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Synergistic Effect of *Trichoderma reesei* and *Fusarium solani* on growth and yield of Barley plant

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ABSTRACT

Chemical fertilizers are known to improve plant yield but on the cost of environment, soil health as well as human health. Thus, alternatives of chemical fertilizers are the demand of time. Plant growth-promoting fungi (PGPF) are using nowadays as an alternative of chemical fertilizers to improve the growth, yield, and plant nutrient uptake. They are also involved in the improvement of rhizosphere fertility, without compromising environment and human health. A total of seven fungal isolates were obtained from rhizospheric soil of two crop plants *viz*., turnip and wheat. Fungal strains were examined for their *In vitro* plant growth promotional traits. A total of two strains found positive for 4 or more plant growth promoting traits. These potential fungal strains were characterized by amplification and sequencing of ITS region and were identified as *Trichoderma reesei*, and another was identified as *Fusarium solani.* Our current study is aimed to screen the effect of single inoculation as well as paired fungal inoculations and to provide evidence for the synergistic effects of paired isolates having high impact on growth and development of barley plant.

Key words: *Plant growth promoting fungi, Trichoderma, Fusarium, Barley, Rhizospheric soil*

Introduction

Barley (*Hordeum vulgare*) is considered as world's fourth largest crop grown after maize (*Zeamayz),* rice (*Oryza sativa*), and wheat (*Triticum aestivum*) and is grown as a Rabi crop (Kebede *et al.,* 2019). This crop is generally cultivated in arid and semi-arid areas. Barley has a considerable economic impact due to its widespread distribution and use. More than 140 million tonnes (Mt) of barley are typically harvested annually on almost 50 million hectares (Mha) of land worldwide (Tricase *et al.,* 2018). However, a variety of biotic and abiotic stressors make it difficult to increase barley output which make it difficult to produce higher amount of Barley. But due to growing demand for the barley crop for benefiting the whole population of the world in an environmentally-safe and cost-effective mean have led to a keen interest and necessity regarding usage of chemical fertilizers. The chemical fertilizers are generally utilized to get higher yields of crops and increase the nutrients availability in organic manures (Ayalew and Dejene, 2012). Therefore, these organic fertilizers help the plant to cope up with various environmental conditions and protect them from various plant pathogenic attacks. However, the excessive use of these chemical fertilizers can lead to changes to various aspects of the crop causing seri-

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ous damage to the environment, acidification to soil crust, lowering the amount of potential micro-organisms (Bisht and Chauhan, 2020). These potentially dangerous compounds can survive and build up in natural environments. One strategy to solve this issue is switching from chemical solutions to biological ones, which are thought to be more environmentally friendly in the long term. These biological agents play a vital role in agriculture, encouraging the circulation of nutrients in plants and minimizing the use of chemical fertilizers. In reference to this, there are multiple numbers of beneficial fungal genera's such as *Trichoderma, Aspergillus, Penicillium*, and *Fusarium* promoting the plant growth and development as well as protecting the crops from different diseases. However, species of *Trichoderma* and *Fusarium* such as *Trichoderma reesei* and *Fusarium solani* have wide ranging applications in field of agricultural sciences. Genus *Fusarium* is capable of growing on a variety of substances and is prevalent in soil and organic substrates. They are non-pathogenic isolates which act as plant growth promoters as well as biocontrol agents. Similarly, Genus *Trichoderma* are is one of the important plant growth promoters which mediate beneficial changes in plant development and health of the plant (Hossain and Sultana, 2020). In view of the above, the current research work was carried out to screen the effect *Trichoderma reesei* and *Fusarium solani* as plant growth promoting fungi and their impact on the Barley plant growth and sustainable development.

Materials and Methods

Collection of soil sample

For the purpose of present study, soil samples were collected from rhizospheric soil samples of crops turnip and wheat from adjacent agricultural fields of Chandigarh which lies between coordinates of 30.7688° N, 76.5746° E. The soil samples were kept in sterilized poly bags and transferred from field to laboratory for further research.

Isolation of Fungal isolates from soil samples

The isolation of fungi from the collected soil samples was carried out by serial dilution method and culturing them on Potato Dextrose Agar medium. Medium plates containing fungal isolates were incubated at 28±1°C for 48-72 hours. Fully grown colonies were isolated, purified and grown individually.

Identification and characterization of fungal isolates

The morphological of fungal isolates was done on the basis of their colony morphology such as size, color, shape of the fungal colonies. Additionally, the microscopic characterization was also done on the basis of the structure of hyphae and the type of conidiation by using lacto-phenol cotton blue stain.

