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ORIGINAL RESEARCH ARTICLE

Effect of Seed Priming with Native PGPR on its Vital Seedling and Antioxidant Enzyme Activities in *Curcuma longa* (L.)

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ABSTRACT

An experiment was conducted on *Curcuma longa* to study the effect of seed treatments with native PGPR (plant growth promoting rhizobacteria) on its seedling growth and antioxidant enzyme activities. The vital seedling parameters such as germination percentage and vigour index were improved. *Azospirillum lipoferum* (AUAL27) treatment influenced maximum of 70%, *Pseudomonas fluorescens* (AUPF25) of 65% and compared with control 35% germination, where as *P. fluorescens* (AUPF25) and *A. lipoferum* (AUAL27) showed vigour index value of 870, 950 respectively with *C. longa*. There was significant difference in the total rhizosphere population between rhizosphere and non-rhizosphere soils of *C. longa*. The activities of antioxidant enzymes such as Superoxide dismutase (SOD), Peroxidase (POX) and Catalase (CAT) were increased to a significant extent due to the treatments with PGPR. The antioxidant enzymes activities in root were found to be high using AUAL27 (5.8U/mg, 3.5U/mg and 0.23U/mg for SOD, POX and CAT respectively), while 5.5U/mg, 3.0U/mg and 0.22U/mg SOD, POX and CAT respectively antioxidant activity were found in shoot.

Key word: *Curcuma longa*, PGPR, Antioxidant enzyme and Seed priming.

1. INTRODUCTION

Turmeric has a wide range of essential application like food and pharmaceutical products, cosmetics and textile industries [1]. As agriculture is concerned, it is indeed necessary to focus on plant growth, rhizosphere microorganisms and soil health. These factors are largely depending upon on biological features. Stimulating effects of fungal elicitors are complicated and could be related to the interactions between fungal elicitors and plant cells, elicitors signal transduction and plant defense responses [2]. Certain secondary metabolite pathways in plant are induced by infection with microorganisms [3]. Plant growth promoting bacteria is an important biological process that major role in satisfying the nutritional requirements of plants.

Plant are equipped with oxygen radical detoxifying enzymes such as Superoxide dismutase (SOD), Peroxidase (POX), Catalase (CAT) and antioxidant molecules like Ascorbic acid and reduced Glutathione in order to survive under stress conditions [4]. Generation of reactive oxygen species (ROS) such as superoxide, H_2O_2 and Hydroxyl molecules causes rapid cell damage by triggering off a chain reaction [5]. Plants under stress produce some defense mechanisms to protect themselves for the harmful effect of oxidative stress. ROS scavenging is one among the common defense responses against provided by an integrated system of non-enzymatically reduced molecules like Ascorbate and Glutathione and Enzymatic Antioxidants [6].

Major activities of turmeric chemical constituents include significant antioxidant capability. Potent fungicidal activities against phytopathogenic fungi have been demonstrated in greenhouse settings [7]. Chowdhury *et al.* [8] showed insecticidal properties of turmeric extracts. Much work has been carried out on the antioxidant and related anticancer activities of compounds derived from turmeric rhizomes. The curcuminoids i.e., curcumin (1.7-bis (4-hydroxy -3-methoxyfenil)-1,6-heptadiene-3,5-dione), demethoxy – curcumin , and bis-demethoxy-curcumin are major compounds of turmeric. However, they are readily decomposed when exposed to bright light [9], high temperature or oxidative conditions [10].

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Curcuma longa Linn. (family: Zingiberaceae), commonly known as turmeric, is one such perennial herb. Its rhizome importance also used as a coloring agent for textiles, pharmaceuticals, confectionary and cosmetics [11]. The major practical problem in the cultivation of *C.longa* is the poor germination percentage at field level. To the best of our knowledge, no information on the germination, seedling vigour and antioxidant potential of *C.longa* under PGPR treatment is available. The purpose of study was to provide additional information on the germination, seedling vigour and antioxidant constituents of *C.longa* under seed priming with native PGPR such as *Azospirillum lipoferum* (AUAL27) and *Pseudomonas fluorescens* (AUPF25).

2. MATERIALS AND METHODS

Seed collection and PGPR organisms isolation:

The seeds of turmeric (*C.longa*) were collected from Horticulture Farm of Annamalai University, Tamil Nadu, India. The Rhizosphere and Non-rhizosphere soil sample collected from C.*longa* rhizosphere region. The population of total PGPR organisms was estimated as standard microbiological test. *Azospirillum lipoferum* (AUAL27) is isolated from the surface sterilized roots of *C.longa* and *Pseudomonas fluorescens* (AUPF25) isolated from the rhizosphere soil samples of *C.longa*.

