Advances in Research

10(6): 1-12, 2017; Article no.AIR.34227
ISSN: 2348-0394, NLM ID: 101666096

Antagonistic ACC Deaminase Producing *Pseudomonas fluorescens* with Polymer Seed Coating for the Management of Rice Fallow Black Gram Diseases

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Authors' contributions

This work was carried out in collaboration between all authors. Author MK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KR, IJ, PL and DS managed the analyses of the study. Author IJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2017/34227
Editor(s):
(1) Magdalena Valsikova, Horticulture and Landscape Engineering, Slovak University of Agriculrure, Nitra, Slovakia.
Reviewers:
(1) Dusit Athinuwat, Thammasat University, Thailand.
(2) Bernard R. Glick, University of Waterloo, Canada.
Complete Peer review History: http://www.sciencedomain.org/review-history/20274

Original Research Article

Received 19th May 2017
Accepted 21st July 2017
Published 29th July 2017

ABSTRACT

Plant growth promoting bacterial (PGPB) strains of *Pseudomonas fluorescens* were tested for their capacity to overcome the water stress under rice fallow condition in black gram plants. Among the different bacteria used, *P. fluorescens* TRRI-1 were exhibited the highest antagonistic activity, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase positive reaction to PCR amplification, increased the vigour index, Germination, Seedling dry matter production, No. of nodules in black gram seedlings grow out test and pot culture. Quantitative and qualitative analyses of stress-related enzymes indicated the greater activity of catalase and peroxidase in black gram plants bacterized with *P. fluorescens* TRRI-1 when compared to untreated plants. The greater accumulation of proline was recorded in TRRI-1 treated plants compared to untreated plants. The greater activity of

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stress-related enzymes in green gram plants mediated by PGPB could pave the way for developing drought management strategies. The field trials revealed the greater reduction in disease incidence and increase yield by black gram plants treated with *P. fluorescens* TRRI-1compared to untreated plants. The promising role of antagonistic and ACC deaminase from Ps. fluorescens strain TRRI1 in alleviating biotic and abiotic stresses has been concluded in black gram plants.

Keywords: Rice fallow pulses; plant growth promoting bacteria; Pseudomonas fluorescens; water stress.

1. INTRODUCTION

Black gram is one of the important pulses grown in both Kharif and Rabi seasons. Black gram is being cultivated under irrigated, rainfed as well as rice fallow condition, after the harvest of samba/thaladi paddy. Rice fallow black gram grows in the residual soil moisture, which is broadcasted 7-10 days before the harvest of paddy crop and allowed to germinate and grow. However, yield recorded in this ecosystem is highly variable mostly and depends on the management practices followed. The yield ranged from 200-400 kg/ha. The poor yield of rice fallow black gram is attributed by many factors. Among the various factors cause for poor yield of black gram, occurrence of disease and drought is one of the important factors for reducing yield. As there is no field preparation for sowing, the crop residue and weed are severe as source for the inoculum. Important diseases noticed in the rice fallow black gram are root rot, leaf spot, powdery mildew, viral diseases like yellow mosaic and leaf crinkle.

The eco-friendly approaches such as biological control and host resistance induction have gained much attention in the past decade as a way of reducing the use of chemical products in agriculture. In these circumstances, use of microbes could play an important role in the management of biotic and abiotic stresses. Among different microbes, PGPR bacteria may play important beneficial roles in the metabolism and physiology of the host plant, including promotion of plant growth, inhibiting strong fungal activity, accumulation of pathogenesis related protein, deposition of cell wall barrier, inhibit growth of pathogens [1]. Fluorescent pseudomonads have received attention throughout the global science because of their catabolic versatility, excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites. Hence, the present study was undertaken to test the efficacy of the endophytic *P. fluorescens* TRRI 1 formulation for the management of rice fallow black gram diseases. On the other hand, ethylene is synthesized in plant tissue from precursor 1-aminocyclopropane-1-carboxylic acid (ACC) during biotic and abiotic stress conditions. Which in turn retarded root growth and caused senescence in crop plants [2]. Interestingly, PGPR strains possess the enzyme ACC deaminase can cleave the plant ethylene precursor ACC and thereby lower level of ethylene in a developing seedling or stressed plant [3]. The ACC deaminase containing biocontrol agents markedly lowered the level of ACC in the stressed plants. Thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant.