Plant growth promoting characterization

These isolates were assessed for their ability of showing various promote plant growth traits. Various plant growth promoting tests *viz*. Phosphate solubilizing test, Zinc solubilizing test, Indole acetic acid production test, hydrogen cyanide test and siderophore production test were performed.

Molecular Identification

The fungal isolates showing various plant growth promoting traits were further selected for molecular identification using sequencing of ITS region of genome. The amplification was carried out using forward and reverse primers as ITS 1 (F) 5′-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2(R) 5′-GCTGCTTCTTCATCGATGC-3'.To assess the degree of similarity, the obtained fungal sequences were compared by $BLAST_N$ search against the NCBI database.

Results

Physico-chemical properties of soil

The soil's physical and chemical characteristics were studied where electrical conductivity (EC)of wheat soil sample was recorded as 29mS/m and pH was reported to be basic in nature (pH>7.5). Similarly, the organic carbon (C) in soil was 0.39% with total nitrogen (N) as 0.029% and organic matter content measured as 0.69%.While as in turnip soil sample, the soil electrical conductivity was measured as 36mS/m and pH was alkaline (pH>7.3). Additionally, the total organic carbon (C) percentage was 0.56% with nitrogen percentage as 0.04% as well as organic matter content as 1.02% respectively (Table 1).

Colony morphology and microscopic examination

The morphology and microscopic characteristics of fungal isolates was studied on the basis of various shape, color and sizes on medium plates. The mor-

Table 1. Physico-chemical properties of rhizospheric soil samples

S.N.	Soil Sample	pH	Electrical conductivity	Organic Carbon (%)	Organic Matter (%)	Total Nitrogen (%)
1.	Turnip	7.3	0.36	0.56	1.02	0.04
2.	Wheat	7.5	0.29	0.39	0.69	0.029

phology of isolates such as N1K1 and 17F showed pale yellow, radial shaped colonies and had filamentous structure with presence of conidiophores under light microscopic examination which were recognized as *Aspergillus* specie. Similarly, isolates *viz.*, P1S1, P2S1 and 14F exhibited round, light green colony characteristics as well as showed branched and un-branched filamentous hyphae under microscope was identified as *Trichoderma*. Moreover, isolate 11F colony appeared as white, cotton-like colony and microscopic structure as septate hyphae with stout macro conidia's and was identified as *Fusarium.* In addition, isolate 3F appeared as light green, wooly colonies and under microscopical structure as branched hyphae with conidiophores and was identified as *Penicillium*.

Plant Growth Promoting (PGP) traits of isolates

The isolates were examined for all plant growth promoting traits, where out of seven isolated strains only one isolate (14F) showed positive results for all the PGP traits such as HCN production, IAA Production, phosphate solubilizing test and siderophore production test as well as Zinc solubilizing test while as another isolate (11F) gave positive results for three tests out of total five tests, shown in (Table 2). This study validates the findings of several researchers that concluded *Fusarium solani* and *Trichoderma reesei* exhibit various plant growth promoting (PGP) traits.

Molecular identification of potential fungal isolates

The fungal isolates such as 11F and 14F were further subjected to molecular characterization at species level. The obtained fungal sequences were compared by $BLAST_N$ search against the NCBI database to assess the degree of similarity. Based on the sequences containing ITS region, the isolate 11F was identified as *Fusarium solanii* with 100% similarity percentage in comparison with the reference sequences. Similarly isolate 14F showed 99% similarity and was identified as *Trichoderma reesei.* The ITS gene region containing sequences were submitted to NCBI portal and accession number were provided for isolate 11F as OQ581866 and isolate 14F as OQ581865 respectively.

Phylogenetic studies

A phylogenetic tree was constructed using CLUSTALW program (MEGA X 10.2.6 Version), the bootstrap of 1000 replications was used to test the phylogeny. The fungal isolates phylogenetic analysis showed that the isolates were grouped in manners that closely resembled one another. The evolutionary studies showed that one isolate was closely related to Genus *Trichoderma* with 99-100% similarity. In addition, another isolate studied was closely

Table 2. Qualitative plant growth promoting traits of the fungal isolates

Isolates	IAA production (mg/ml)	Phosphate solubilizing test	HCN production test	Zinc solubilizing test	Siderophore production test
14F	+	+	+	+	+
11F	+	+	–	+	–

Table 3. Inoculation effect of *Trichoderma reesei* and *Fusarium solanii* in single and in combination on barley plant

Treatment	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Seeds/plant
Control	54.4 ±1.4	2.11 ±0.20	0.78 ±1.1	39
14F	67.33±2.3	3.02 ±1.2	1.2 ±1.7	64
11F	61.23±1.5	2.16±0.25	1.1±1.4	72
14F+11F	69.28±2.5	3.07±1.5	1.7±1.8	79

related to Genus *Fusarium* with 98-99% similarity presented in (Fig. 1).

