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The split root system : a tool to validate *Trichoderma* species inducing systemic resistance in green beans infected by *Rhizoctonia solani*

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Abstract

In the present study we evaluate the effect of root dips of *Trichoderma* suspensions in inducing systemic resistance (ISR) in green beans against *Rhizoctonia* solani using split root system (SRS) technique. The approach of SRS is to separate green beans roots in two sections and sow them in different compartments. A half roots section is dipped with *Trichoderma* suspension and installed in one compartment whereas *Rhizoctonia* is inoculated in the other half roots and sown in the second compartment. Results obtained of reducing disease incidence in green beans demonstrated that *Trichoderma* treatments showed a potential to induce systemic defense in green beans against *Rhizoctonia.* The lowest disease incidence in root units (DI-RU = 8.3%) was observed in green beans treated with *T. reesei*. *Rhizoctonia* disease control by *Trichoderma* spp. has been interpreted as a systemic resistance response primed in the pathogen host. This approach may open new perspectives towards cost effective tools to screen ISR in *Trichoderma* root dips to be used for propagated materials treatment during transplantation.

Keywords: ISR, Plant-pathosystem, soil-borne plant pathogens, Trichoderma.

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1. Introduction

Control of crops diseases caused by soil-borne phytopathogens was tightly related to the suppressive soil microbial content and the capacity of microbial population living in the rhizosphere to antagonize and parasitize the pathogen. Soil-borne disease suppression is primary related to mechanisms involved by the soil beneficial microbial population. These mechanisms are competition for space and nutrient, hyperparasitism, production of inhibitory secondary metabolite and inducing systemic resistance (ISR) in plants (Figure 1) [1]. Induced systemic resistance (ISR) attracted much attention as a sustainable biocontrol strategy of soil-borne phytopathogens. Plant ISR are induced by beneficial soil-borne microbes like *Trichoderma* that trigger defense mechanisms in pathogens host [2-4].

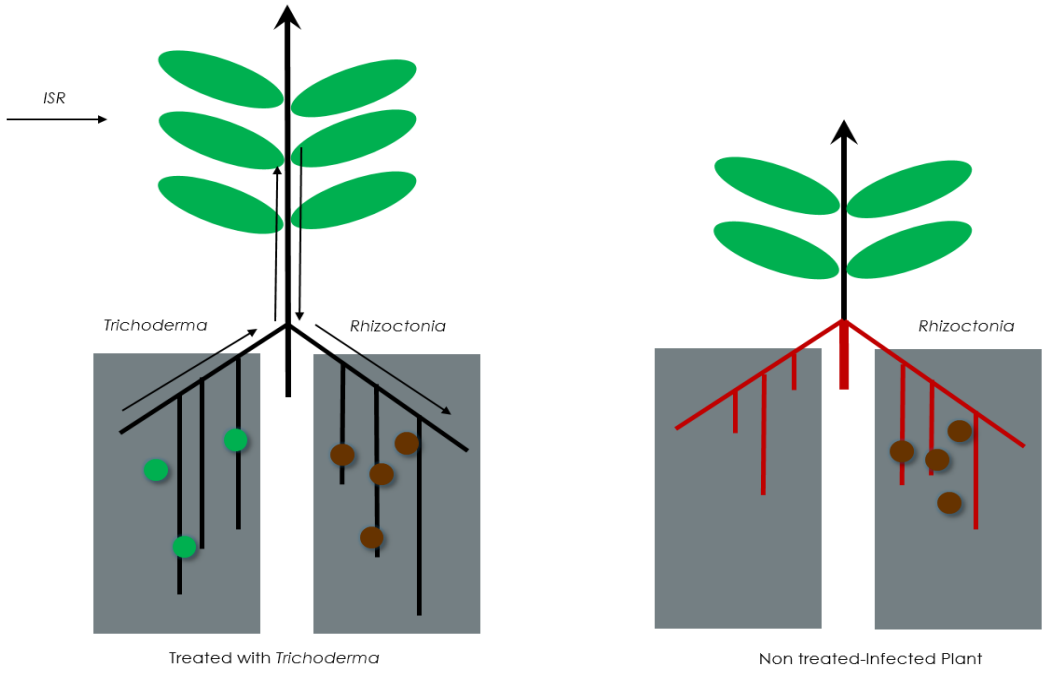

Figure 1: *Split Root System in plant*

Different studies demonstrated the effectiveness of different microorganisms like *Trichoderma* spp, *Bacillus pumilus* and *pseudomonas fluorescens* on stimulating ISR in crops like rice suppressing blast disease and in tomato against *Phytophtora infestans* [5-6]. *Trichoderma* spp. stimulate ISR by triggering and regulating Jasmonate (JA) and Ethylen (ET) signaling pathways and activating a primed state in plants. Actually, *Trichoderma*-plant interaction and root colonization involving systemic defense mechanisms mainly occurred at the level of plant cells. Hence, the Split Root System (SRS) is the most affordable approach in demonstrating plant defense signaling patterns and therefore the occurrence of ISR in pathogen host [7]. In this study, we attempt to evaluate if systemic resistance is primed in green

beans-*Rhizoctonia* pathosystem when *Trichoderma reseei* and *Trichoderma afro-harzianum* are root dipped in green beans using split root system method.

