

Enhancement of Rice Growth and Production of Growth-Promoting Phytohormones by Inoculation with *Rhizobium* and Other Rhizobacteria

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Abstract: Excessive use of chemical fertilizers & repetition of same crops not only causes pollution but also a source of depletion of soil nutrients, ultimately reducing rice yield in the world. Co-Inoculation of various strain of bacteria are usually applied to overcome this problem. In present study, PGPR were isolated from washed-surface sterilized roots of rice (*Oryza sativa* L.) field near Gujranwala city in Pakistan. The isolates were identified on the basis of their morphological and biochemical characteristics. Two of the PGPR isolates were found belonging to *Enterobacter* spp. while the other two isolates were *Azospirillum* spp. The two rice cultivars Basmati and Super Basmati were inoculated with bacterial isolates alone and in combination with *Rhizobium* and *Azospirillum* respectively and their growth responses were examined. Our results confirmed the beneficial effects of co-inoculation with sufficient increase in root length, shoot length and fresh and dry weight of plants, when compared to single inoculation or uninoculation. All the Isolated strains were found capable of producing phytohormones i.e. indole-3-acetic acid (IAA) and gibberellic acid (GA). The dual inoculation of rice with *Rhizobium* and *Azospirillum* produced higher IAA & GA as compared to single inoculation. Furthermore IAA production by the *Rhizobium* and PGPR cultures with tryptophan was more prominent than single inoculation.

Key words: Rice • Co-Inoculation • Plant growth • Phytohormones • *Azospirillum* • *Rhizobium*

INTRODUCTION

In Pakistan, rice occupies about 25 % of the cultivated area in the summer moon-soon season. There, the commonly rice varieties are Super Basmati, Basmati 385, Basmati 370 and IRRI 6, Super Kernel and Kernel. It is a versatile cash crop and Pakistan exports more than one million tons of rice annually, which is 10% of the total world rice trade [1]. Rice is one of the most important cereal crops after wheat, because more than half of the world's population depends on rice for its basic diet [2-3]. Future demand for rice is expected to increase in a rate that at least just following the expected population growth in the next 20 years regardless of that which may be attributed to increase in family life standard and purchase

power. Rice is the most important food crop of the developing world and is the staple food for 2.7 billion people in Asia and for many millions in Africa and America [4]. To feed the fast increasing global population, the world's annual rice production must increase from the present 520 million tons to 760 million tons by the year 2020 [5].

Nitrogen is an important key input factor in rice farming in those areas where most of the field crops are external-N dependent. The environmental benefits from reducing nitrogen runoff are likely to be more important than the financial benefits for farmers due to reduced mineral N consumption. Because, a large part of rice is grown under irrigated or in rain fed environments with continuous flooding, it will be important to find out if

biological nitrogen fixation can contribute to crop performance under these flooded conditions. Furthermore, increasing the use of chemical fertilizers for crops is not only expensive but also creates environmental problems. Therefore, it is important to use different mixture of beneficial bacteria (biological fertilizer) to enhance yields. The beneficial impacts of PGPRs on plant growth and performance were found due to direct plant growth promotion activity by production of plant growth regulators which not only enhance the uptake of soil nutrients but also the fresh & dry weight of plants [6]. Further studies have shown that the beneficial effects of microbes on plants growth can be enhanced by co-inoculation of PGPR's with other microorganisms [7]. Apparently, co-inoculation allows plants to have more balanced nutrition based on improved absorption of nitrogen, phosphorus and other macro and micro-nutrients. Previously many researchers have reported the Synergistic effect of plant growth promoting rhizobacteria and nitrogen fixing bacteria on growth and yield of different plants [8-9]. Due to increasing the demands of rice there is need to improve the production of this crop. There were many studies conducted to see the effect of PGPR on different crops but only few reports are available on rice with co-inoculation effect.

The present study aimed to isolate PGPR from protected zones inside roots of rice plants collected from Gujranwala, Pakistan. Secondly, we identified these PGPR by morphological and biochemical tests. Finally, we compared the role of growth regulating hormone indole acetic acid (IAA) and gibberellic acid (GA) by stimulating rice growth under controlled conditions when inoculated alone or in-combination with *Rhizobium*.