Inoculant on the outside of seed may be knocked off during seeding and/or desiccates and dies before making it into the soil. Polymer coating has been used to improve inoculants viability and shelf life with legume seed. Polymer is a film coating chemical normally applied over seeds without significantly increasing the size or weight of seed. The film formulations consist of a mixture of polymer, plasticizer and colourants [4] that are commercially available as ready to use liquids or as dry powders [5]. This kind of plasticizer polymer form flexible film that prevents dusting off and loss of biocontrol agent/fungicide during handling and is readily soluble in water (hydrophilic) so as not to impede with normal germination [6]. Film coating provides protection from the stress imposed by accelerated ageing, which include fungal invasion. Keeping this view, current study is aiming to manage the diseases in the rice fallow black gram by using ACC deaminase producing *Pseudomonas* sp with polymer coated seed.

2. MATERIALS AND METHODS

2.1 Plant Growth-promoting Rhizobacteria Strains

Rice rhizosphere soil samples were collected from the rice ecosystems of Tamil Nadu, India. The fluorescent pseudomonad strains such as

Pseudomonas fluorescens TRRI1-TRR20 were isolated from the rhizosphere soil samples by using the serial dilution technique in Kings' B (KB) medium (peptone 20 g; $MgSO_4$ 1.5 g; K_2HPO_4 1.5 g; glycerol 10 ml; agar 20 g and distilled water 1 l) [7]. To confirm strains as *Pseudomonas* sp. 16S-23S rRNA intervening sequence-specific internal transcribed spacers (ITS) IF (AAGTCGTAACAAGGTAG); ITS2R (GACCATATATAACCCCAAG) primers were used to get an amplicon size of 560 bp [8]. These isolates were maintained at -80℃ with 50% glycerol. *Pseudomonas fluorescens* ACC deaminase positive strains, TDK1 and PY15 were kindly provided by Dr. D. Saravanakumar, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. *Pseudomonas fluorescens* strain Pf1 was obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India.

2.2 *In vitro* Screening of Bacterial Strains against *M. phaseolina*

For *in vitro* screening of bacterial strains against *M. phaseolina*, the bacterial isolates were streaked on one side of a Petri dish (1 cm from the edge of the dish) with PDA medium and a mycelia disc (8 mm diameter) of 3-day-old culture of *M. phaseolina* was placed on the opposite side of the Petri dish perpendicular to the bacterial streak [9]. The dishes were incubated at room temperature (28±2℃) for 4 days and the mycelia growth of the pathogen was measured.

2.3 Amplification of 1-aminocyclopropane-1-carboxylic Acid Deaminase

The ACC deaminase gene of *Pseudomonas* was amplified with sequence-specific forward (5´-ATGAACCTGCAACGATTC-3´) and reverse (5´-TCAGCCGTCTCGGAA GAT-3´) primers [10]. PCR reactions were carried out in 20 µl reaction mixture containing 10x buffer (with 2.5 m mol l^{-1} $MgCl_2$), 2 µl; 2 m mol l^{-1} dNTP mixture,2 µl; 2 mol l^{-1} primer, 5 µl; Taq DNA polymerase,3 U; H_2O, 8 µl and 50 ng of template DNA samples were amplified on DNA thermal cycler (Eppendorf Master Cycler Gradient). The PCR programme consisted of an initial denaturation at 94℃ for 5 min followed by 3 0cycles of 94℃ for 60 s, 58℃ for 60 s and 72℃ for 60 s with final extension of 10 min.

2.4 Efficacy of *Pseudomonas fluorescens* to Promote Plant Growth under *In vitro*

Fluorescent pseudomonad strains were grown on KB broth with constant shaking at 150 rev min^{-1} for 48 h at room temperature (28 ± 2℃). The bacterial cells were harvested and centrifuged at 4000 g for 15 min and resuspended in sterile water. The concentration was adjusted using a spectrophotometer to approx. 10^8 CFU ml^{-1} (OD_{595}= 0.3) and used as bacterial inoculum [11]. The bacterial suspensions of the fluorescent pseudomonads prepared were tested for their plant growth-promoting activity, which was carried out by the standard roll towel method [12]. Black gram seeds (var. ADT-3) were used for testing the efficacy of plant growth promotion by fluorescent *pseudomonas* strains under *in vitro*. Black gram seeds soaked in 10 ml of the bacterial suspension (10^8 CFU ml^{-1}) for 2 h were blot-dried, placed in wet blotters and two third of the blotter was immersed in water solution and incubated in growth chamber for 15 days. The seeds soaked in sterile water served as control. The germination percentage of seeds was recorded and the vigour index was calculated using the following formula: vigour index = percent germination x·seedling length (shoot length + root length) [13]. Five seedlings were taken randomly from each *Pseudomonas* treatment and their fresh weight was recorded. Then, the seedlings were kept in the hot-air oven for 7 days at 55℃ for complete desiccation, and dry weight of the seedlings was recorded and individual seedling fresh weight and dry weight were calculated.

