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Bipartite interaction effect of *Trichoderma* on the root architect of Rice

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Abstract
Microorganisms play an essential role for nutrition management, disease prevention, and to promote plant growth. By eradicating plant diseases, the mycorrhizal fungi, some plant-growth-promoting rhizobacteria (PGPR), and fungi like *Trichoderma* spp. and *Piriformospora indica* can encourage plant growth. Some strains of *Trichoderma* that are rhizosphere-competent directly influence plants by increasing their capacity for growth, nitrogen uptake, and fertiliser utilisation. The focus of the current study is on the effects of ten different *Trichoderma* spp. (IRRI-1, IRRI-2, IRRI-3, IRRI-4, IRRI-5, IRRI-6, TH3 TV12 T_{14} 94a) on the root system morphology of four rice varieties (IR-64, Sahbhagi dhan, RRF-75, and Indira barani dhan-1). The rice genotypes IR64 and RRF75 with isolate T_{14}, Sahbhagi dhan with IRRI5, and Indira barani dhan with TV12 all showed the maximum root length. Following treatment with isolates IRRI-6, IRRI-4, IRRI-6, and IRRI-5, respectively, maximum shoot lengths were seen in IR-64, Sahbhagi dhan, RRF-75, and Indira Barani dhan. Spectrophotometric analysis of these isolates revealed a range of responses for the ACC deaminase activity test; isolate TV12 displayed the greatest activity (10.46 g/ml). Additionally, distinct gene-specific primers were used for the PCR-based amplification of several *Trichoderma* isolates. This research investigation will encourage breeders to select the desired genotypes on the basis of root architecture for drought tolerance.

Keywords: *Trichoderma*, ACC deaminase, *ethylene biosynthesis*

1. Introduction
The security of the world's food supply is under stake from plant diseases and pests. Overuse of chemical pesticides is a widespread practise to mitigate the impacts of bacterial and fungal pathogen-induced plant illnesses. The need to boost agricultural yields to feed an expanding population is a major challenge as we work towards more sustainable agriculture. The use of this method to control plant diseases, promote plant growth and performance, and boost output has been shown to be environmentally friendly. Microbes are viewed as a practical, alluring economic strategy for sustainable agriculture, culminating in a "win-win" scenario as biofertilizers and biopesticides. Through a variety of ways, these helpful bacteria stimulate plant growth in the rhizosphere and endo-rhizosphere (Saxena *et al*. 2005) [5]. However, the importance of microbes in the management of biotic and abiotic pressures is growing. Recent reviews (Venkateswarlu *et al*. 2008; Yang *et al*. 2009) [7, 3] of the PGPR activity produced tolerance to abiotic stressors are available. Additionally, ACC deaminase, which breaks down ACC, the immediate precursor of the plant hormone ethylene, to create -ketobutyrate and ammonia, can increase plant growth (Todorovic & Glick, 2008) [6]. When plants are exposed to abiotic challenges, such as pathogen attack, ethylene is a key signalling chemical that inhibits plant development. Longer roots and less suppression of plant growth owing to environmental or pathogen-induced stress are the outcomes of inoculating plants with PGPR producing ACC deaminase (ACCD), which lowers ethylene levels. Microorganisms may be crucial in managing stress, and strategies for their effective use in agricultural productivity are being explored.

2. Materials and Method
The root system morphology of the rice lines IR 64 (Drought sensitive line), Sahbhagi Dhan (drought resistant line), RRF 75 (Drought resistant line), and Indira Barani Dhan (drought resistant line) was examined in 10 genetically pure isolates of *Trichoderma* spp.

2.1 Acquiring Washed Roots

The first step is to get cleaned roots. If plants are grown in a solid media, it is the trickiest and most time-consuming step of the experiment. To completely remove the soil, the roots were washed twice with tap water. For root scanning, these roots were kept in 25% spirit.

2.2 Making Ready the Roots for Scanning

On the scanner, roots are placed in acrylic trays with water. This enables the roots to be positioned in a way that minimises overlap and root crossing. Roots were planted using plastic forceps.

2.3 Looking at Roots

With an approved scanner that allows the roots to be suspended from above and below while being scanned, Win Rhizo software is utilised for the best results. Regent's documentation system uses "Dual Scan" as its key feature to lessen shadows on the root image. Lower resolution greatly increases scanning, especially if the samples call for large trays. Images that were grayscale were used for root length studies.