MATERIALS AND METHODS

The entire chemical used in this study was obtained from Plant Sciences Department Quaid-i-Azam University Pakistan. The different *Rhizobium* strains (E7, E8, E10) were also obtained from above said department.

Isolation, Identification and Characterization of the Plant Growth-Promoting *Rhizobacteria* (PGPR): Rice (*Oryza sativa* L.) plants were uprooted from different farmer's field at Kalashah kaku, Kamonkey and Wazirabad, District Gujranwala, Pakistan, washed thoroughly with sterile distilled water to remove adhering soil particles, surface sterilized and homogenized in sterile distilled water (1.0 g fresh root / 9 ml water). 100 µl of root homogenates was inoculated into a glass vials containing

5 ml nitrogen-free combined carbon medium (CCM) and incubated at 30°C for 48 hours. The vials showing bacterial growth were used to inoculate plates of the same medium to get pure colonies. The serial dilutions prepared from homogenized root material were transferred on LB agar plates. The root material was also transferred on YMA medium plates for isolation of PGPR strains. The bacterial isolates were identified on the basis of their cellular, cultural and biochemical characteristics.

Colony and Cell Morphology: Bacterial strains from overnight grown cultures in YMA broth were spread on YMA agar plates and incubated at 30°C for 24 h. After 24 h the color and shape of colonies was noted. Cell motility and shape of single colony was observed under light microscope (Nikon, Japan).

Gram Staining

Gram Staining Was Done by the Method of Vincent [10].

Oxidase Test: Oxidase test was performed to determine the presence of oxidase enzyme in bacterial isolates (Steel, 1961). Kovac's reagent (1% N, N, N.N-tetramethylethylenediamine) was dissolved in warm water and stored in dark bottle. A strip of filter paper was dipped in this reagent and air-dried. With the help of sterile wire loop, one-day-old rhizobial colonies from agar plates were transferred on this filter paper strip. The oxidase positive colonies turned lavender colored which became dark purple to black in color within 5 min.

Catalase Test: This test was performed to study the presence of catalase enzyme in bacterial colonies. Rhizobial colonies (24 h old) were taken on glass slides and one drop of H₂O₂ (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

Miniaturized Identification System (QTS 24):

Physiological and biochemical tests of *Rhizobium* isolates were performed using QTS-24 miniaturized identification system (DESTO Laboratories Karachi, Pakistan) following the method [11]. For these tests 24 h old bacterial cultures were used and results were noted after 18 h of incubation at 30°C.

Plant Growth and Re-Inoculation Studies: Rice seeds were surface-sterilized with 0.1% HgCl₂ for 2-3 minutes followed by subsequent washing in sterilized distilled water and sown in autoclaved soil in earthen pots of the dimensions 24 length x 30 cm diameter. The pots were sterilized with 10% Chlorox prior to being filled with soil.

After 7 days of germination, seedlings were inoculated with 1ml (10^6 cells per ml) of the bacterial strains of *Rhizobium* and *Azospirillum* grown in YMA broth. The soil was inoculated by using disposable sterile plastic syringes to inject the bacterial suspension to 2-3 inches below soil surface while avoiding contamination of the above ground plant tissues. The control (uninoculated) plants were treated with distilled water.

Determination of IAA and GA Contents in Plant Roots and Leaves: The roots and leaves of rice plants were collected 30 days after sowing (3 weeks after inoculation) for the extraction of GA and IAA, purified extract was re-dissolved in methanol and analyzed by HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) using a C-18 column (39 x 300 mm, Merck column) with methanol: acetic acid: water (30:1:70) as the mobile phase, a flow rate of 0.5 ml / min (15 min / sample) and UV detection [12]. Identification and quantitation were based on comparison to retention time and peak area of authentic standards (Sigma Chemical Company USA).

