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PLANT PHYSIOLOGY

# Seed bio-priming with *Trichoderma asperellum* effectively modulate plant growth promotion in pea

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**Abstract**

Seed biopriming is an advance technique of seed treatment that involves application of beneficial microorganisms on seed surface followed by seed hydration. Seed bioprimig is an ecological management strategy to control many seed and soil-borne pathogens which provide an alternative to chemical treatment. Seed biopriming enhance the initial step of plant development by increased seed germination and provide protection before seedling emergence. *Trichoderma* spp. is widely used as biocontrol agent that enhance plant growth as well as inhibits phytopathogens. In the present study, effectiveness of biopriming with *T. asperellum* BHUT8 evaluated for plant growth promotion effect in pea. The results showed enhancement in plant growth in the treated plants as compared to control. There was increase in shoot length, root length, number of leaves, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight by 35.29, 96.49, 28.13, 36.10, 146.26, 30.17 and 77.20 %, respectively, as compared to the control.

**Highlights**

- Seed Treated with $2.26 \times 10^7$ spores $ml^{-1}$ suspension and bioprimed for 24h
- Seed biopriming effectively enhance plant growth in pea

**Keywords:** Biopriming, pea, *Trichoderma asperellum,* plant growth promotion

Biological seed treatment is the most effective method to protect seeds from soil-borne pathogens at early stage of plant development (Singh *et al*. 2013a; Jain *et al*. 2013) and also effectively reduce the dependence on chemical fungicide for diseases management (Challan *et al*. 1997; Pill *et al*. 2009). Seed bio-priming is an advanced and prominent technique used to persuade plant health and stress tolerance. Most importantly, this ecological approach protects the seeds against various seed and soil borne pathogens by suppressing incidence of a disease (Harman and Taylor 1988; Jenson *et al*. 2004). Seed biopriming involves seed coating with a myriad of beneficial agriculturally importantly microbes leading to rapid and uniform seed colonization (Challan *et al*. 1997). This promising biological seed treatment method provides enhanced stress tolerance to the seeds before germination (Harman and Taylor 1988; Jenson *et al*. 2004). However, if seeds are infected with unwanted indigenous microorganisms, they will proliferate during priming and may decrease the survivability of useful microbes (Wright *et al*. 2003), thus it is necessary to disinfect the seeds before priming (Pill *et al*. 2009).

*Trichoderma* spp. is one of the most common free-living saprophytic fungi in rhizosphere which widely occupy the major share of fungal biocontrol agents in biopesticide industry (Woo *et al*. 2014). *Trichoderma* spp. not only promotes plant growth and development but also have broad-spectrum antagonistic activities against various soil borne

phytopathogens (Singh *et al.* 2013b, Keswani *et al*. 2014). Due to above reasons, *Trichoderma* has been widely used for seed treatment (Taylor *et al*. 1991; Pill *et al*. 2009; Jain *et al*. 2014).

Seed treatment is very effective and economical compared to drenching as it requires small volume of inoculums (Bennett *et al*. 1992; Pill *et al.* 2009). In seed treatment, resting spores or conidia of *Trichoderma* are applied on the seed surface which must get geminated prior to interaction with pathogens. The presence of an active fungal agent in the spermoplane or spermosphere in enough quantity is must to protect geminating seeds (Pill *et al.* 2009). It was reported that *Pythium* species infect the seeds in less than 4 h after sowing while spore of *Trichoderma* needs nearly 12 h to germinate (Taylor *et al*. 1991; Pill *et al*. 2009). Considering to this, bio-priming is considered as the most effective method of seed protection. The main objective of the present work is to evaluatione the response of seed biopriming with *T. asperellum* BHUT8 on plant growth promotion in pea.

## Materials and Methods

### *Plant material*

In the present study seeds of pea (*Pisum sativum*) cv. NBR- Ruchi (Noble Seeds Pvt. Ltd., Khera Kalan, Delhi) were used.

### *Fungal strain and preparation of spore suspension*

*Trichoderma asperellum* BHUT8 (Accession no. KU533735) was grown on PDA for 7 days at 28 ± 2°C. The spores were harvested in sterilized saline (NaCl 0.85 %) and filtered with sterile muslin cloth. OD was observed at 600 nm with OD of 1.026 contained $2.26 \times 10^7$ spores $ml^{-1}$. The spore suspension was centrifuged at 10,000 rpm for 10 minutes. The pellet was resuspended in same volume of autoclaved 1.5 % CMC (Carboxymethylcellulose) (Jain *et al.* 2012).

