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Effect of Improved *Trichoderma harzianum* on Growth and Resistance Promotion in Bean Plant

Sahar Mahmoodian[1]
https://orcid.org/0000-0001-8604-6859

Mojegan Kowsari*[2]
https://orcid.org/0000-0002-6858-4052

Mostafa Motallebi[1]
https://orcid.org/0000-0002-8134-7799

Mohammadreza Zamani[1]
https://orcid.org/0000-0002-7165-2343

Zahra Moghaddasi Jahromi[1]
https://orcid.org/0000-0002-1780-5791

[1]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; [2]Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

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*Correspondence: Kowsari@abrii.ac.ir, Phone: 026-32703536 (M.K.).

HIGHLIGHTS

- *Trichoderma* inoculation increased the total biomass, seedling height, root length, root area, root diameter, number of root tips, and number growth potential of seedlings.
- *Trichoderma* inoculation increases the resistance of bean plant to *Rhizoctonia* pathogen.
- Among improved isolates of *Trichoderma*, the ChBD13 and ChBD15 showed better performance in terms of plant growth.

Abstract: Different species of *Trichoderma harzianum* have been used mainly as an antagonist against a number of plant diseases. There is a direct relationship between the biocontrol potential of these fungi and the rate of enzymatic degradation of the cell walls of pathogens. Following the successful production of chimeric chit 42 via protein engineering in the previous work of authors, the resulting recombinant protein was transferred to *T. harzianum*. In this study presence and stability of chimeric Chitinase (Chit42-ChBD) was verified then growth stimulating and biocontrol potential of the six improved isolates were evaluated on bean plants under laboratory and greenhouse conditions compared to the wild type. Among engineered isolates, Chit42-ChBD3 with 53.83% growth inhibition on *Rhizoctonia* showed the highest growth rate and sporulation compared to the wild type in the dual culture test. The results showed that ChBD15 and ChBD13 had better performance in terms of increasing nutrient uptake and root growth thereby enhancing the growth

rate and yield performance of the whole plant compared to the plants treated with wild type and non-*T. harzianum* treated plants.

Keywords: Chimeric chitinase; *Trichoderma*; Biocontrol; Growth stimulator; *Rhizoctonia sp.*

INTRODUCTION

The increasing need of the current society for agricultural products and the quality of products has led to an increase in the use of fertilizers and chemical pesticides, which results in serious environmental pollution. The use of biocontrol agents against plant pathogens is one of the ways to reduce these destructive effects [1].The use of biological agents such as antagonistic microorganisms is one of the prominent and nonchemical ways to control plant diseases. In this regard, *Trichoderma* species are able to parasitize phytopathogenic fungi [2]. *Rhizoctonia* root rot of bean is the most common soil-borne disease known throughout the world, and the severity of its damage has been reported as high as 15% [3]. It is also difficult to control because it is caused by soil-borne pathogens that are difficult to manage. The efficacy of biocontrol agents is related to their compatibility, fortifying the plants with improved nutrient uptake and disease control [4].

Trichoderma strains can colonize plant roots through mechanisms similar to mycorrhizal fungi. They stimulate plant growth by producing compounds as well as improving plant defense mechanisms [5]. Some of *Trichoderma* characteristics, including capacity to *altering* the Rhizosphere, potent aggressiveness against phytopathogenic fungi, survival under unfavorable conditions and the promotion of plant growth as well as defense mechanisms can contribute to successful control of plant diseases. These advantages have made *Trichoderma* an omnipresent genus that is able to survive in any habitat and at high population densities [6]. Rhizosphere infestation of *Trichoderma* helps the plant to thoroughly increase nutrient/fertilizer availability [7] and seed germination [8]. As major hydrolytic enzymes, chitinases play an essential role in the cell wall hydrolysis of fungi and the biological control procedure [9,10]. Among different chitinases, chit42 is crucial for biocontrol function against phytopathogenic fungi. The Chitinase-binding domain, which is absent in chitinase 42 of *T. harzianum*, seems to enhance the binding of enzyme to substrate and allows for binding to an insoluble substrate. Kowsari and coauthors (2014) attempted to enhance the activity of this the enzyme through purification of ChBD enzyme chitinase of 18-10 *T.atroviride* and adding them by a linker to the amino-terminal sequence of Chitinase 42 just after peptide signal site using protein engineering by SOEing PCR, after which the chimera protein-producing gene was transferred to *T. harzianum* [11]. Limón and coauthors (2001) produced hybrid chitinases with more affinity to chitin by fusing the catalytic domain from *T. harzianum* Chit42 fungal Chitinase to a fungal cellulose-binding domain [12]. In this study, the performance of six engineered strains of *Trichoderma* and a wild type strain was assessed in bean plants in terms of promoting common bean growth plants and decreasing the incidence of *Rhizoctonia* root rot under both *in vitro* and greenhouse conditions.