Detection of Plant Growth Hormones Produced by Bacterial Isolates: Five bacterial strains (*i.e.* three strains of *Rhizobium* spp obtained from Plant physiology lab. and two strains of *Azospirillum liipoferum* L, *Enterobacter* isolated from field-grown rice roots were analyzed for production of indole acetic acid (IAA) and gibberellic acid (GA) in pure culture. Rhizobia and PGPR were grown in yeast mannitol broth and CCM liquid media (without sodium lactate and para-amino-benzoic acid, Ammonium chloride (1.0 g / L), tryptophan (100 mg / L; precursor for IAA biosynthesis) and root exudates (2 ml, 4 ml and 6 ml) were added to CCM and tryptophan (100 mg / L) was added to yeast mannitol medium. Growths media (100 mL) was inoculated into 24 day-old bacterial cultures and incubated on a shaker at 30°C. Cultures were harvested after one week by centrifugation at 10,000 RPM. Phytohormones were extracted from the supernatant fluid as describe and were identified and quantified by HPLC as described above [13]

Plant Shoot and Root Length: Plant root and shoot length was measured with a centimeter scale from base of stem till the base of apical leaf and from base of stem till root tip respectively.

Fresh and Dry Weight of Root and Shoot: Root and Shoot were kept in oven at 70°C for 24 hours. Dry weight of root and shoot were recorded.

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) and the significance of mean values was tested at 5% significance level by Duncan's Multiple Range Test [14].

RESULTS

During current investigations, we studied the effect of PGPR isolates from Kalashah Kaku and Gujranwala Pakistan with rhizobial co-inoculation on growth of rice plants. Our results demonstrate the possibility of all above mentioned outcomes. Enrichment cultures of *Rhizobacteria* were obtained using N-free semi-solid medium inoculated with suspensions which were prepared by homogenization of rice roots collected from Kalashah Kaku and Gujranwala sites in Pakistan (as described in material and methods). A total of 12 bacterial isolates were obtained from rice roots out of which only four isolates grew well in N-free semisolid medium and were selected for further studies on the basis of Morphological and culture characteristics, as other were re-isolation of similar strains as shown in Figure 1. Morphological characterization and biochemical tests (QTS-24 kit) of these bacteria showed that they belong to different bacterial genera including *Azospirillum* and *Enterobacter*. Isolates 1 and 4 were identified as *Azospirillum* on the basis of their typical spiral motility and nitrogen fixing ability. These two isolates were further studies on the basis of cell length (having more than 5 μ m in LB broth) as well as its carbon utilization. These isolates showed catalase and oxidase test positive because these used glucose and produce acid. The cells of Isolates 2 and 3 were gram negative, short, medium sized, motile rod shaped. Further these isolates were facultative, anaerobic having both respiratory and fermentative type of metabolism. These isolates have ability to used glucose as well as carbohydrates with production of acid. Isolates 1 and 4 produced off-white colonies as shown in Fig. 1. (a) and the other two produced yellow-pigmented colonies on LB agar plates & broth culture in Vials as shown in Fig. 1. (b) The rod-shaped cells of isolates 1 and 4 were greater than 4 μ m in length when grown in LB broth and showed spiral-type motility, typical of *Azospirillum* strains. The rod-shaped cells of isolates 2 and isolates 3 were 0.5-1 μ m in diameter and 1-4 μ m in length and grew as facultative anaerobes. The variation in morphological characteristics of different isolated were observed keenly during the study. Moreover these results plus various biochemical tests (Table 1) were compared to bacterial genera described in Bergey's Manual of Determinative

Table 1: Morphological and biochemical characteristics of the bacterial isolates obtained from rice roots