2.5 Preparation of Talc-based Formulations of *Pseudomonas* Strains

A loopful of Pseudomonas was inoculated into the KB broth and incubated in a rotary shaker at 150 rev min)1 for 72 h at room temperature (28 ± 2℃). After 72 h of incubation, the broth containing 9 x 10^8 CFU ml^{-1} was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the purified talc powder (sterilized at 105℃ for 12 h), calcium carbonate, 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC), 10 g (adhesive) were mixed under aseptic conditions following the method described by Nandakumar et al. [14]. The product was shade dried to reduce the

moisture content below 20% and used for application. At the time of application, the population of bacterium in talc formulation was checked to2.5 to 3 x 10^8 CFU g^{-1}.

2.6 Seed Treatment and Viability of *Pseudomonas* on Seed

Seeds of Blackgram cv. ADT 3 obtained from Tamil Nadu Rice Research Institute, Aduthurai and the seeds were soaked in different *Pseudomonas* strains (10 ml of 10^{-8} CFU bacterial broth + 250 ml water + 1 kg seed) 30 min and subsequently shade dried to bring back its original moisture content. Then, the seeds were film coated with black polykote (3 g/kg) (Hitron Herbal Seed Coat Pvt Ltd, Coimbatore) and shade dried for 24 h to bring back its original moisture content. Numbers of colony forming units (CFU)on seed were determined by serial dilution plating onto King's B agar.

2.7 *In vitro* Antagonism of *Pseudomonas* spp against *M. phaseolina*

To study the *in vitro* antagonistic activity of strains of *Pseudomonas* spp*.* against *M. phaseolina*, a nine mm mycelial disc from actively growing colony of *M. phaseolina* was placed at one side of the Petri plate containing PDA medium. After 12 h of incubation, *Pseudomonas* spp*.* antagonists were streaked opposite to the fungal disc. PDA inoculated with the pathogen alone was used as a check. Three replications were maintained for each bacterial antagonist [15]. Observations were taken after complete growth of pathogen in the control plate for the presence of inhibition zone nearer to the bacterial streak.

2.8 Greenhouse Studies

Various *Pseudomonas* strains polymer coated seeds were assessed for their efficacy on the seedling and root growth of black gram in green house conditions. A pot culture study was undertaken with the following treatments (Table 3) by using completely randomized design with four replications. Soil collected from a rice fields in Tamil Nadu Rice Research Institute, Aduthurai, India was air-dried, homogenized using are volving jar mill and sterilized using a steam sterilizer for 3 h at 85℃. Pots (40 cm diameter) were filled with soil (7 kg). Thirty treated black gram seeds with *P. fluorescens* formulations were sown (depth 2 cm; spacing 2×3 cm) in each pot. Control (soil + untreated seeds) was also included. Determination of black gram Seedlings Growth Characteristics Sixty days after sowing, the seedlings Plant bio mass (g/plant), Plant height (cm), No. of branches, No. of pods/plant, No. of seeds/pod and Seed yield (g/plant) were determined. Experiment were performed in 7 treatments each with 3 replications.

Another set of experiment with above treatment were maintained for studying the stress related enzymes and proline accumulation in black gram. After sowing the seed, the plant samples were collected at 24 h interval up to 12 th day. One gram of the leaf sample homogenized with 1 ml of sodium phosphate buffer (0.1 M) (pH 7.0) was used for the estimation of catalase (CAT) and peroxidase (PO). Assay of Catalase, PO activity and Proline content was carried out as per the procedure described by Barber [16], Hammerschmidt et al. [17], Bates et al. [18] respectively.

2.9 Field Study

Pseudomonas polymer coated black gram seeds (var. ADT-3) were broadcast in the standing crop 7 days before the harvest of the paddy crop uniformly under optimum soil moisture conditions so that the seeds should get embedded in the waxy mire. Thirty days after sowing, the talc-based bioformulations were applied at 2.5 kg ha^{-1} as foliar spray. Two field trials were conducted at TRRI, Tamil Nadu Agricultural University, Aduthurai and Farmers field, Aduthurai, A randomized block design was used in the experiments with plot size of 10x10 m^2 and five replications were maintained for each treatment. Observations were made on the incidence of yellow mosaic virus, root rot, powdery mildew and seed yield were recorded.

2.10 Statistical Analysis

The *in vitro* and field trial data were analysed using the IRRISTAT version 92-1 program developed by the biometrics unit, International Rice Research Institute, Metro Manila, The Philippines. Plant growth and yield data were analysed independently by trial. Data were subjected to analysis of variance (ANOVA). The treatment mean values were compared by Duncan's multiple range test (DMRT) [19].