2.4 Examining Scanned Documents

The software uses thresholding to distinguish between what is root and what is not root at the moment of root evaluation (Each pixel is distinguished as either root or not root depending on its grayscale value, which is why shadows in photos are problematic).

2.5 Qualitative estimation of ACC Deaminase activity

1. The harvested *Trichoderma* cells were centrifuged at 3000 g for 5 min, washed twice with sterile 0.1 M Tris-HCl (pH 7.5), and then re-suspended in 1 ml of the same solution. On Petri plates containing modified synthetic medium (SM; Yedida *et al*., 1991) supplemented with 3 mM CCA as the only source of nitrogen, the collected cells were injected.
2. Synthetic medium served as both a positive control with (NH4)2SO4 (0.2% w/v) and a negative control with ACC absent.
3. The inoculated plates underwent a 72-hour incubation period at 28 °C.
4. Positive controls were chosen based on the growth of isolates using ACC as a nitrogen source since the growth of isolates on plates supplemented with ACC was deemed to be negative.

2.6 Quantitative Estimation of ACC Deaminase activity

The production of α-ketobutyrate and ammonia produced by the cleavage of ACC by ACC deaminase concludes the ACC deaminase activity to be consistent (Honma and Shimomura, 1978; Penrose and Glick, 2003) [2, 4]. For the ACCD activity determination in *Trichoderma*, 20 µl of spore suspension was cultured on a synthetic medium (SM) for 48 hours. The mycelia was then washed and shifted to 5ml of SM without ammonium and with 0.3-3 mM ACC. The culture was again suspended in half volume of Tris buffer 0.1 M (pH 8.5) and homogenized at the end of the induction period. In 200 µl of aliquot, toluene (25µl) was added and vortexed vigorously for 30s. After that ACC (20 µl of 0.5 M solution) was added and mixed and after an incubation period of 15 min at 30°C, 0.56N HCl 1 ml was added. Lysates were centrifuged (10000g, 10 min) and 1ml of the supernatant was mixed with 800 µl 0.56N HCl and 300 µl of 2, 4-dinitrophenylhydrazine (0.2g in 100 ml of 2N HCl). The mixture was then incubated for 30 min at 30 °C, after which 2 ml of 2N NaOH was added. ACCD activity was assessed quantitatively by measuring the amount of α-ketobutyrate formed by the deamination of ACC. ACCD activity was expressed in µmol of α-ketobutyrate/ mg protein/hr at 540 nm.

2.7 Polymerase Chain Reaction (PCR) analysis of Isolates of *Trichoderma spp*

A primer pair (AcdS-F GGCAAGGTCGACATCTATGC, AcdS-R GGCTTGCCATTCAGCTATG with an expected product size of 1000 bp) was used for PCR based DNA fingerprinting for the analysis of 10 isolates of *Trichoderma* spp. 5% non-denaturing PAGE was performed to separate the PCR amplified products.

3. Results

3.1 Analyzing root system morphology against candidate *Trichoderma* isolates

In the current investigation, ten *Trichoderma* isolates were assessed to determine their impact on the root morphology of the chosen genotype/rice lines. *Trichoderma* isolates responded differently to four different genotypes of rice.

In numerous different *Trichoderma* isolate vs. rice genotype treatment combinations, a growth in root length relative to control was seen. We used the root scanner machine Epson Perfection V700/V750, 3.81 Version, WinRhizo Reg 2009 to scan the roots derived from all of the treatment combinations because it had been observed that *Trichoderma* seed treatment contributed to increased root length. Observations were made on a number of root parameters, including total root length, root volume, tips, and forks (Fig.1). Maximum root length is displayed in IR-64 and RRF-75 by IRRI-4. Sahbhagi and Indira Barani dhans are produced by T14 and IRRI-3, respectively.

The TV12 treatment produced the greatest root volume in the IR-64 and Sahbhagi dhan samples. While IRRI-6 displayed the best result in RRF-75. Indira Barani Dhan demonstrated the best performance for IRRI-2 isolates. The treatment of IRRI-5, T14, IRRI-6, and IRRI-3, respectively, resulted in the highest root tip count in IR-64, Sahbhagi Dhan, RRF-75, and Indira Barani Dhan. The number of forks increased in the treatments with various *Trichoderma* isolates in the IRRI-3 seen in RRF-75 and Indira Barani Dhan as compared to control. While IRRI-6 has the most forks in the sahbhagi dhan isolate, IRRI-2 has the greatest effects in IR-64. As a result of our findings, we hypothesised that *Trichoderma* therapy would work through a variety of methods and trigger unique genes in plants that would lead to improved root growth.