Tests/Strains	Isolate 1	Isolate 2	Isolate 3	Isolate4
Colony Morphology	Off-white, rounded flat	Yellwish, rounded flat	Whitish rounded centre dark	Off-white, rounded flat
^a Cell Morphology	Plump rods	Short rods	Short rods	Plump rods
Catalase	+	+	+	+
Oxidase	+	-	-	+
^b Ortho-nitro-phenyl β-				
D-galactopyranoside	-	+	+	-
*Sodium citrate	+	-	-	+
*Sodium malonate	+	-	-	+
*Lysine decarboxylase	-	-	-	-
*Arginine dehydrolase	-	-	-	-
*Ornithine decarboxylase	+	-	-	+
*H ₂ S Production	-	-	+	+
*Urea hydrolysis	+	-	-	+
*Tryptophan deaminase	-	+	+	-
*Indole	-	+	-	+
*VogesProskauer	-	-	-	-
*Gelatin hydrolysis	+	+	+	-
*Acid from glucose	+	+	-	-
*Acid from maltose	+	+	+	+
*Acid from sucrose	-	-	-	+
*Acid from mannitol	+	+	+	-
*Acid from *arabinose	+	+	+	+
*Acid from *rhamnose	+	+	+	+
*Aid from sorbitol	-	+	+	-
*Acid from inositol	-	+	+	-
*Acid from adonitol	-	+	+	-
*Acidfrommelibiose	+	+	+	+
*Acid from raffinose	-	+	+	+
*Cytochrome oxidase	+	-	+	-
*Nitrate reduction	-	+	+	-
*N ₂ gas production	-	+	+	-
Gram reaction	-	-	-	-
Motility ARA	+	+	+	+
Organisms Identified	<i>Azospirillumlipoferum</i> .	<i>Enterobacter</i> sp.	<i>Enterobactersp</i>	<i>Azospirillumlipoferum</i>

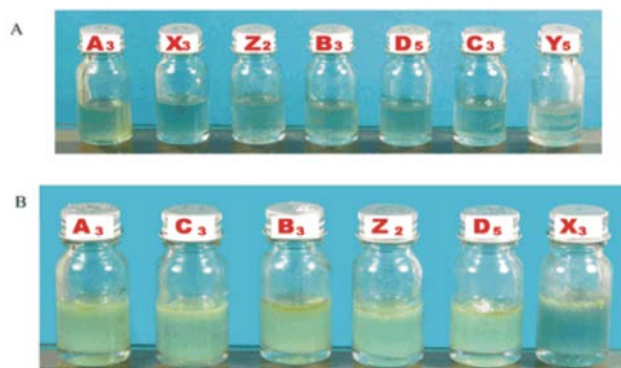


Fig. 1: Isolation of PGPR from different soil sample (Gujaranwala Kalashah kaku Pakistan)

Bacteriology (Holt *et al.*, 1994), indicating that the characteristics of isolates 1 and 4 matched *Azospirillum* sp and isolates 2 and 3 matched *Enterobacter* sp. Additionally, The isolates 1 and 4 accumulated

poly-hydroxybutyrate granules, which were also identified in *Azospirilla*. We assume that our isolates showed similar characteristics as used by Bergey's to distinguish these two species of *Azospirillum*.