3. RESULTS AND DISCUSSION

Globally, crop growth protection and health is continuously challenged by emerging, re-emerging and endemic plant pathogens. Rhizospheric microbes that suppress plant pathogens could be used as biocontrol agents and may be considered as alternative to chemical pesticides. There are several mechanisms for beneficial microorganisms to protect plants from biotic and abiotic stress. Plant growth promoting rhizobacteria (PGPRs) are known as beneficial bacteria for plant growth and yield. One PGPR group are the ACC deaminase positive (ACC+) bacteria which degrade 1-aminocyclopropane-1- carboxylic acid (ACC), the plant produced per-cursor to ethylene. Studies have shown that ACC+ bacteria, in association with plant roots, can improve plant growth under abiotic stress (e.g., drought, salinity, heavy metals) by reducing concentrations of stress ethylene. Current study, growth promotion by ACC positive fluorescent pseudomonads polymer coated black gram under *in vitro* condition showed improvement in plant growth parameters over untreated seeds. In this study, we evaluated the ACC positive fluorescent pseudomonads under rice fallow condition black gram cultivation. Rice fallow black gram grows in the residual soil moisture, which is broadcasted 7-10 days before the harvest of paddy crop and allowed to germinate and grow. Major problem in rice fallow crop is drought and occurrence of disease. This study demonstrates the effectiveness of rhizobacteria containing ACC deaminase for inducing drought tolerance and consequently improving the growth of black gram plants under rice fallow (water stress) conditions.

3.1 Effect of Biocontrol Strains on Radial Mycelial Growth of *M. phaseolina*

Antagonistic activity of *Pseudomonas* strains exhibited diversified percent inhibition against *Macrophomina phaseolina*. TRRI1 strain recorded maximum inhibition against *M. phaseolina* (44.4%) followed by TRRI 7 and TRRI 15 (41.1%). Top 10 TRRI strains were selected for further screened for ACC production (Table 1). In this study, *in vitro* assessment dual plate assay resulted in varied in inhibition zone after 3 d of inoculation of *P. fluorescens* isolates against *M. phaseolina*, which suggested the extra-cellular secretion of antifungal by these pseudomonads. Some workers have suggested a significant role for secondary metabolites such as antibiotics, siderophores of *Pseudomonas* in suppression of fungal pathogens [20-23].

Table 1. Efficacy of different *Pseudomonas* isolates against the growth of *M. phaseolina* *In vitro*

***Pseudomonas* isolates**	**Growth of *M. Phaseolina***	**Per cent inhibition over control**
TRRI -01	4.7^{a}	44.4^{a} (41.8)
TRRI -02	8.5^{gh}	5.6^{h} (12.4)
TRRI -03	6.8^{d}	24.4^{cde} (29.5)
TRRI -04	8.1^{fg}	10.0^{fgh} (18.1)
TRRI -05	7.7^{ef}	14.4^{efg} (22.1)
TRRI -06	5.6^{bc}	37.8^{ab} (37.9)
TRRI -07	5.3^{b}	41.1^{a} (39.9)
TRRI -08	8.6^{gh}	4.8^{h} (11.4)
TRRI -09	8.5^{gh}	5.6^{h} (12.4)
TRRI -10	6.0^{c}	33.3^{abc} (35.2)
TRRI -11	7.4^{e}	17.8^{def} (24.8)
TRRI -12	7.4^{e}	17.8^{def} (24.8)
TRRI -13	8.3^{g}	$7.8g^{h}$ (15.6)
TRRI -14	6.6^{d}	26.7^{bcd} (31.1)
TRRI -15	5.3^{b}	41.1^{a} (39.9)
TRRI -16	6.8^{d}	24.4^{cde} (29.5)
TRRI -17	8.2^{fg}	8.9^{fgh} (16.9)
TRRI -18	5.6^{bc}	37.8^{ab} (37.9)
TRRI -19	5.3^{b}	41.1^{a} (39.9)
TRRI -20	8.1^{fg}	10.0^{fgh} (18.1)
Control	9.0^{h}	0.00

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

3.2 Amplification of 1-aminocyclopropane-1-carboxylic Acid Deaminase

ACC deaminase genes from the *P. fluorescens* strains were amplified using PCR with gene-specific primers. Among the ten isolates tested, *Ps. fluorescens* strain TRRI1, TRRI15 and TRRI 19 amplified the product of 700 bp (Fig. 1) and these three strains were further characterized for the plant growth promotion studies. ACC deaminase activity was more widely present in different species of *Pseudomonas* [2,24-27].

3.3 Effectiveness of *Pseudomonas* Strains in Promoting Plant Growth

The biocontrol *Pseudomonas* strains TRRI 1, TRRI 15 and TRRI19 produced green gram seedlings with a significantly higher vigour index, 7426.6, 6942.2 and 6874.3, respectively.