Experiment 1: **Study of Germination (%) and seedling vigour index**

To find out the effect of these isolated *Azospirillum lipoferum* and *Pseudomonas fluorescens* of the Germination and vigour index of C.*longa* , Micro rhizomes were treated with Rhizobacterial broth culture of (with an initial population 10^7cells/ml) *Azospirillum* and *Pseudomonas fluorescens* as separate treatments. The seeds were treated for 30 min and then shade dried. Then these inoculated seeds were for the germination rate using paper towel method [12]. The germination percentage was calculated from 10 days after showing (DAS) to 14 DAS. The morphological parameters like shoot length and root length were measured on 30 DAS. The vigour index (VI) of the seedling was estimated as suggested by Abdul Baki and Anderson [13].

VI=RL+SL×GP

Where; RL is root length (cm), SL is shoot length (cm) and GP is germination percentage

Experiment 2: **Study of antioxidant enzyme extractions and assays**

1. Superoxide dismutase (SOD)

The activity of SOD was assayed as described by Beauchamp and Fridovich [14]. The reaction mixture contained 1.17×10^{-6}mol/L riboflavin, 0.1 mol/L methionine, 2×10^{-5}mol/L KCN and 5.6×10^{-5}mol/L nitroblue tetrazolium (NBT) salt dissolved in 3ml of 0.05 mol/L sodium phosphate buffer (pH 7.8). Three milliliters of the reaction medium was added to 1 ml of enzyme extract. Illumination was started to initiate the reaction at 30°C for 1 h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U/mg protein. One U is defined as the change in 0.1 absorbance per hour per mg protein.

2. Peroxidase (POX)

POX was assayed by the method of Kumar and Khan [15]. Assay mixture of POX contained 2 ml of 0.1 mo/L phosphate buffer (pH 6.8), 1ml of 0.01 mol/L pyrogallol, 1ml of 0.005 mol/L H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1 ml of 2.5 mol/ L H_2SO_4 the amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 mol/L H_2SO_4 at zero time. The activity was expressed in U/ mg protein. One U is defined as the change in the absorbance per 0.1 min per mg protein.

3. Catalase (CAT)

The activity of CAT was measured according to the method Chandlee and Scandalios [16] with small modification. The assay mixture contained 2.6 ml of 50 mmol/L potassium phosphate buffer, 0.4 ml of 15 mmol/L H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/ mg protein. One U is defined as 1 mmol/L of H_2O_2 reduction per min per mg protein. The enzyme protein was estimated by the method of Bradford [17] for all the enzymes.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dencan's Multiple Range Test (DMRT).

3. RESULTS

Significant difference were observed in germination rate, root length, shoot length, dry matter production and vigour index with Azospirillum or Pseudomonas between treated seeds of *C.longa* when compared with uninoculated control. The maximum germination

70% was recorded in *A. lipoferum* (AUAL27) treatment followed by *P. fluorescens* (AUPF25) 65%. The native isolates of *A. lipoferum* (AUAL27) and *P. fluorescens* (AUPF25) significantly increased the germination rate in *C.longa* which was 70% as against 35% recorded by untreated control.

Treatments with these Rhizobacterial resulted in significantly higher dry matter production for (AUAL 27) 37mg and (AUPF25) 36mg than control. No significant difference in the dry matter production between *A. lipoferum* (AUAL27) and *P. fluorescens* (AUPF25) treatment in *C.longa*.

Significant difference in the population of total *Azospirillum lipoferum* (AUAL27) and *Pseudomonas fluorescens* between Rhizosphere and Non-rhizosphere soils of *C.longa* was found. The rhizosphere soils sample of *C.longa* population 70.22×10^3 CFU /g. The population of *A. lipoferum* (AUAL27) was also found to be high in rhizosphere soils 19×10^3 followed by *P. fluorescens* (AUPF25) 17×10^3.

There was a significant increase in SOD, POX and CAT activities under *A. lipoferum* (AUAL27) and *P. fluorescens* (AUPF25) treatments. The SOD activities of *C.longa* treated with *A. lipoferum* and *P. fluorescens* in root and shoot 6 U/mg proteins. POX (3.4 U/mg protein in root sample >3.0U/mg protein in shoot sample) and CAT (0.22 U/mg protein in root sample) activities high compared with control.

Table 1: Effect of *Azospirillum lipoferum* (AUAL27) and *Pseudomonas* fluorescens (AUPF25) seed treatment on seedling parameters of *C.longa*

Treatment	Germination (%)	Root length(cm)	shoot length(cm)	Dry matter production(mg/seedling)	vigor index
Untreated	35	3.5	3.1	28	320
Azospirillum lipoferum	70	5.6	5.2	37	950
Pseudomonas Fluorescens	65	5.1	4.8	32	870

Table 2: Population of Total Rhizobacteria, *Azospirillum* and *Pseudomonas* individually on the rhizosphere region of *C.longa*

Soil sample	Total rhizosphere population ($\times10^3$ CFU/g)	*Azospirillum lipoferum* ($\times10^3$ CFU/g)	*Pseudomonas fluorescens* ($\times10^3$ CFU/g)
Rhizosphere	56	19	17
Non Rhizosphere	27	12	11