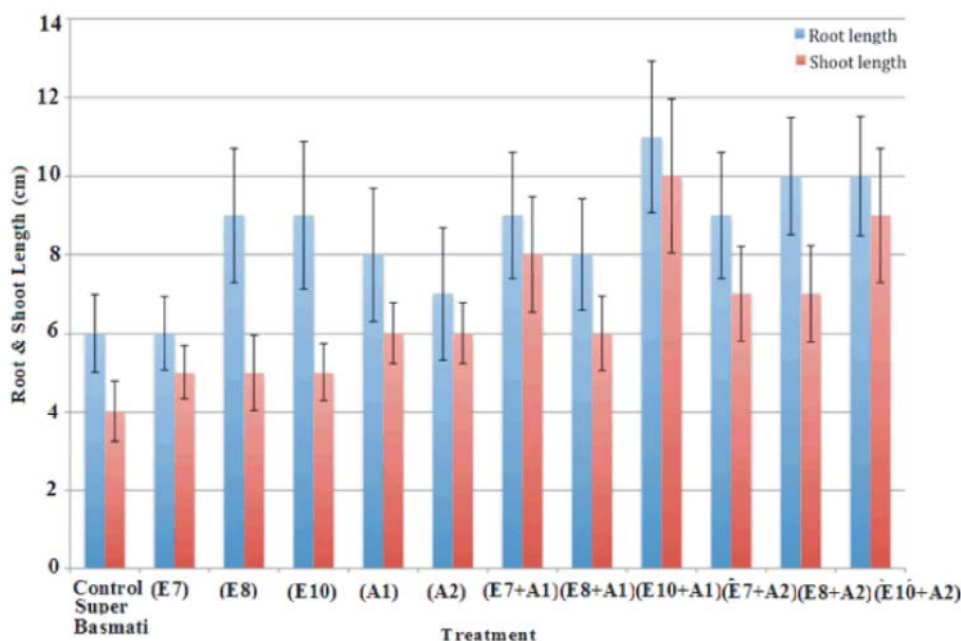


Fig. 2: Effect of *Rhizobium* (E7, E8, E10) and PGPR isolates alone & in combination on root length; shoot length, fresh weight and dry weight of root and shoot rice cv. Super Basmati

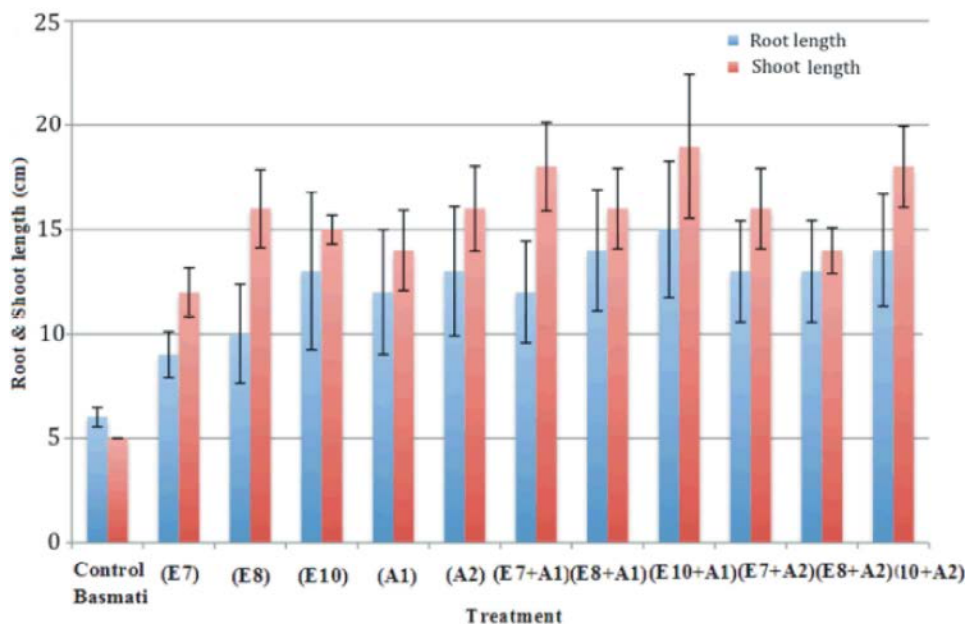


Fig. 3: Effect of *Rhizobium* (E7, E8, E10) and PGPR isolates alone & in combination on root length, shoot length, fresh weight and dry weight of root and shoot of cv. Basmati rice

In order to study the effects of single and co-inoculation on growth, plants were treated with these isolates in several combinations (for details please see material and methods). All the plants were harvested after 4 weeks and several plant growth parameters were recorded. According to Results presented in Fig. 2 and Fig. 3 revealed that inoculation of rice seedling roots

under genotobiotic conditions with *Rhizobium* and PGPR isolates alone and in combination. Our results showed the stimulation of plant growth in all the varieties tested. Inoculated seedlings had greater plant height and stem width as compared to control. The effect of *Rhizobium* strain E10 was more efficient, the response of *Azospirillum* strain was variable with the cultivars.