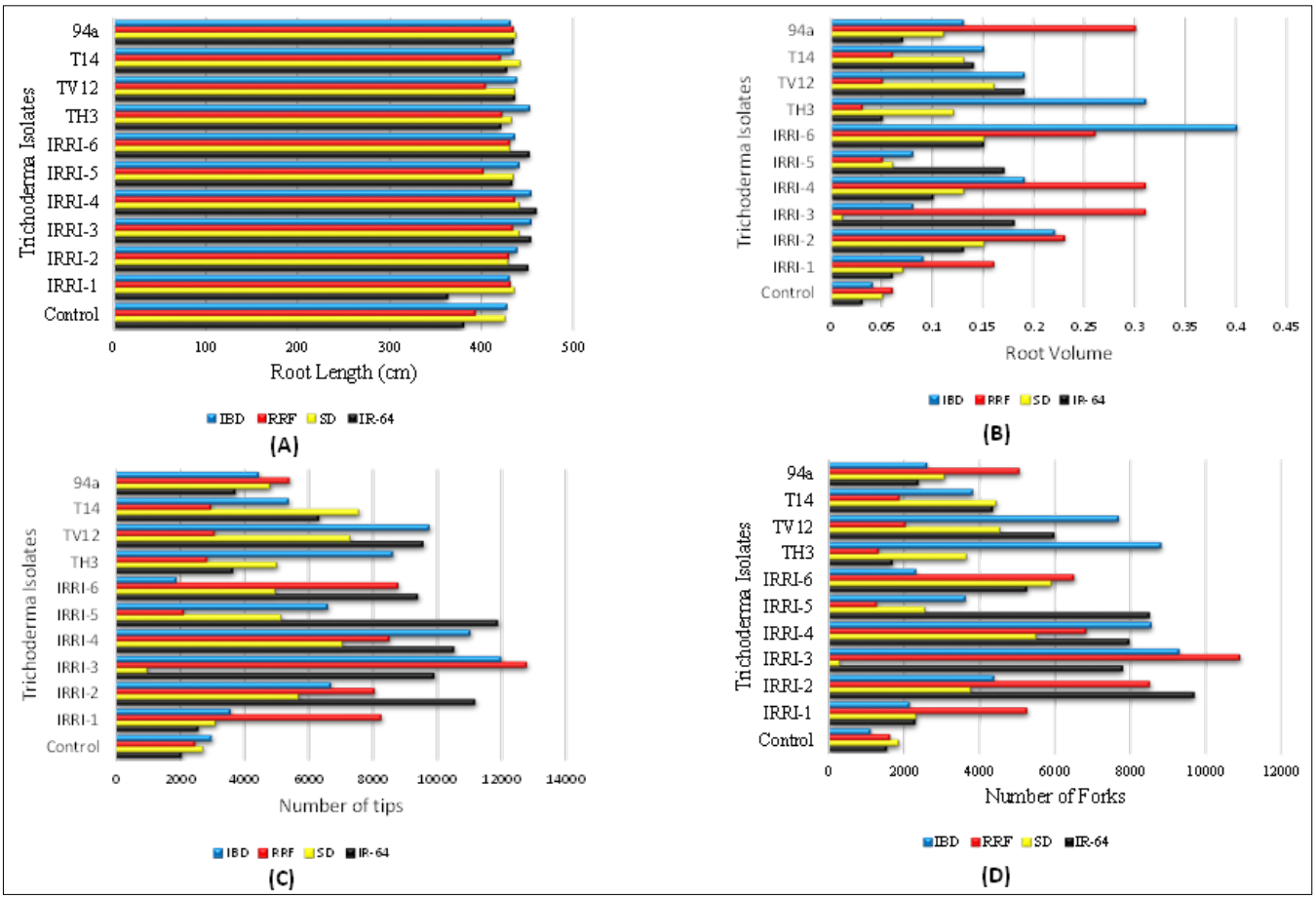

Fig 1: Histogram showing effect of treatments on (A) root length (B) root volume (C) root tips (D) root fork of rice genotypes

3.2 Qualitative assay for ACC deaminase activity

Based on the enrichment method, where ACC was employed as the sole nitrogen source, all 10 isolates were tested for ACC deaminase. Since all of the isolates could grow on Petri plates containing synthetic media with ACC as the only nitrogen source and plates containing $(NH_4)_2SO_4$, which served as a positive control, it was determined that all of the samples were positive. On plates with solely synthetic media devoid of ACC, which served as the negative control shown in Fig. 2, the isolates were unable to develop.

