Inoculum of *S. rolfsii* was multiplied on Potato Dextrose Agar (PDA). For this purpose, *S. rolfsii* grown for 25 days on 10 plates was blend in liquidizer for five min, diluted in 10 liters of distilled water, and used as inoculum. One hundred milliliters of inoculum per tray was used for soil infestation before planting.

After emergence infected plants of the three crops were uprooted and were examined every 48 hours for the symptoms. Periodically, a few infected plants were used for the isolation of the pathogen to confirm the visual identification of the disease. The experimental design was randomized blocks with eight to ten replications. Data were compared by analysis of variance.

Soybean. Treatments of soybean experiment were: T1 = seeds not treated and soil not infested; T2 = seeds not treated and soil infested with *S. rolfsii*; T3 = seeds treated with shale water and soil infested with *S. rolfsii*.

Common bean and cotton. Treatments for common bean and cotton were similar to the soybean experiment except that for these crops one treatment was added where, T4 = seeds treated with fungicide (Baytan 0.500 µl/250 gr, Monceren 0.75 gr/250 gr, Euparen 0.36 gr/250 gr) and the soil infested with *S. rolfsii*. This treatment was added considering the fact that some farmers in Brazil treat the seed with fungicide.

3. Results

In *in vitro* test, the growth of *R. solani* mycelium in Petri plates containing PDA with shale water was completely checked at the concentration of only 3% (**Figure 3**).

Soybean. The average number of soybean plants infected with *R. solani* in treatment T2 with un-treated seeds was much higher than the treatment T3 where seeds were treated with shale water. This represented a control of over 41% of the disease (**Table 1**). In this experiment the un-treated seeds with un-infested soil showed some infected plants due to some naturally existing soil borne inoculum. In this case, isolations of the pathogen were not made to confirm the identity of the causal organism.

In case of *S. rolfsii*, seeds treated with shale water controlled about 84% of the disease as compared to the untreated seeds (**Table 2**).

Common bean. Two experiments conducted in the greenhouse were with soil naturally infested with *R. solani*. In the first experiment 400 seeds were treated with shale water and the other 400 were un-treated. In this ex-

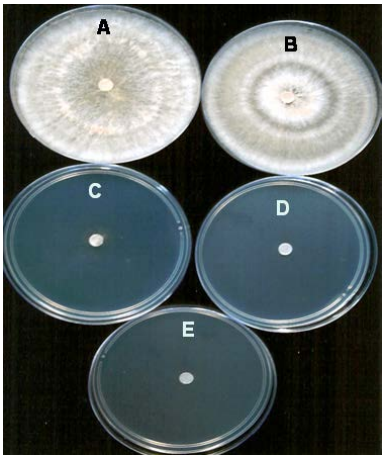

Figure 3. Effect of shale water concentration on the development of *R. solani* on PDA culture medium. A = check-without shale water; B = 1% shale water; C, D, E = 3%, 5%, 10% shale water respectively.

Table 1. Effect of soybean seed treatment with shale water on the severity of root rot caused by *Rhizoctonia solani*, under greenhouse conditions.

Treatment*	Average number of infected plants with *R. solanii*, 28 days after planting**
T1—Seeds not treated and the soil not infested with *R. solani*	3.85 c
T2—Seeds not treated and the soil infested with *R. solani*, before planting	48.48 a
T3—Seeds treated with shale water (5%) and the soil infested with *R. solani*, before planting	28.40 b

*Sterilized soil was infested with 100 ml of inoculum of *R. solani* cultured on autoclaved sorghum seeds; **Average of 10 repetitions, each one with 40 plants. Treatments with similar letters do not differ with each other. Tukey 5%. MSD = 13.258; CV = 23.4246.

Table 2. Effect of soybean seed treatment with shale water on the severity of root rot caused by *Sclerotium rolfsii*, under greenhouse conditions.

Treatment*	Average number of infected plants with *S rolfsii*, 28 days after planting**
T1—Seeds not treated and the soil not infested with *S. rolfsii*	0.00 c
T2—Seeds not treated and the soil infested with *S. rolfsii*, before planting	50.95 a
T3—Seeds treated with shale water (5%) and the soil infested with *S. rolfsii*, before planting	7.81 b

*Sterilized soil was infested with 100 ml of inoculum of *S. rolfsii* cultured on Potato Dextrose Agar, per tray; **Average of 10 repetitions, each one with 40 plants. Treatments with similar letters do not differ with each other. Tukey 5%. MSD = 9.23; 41.319.

periment the average number of infected plants went on increasing gradually till 50 days after sowing when the average difference between treated and un-treated plots was very high representing control of about 91% of the disease (**Figure 4**).