2. Materials and methods

2.1. Obtaining fungi isolates (test pathogenic and Trichoderma) cultures

Rhizoctonia solani was provided from mycotheque of mycology laboratory at IAV CHA, Ait-melloul in Morocco. We tested ISR for two *Trichoderma* species *T. afro-harzianum,* and *reseei* respectively [8] for their ability to prime ISR on green beans against *Rhizoctonia solani*.

2.2. Split root system: splitting roots for different treatment in compartments

Split Root System (SRS) was generated as described by Kassaw and Frugoli 2012 and Larrainzar *et al*. 2014. Seeds of green beans were first germinated on 77 peat trays for 3 weeks until the seedlings' main root is developed. Main root was cut afterwards to develop more secondary roots. Seedling roots were separated into two root sections 1 and 2 which are split at the level of separating section (Ss) and sown on two nursery bags as showed in figure 2 [9-7]. Roots of each cultivar are developed in two black plastic nursery bags of 2.5 L volume. Each plastic bag was cut to 2-3 cm depth at the level of the root separating section (Ss) placement. In this way, all roots will be well covered with the substrate mixture when sowing. Each two bags were sealed together at the level of the root separating

Figure 2: Two roots halves separately co-inoculated in SRS model (Kassaw and Frugoli, 2012). SS: separating section where seedling roots were separated into two root sections 1 and 2; S1: Section 1st inoculation: One halves roots in section 2 with two corn seeds infected with *Rhizoctonia solani*; S2: 2nd inoculation: the other halves roots in section B dipped in *Trichoderma* suspension.

2.3. Inoculation of one roots compartment of green beans with *Rhizoctonia* sclerotia

Rhizoctonia inoculum was prepared using infected corn seeds with *Rhizoctonia* mycelium as adopted by Cardoso and Echandi (1987) [10]. Green beans' roots of section 1 were inoculated using two infected corn seeds. Therefore, the inoculation in section 1 (S1) contains 2 corn seeds infected with *Rhizoctonia* at transplanting as shown in figure 2. The other section (S2) contains the inducer treatment with roots dipped in a suspension of *Trichoderma* spp. for 15-20 minutes as described byKhan *et al*. (2004)and Medeiros *et al*. (2016) [11-12].

2.4. Inoculation of the other roots compartment of green beans with *Trichoderma* spores suspension

Green beans' roots of section 2 (S2) were dipped in spores' suspension of 10^6 conidia/mlof different *Trichoderma* spp. for 15 to 20 min. *Trichoderma* spp. used for Induced Systemic Resistance (ISR) treatment are: *T. afro-harzianum* (T1)and *T. reseei* (T2). Tm1 was designed for non inoculated healthy cultivars (negative control). Tm2 was designed for cultivars inoculated with pathogen only (positive control). TC designed for treatment of cultivars inoculated with *Trichoderma harzianum* extracted from a commercial product to be compared with other *Trichoderma* isolates [10-11-7].

2.5. Disease evaluation

Diseases assessment was estimated by measuring Disease Incidence (DI) as described in previous studies and reported in the equation 1 [13-14].

$$Disease\ Incidence\ (DI) = \frac{number\ of\ infected\ plant\ units}{number\ of\ plants} * 100 \qquad (1)$$

Disease incidence was measured in the above ground units (DI-AU) and root units (DI-RU) of every plant. Cankers and lesions are the main symptoms to be determined in green beans roots and stems infected with *Rhizoctonia* sclerotia. Plant parameters calculated in this experiment were plant height (PH), Pods number (Pods N°), plant dry weight (PDW) and roots dry weight (RDW).

2.6. Experimental design

Experimental design of SRS was organized in three randomized complete blocs with three replicates in each experimental unit. That is, six bags in each experimental unit and for each treatment. Notes that;

Corn seeds were placed near to beans' roots at the moment of transplanting and all seeds are surface disinfected for 30 seconds (s) in 1.5 % sodium hypochlorite and rinsed with tap water at least three times then dried under laminar flow hood.