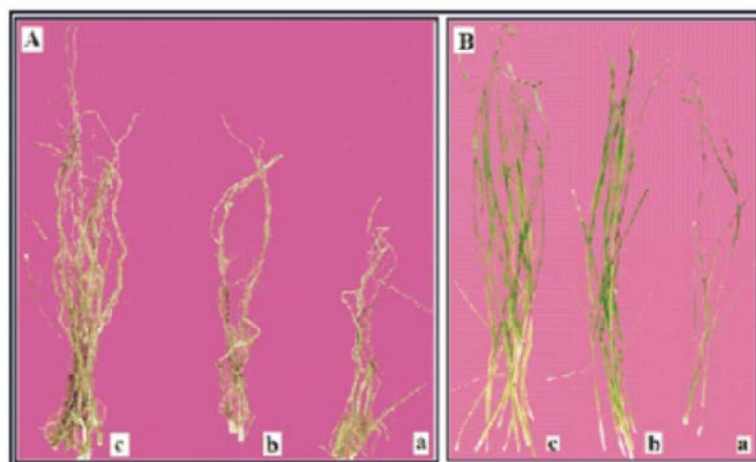


Fig. 4: Effect of PGPR and Rhizobium on root & shoot length in single and co-inoculation

Table 2: IAA and GA content of root and shoot of rice cv Super Basmati following inoculation with *Rhizobium* (E7, E8, E10) and PGPR isolates. Each value represents mean +stdev of 5 plants:

Treatment	IAA (Root) $\mu\text{g/ml}$	IAA (Shoot) $\mu\text{g/ml}$	GA (Root) $\mu\text{g/ml}$	GA (Shoot) $\mu\text{g/ml}$
Control	27 G	41 G	36 D	44 F
E ₇	29 G	62 D	110 E	89 BCD
E ₈	37 AB	79 E	119 C	94 B
E ₁₀	41 D	91 C	139 F	100 A
A1	35 B	70 F	130 G	88 D
A2	39 AB	60 D	120 C	78 E
E7+A1	42 AB	43 G	180 A	93 B
E8+A1	50 F	110 A	182 A	91 BD
E10+A1	70 E	90 C	210 H	99 A
E7+A2	45 D	90 C	271 B	84 CD
E8+A2	60 C	89 C	273 B	90 B
E10+A2	61 C	99 A	299 H	95 A

Table 3: Effect of *Rhizobium* (E7, E8, E10) and PGPRisolates 1,2 on the production of IAA and GA from Root and shoot of cv.Basmati. Each value represents mean +stdev of 5 plants.

Treatment	IAA (Root) $\mu\text{g/g}$	IAA (Shoot) $\mu\text{g/g}$	GA (Root) $\mu\text{g/g}$	GA (Shoot) $\mu\text{g/g}$
Control	21 H	30 H	70 E	51 E
(E ₇)	38 E	58 E	108 D	53 E
(E ₈)	27 G	73 D	121 F	62 CD
(E10)	44 AC	74 D	111 D	59 D
A1)	40 CDE	57 E	145 AB	60 CD
(A2)	39 DE	63 F	136 C	64 CD
E7+A1	47 AB	111 BC	149 A	81 F
E8+A1	47 AB	120 A	211 G	73 A
E10+A1	59 F	109 C	231 H	65 BC
E7+A2	46 A	99 G	140 BC	69 A
E8+A2	45 A	110 B	190 I	73 A
E10+A2	51 B	116 A	201 I	70 AB

However, all the strains promoted growth of the rice cultivar, cv. The root length, shoot length as shown in Fig. 4 was increase more in double inoculation as compare to single and control. Furthermore the fresh and dry weight of roots and shoots were increased by above mention inoculation.

Interestingly, the magnitude of stimulation was invariably greater in co-inoculated treatments as compared to single treatment. The roots of all the cultivars responded more to the applied inocula than did the shoots. The maximum increase in root length was observed when *Rhizobium* E10 was co-inoculated with

Table 4: Effect of *Rhizobium* (E7, E8, E10) and PGPR isolates 1,2 on the production of IAA with & without Tryptophan.