Considering the success of the first experiment, in the second experiment we used the same lot of naturally infested soil and used 2000 seeds treated with shale water and the other 2000 seeds un-treated. In this experiment the results were very much similar to the first experiment. The average number of infected plants in un-treated plots was 13.5 whereas in the treated plot it was 2.5. Representing over 81.48% control of the disease, which was comparable with the first experiment (**Figure 4** and **Figure 5**).

The severity of *S. rolfsii* in common bean was also reduced due to SAR induced by shale water seed treatment. The reduction in *S. rolfsii* infection was over 57% in T2 due to seed treatment with shale water in relation to un-treated seeds of T3 (**Table 3**). Seed treatment with mixture of shale water and fungicide gave lower number of infected plants as compared to seed treated with shale water alone, but the difference was not statistically

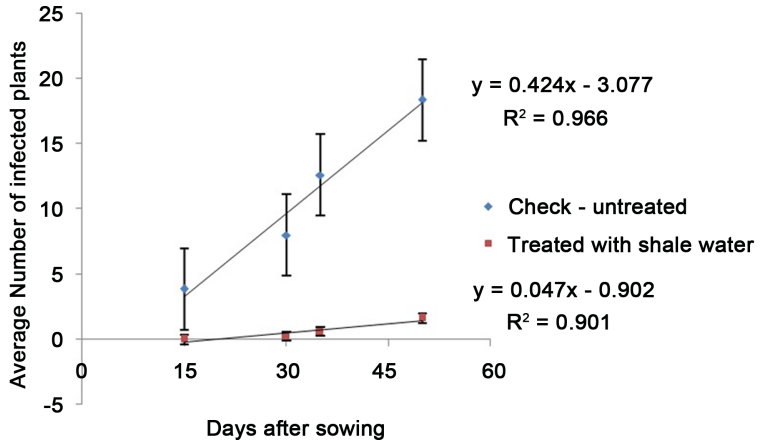

Figure 4. Effect of seed treatment of common bean with shale water (5%) on the progress of root rot caused by *R. solani*, under greenhouse conditions. Average of eight replications. Tukey 5%, MSD = 15.001, CV 3.701.

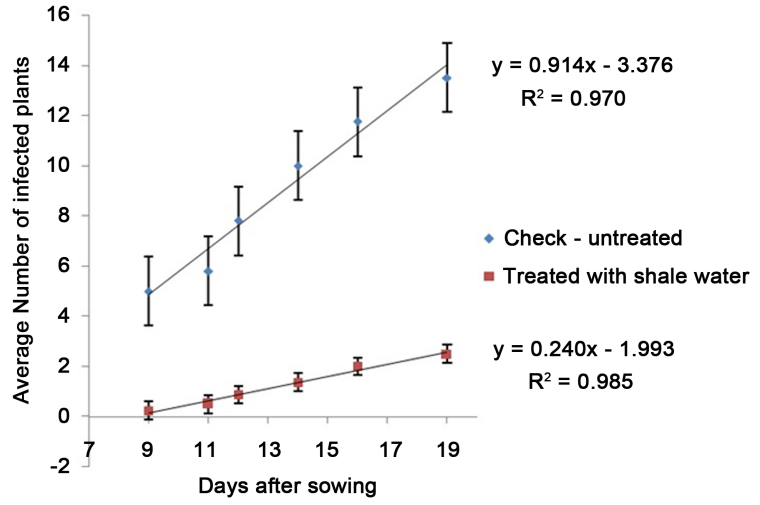

Figure 5. Effect of seed treatment of common bean with shale water (5%) on the progress of root rot caused by *R. solani*, under greenhouse conditions.

Table 3. Effect of seed treatment of common bean with shale water on the severity of root rot caused by *Sclerotium rolfsii*, under greenhouse conditions.

Treatment*	Average number of infected plants with *S. rolfsii*, 30 days after planting**
T1—Seeds not treated and the soil not infested with *S. rolfsii*	2.22 c
T2—Seeds not treated and the soil infested with *S. rolfsii*, before planting	82.91 a
T3—Seeds treated with shale water (5%) and the soil infested with *S. rolfsii*, before planting	35.16 b
T4—Seeds treated with fungicide and the soil infested with *S. rolfsii*, before planting	26.61 b

*Sterilized soil was infested with 100 ml of inoculum of *S. rolfsii* per tray, cultured on Potato Dextrose Agar for 25 days; **Average of 8 repetitions, each one with 50 plants. Treatments with similar letters do not differ with each other. Tukey 5%. MSD = 15.75; 30.78.

significant. In this experiment also the un-treated seeds with un-infested soil showed some infected plants due to the naturally existing soil-borne inoculum.