3. Results and discussion

3.1. Induced Systemic Resistance (ISR) of *Trichoderma afro-harzianum* and *reesei* on green beans

Using split root system (SRS) technique explained in material and methods, it seems that *Trichoderma* spp. effectively have the potential to control *Rhizoctonia* disease in green beans plants while systemically their two root halves were separately inoculated; half with *Trichoderma* and the other half with the pathogen. The split root system is designated (*Trichoderma*/*Rhizoctonia)* in this paper. Comparing disease incidence in roots (DI-RU) of different *Trichoderma* treatments and Tm2 controls in table 2 only 8.3 % disease incidence was recorded in *Trichoderma*/*Rhizoctonia* SRS when treating green beans with *T. reesei* (T2) while DI-RU recorded for T1 (*T. afro-harzianum)* and TC (commercial *Trichoderma*) was 16.6 % and 25.0 % respectively. In addition, disease incidence recorded in different treatments was significantly lower compared to that of Tm2 with DI-RU = 100 %, P= 0.000. Moreover, RDW data support the DI-RU results. The highest RDW of green beans was observed in T2 treatment with 5.5 g which is fairly compared to RDW = 5.7 g of healthy plants in Tm1.

T1 and TC treatments showed the lowest RDW = 3.9 g and RDW = 2.5 g respectively whereas RDW = 1.9 g is recorded in Tm2. RDW data suggest that *Trichoderma spp* maintain and improve green beans development though roots were separated in different compartment with different components; S1: *Trichoderma* spore suspension and S2: *Rhizoctonia* corn seed inoculum (see figure 3). It may be reasonable to assume from root disease incidence results that *Trichoderma* treatments have the potentialto induce systemic resistance in green beans cultivars by probably activating defense mechanism in the *Rhizoctonia* roots attacked half (S2). Green beans cultivars infected with *Rhizoctonia* only (Tm2) indicated damaged stem and root. Cankers and lesion were also observed in figure 4. While in T2 treated green beans no symptom was observed. It can be inferred from results obtained of different plant parameters assessed in *Trichoderma*/*Rhizoctonia* that *Trichoderma* treatments have the potential to maintain plant development. PDW in *Trichoderma*/*Rhizoctonia* of green beans treated with *reesei* (T2) has been maintained with an average weight of 33.0 g compared to healthy Tm1 with an average PDW = 32.6 g (table 2). Nevertheless, the induction of defenses did not seem to affect cultivars reproduction since Pods number values in SRS treatments (*Trichoderma*/*Rhizoctonia*) were not significantly different to those in Tm2 controls. Pods numbers registered for different *Trichoderma*/*Rhizoctonia* SRS and Tm2 controls were fairly equal.