MATERIAL AND METHODS

Fungal Strains and Culture Conditions

In the current study, seven isolates of *T. harzianum* were used. Genetically modified strains of *T. harzianum* (Chit42- ChBD3, Chit42- ChBD6, Chit42- ChBD7, Chit42- ChBD11, Chit42- ChBD13, Chit42-ChBD15) as well as the wild type [*T. harzianum* (T8-7) and *R. solani* (AG-4)] were provided by Agricultural Biotechnology Research Institute of Iran (ABRII) type culture collection and National Institute for Genetic Engineering and Biotechnology (NIGEB). The fungi isolates were cultured on Potato Dextrose Agar (Sigma Aldrich, USA) and were incubated at 27± 3 ºC for 5-7 days. After successive transplanting emerged colonies in the sterile dishes containing PDA medium, they were purified using Bastakoti and coauthors (2017) method [2].

Plant Material

Bush bean (*Phaseolus vulgaris* L.) seeds were obtained from Khomeyn Agricultural Research Center (Iran).

Construction of Chimeric Chitinase

The chimeric chitinase was constructed via Splicing by Overlap Extension (SOEing) PCR. For this reason, the chimeric gene containing ChBD+linker at the N-terminal end of Chit42 cDNA from *T. atroviride* (DQ022674), i.e. the fragment containing Chit42 cDNA was amplified using specific PCR primers [11].

Confirmation of the Presence and Stability of the Chimera Chitinase Gene

To confirm the presence of the 237 bp fragment in the engineered isolates (a previous work by the present authors), polymerase chain reaction **(**peQlab, United States) was carried out, followed by DNA extraction of fungal hyphae [13].

The thermal cycling conditions by primers [(PF1-R3*xba1*) and (PF1-R3chit42)] were as follows: one cycle at 94 ˚C for 15 min, forty cycles of amplification were performed, each cycle consisting of 1 min at 94 ˚C, 1 min at 62 ˚C, and 1 min at 72 ˚C, followed by an additional cycle of 10 min at 72 ˚C (Table 1).

Table 1. The sequence of primers used to confirm the chimera chitinase 42 gene

Primers	Sequence 5 to3	5 poly cloning site & Tail
PF1	GCTCTAGAATGTTGGGTTTCCTCGGAAAG	*Xba*1
R3xba1	GCTCTAGACTAGTTGAGACCGCTTCGG	*Xba*1
R3chit42	CGCCTCCGTTGATATAAGCC	

In vitro Biocontrol Activity

The antagonistic activity of *Trichoderma* species against *R. solani* was evaluated in a dual culture assay using the PDA medium. Each treatment was carried out with three plates per replication for 72 hours. Periodical observations on the growth of *Trichoderma* species and their ability to colonize the pathogen were made, and the percentage inhibition of pathogen mycelial growth was calculated using the following formula [14].

$$\text{Inhibition of growth (\%)} = \left(x-\frac{y}{x}\right) \times 100 \quad (1)$$

Where X = mycelia growth of the pathogen in the absence of *Trichoderma* (control) and Y = mycelia growth of the pathogen in the presence of transformants [11].

Greenhouse Assays

Greenhouse experiments were performed in 2015 at the experimental greenhouse of the Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

Bean seeds (10 seeds per pot) were sown in pots (10 × 10 × 12 cm) containing sterile soil. The experimental design consisted of the following four treatments in a randomized complete block design with five replications: T0P0 (without application of *Trichoderma* and without pathogen), T0P1 (without application of *Trichoderma* and with pathogen inoculation), T1P0 (with *Trichoderma* and without pathogen inoculation), and T1P1 (with *Trichoderma* and with pathogen inoculation).

Seed treatment: *P. vulgaris* seeds were initially surface sterilized with 1% hypochlorite solution for 1 min and then rinsed in sterile distilled water. The seeds were then treated with a spore suspension of *T. harzianum* and shaken for half an hour in the prepared suspension (10^6 CFU.mL^{-1}).

Preparation of inoculum: The inoculums were prepared according to Pugliese [15]. The *Trichoderma* isolates were propagated on sterilized wheat and wheat bran medium (1%w/w), and the pathogen was propagated on a sterilized wheat medium (1%w/w) and incubated at 30 °C for 10–15 days. The inoculum of *Trichoderma* isolates was added into potting soil 4 days before sowing. We added the inoculum of *R. solani* to the pots, 4 days after sowing.

In Vivo Biocontrol Activity of Trichoderma Species against R. Solani

Germination was completed about five days after sowing, and root rot symptoms were evaluated in two leaf stages, a month after planting, at the early reproductive growth stage, and at harvest according to protocol reported by Kim and coauthors [16]. After uprooting the plants, they were washed with tap water. The roots were categorized using a 0–5 scale where 0 = healthy plant (no infection), 1 = 20% infected root,

2 = 40% infected root, 3 = 60% infected root, 4 = 80% infected root, 5 = 100% infected or completely dead plants depending on the appearance of elongated, red-brown lesions on roots (Figure 1).