### *Seed bio-priming with T. asperellum BHUT8*

The seeds of pea were surface sterilized with 1.5 % sodium hypochlorite (NaOCl) for 5 minutes and rinsed thrice with autoclaved distilled water and dried under laminar air flow on autoclaved blotting paper (Jain *et al*. 2012). The surface sterilized and dried seeds were treated by soaking in the spore suspensions prepared as described above and seeds treated with CMC only acted as control. The seeds were dried under sterile air stream in laminar air flow for 2 h (Singh *et al*. 2013a). The seeds were placed in the moist chamber at 98 % relative humidity and 28 – 30°C, and maintained for 24 h (Jensen *et al.* 2004).

### *Pot Trial*

A soil mixture of vermicompost and sandy soil (1:3) was filled in the autoclavable polypropylene bags and sterilized for three consecutive days at 15 lb pressure for 30 min in an autoclave and was filled in 150 ml thermocol glass. The bioprimed pea seeds were grown under controlled environment in a plant growth chamber (Model LGC – 5301, Daihan Labtech Co., LTD) with light duration of 14 h and dark 10 h having relative humidity range of 50 – 70 %. Air temperature of the plant growth chamber ranged from 22 to 28°C, while night temperature was maintained at 18°C. The plants were grown in thermocol pots of 150 ml volume with 10 replicates for each treatment and a control set was maintained. 4 seeds per pot was sown and irrigated manually every alternate day. Plants were harvested from each control and treatment set after 15 days of germination and the samples were analyzed for plant growth promotion parameters such as shoot length, root length, number of leaves, fresh weight and plant biomass. Fresh weight was measured immediately after harvesting while for measuring dry biomass, plants were dried in oven at 60°C for 48 h.

## Statistical Analysis

Statistical analyses were performed using SPSS 16.0. To assess the potential of fungal isolate on plant growth, standard errors of means (SE) for each treatment were calculated.

## Results and Discussion

Seed priming is an important tool to improve emergence of crops, especially under the stress conditions (Pill *et al*. 2009; Rakshit *et al.* 2014). Earlier works showed that combining seed priming with biocontrol agent application ultimately resulted in improvement in crops and different methods were utilized for biopriming (Harman and Taylor, 1988; Callan *et al.* 1991; Jensen *et al*. 2004). *Trichoderma*


**Fig. 1: Plant growth promotion in pea after seed boipriming with Trichoderma asperellum BHUT8. A: Control plants; B: Treatment (Bio-primed).**