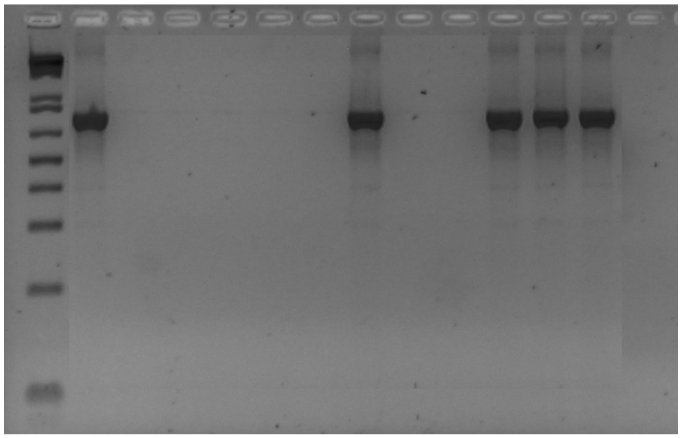

Fig. 1. ACC deaminase producing TRRI isolates + TRRI 1, TRRI 15, TRRI 19, + strai 2 control

The reference biocontrol strains Pf1, TDK1 and Py15, whose vigour index was 7010.5, 7038.4 and 7024.3 respectively. Interestingly, TRRI1 strain produced seedlings with a high vigour index, Seedling dry matter production, No. of nodules and a higher germination percentage, than seedlings treated with the other two strains. The untreated control seedlings had the lowest vigour index, 6435.4 (Table 2). Visual observation from this study, we observed early germination (within 2 days) in all the *pseudomonas* polymer coated seeds as compared to the normal seeds (data not shown here). From this screening, we had chosen TRRI-1 strains for our further studies.

3.4 Glasshouse Study

TRRI-1 strain that had been most effective in inhibiting *M. phaseolina* mycelial growth and in promoting green gram seedling growth was selected for pot culture studies. From the pot culture study, yield-attributing characters such as plant bio mass (g/plant), plant height (cm), No. of branches, No. of pods/plant, No. of seeds/pod and seed yield (g/plant) were recorded. Significantly greater number of yield-attributing parameters were recorded from the plants treated with *P. fluorescens* strain TRRI1 bioformulation when compared with untreated control and other *Pseudomonas* treatments. *P. fluorescens* strain TRRI1 treated plants recorded with higher as plant bio mass, plant height, No. of branches, No. of pods, No. of seeds and seed yield in pot culture (Table 3).

Inoculation with rhizobacteria having ACC deaminase activity resulted in the development of a better root system, which subsequently affected shoot growth positively [28]. Inoculation with ACC-deaminase containing bacteria promotes root growth of developing seedlings of various crops [29]. The differences in plant growth promotion among the isolates are also attributed to their individual rhizospheric competencies and hydrolyzing the ACC synthesized in roots.

The activation of metabolic activity of seed could also be due to hydrophilic polymer present in the coating material, which might improve the rate of water uptake by the seed [30] leading to early germination and better seedling establishment, which might also help in better plant height. Kavitha et al. [31] also confirm the coating with hydrophilic polymer was regulates the rate of water uptake, reduce imbibition damage and improve the emergence of sorghum seeds.

3.5 Activity of Water Stress Related Enzymes and Proline Accumulation in Black Gram

The activity of peroxidise and catalase in black gram plants treated with *Pseudomonas* strains was observed at different time intervals. A greater activity of catalase and peroxidise was recorded in all the plants receiving the biocontrol treatment as compared with untreated plants. The increase lasted for 5-7 days with all biocontrol agents, after which there was a decline. The plants treated with *P. fluorescens* TRRI-1 showed the greater activity of catalase (Fig. 2) and peroxidase (Fig. 3) against control. These enzymes tend to detoxify the toxic H2O2 accumulated during the water stress conditions. Proline accumulation was found to be higher in

TRRI1 treated plants against control followed by TDK1treatment. In contrast, the proline accumulation in control plants was observed steady lower level (Fig. 4).