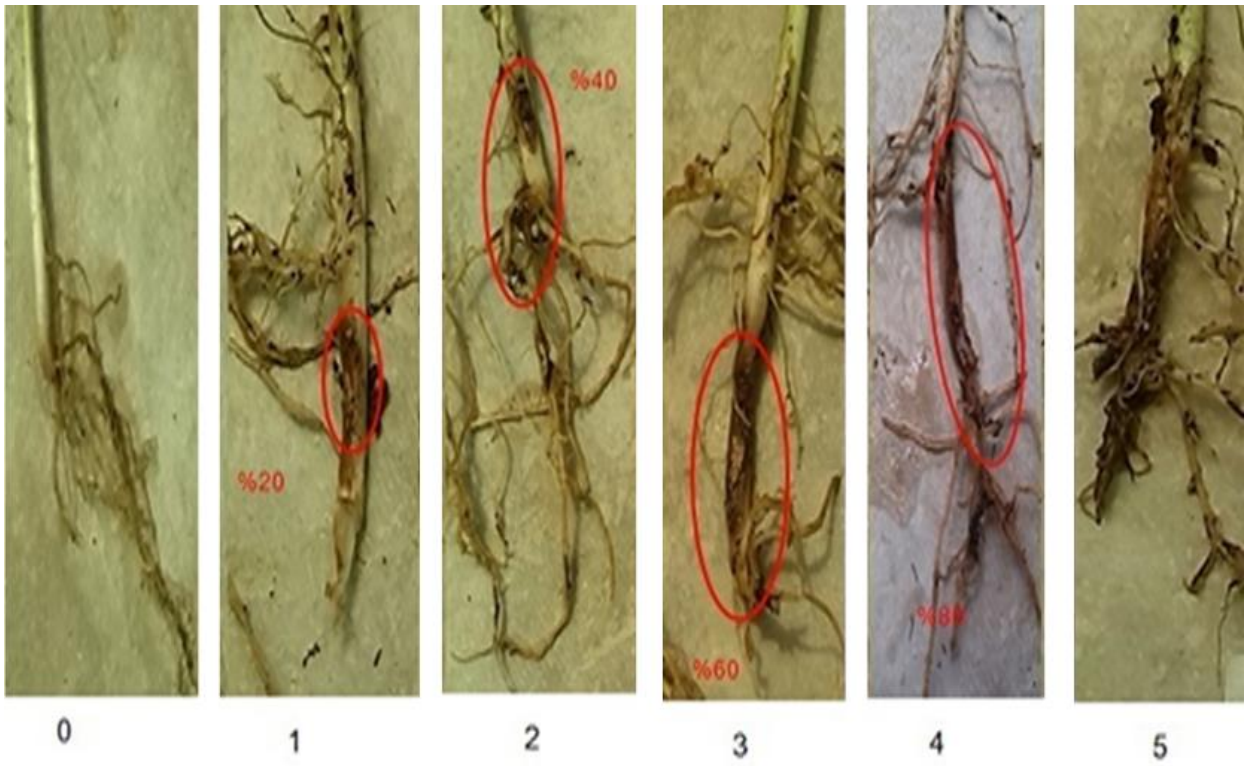

Figure 1. Investigation of Root rot Severity of beans (%) under greenhouse condition at Germination stages. 0 = healthy plant (no infection), 1 = 20% infected root, 2 = 40% infected root, 3 = 60% infected root, 4 = 80% infected root, 5 = 100% infected or completely dead.

Growth Analysis of Bean in Greenhouse

Morphological traits such as dry and fresh weights of the root, fresh weights of leaves and stems. The number of pods per plant and the number of seeds per pod of the impregnated pots were determined at the harvesting stage. Five replicates were made for the measurements.

To analyze the rate of photosynthesis, Chlorophyll was extracted using the Arnon (1956) method as follows [17].

Chlorophyll Extraction

To compare the chlorophyll content in the leaves of inoculated pots with engineered *Trichoderma* fungi and wild isolates, 0.25 g fresh leave (the first adult leaf from the stem tip) was chopped with scissors in triplicate, 5 mL water was added to it, was homogenized in a mortar, and the mixture reached 25 mL volume in a volumetric flask using distilled water. 0.5 mL of the sample was mixed with 4.5 mL of 80% acetone and centrifuged (High-Speed Universal Centrifuge, PIT320, Iran) for 15 minutes at 3000 rpm. *Rhizoctonia* and *Trichoderma* plants without inoculation were used as control. The supernatant was isolated and its absorbance was read at 645 and 663 nm using a spectrophotometer (AquaLabo, Iran). The chlorophyll content was measured using the following formula [17].

$$\text{Total Chl (g.Lit-1)} = (0.0202) \times (\text{OD } 645) + (0.00802) \times (\text{OD } 663) \quad (2)$$

Statistical Analysis

Data were analyzed by one-way ANOVA using SAS 9.1.3 statistical software packages. Treatment means were separated by Duncan's multiple range test at the 5% level of significance.

RESULTS

Confirmation of the Presence and Stability of the Chimera Chitinase Gene

The presence and stability of chimeric chitinase in *Trichoderma* strains were confirmed using PCR analysis. Within 2 years, the fungi containing chimeric genes were sub-cultured on the PDA medium. Then, using specific primers [(PF1-R3*xba1*) and (PF1-R3chit42)], the presence of the domain, a fragment of approximately 237 bp, was confirmed by PCR technique (Figure 2 a, b).