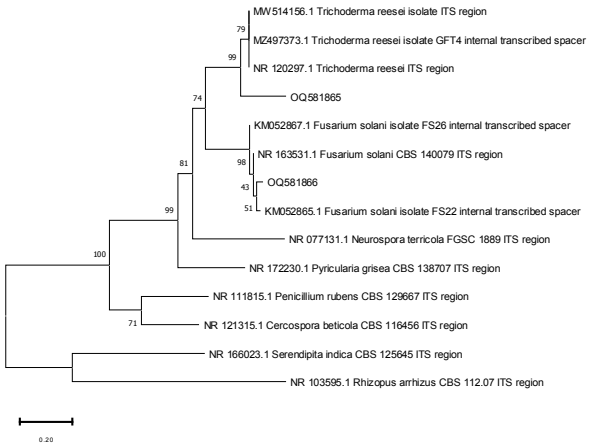

Fig. 1. Phylogenetic tree (N-J tree) showing the relationship between *Trichoderma* and *Fusarium* isolates and other type strains based on ITS region DNA sequences for structure analysis; bootstrap values derived from 1000 replicates.

Effect of treatment of *Trichoderma pleuroticola* and *Aspergillus alboviridis* on Barley

The treatment of fungal strains including *Trichoderma reesei* and *Fusarium solani* was carried out on Barley seeds of variety PL-426. The inoculation of selected fungal isolate such as 14F showed an increased plant growth and development in terms of total plant length, plant dry weight, wet weight as well as the seed numbers in comparison to untreated plants. Similarly, inoculation of fungal isolate 11F on barley plants showed improved plant growth and increased number of seeds/per plant as compared to the control plant seeds. However, the combined treatments of isolates 11F and 14F on barley plants in controlled concentrations provided a synergistic effect on the overall growth of the barley plants *viz.* Plant length (cm), fresh as well as dry weight of root/shoot weight and higher amounts of seeds were produced/plant.

Discussion

The aim of this study was isolation of potential plant growth promoting fungi. Plant growth promoting microorganisms are type of non pathogenic organisms which are present in nature that enhance the plant health. In this study, seven fungal isolates were obtained from rhizospheric soils of Wheat and Turnip. Isolates were further examined for their plant growth promotional traits. IAA is a naturally available and most prevalent phytohormone, which is essential for plant growth. IAA hormone plays a key role in various developmental events such as regulating root elongation, the formation of xylem and phloem tissues, and the process of plant development (Caumon and Vernoux, 2023). It is important for the initiation of primary and secondary roots inducing the lateral root growth, adventitious roots, and a dose-dependent increase in the length of root hairs which are generated from the epidermis. In absence of auxins, the cells cannot divide or expand, which prevents the growth of the shoot. In addition to this, the absence of IAA also leads to inhibition of many processes such as cell development, germination process, root elongation, formation of vascular bundles and so on.In our study, out of the total 7 fungal isolates, only two isolates i.e.; 11F, 17F showed positive results for the production of IAA. Our results are in agreement with the results of different researchers (Islam and Datta, 2017; Ikram *et al.,* 2019). Hydrogen cyanide is a gaseous acid with glycine, threonine, and serine as radioisotope precursors. It is produced in nature by various microbes including fungi; bacteria as well as algae. Hydrogen cyanide often functions as a biocontrol agent in agricultural systems to increase growth production due to its considerable toxicity against phytopathogenic agents (Pandey *et al.,* 2023). In addition to this, hydrogen cyanide (HCN) serves as a messenger molecule that regulates some metabolic activities in case of plants. The deficiency of Hydrogen cyanide in plants can led to different herbivores and other pathogenic attacks and similarly the plant metabolism will be affected. In our work, all the seven isolates were tested for the production of Hydrogen cyanide and only one isolate (14F) found positive for HCN production, while as isolate (11F) tested negative for HCN production. Our study is in agreement with (Lalngaihawmi, 2019). Zinc (Zn) has been identified as an important element for plants and is essential for growth of plants (Pandey *et al.,* 2018a). It also comprises of the component which are required in smaller quantities that is necessary for the actions of many enzymes and proteins (Chand and Kumar, 2020; Chitramani, 2020; Sharma and Kumar, 2020). It plays an important role in promoting multiple metabolic and physiological processes in plants and encourages growth, develop-