Table 2. Effect of polymer coating on germination and plant growth of blackgram var. ADT3 *in vitro* in grow out test

Treatments	Germination (%)	Seedling dry matter production (mg/seedling)	Vigour index	No. of nodules/ plant
TRRI1+ PC	95.3[a]	79.00[a]	7426.6[a]	12.27[a]
TRRI15+ PC	93.6[ab]	75.25[bc]	6942.2[c]	10.32[c]
TRRI19+ PC	93.6[ab]	75.13[bc]	6874.3[c]	10.11[c]
Pf1 + PC	94.3[a]	76.20[b]	7010.5[b]	12.21[a]
TDK-1+ PC	94.0[a]	76.50[b]	7038.4[b]	12.23[a]
PY15 + PC	93.0[ab]	76.35[b]	7024.3[b]	11.20[b]
Polymer coating	90.3[c]	73.20[d]	6688[d]	9.10[d]
Water soaking	90.6[c]	74.25[d]	6683.3[d]	8.88[e]
Untreated control	88.0[d]	71.50[e]	6435.4[e]	8.40[e]

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

Table 3. Effect of polymer coating on germination and plant growth of blackgram var. ADT3 under pot culture

Treatments	Plant bio mass (g/plant)	Plant height (cm)	No. of branches	No. of pods/plant	No. of seeds/pod	Seed yield (g/plant)
TRRI + PC	7.00[a]	46.35[a]	6.13[a]	20.6[a]	6.9[a]	20.3[a]
Pf1 + PC	6.12[c]	43.60[b]	5.20[b]	17.3[c]	5.4[c]	18.4[b]
TDK-1+ PC	6.54[b]	43.84[b]	5.98[b]	18.4[b]	6.2[b]	18.8[b]
PY15 + PC	6.20[c]	43.20[b]	5.00[b]	16.5[d]	5.8[c]	18.2[b]
Polymer coating	5.50[d]	41.50[d]	5.13[b]	14.4[e]	5.1[d]	16.8[d]
Seed treatment with Carbendazim (2 g/kg)	5.46[d]	42.18[c]	5.40[b]	16.8[d]	5.7[c]	17.6[c]
Control	5.20[d]	41.20[d]	5.12[b]	14.6[e]	5.0[d]	15.3[e]

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

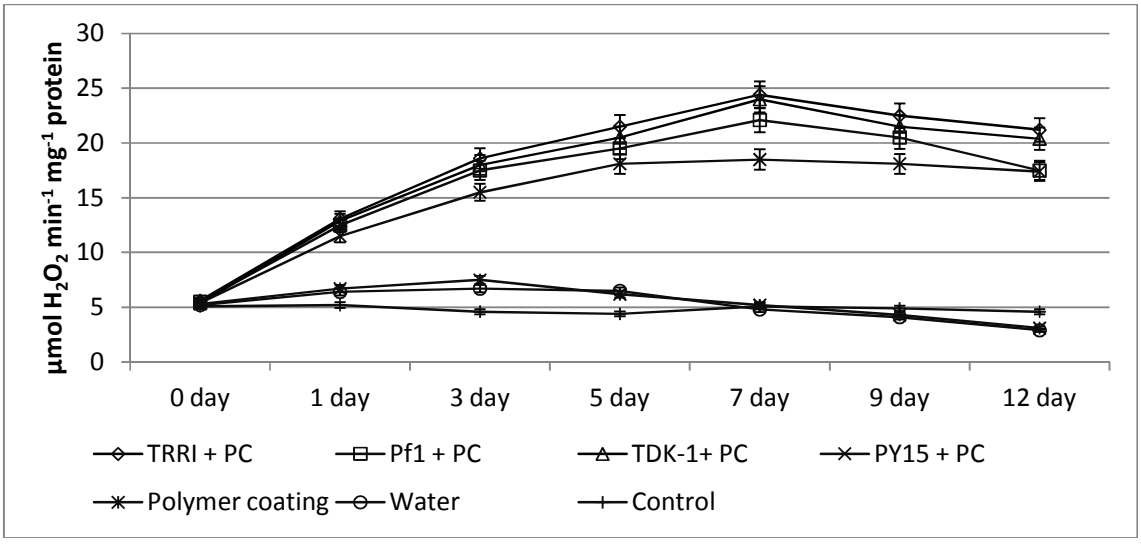

Fig. 2. Catalase activity in green gram plants treated with polymer coated *Pseudomonas*
Three samples were analysed for each replication and each treatment consisted of three replications