Two bands of 1512, 1275 bp, and a single band of 1275 bp were obtained in transgenic and wild type strains with (PF1-R3*xba1*) primers, respectively (Figure 3).

In addition, the bands of 1081 bp were amplified with (FP1-R3chit42) primers from chimeric gene in the transgenic strains while smaller fragments were amplified from the wild chit42 allele, both in the wild type and in the transgenic strains. The presence of ChBD fragment in the chitinase gene of optimized isolates was confirmed by PCR results.

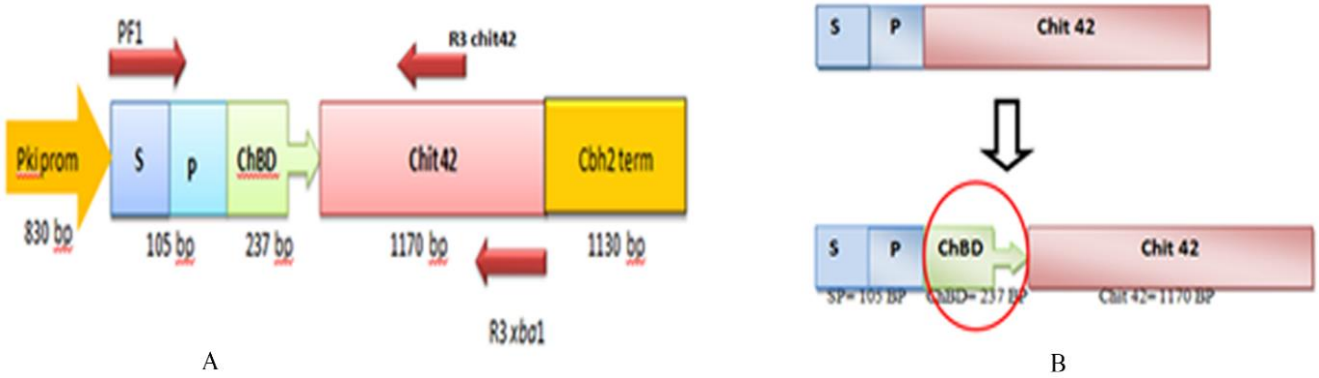

Figure 2. A *T. atroviride* Chitinase 18-10 fused to N-terminal end of Chit 42 just after signal peptide domain, B Schematic of chimeric Chitinase 42 gene with primer (PF1 / R3chit 42) and (PF1 / R3*xba*1).

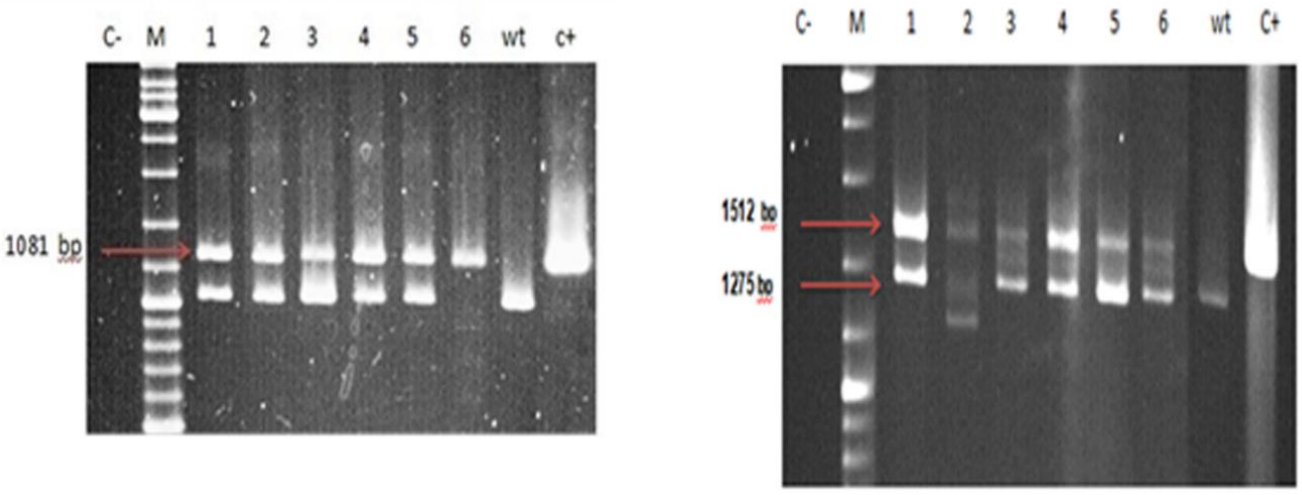

Figure 3. PCR Analysis of chimeric Chitinase gene stability in *Trichoderma* isolates with primers (PF1/R3*xba*1) and (PF1 / R3chit 42). C-: sample without DNA, M: Ladder mix 100bp, 1: Chit42- ChBD3, 2: Chit42- ChBD6, 3: Chit42- ChBD7, 4: Chit42- ChBD11, 5: Chit42- ChBD13, 6: Chit42- ChBD15, Wt: *T. harzianum* (T8-7), C+: PEJT plasmid.