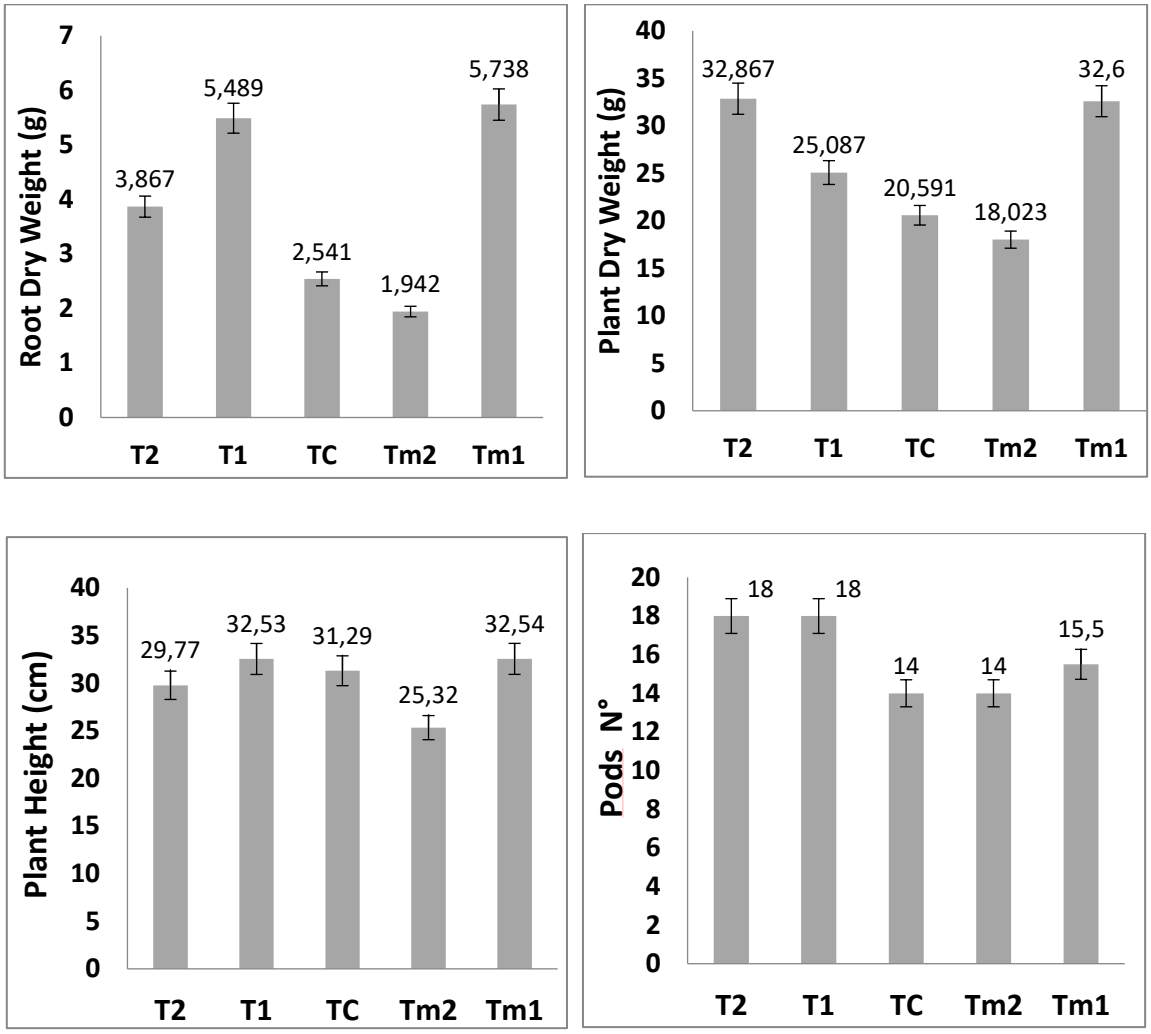

Figure 3: Evaluation of biocontrol efficacy of *Trichoderma*spp. on plant development parameters on ISR *Rhizoctonia*-green beans pathosystem. Plant development parameters measured were; Root dry weight (RDW), Plant dry weight (PDW), Plant Height (PH), and Pods Number (Pods N°).

Figure 4: *Trichoderma*/*Rhizoctonia* SRS in infected green beans; Tm2: infected controls with in 1: brown cankers in stem and in 2: rots in roots; T2: green beans treated with *T. reesei*. S1 and S2 are halves roots inoculated separately with *Rhizoctonia solani* and *Trichoderma* suspension respectively.

Our results match well with ISR results in previous research work. As reported by Khan *et al*. (2004), *Trichoderma hamatum* triggered induced systemic resistance in cucumber cultivars when infected with *Phytophthora*. In Khan *et al*. study, substrate amended with heated compost and *Trichoderma* showed the suppression of blight disease in cucumber. It was reported in Khan *et al*. (2004) and Yedidia *et al*. (1999) research that *T. hamatum* and *T. harzianum* effectively activated ISR in cucumber [11-16].
It was reported in ISR review [7] that both local and systemic responses raise during pathosystem and antagonist interaction. It was considered that shoot to root and root to shoot reaction are crucial signals to clearly identify which responses is targeted. Therefore, developing split root system (SRS) technique where shoot and roots were compartmentalized may lead to conclude ISR mechanism of the antagonist candidates used. In fact, to determine if antagonists *T. afro-harzianum* and *reseei* primed ISR responses in green-beans, antagonistic effect was recorded on halve root compartment inducing resistance stimulus in the other halve root compartment [17-21]. Therefore, the control of disease in overall plant may be interpreted as systemic responses primed in the host plant [7-16].

Conclusion

In summary, split root system SRS method permitted to demonstrate the systemic long distance responses towards disease and pathogen causing it. Therefore, long term regulation may be translated by root to shoot long signal walking during *Trichoderma*-plant interaction. SRS is considered a reference tool in analyzing the local and systemic nature of antagonist-pathosystem signaling networks. However, it is important to mention that the production system used (2.5 L black plastic bag) may be one of the limitation that did not allow the effective exhibition of ISR response on plant development such as the case of Pods production.

All in all, SRS has been effectively applied to study ISR of *Trichoderma* spp. in *Rhizoctonia*-green beans pathosystem. SRS technique allowed us adapting two differential treatments (*Trichoderma/Rhizoctonia*) in two independent roots system in the same host. This approach help us developing consistent evidence about the action of *Trichoderma* spp. not only as an antagonist but also as inducer of host immunity.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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