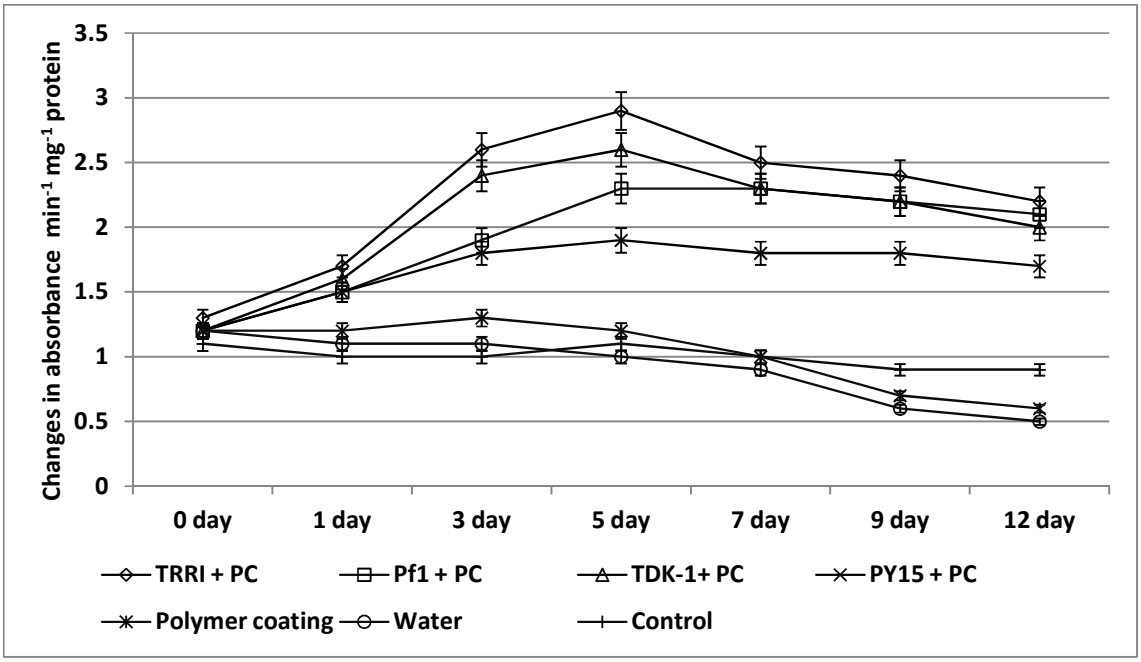

Fig. 3. Peroxidase activity in green gram plants treated with polymer coated *Pseudomonas*
Three samples were analysed for each replication and each treatment consisted of three replications

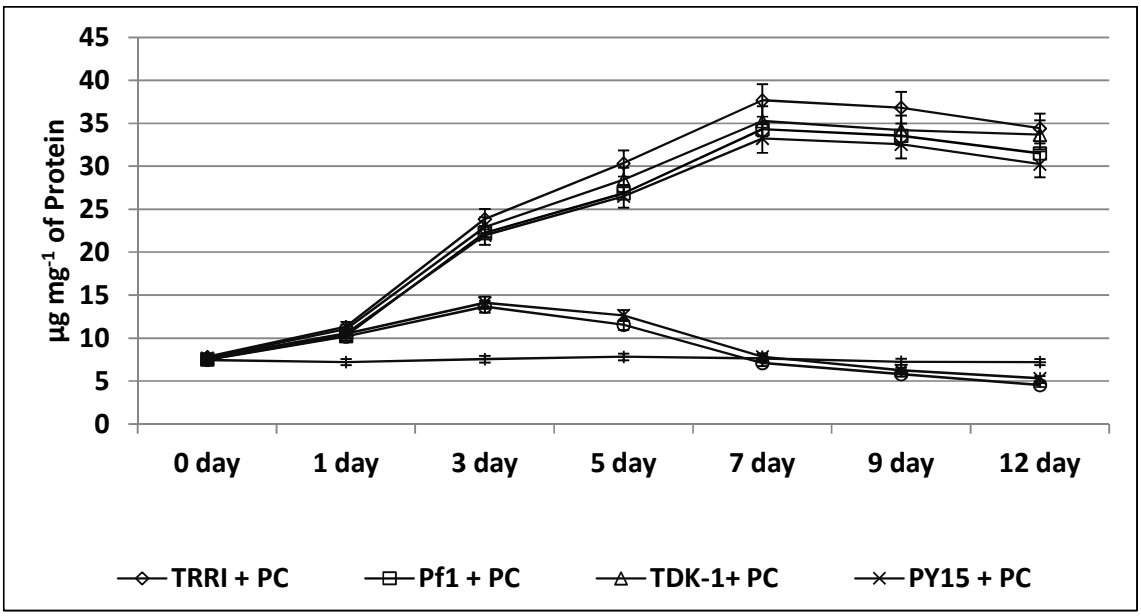

Fig. 4. Proline accumulationin green gram plants treated with polymer coated *Pseudomonas*
Three samples were analysed for each replication and each treatment consisted of three replications