In Vitro Biocontrol Activity

The dual culture of the fungal pathogen with engineered strains of *Trichoderma* cause to a reduction in radial growth of pathogen after 72 hours. This can prove biocontrol effect of these strains. The engineered strain Chit42-ChBD3 with a maximum inhabitation of 80% in growth followed by Chit42-ChBD6, Chit42-ChBD11, and wild type were considered as the best biological agents. All *Trichoderma* isolates had overgrown the colony of *R. solani* in different degrees (Figure 4).

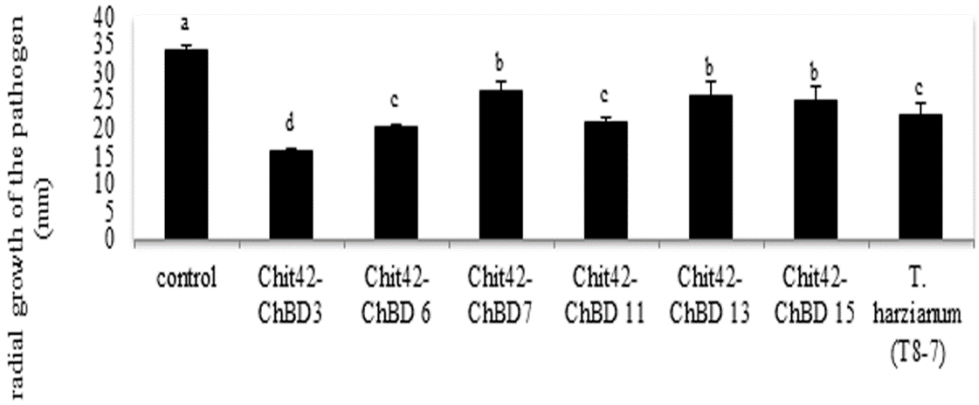

Figure 4. Macroscopic evaluation (observation) of in vitro dual culture of *Trichoderma* and *Rhizoctonia* strains. At 72 hours of culture. Control petri dishes inoculated with *Rhizoctonia*. Each value is mean of three replicates ± SE. Statistically significant differences (Duncan's Multiple Range test at p. value ≤ 0.05).

In Vivo Biocontrol Activity

The results of root rot percentage affected by *R. solani* in the presence of *Trichoderma isolates* indicated that the pots treated with Chit42- ChBD3, Chit42- ChBD7, and Chit42- ChBD15 isolates at different times of growth had minimum disease symptoms while, on average, the wild type isolate had maximum disease symptoms in bean root, especially at the time of harvest (Table 2).

Table 2. Root rot Severity of beans (%) under greenhouse condition at various stages of growth. Each value is mean of five replicates ± SE. statistically significant differences (Duncan's Multiple Range test at p≤0.05).

Harvest	Early reproductive growth	A month after planting	Two leaves	*Trichoderma* isolates
35-40^{c}	25^{cd}	35 ^{bcd}	40^{bc}	Chit42- ChBD3
35-40^{c}	30^{bc}	≤ 30^{d}	45-50^{b}	Chit42- ChBD6
35-40^{c}	20^{d}	≤ 30 ^{ed}	40^{bc}	Chit42- ChBD7
60-70^{b}	30^{bc}	38^{bc}	45-50^{b}	Chit42- ChBD11
35-40^{c}	40^{b}	40^{b}	40^{bc}	Chit42- ChBD13
35-40^{c}	25^{cd}	30^{cd}	45-50^{b}	Chit42- ChBD15
60-70^{b}	25^{cd}	30^{cd}	40^{bc}	*T. harzianum (*T8-7)
0^{d}	0^{e}	0^{e}	0^{c}	Control (plant)
100^{a}	100^{a}	100^{a}	100^{a}	Control (with pathogen)

Growth Analysis of Bean in Greenhouse

The results showed that the growth of bean roots in pots treated with *Trichoderma* isolates were significantly promoted in terms of weight and volume (Figure 5 and Table 3). The fresh weight of roots in pots treated with *Trichoderma* isolates was significantly increased compared to those grown in pots without treatment (Table 3).

The isolates Chit42- ChBD3, Chit42- ChBD7 and Chit42- ChBD13, and Chit42- ChBD15 showed an average 67% increase of fresh weight compared to the control, but the difference in weight was not statistically significant. In the experiments with a pathogen, root fresh weight in the treated pots with engineered isolates was approximately 60% higher than those of wild and control isolates. The highest root dry weight was obtained in optimized isolates of Chit42- ChBD13 and Chit42- ChBD15 with 70% increase compared to control, which were chosen as the best isolates of engineered *Trichoderma* in root growth (Table 4).