Fig 1: POX Activity

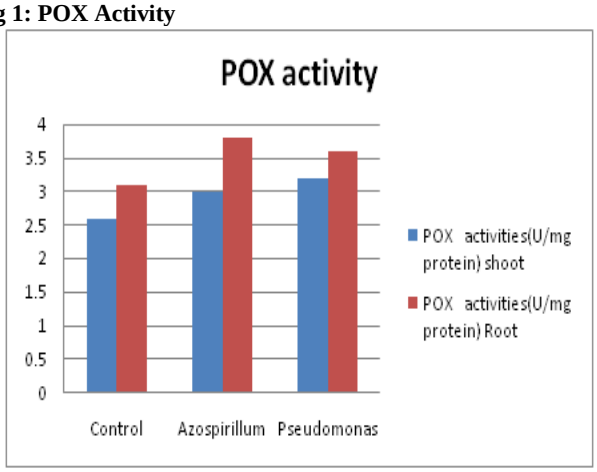


Fig 2: SOD Activity

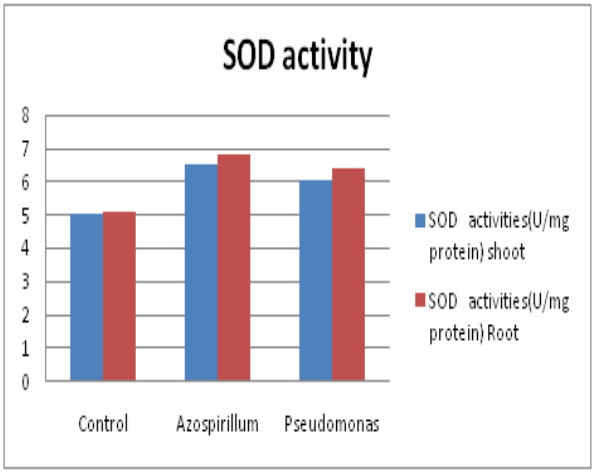
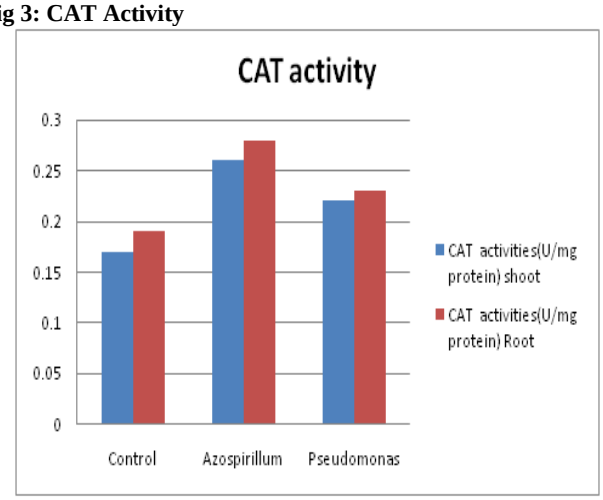


Fig 3: CAT Activity



4. DISCUSSION

The seed treatment with native isolates of *Azospirillum lipoferum* (AUAL27) and *Pseudomonas fluorescens* (AUPF25) increased the germination (%), root length, shoot length and vigour index of *C.longa*. The occurrence of *Azotobacter* in and around the root systems of crop and beneficial effect upon inoculation have been well established [18]. In the present study the increased seedling parameters in *C.longa* may be due to the production of growth hormones (Auxins, Gibberellins and Cytokinins) by the

heterotrophic nitrogen-fixation bacteria. It is worth nothing that earlier research determined that the increase in plant growth observed on inoculation with *Azospirillum* was not caused by nitrogen fixation, but bacterial production of plant hormones [19].

In the present study, isolates of *Azospirillum lipoferum* (AUAL27) and *Pseudomonas fluorescens* (AUPF25) were tested for their effect on seedling parameters of *C.longa* and results clearly indicated that the native isolates significantly improved the germination and related seedling parameters .this conformities earlier report by Govindarajan and Kavitha [20] that the *Azospirillum lipoferum* was isolated to paddy seedling under auxenic conditions.

There was a significant increase in SOD, POX and CAT activities under *Azospirillum lipoferum* (AUAL27) and *Pseudomonas fluorescens* (AUPF25) treatments. It catalysis the dismutation of superoxide anion radical with great efficiency, resulting in the production of H_2O_2 and O_2 [21], in our study an increased level of SOD activity was found. Variations are recorded in antioxidant enzyme activities under various stresses and treatments of pea [22] showed that a reduction in POX activity in radish. We observed an increased in CAT activity in *C.longa* seedling subjected to *Azospirilum lipoferum* (AUAL27) and *Pseudomonas fluorescens* (AUPF25) treatments. The level of antioxidative response depends on the species, the development and metabolic stat of the plant, as well as the duration and intensity of stress [23]. It is well known that treatment of plants with elicitors, or attack by incompatible pathogens, causes an array of defense reactions, including the accumulation of a range of plant defensive secondary metabolites [24].

5. CONCLUSION

From the results it can be concluded that the application of PGPR native isolates such as *Azospirillum lipoferum* and *Pseudomonas fluorescens* could be well used to promote growth and plants innate antioxidant defense potentials in turmeric.

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