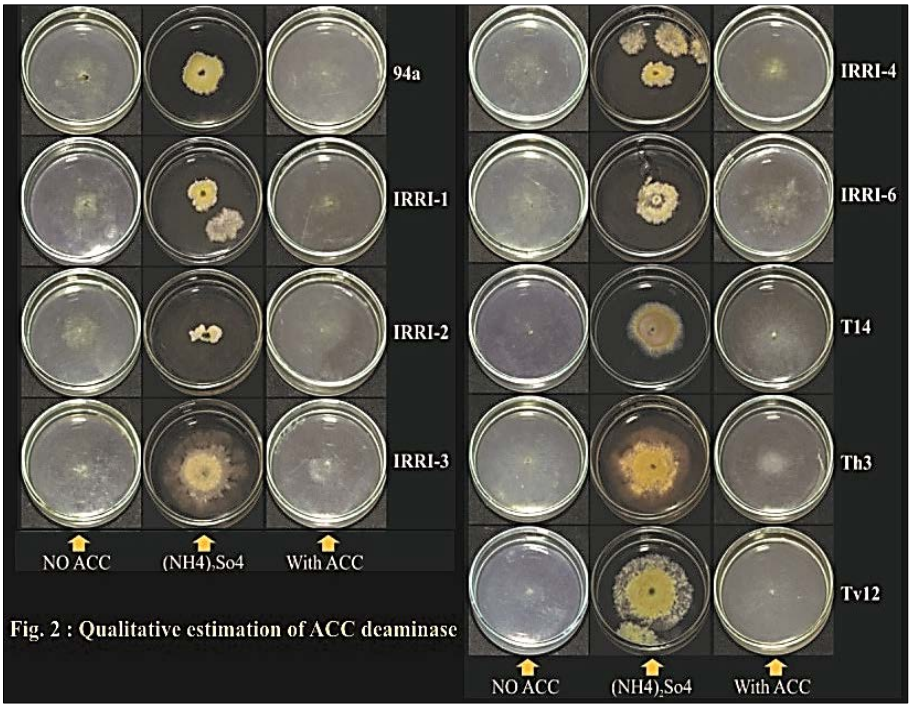

Fig 2: Qualitative estimation of ACC Deaminase

3.3 Quantitative estimation of ACC deaminase

All of the isolates were chosen for quantitative measurement of enzyme activity because they could all grow on petri plates receiving synthetic medium supplemented with ACC. By measuring the amount of -ketobutyrate produced during the deamination of ACC by the enzyme ACC deaminase, the ACC deaminase activity was evaluated. All of the isolates displayed activity between 10.463.92 and 2.371.37 mol/mg protein/h for ketobutyrate. The isolates were divided into groups that produced high, medium, and low levels of ACC deaminase activity. The isolates IRRI-1, IRRI-2, IRRI-3, and TV12 were put in the group with high enzyme activity, whereas IRRI-4 was put in the group with medium enzyme activity. Isolates IRRI-6, TH3, T_{14}, and 94a were also mentioned in the group with low enzyme activity. The ACC deaminase activity was highest in isolation TV12 (10.463.92 mol ketobutyrate/mg protein/h) and lowest in isolate T_{14} (2.371.37 mol ketobutyrate/mg protein/h) (Fig. 3).