Results of fresh stem weights at early stages of bean growth showed that the optimized isolates of Chit42- ChBD11 and Chit42- ChBD15 in the absence of pathogens and the isolate Chit42- ChBD13 in the presence of pathogen had the highest growth rates compared to wild isolates (Figure 2, 3). In the study of changes in total chlorophyll content and fresh weights of leaves in optimized isolates compared to controls

and wild isolates, the results showed that in the presence and absence of pathogens, the isolate Chit42-ChBD15 and Chit42- ChBD13, Chit42- ChBD15, and Chit42- ChBD7 had the highest number of leaves with an increase of 34% compared to control, respectively. Also, isolate Chit42 ChBD13 had the highest total chlorophyll content (Figure. 2, 3). Comparison of the crop yield between plants treated with optimized and wild *Trichoderma* isolates revealed that the plant with isolate Chit42- ChBD15 had the highest number of pods per plant and the highest number of seeds in both non-pathogenic and pathogenic conditions. The plant treated with optimized isolate in the presence of the pathogen, indicate an increase of nearly 49% in the number of bean pods per plant. Isolates Chit42- ChBD6 and Chit42- ChBD11 also had an acceptable yield in the presence of the pathogen (Figure 2, 3).

Table 3. The results of morphological characteristics analysis of bean plant inoculated with *Trichoderma* strains. Pots (1 to 6) containing seeds treated with engineered *Trichoderma* strains. Pots (7) containing seeds treated with wild type *Trichoderma* strains Pots (8) containing non-treated seeds. Each value is mean of five replicates ± SE. statistically significant differences (Duncan's Multiple Range test at p≤0.05).

Pots	*Trichoderma* isolates	Fresh weight root (g)	Dry weight root (g)	Fresh weight stem (g)	Fresh weight leaf (g)	Chlorophyll g/L	Number of pods/per plant	Number of seed/ per pod
1	Chit42- ChBD3	42^{a}	6.75^{c}	10.1^{ab}	9.25^{e}	0.001505^{ab}	9^{ab}	3.42^{bc}
2	Chit42- ChBD6	17.53^{e}	6.7^{c}	9.85^{bc}	12.38^{ed}	0.001305^{b}	7^{ab}	3.3^{c}
3	Chit42- ChBD7	35.64^{b}	6.97^{c}	8.67^{d}	12.33^{ed}	0.001295^{b}	6^{bc}	4.47^{a}
4	Chit42- ChBD11	33.15^{c}	8.82^{b}	10.33^{a}	18.87^{bc}	0.001458^{ab}	8.5^{abc}	3.72^{abc}
5	Chit42- ChBD13	27.82^{d}	10^{a}	8.92^{d}	17.51^{bcd}	0.001422^{ab}	8^{bc}	3.8^{abc}
6	Chit42- ChBD15	33.52^{c}	11.79^{a}	10.36^{a}	25.76^{a}	0.001429^{ab}	12^{a}	4.0^{a}
7	*T. harzianum* (T8-7)	11.85^{f}	5^{d}	9.65^{c}	18.43^{bcd}	0.001532^{a}	5^{c}	3.84^{abc}
8	Control (plant)	11.62^{f}	2.98^{e}	9.748^{c}	24.08^{ab}	0.001426^{ab}	11.66^{ab}	3.63^{bc}

Isolates identified with same letters in each column indicate no significant difference at p≤0.05.

Table 4. The results of morphological characteristics analysis of bean plant inoculated with *Trichoderma* strains and *Rhizoctonia*. Pots (9 to 14) containing seeds treated with engineered *Trichoderma* strains and *Rhizoctonia*. Pots (15) containing seeds treated with wild type *Trichoderma* and *Rhizoctonia*. Pots (16) inoculated with *Rhizoctonia*. Each value is mean of five replicates ± *SE*. statistically significant differences (Duncan's Multiple Range test at p≤0.05).

Pots	*Trichoderma* isolates	Fresh weight root (g)	Dry weight root (g)	Fresh weight stem (g)	Fresh weight leaf (g)	Chlorophyll g/L	Number of pods/per plant	Number of seed/ per pod
9	Chit42- ChBD3+R	27.98^{ab}	6.44^{d}	8.61^{ab}	18.99^{b}	0.001612^{bc}	10.8^{b}	3.53^{ab}
10	Chit42- ChBD6+R	31.53^{a}	7.39^{c}	8.946^{ab}	18.11^{b}	0.001472^{c}	19.2^{ab}	3.36^{ab}
11	Chit42- ChBD7+R	31.3^{a}	7.25^{c}	9.364^{ab}	28.77^{a}	0.001745^{b}	10^{b}	2.35^{c}
12	Chit42- ChBD11+R	23.3^{abc}	9.15^{b}	9.546^{ab}	6.99^{d}	0.001596^{bc}	11.4^{ab}	3.58^{ab}
13	Chit42- ChBD13+R	38.99^{a}	10.91^{a}	12.03^{a}	28.36^{a}	0.001949^{a}	9^{b}	3.94^{a}
14	Chit42- ChBD15+R	39.77^{a}	11.19^{a}	9.572^{ab}	24.7^{a}	0.00155^{bc}	19.75^{a}	3.70^{ab}
15	*T. harzianum* (T8-7)+R	12.53^{c}	4^{e}	9.36^{ab}	12.09^{c}	0.001613^{bc}	7.2^{b}	2.84^{ab}
16	Control (with pathogen)	11.5^{c}	2.8^{f}	5.398^{b}	17.35^{b}	0.001501^{c}	10^{b}	3.6^{ab}

Isolates identified with same letters in each column indicate no significant difference at p≤0.05.