ment, and production. Zinc (Zn) controls a number of biological and molecular events that help crops escape stress caused by drought. Zn deficiencies develop abnormalities in plants and in case of acute zinc deficiency growth, various signs such as chlorosis of the leaves, spikelet sterility, small leaves. The results of our study, isolates 11F and 14F showed positive results for zinc solubilizing test. Our recent work is in agreement of the recent work of scientific communities (Hong *et al.,* 2010) and other researchers who studied Zinc production in *Trichoderma* species (Ali *et al.,* 2022; Shobha *et al.,* 2020). Phosphate is considered as the second most important macronutrient in plants which have a useful impact on plant development and growth after nitrogen (Bechtaoui *et al.,* 2021). The most vital role of phosphate is the regulation of multiple enzymes (Xu *et al.,* 2018). Phosphorus is an essential component of numerous energy rich products involving guanosine triphosphate (GTP), adenosine triphosphate (ATP) as well as of phosphoenol pyruvate. The unavailibity of phosphate in plants can change metabolic activities as well as alter the translocation of carbohydrates like organic acids and soluble sugars (Meng *et al.,* 2021). In our study, a total of one fungal strain found able to solubilize phosphate compound, i.e.; 14F, whereas isolate 11F gave negative results for phosphate solubilizing test. The same findings were examined by researchers (Mahato *et al.,* 2018) and other researchers also examined the results for phosphate solubilization by *Fusarium* spp. (Elias *et al.,* 2016). Siderophores are known as secondary metabolites which are synthesized by various organisms to chelate iron from adjacent surroundings allowing the element to be used by the cells (Albelda-Berenguer *et al.,* 2019). The main role of siderophore in plants is to scavenge iron and store the iron in the cells. This secondary metabolite has an importance in plants performing certain functions such as photosynthesis, growth promotion in plants, respiration (Singh *et al.,*2022). In our examination, from total of seven fungal isolates, i.e. 11F, 17F, 14F, 3F, P2S1, P1S1 and N1K1 only two strains such as 11F, 14F showed positive results for the siderophore production. Our current studies are in agreement with various researchers (Lehner *et al.,* 2013; Pandey *et al.,* 2018b; Pandey *et al.,* 2018c).

In our study fungal isolates were isolated from rhizospheric soils of two crop plants. Isolates were screened for their *in vitro* plant growth promotional activities. Out of total seven, two isolates found having multiple plant growth promoting traits and were identified as *Fusarium solanii* and *Trichoderma reesei* using sequencing of ITS regions. These potential fungal strains were inoculated in barley seeds variety PL-426;a popular variety of Barley grown in Punjab. The barley seeds upon inoculation with varied fungal isolates showed plant growth promoting effects such as plant height and weight (in terms of dry weight and wet weight) as well as production of seeds per plant. On treatment of barley seeds with isolate 14F overall plant growth was analyzed, such as plant length was measured as 67.33±2.3cm which was higher in comparison to un-treated plants as 54.4 ±1.4cm. Similarly, plant root dry weight as well as shoot was also measured higher than the control plants presented in (Table 3). Additionally, the amount of seeds produced recorded were (64/plant) greater than the control plants presented in (Table 3). Subsequently, barley plants inoculated with isolate 11F showed increased plant length as 61.23±1.5 as compared to control plants. Further, plant fresh/dry weights of root as well as shoot were higher than the un-treated plant and the seed production (72/plant) was increased on treatment with fungal isolate. Although, combined treatment of isolates 11F and 14F showed comparatively more growth than individual treatments as compared to control plants. The plant height increased by 69.28±2.5cm as well as fresh/dry weight of plants was also shown higher in amount (Table 3). Subsequently, more number of seed production was observed by 79/plant in comparison to control plants. It is therefore an effective combined strategy that may benefit the plant growth and development as well as be the best alternative for chemical fertilizers.

Conclusion

In current study, a total of two potential isolates were isolated from turnip and wheat rhizosphere and were analyzed on the basis of morphological and molecular characterization, as *Trichoderma reesei* and *Fusarium solani.* On treatment of these fungal strains individually and in combination to selected variety of barley plant, an increase in plant growth in terms of length as well as weight of plant was observed. Similarly, the seed production per plant in comparison to the un-treated plants also increased. In our study, we showed the positive impact of individual and combined inoculations and analyzed their plant growth promoting effects separately.

However, the application of these potential fungal strains in open agricultural field trails are required to uncover the potential of isolates for plant growth promotion and yield enhancement.

Statements and Declarations

There was no funding provided to carry on this research work. Authors are declaring that they have no conflict of interest.

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