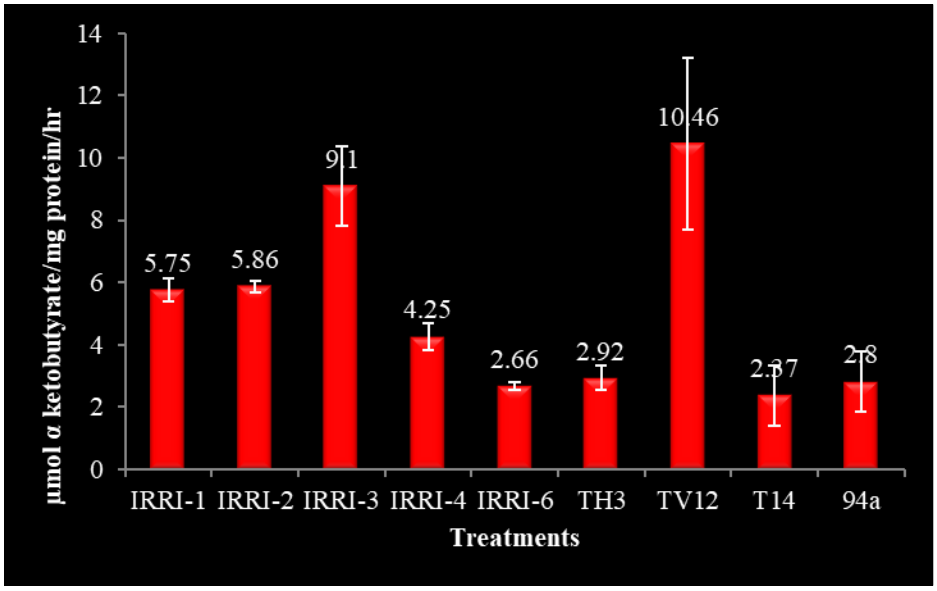

Fig 3: Bar Graph showing quantitative estimation of ACC deaminase

3.4 *In vitro* detection of ACC deaminase activity using gene-specific primers

Utilizing the primer pair acdS, isolates were screened for the presence of the acdS gene. According to the PCR analysis's findings using the primer pair AcdS-F & AcdS-R, all *Trichoderma* isolates yielded a DNA fragment of about 1500 bp in size. A 1.5 to 1.7 kb gel band produced by amplification of the acdS gene serves as evidence of the gene's existence. All of the isolates also contained a second band that measured 1270 bp (Fig. 4). It is well recognised that ACC deaminase-producing microorganisms promote the growth of a variety of plants, particularly in stressful conditions including flooding, heavy metals, salt, and drought.

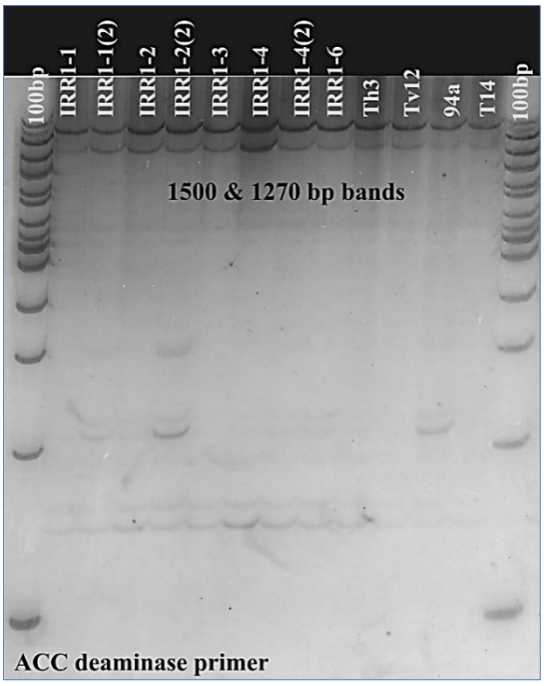

Fig 4: Gel picture showing ACC deaminase activity using gene-specific primers in *Trichoderma* strains

4. Discussion

4.1 Analyzing root system morphology against candidate *Trichoderma* isolates

In the current investigation, ten *Trichoderma* isolates were assessed to determine their impact on the root morphology of the chosen genotype/rice lines. It has been found that *Trichoderma* seed treatment results in longer roots. *Trichoderma* isolate TV12's seed treatment on IR-64 and Sahbhagi dhan reveals highest root length. Similar to how IRRI-2 displays the maximum root length in Sahbhagi Dhan and IRRI-6 displays it in Indira Barani Dhan.

4.2 Screening of isolates of *Trichoderma* spp. for ACC deaminase activity

All of the isolates displayed activity between 10.463.92 and 2.371.37 mol/mg protein/h of Ketobutyrate. The ACC deaminase activity was highest in isolation TV12 (10.463.92 mol ketobutyrate/mg protein/h) and lowest in isolate T_{14} (2.371.37 mol ketobutyrate/mg protein/h).

4.3 Molecular assessment of ten isolates of *Trichoderma* spp

To characterise *Trichoderma* strains and identify the ACC deaminase gene using a primer, PCR-based fingerprinting is used. With the AcdS-F & AcdS-R primer pair, PCR was used to check for the presence of the acdS gene, and the results showed that all *Trichoderma* isolates included a DNA fragment of about 1500 bp in size. All *Trichoderma* isolates demonstrated homogeneity after PCR amplification using ACC primers, showing that each one may manufacture ACC deaminase.

5. Acknowledgements

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