The scavenging enzymes are known as antioxidant enzymes and includes peroxidase and catalase [32]. The antioxidant enzymes have the ability to remove free radicals and prevent damage to the membranes and DNA. An earlier study demonstrated that mechanisms that reduce oxidative stress indirectly play an important role in drought tolerance [33]. Kohler et al. [34] demonstrated the greater activity of antioxidant catalase in lettuce plants under

severe drought conditions when inoculated with PGPR. It is interesting to note in the current study that PGPB strain *P. fluorescens* TRRI -1 increased the accumulation of catalase and peroxidise when black gram plants were exposed to water stress. Further, the accumulation of proline in plants acts as anosmatic and helps to maintain the water potential of plants under stress which in turn facilitate the plant to extract water from soil [35]. It also acts as a storage compound for protein synthesis. Whenever a plant experiences a stress, inhibition of starch biosynthesis takes place and proline accumulation will be used as a source of carbon and nitrogen for the survival of plant [36]. Kohler et al. [34] reported the highest accumulation of proline in the plants inoculated with PGPR under severe drought conditions. Thus, itis also assumed in the current study that accumulation of proline in black gram plants might attribute to the bacterization of *P. fluorescens* TRRI-1.

3.6 Field Study

Chemical treated plot were observed the lowest disease incidence of yellow mosaic virus, dry root rot and powdery mildew. But the greatest reduction in disease incidence was also observed in plots treated with TRRI-1 followed by TDK-1 as compared with the untreated control. The biocontrol agents not only reduced disease incidence but also increased yield compared to chemical treated and control plots. *Ps. fluorescens* strain TRRI 1treatment recorded the highest yield which is significantly higher from all other treatments. Lowest yield was recorded in the untreated control plots (Table 4). The results of the field studies revealed that the seed treatment with ACC deaminase producing plant growth promoting *Pseudomonas* strain TRRI-1 significantly reduced the incidence of yellow mosaic, dry root rot and powdery mildew diseases. Similar report of use of biocontrol agents was reported by several workers [37-40]. The *P. fluorescens* strains reduced the disease incidence through several mechanisms including production of lytic enzymes [20], siderophores [21], salicylic acid [22] and hydrogen cyanide [23].

Although plant growth-promoting bacteria use a number of different mechanisms to promote the growth of plants [41], arguably, the bacterial trait that is key in facilitating plant growth is the possession of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and α-ketobutyrate [42]. By decreasing ACC levels in plants, ACC deaminase-producing organisms decrease plant ethylene levels [28,43], PGPRs having ACC deaminase activity help plants to withstand stresses (biotic or abiotic) by reducing the level of stress ethylene [3,28,44-45]. Glick et al. [28], reported that ACC deaminase-producing plant growth-promoting bacteria first bind to the surface of a plant (usually seeds or roots), although these bacteria may also be found on leaves and flowers or within a plant's internal tissues (i.e., as an endophyte). Polymer present in the seed coating material have also helped in higher rate of water uptake in turn, resulted in the early germination with more seedling vigour and better stand establishment [46], which ultimately led to better growth and productivity of black gram.

Table 4. Effect of polymer coating on disease incidence of blackgram var. ADT3 under Rice fallow condition

Treatments	YMV (PDI)	Root rot (DI)	PMD (PDI)	Yield (kg/ha)	Per cent increase over control
TRRI + PC	17.72^{b}	5.76^{a}	29.16^{b}	523.12^{a}	32.66
Pf1 + PC	20.12^{b}	5.92^{a}	31.88^{c}	486.80^{b}	23.45
TDK-1+ PC	18.92^{b}	5.84^{a}	30.52^{c}	498.08^{b}	26.31
PY15 + PC	19.52^{b}	5.88^{a}	31.20^{c}	463.16^{c}	17.46
Carbendazim seed treatment (2 g/kg) and foliar spray (0.1%)on 35th day	13.65^{a}	5.53^{a}	21.11^{a}	458.40^{c}	16.25
Polymer coating	30.52^{c}	6.58^{b}	49.40^{d}	414.60^{d}	5.14
Control	31.92^{c}	6.64^{b}	52.67^{d}	394.33^{e}	0.00

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

4. CONCLUSION

The current study concluded that inoculation with the polymer coated ACC deaminase containing PGPR caused significant alleviation of biotic and abiotic stress and consequently improving the yield of the black gram. Formulation of PGPR bacteria may have practical application in biological promotion of plant growth characteristics which can potentially replace the use of chemical fertilizers. The use and application of such bioformulations in the fields can result in the reduction of application of harmful chemicals, protect the environment and biological resources and can be an important component of integrated pest management (IPM) that can help the growers to achieve a sustainable agricultural system.

ACKNOWLEDGEMENT

The authors thank DST-FIST Facility and UGC-SAP-DRS1 Facility in the Department of Plant Pathology, TNAU, Coimbatore for conducting this experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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