Treatment	IAA without tryptophan			IAA with tryptophan		
	2ml	4ml	6ml	2ml	4ml	6ml
Control	00	00	00	00	00	00
Rhizobium (E7)	0.08	0.11	0.23	0.43	0.33	0.67
Rhizobium (E8)	0.09	0.10	0.20	0.34	0.46	0.55
Rhizobium(E10)	0.07	0.11	0.19	0.56	0.69	0.73
Azospirillum (1)	0.10	0.13	0.31	0.54	0.71	0.81
Enterobacter(2)	0.12	0.10	0.34	0.50	0.70	0.89
Rhizobium (E7) + 1	0.17	0.20	0.36	0.89	0.98	1.17
Rhizobium (E8) + 1	0.19	0.19	0.32	0.98	1.10	1.31
Rhizobium(E10)+ 1	0.20	0.21	0.40	0.99	1.11	1.28
Rhizobium (E7) + 2	0.16	0.18	0.38	0.90	0.98	1.19
Rhizobium (E8) + 2	0.18	0.24	0.29	0.81	1.45	1.29
Rhizobium(E10)+ 2	0.19	0.18	0.43	1.00	1.21	1.21

Azospirillum (A1) for both Super basmati and Basmati rice varieties. This increase in plant growth following inoculation is possibly attributed to the stimulatory effect of microbes that produce or induced plant growth regulators. It is suggested that growth regulators increase root length and absorption of nutrients leading to overall improved growth of the plant. However, in order to confirm that increase in plant growth indicators is due to change in plant growth regulators, we measured the IAA and GA content of these plants. Our results showed that there were also significant increases (Table 2 and 3) in the IAA and GA contents of both roots and shoots of inoculated plants, similarly GA content in roots inoculated with E10 was particularly strongly increased. The result presented in Table 4 indicated that both the *Rhizobium* sp. and *Azospirillum* sp. has capacities to convert tryptophan to IAA but the *Azospirillum* sp. appeared to be more efficient than the *Rhizobium* sp. The co-inoculation of these two isolates acted synergistically to produce the highest quantity of IAA in culture.

In order to see precursor of IAA which presented in Table 6 indicated that all the *Rhizobium* and *Azospirillum* strains produced IAA *in vitro* culture both alone and mixed culture. This capacity was increased when tryptophan was added to the culture medium. *Rhizobium* strain E10 was more efficient in converting tryptophan to IAA.

Co-inoculation of several forage legumes by *Rhizobium* and *Azospirillum* was found to promote nodulation and nitrogen fixation more efficiently than does single inoculation. Our results clearly showed that dual inoculation has positive effects on plant growth, which could be explained by an enhancement of root growth due to IAA production, resulting in improved

efficiency of minerals and water uptake in inoculated treatments. Gibberellic acid was produced by the microbial cultures with a ranking of the abundance in production being *Rhizobium* E10 strain > the two isolates of *Azospirillum* > the other 2 strains of *Rhizobium* tested.

Control = non-inoculated; E7, E8, E10 = *Rhizobium* strains from QAU Plant Sciences laboratory.

A1 = Strain of *Azospirillum lipoferum* isolated (Kalashah Kaku) from rice roots, A2 = Strain of *Enterobacter* isolated (Gujranwala) from rice roots. E7+A1 = Co-inoculation of *Rhizobium* strain (E7) and *Azospirillum* (A1), E8+A1 = Co-inoculation of *Rhizobium* strain (E8) and *Azospirillum* (A1), E10+A1 = Co-inoculation of *Rhizobium* strain (E10) and *Azospirillum* (A1), E7+A2 = Co-inoculation of *Rhizobium* strain (E7) and *Enterobacter* (A2), E8+A2 = Co-inoculation of *Rhizobium* strain (E8) and *Enterobacter* (A2), E10+A2 = Co-inoculation of *Rhizobium* strain (E10) and *Enterobacter* (A2).

DISCUSSION

Many researchers previously isolate PGPR from soil and identified by Morphological characterization and biochemical tests (QTS-24 kit) which showed that they belong to different bacterial genera including *Azospirillum* and *Enterobacter* as they showed maximum common characteristics with these genera [15]. The variation in morphological characteristics of different isolate and various test have been observed during the study was similar to that reported previously by [16]. Isolation of *A. lipoferum* has often been reported from maize while *A. brasilense* has been commonly obtained from wheat and rice [17]. During previous investigations, it has been suggested that the effects of rhizobial co-inoculation on plants growth such as germination rate, radicle growth, dry matter production, disease control, leaf area, chlorophyll content, drought resistance, radicle and shoot weight depend on the particular combinations used [18]. These interactions have been ranging from the very compatible, resulting in positive effects [19-21]. The less compatible as a consequence of neutral effects [22-24]. to the incompatible resulting in a detrimental effect or demonstrated better results from single inoculations over than the dual inoculations [25]. During current investigations, we studied the effect of rhizobial co-inoculation on growth of rice plants like Increases of IAA and GA in the roots and shoots of rice varieties due to inoculation have previously been reported [26]. Our results demonstrate the possibility of all above mentioned outcomes.