Figure 5. Comparison roots inoculated with engineered *Trichoderma* with control. Left to right: Chit42- ChBD3, Chit42- ChBD6, Chit42- ChBD7, Chit42- ChBD11, Chit42- ChBD13, Chit42- ChBD15, control.

DISCUSSION

In vitro and in vivo investigation of antagonistic effects of *Trichoderma*, i.e., the genetically modified *Trichoderma* demonstrated promoted growth compared to the wild type and that the antagonism of *Trichoderma* strains typically correlated with the secretion of enzymes degrading fungal cell wall, including chitinase type *Trichoderma*. Hashimoto and coauthors (2000) reported that the chitinase containing the ChBD domain can efficiently degrade chitin compared to chitinase lacking the ChBD domain [18]. Contreras-Cornejo, and coauthors (2009) evaluated the role of auxin in modulating the growth of Arabidopsis seedlings treated with *Trichoderma*. They reported that treating plants with *Trichoderma* improves biomass production and incites the growth of lateral roots [19]. We evaluate the biocontrol potential of genetically modified *Trichoderma* based on morphological traits, including the fresh weight of root, shoot, and leaf, the number of pod per plant and seed per pod. In this study, genetically modified *Trichoderma* was able to promote plant growth and increase tolerance against *R. solani* as a soil-borne pathogen better than the wild type *Trichoderma* isolate. The plants were treated with wild type and the improved *Trichoderma* (Chit42- ChBD13, Chit42- ChBD15) showed on average about 1.7 and 3.7 fold rises in root dry weight compared to untreated plant, respectively. In the same way, in the presence of *R. solani*, wild type and improved *Trichoderma* (Chit42- ChBD13, Chit42- ChBD 15) were able to increase root dry weight on average 1.4 and 3.8 fold compared to control plants infected with the fungal pathogen, respectively. Colonization of bean root by *Trichoderma* caused the activation of different developmental and defense responses. A plant exposed to *Trichoderma* can efficiently uptake nutrients and utilize fertilizer, which subsequently leads to increased root growth [20]. Also, different studies have proved that the activation of jasmonic acid, auxin, and other pathways can lead to the induction of systemic resistance in plants [20]. Mayo and coauthors, showed that the root dry weight of uninfected bean plants treated with *Trichoderma* increases 1.02 fold compared to untreated plants. While in conditions of fungal infection with *R. solani*, the dry weight of treated plants increases 1.05 fold compared to untreated plants [3].

In the presence and absence of the pathogen, a plant treated with improved *Trichoderma* showed higher performance in terms of increasing fresh weight of plant shoot relative to a plant treated with the wild type *Trichoderma*. Interestingly, when there was no pathogen, the plants treated with improved *Trichoderma* (Chit42- ChBD11, Chit42- ChBD15) showed 1.06-fold rise in fresh weight of shoot compared to the untreated plant. Nevertheless, this rise in shoot fresh weight of an infected plant was 1.7 fold compared to an infected control plant, which is a function of improved growth of the plant encountered with a pathogen. The results obtained from analyzing leaf fresh weight indicated that the infected bean plant treated with *Trichoderma* had a higher weight than the *Trichoderma* untreated plant inoculated with the pathogen. Increased fresh weight can improve photosynthesis efficiency, plant growth and its tolerance against diseases. Infected plants exposed to Trichoderma showed a higher chlorophyll content compared to the plants untreated with *Trichoderma*. Lee and coauthors (2016) demonstrated 71 and 82.1% elevated chlorophyll content in tomato and bean, respectively [21]. Alexandru and coauthors (2013) reported increased content of chlorophyll in infected tomato plant treated with *Trichoderma* [22]. *Trichoderma* can decrease the damage arising from the accumulation of reactive oxygen species in stressed plants, subsequently leading to increased photosynthesis efficiency and improved biotic stresses [23]. To understand the effects of *Trichoderma* treatment biocontrol on bean yield and its components, the number of pods per plant as well as the number

of seeds per pod were recorded from plants and pods at the harvest stage. The application of improved *Trichoderma* significantly increased the number of pods per plant. Abd El-Khair and coauthors (2010) reported 1.7 fold rise in the number of pods for infected plants treated with *Trichoderma* compared with a control infected plant [24].

CONCLUSION

1. *Trichoderma* inoculation increased the total biomass, seedling height, root length, root area, root diameter, number of root tips, and number growth potential of seedlings.
2. *Trichoderma* inoculation increases the resistance of bean plants to *Rhizoctonia* pathogen.