Cotton. Effect of cotton seed treated with shale water on the severity of *R. solani* showed about 86% control of the disease in relation to the untreated seed (**Figure 6**). The greenhouse experiment of cotton with *S. rolfsii*

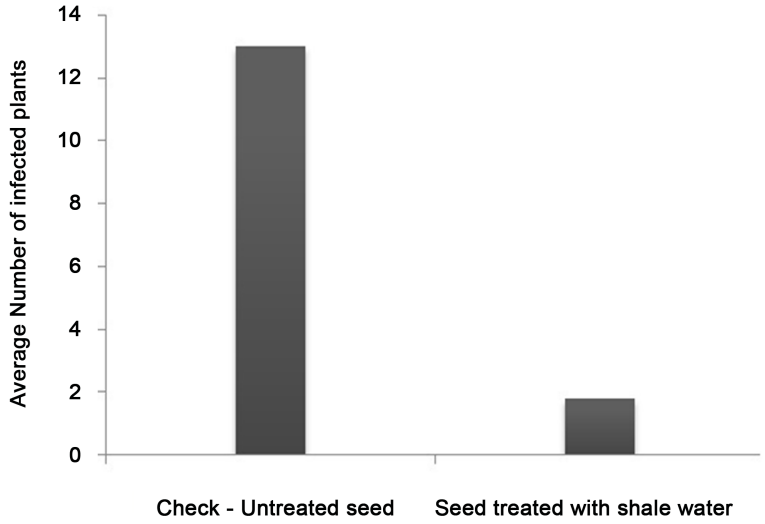

Figure 6. Effect of cotton seed treated with shale water (5%) on the severity of *R. solani*, under greenhouse condition. Average of four replications. Tukey 5%, CV 75.1%.

was not successful since the isolate of *S. rolfsii* was not pathogenic to the cotton cultivar used in this study. With this exception, results of the present investigation gave clear indication of SAR activity of shale water in three crops against *R. solani* and *S. rolfsii.*

4. Discussion

Seeds of all the three crops treated with shale water showed rapid and uniform development of seedlings as compared to the untreated seeds indicating that the shale water had this additional quality other than SAR. In case of soybean the control of *R. solani* was much lower (41%) as compared to the cotton and common bean (**Table 1**). On the other hand, the control of *S. rolfsii* in soybean was as high as 84% (**Table 2**). SAR in cotton and common bean to *R. solani* varied between 86.16% and 91.13%, whereas for *S. rolfsii* in soybean and common bean varied between 84.0% and 57.54%. Similarly, the control of *S. rolfsii* in common bean was much lower as compared to soybean. The SAR activity was evident against both the pathogens but it did not show complete resistance. Nonetheless, it is believed that it may be higher in case of less aggressive isolates of the pathogen.

While proteomic and other related studies are needed to conclusively demonstrate the SAR inducing quality of shale water, in the interim shale water can be used after its official registration in Brazil to reduce the cost of cultivation and yield losses. This is the first report of SAR induced by shale water seed treatment of soybean, cotton and common bean to *R. solani* and *S. rolfsii*. No report in this respect has been encountered in the literature. However, bacterial physiological diversity in the rhizosphere of range plants was reported in response to retorted shale stress (4).

The seed could also be treated with both shale water and fungicide in order to eliminate some of the seed transmitted fungal pathogens. The shale water seed treatments practiced with resistant and moderately resistant soybean cultivars would offer new perspective in reducing the severity of some other diseases and the long term use of such practices would help in reducing yield losses.

In Brazil, seed treatment with shale water for large quantities of seed for big producers (>50 ha), can be performed in the Seed Processing Units (SPU) using 0.4 - 0.5 L/100 kg of concentrated shale water instead of 5% of the 5% diluted shale water. Whether the seed treatment with such a low quantity of concentrated shale water in the SPU would have the same level of SAR activity needs to be further verified. One of the limitations of this technique is that the seed treatment in the SPU cannot be performed using quantities of concentrated shale water larger than 0.5 L/100 kg seed. Besides, the final composition of shale water (EAX) involving distillation process may increase the cost of its production. For small producers (<50 ha), especially the common bean producers, seed treatment with 5% of the 5% diluted shale water could be easily practiced. Since the seed treatment technique is simple it can be used for the pirated seed as well.

5. Conclusions

1) Results of the greenhouse experiments of the present investigation gave strong indication that the Systemic Acquired Resistance—SAR in cotton, soybean and in common bean to *R. solani* and *to S. rolfsii* was induced by shale water seed treatment and was long lasting; 2) SAR in cotton and common bean to *R. solani* varied between 86.16% and 91.13%, whereas for *S. rolfsii* in soybean and common bean it varied between 84.0% and 57.54%; 3) Shale water did not show any phytotoxic effect on the seedlings of any one of the three crops; 4) This is the first report of SAR of the three crops to *R. solani* and to *S. rolfsii* induced by shale water; 5) Patent regarding this investigation is obtained with Petrobras, Brazil, under the number EVP 12/039.

Acknowledgements

The present research was conducted under the financial support of Petrobras, Brazil. Technical assistance was provided by Carla Zandoná, Tanara Novaes and Priscila Alves.

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