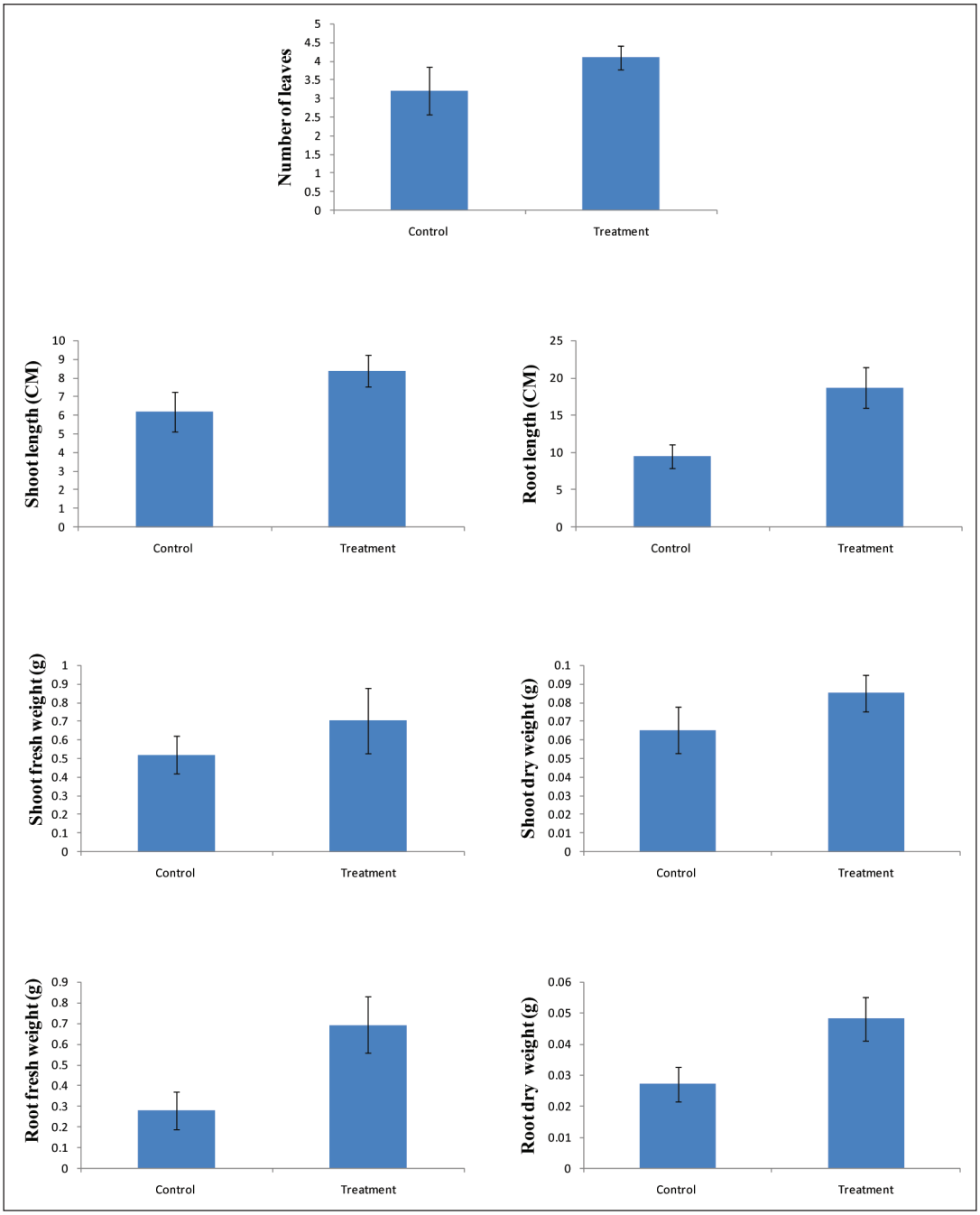


**Fig. 2: Different plant growth promotion parameters analyzed on pea after seed bio-primed with of *T. asperellum.* Results are expressed as means of ten replicates and vertical bar indicate the standard deviation of the means.**

spp. is the most common saprophytic fungus in rhizosophere which act as mycoparasite on pathogenic fungi and on the other hand it stimulates plant growth as well (Singh *et al*. 2013b; Meena *et al.* 2016; Rakshit *et al*. 2015b). *Trichoderma* spp. is a well known biocontrol agent used in seed biopriming (Harman and Taylor, 1988; Pill *et al*. 2009).

In the present study, we evaluated the effect of biopriming of pea seeds with *T. asperellum* BHUT8 on plant growth. The results showed enhanced plant growth in the treatment as compared to the control plants (Figure 1). There was increase in shoot length, root length, number of leaves, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight by 35.29, 96.49, 28.13, 36.10, 146.26, 30.17 and 77.20 %, respectively, as compared to control (Figure 2). Similarly, Saxena *et al*. (2015) reported enhancement in root and shoot lengths along with dry weight of plants with significant increase in the number of leaf in the plants treated with the *Trichodemra* isolate BHUF4. It was well studied that *Trichoderma* spp. enhances plant growth by increasing nutrient uptake (Harman *et al*. 2004; Rakshit *et al.* 2013) along with induction of secondary root development through auxins and indoles production (Contreras-conrnejo *et al*. 2009, 2014a,b). Phosphorus is an important nutrient that is essential for plant growth and development which is generally present in unavailable form. Many microorganisms including *Trichoderma* spp. produce organic acids and phosphatase that solubilise the unavailable phosphate to available phosphate that can be easily absorbed by plants. *Trichoderma* spp. also helps to increase the nitrogen use efficiency in plants (Rakshit *et al*. 2015a).

Singh *et al*. (2013a) studied the effect of triple microbes i.e. *Trichoderma harzianum* THU 0816, *Pseudomonas aeruginosa* PHU 094 and *Mesohizobium* sp. RL 091 on plant growth in chickpea. They reported that the triple microbe combination RL091 + PHU094 + THU0 816 showed enhancement in the plant growth parameters as compared to control and other treatment combinations. The increased in growth parameters may be due to the contribution of substances produced by the three microbes. Biopriming of chickpea and rajma seeds with *Pseudomonas fluorescens* OKC, *Rhizobium* sp. RH4 and *Trichoderma asperellum* T42 enhanced in seed germination and plant growth parameters (Yadav *et al*. 2013; Singh *et al.* 2014). Similarly, Jain *et al*. (2015) investigated application of three microbe consortium in pea that resulted improved growth and yield with reduced disease compared to control and single and double microbe applications following challenge with the pathogen *Sclerotinia sclerotiorum*. Thus we concluded that seed biopriming is a very effective method for seed treatment that ultimately results in enhancing the plant growth in pea.

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