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REFERENCES

1. Zhang F, Ge H, Zhang F, Guo N, Wang Y, Chen L, et al. Biocontrol potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum* in soybean. Plant Physiol Biochem PPB. 2016 Mar; 100:64–74.
2. Bastakoti S, Belbase S, Manandhar S, Arjyal C. *Trichoderma* species as Biocontrol Agent against Soil Borne Fungal Pathogens. Nepal J Biotechnol. 2017 Dec 31; 5:39.
3. Mayo S, Gutiérrez S, Malmierca MG, Lorenzana A, Campelo MP, Hermosa R, et al. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. Front Plant Sci. 2015; 6:685.
4. Harman G, Björkman T, Shoresh M. Changing Paradigms on the Mode of Action and Uses of *Trichoderma* spp. for Biocontrol. Research Information Ltd. 2015.
5. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species--opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2004 Jan; 2(1):43–56.
6. Waghunde RR, Shelake RM, Sabalpara AN. *Trichoderma*: A significant fungus for agriculture and environment. Afr J Agric Res. 2016 Jun 2; 11(22):1952–65.
7. Yedidia I, Shoresh M, Kerem Z, Benhamou N, Kapulnik Y, Chet I. Concomitant induction of systemic resistance to Pseudomonas syringae pv. lachrymans in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. Appl Environ Microbiol. 2003 Dec; 69(12):7343–53.
8. Shoresh M, Harman G, Mastouri F. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annu Rev Phytopathol. 2010 Mar 1; 48:21–43.
9. Guthrie J, Khalif S, Castle A. An improved method for detection and quantification of chitinase activities. Can J Microbiol. 2005 Jul 1; 51:491–5.
10. Ryder LS, Harris BD, Soanes DM, Kershaw MJ, Talbot NJ, Thornton CR. Saprotrophic competitiveness and biocontrol fitness of a genetically modified strain of the plant-growth-promoting fungus *Trichoderma hamatum* GD12. Microbiol Read Engl. 2012 Jan; 158(Pt 1):84–97.
11. Kowsari M, Motallebi M, Zamani M. Protein engineering of chit42 towards improvement of chitinase and antifungal activities. Curr Microbiol. 2014 Apr; 68(4):495–502.
12. Limón MC, Margolles-Clark E, Benítez T, Penttilä M. Addition of substrate-binding domains increases substrate-binding capacity and specific activity of a chitinase from *Trichoderma harzianum*. FEMS Microbiol Lett. 2001 Apr 1; 198(1):57–63.
13. Kullnig C, Mach RL, Lorito M, Kubicek CP. Enzyme diffusion from *Trichoderma atroviride* (= *T. harzianum* P1) to Rhizoctonia solani is a prerequisite for triggering of *Trichoderma* ech42 gene expression before mycoparasitic contact. Appl Environ Microbiol. 2000 May; 66(5):2232–4.
14. Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC. Isolation and Characterization of *Trichoderma* spp. for Antagonistic Activity Against Root Rot and Foliar Pathogens. Indian J Microbiol. 2012 Jun; 52(2):137–44.
15. Pugliese M, Liu BP, Gullino ML, Garibaldi A. Selection of antagonists from compost to control soil-borne pathogens. J Plant Dis Prot. 2008 Oct 1; 115(5):220–8.
16. Kim DS, Cook RJ, Weller DM. *Bacillus* sp. L324-92 for Biological Control of Three Root Diseases of Wheat Grown with Reduced Tillage. Phytopathology. 1997 May; 87(5):551–8.
17. Arnon D, Allen MB. Photosynthesis by isolated chloroplasts. IV. General concept and comparison of three photochemical reactions. Biochim Biophys Acta. 1956; Jun; 20(3):449-61.
18. Hashimoto M, Ikegami T, Seino S, Ohuchi N, Fukada H, Sugiyama J, et al. Expression and Characterization of the Chitin-Binding Domain of Chitinase A1 from *Bacillus circulans* WL-12. J Bacteriol. 2000 Jun 1; 182(11):3045–54.

19. Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J. *Trichoderma virens*, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis. Plant Physiol. 2009 Mar; 149(3):1579–92.
20. Brotman Y, Landau U, Cuadros-Inostroza Á, Takayuki T, Fernie AR, Chet I, et al. *Trichoderma*-Plant Root Colonization: Escaping Early Plant Defense Responses and Activation of the Antioxidant Machinery for Saline Stress Tolerance. PLOS Pathog. 2013 Mar 14; 9(3):e1003221.
21. Lee S, Yap M, Behringer G, Hung R, Bennett JW. Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biol Biotechnol. 2016 Sep 29; 3(1):7.
22. Alexandru M, Lazăr D, Ene M, Şesan TE. Influence of some *Trichoderma* species on photosyntesis intensity and pigments in tomatoes. Romanian Biotechnol Lett. 2013 Jul 1; 18:8499–510.
23. Mastouri F, Björkman T, Harman GE. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology. 2010 Nov; 100(11):1213–21.
24. Abd-El-Khair H, Khalifa RKM, Haggag HE. Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. J Am Sci. 2010; 6(9).