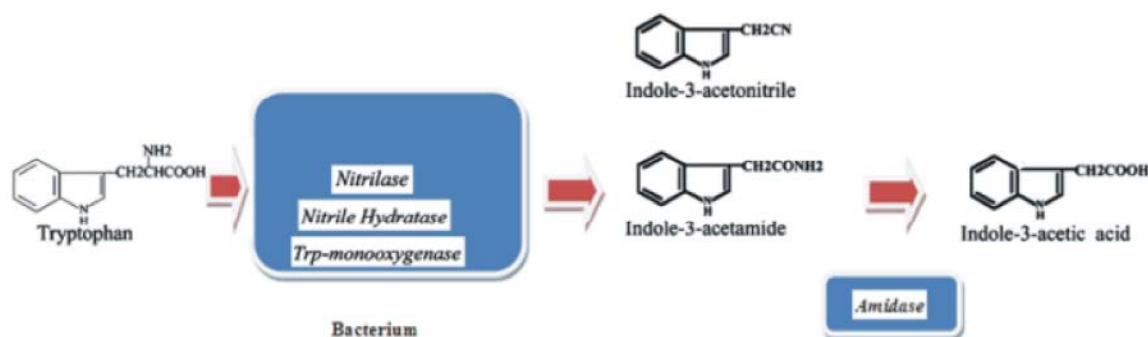


Fig. 5: The figures show synthesis of IAA by Traptophan

This increase in plant growth following inoculation is possibly attributed to the stimulatory effect of microbes that produce or induced plant growth regulators [27]. It is suggested that growth regulators increase root length and absorption of nutrients leading to overall improved growth of the plant [28-29]. This enhancement of single and mixed inoculation has already been reported [30]. However, in order to confirm that increase in plant growth indicators is due to change in plant growth regulators, we measured the IAA and GA content of these plants.

In a previous study, it has been shown that inoculation of wheat plants with rhizobia resulted in increase in root elongation up to 20%, root dry weight up to 13%, shoot elongation 38% and SDW 36%. Similar rate of increase in wheat due to the application of PGPR is also reported [31]. Therefore, our results are in agreement with these results and it can be concluded that the effect of PGPR alone or co-inoculation is not limited to rice plant but it shows influence on other crops.

PGPR strains have been reported to produce IAA either with or without the tryptophan supplement in culture media [32-34]. In order to see precursor of IAA which presented in Table 6 indicated that all the *Rhizobium* and *Azospirillum* strains produced IAA *in vitro* culture both alone and mixed culture. This capacity was increased when tryptophan was added to the culture medium. *Rhizobium* strain E10 was more efficient in converting tryptophan to IAA. The possible pathway of IAA synthesis is summarized in the Fig. 5. The production of IAA by bacteria isolated from rhizosphere of different crops was already reported from wheat, from pearl millet from wheat, maize and rice from peanut [35-36].

This study show that rice roots harbour a variety of PGPRs, including *Azospirillum*, *Enterobacter* and *Rhizobium* strains that are capable of increasing rice plant growth and producing phytohormone growth regulators.

Further results indicate that coinoculation of rice with *Rhizobium* isolates and isolates of *Azospirillum* and *Enterobacter* exhibit synergistic, more stimulating effects when compared to growth responses following inoculation with either *Rhizobium* or *Azospirillum* alone. The mechanism of growth stimulation is mediated by increased IAA and GA production in plants receiving dual inoculation. The best combination of PGPR isolates among those tested was identified as *Rhizobium* strain E10 plus *Azospirillum* strain A1 and this combination of inoculants strains and may be implicated for field studies. These results indicate that the strains isolated from rice field appear more promising for potential biofertilizers in crop fields and can be used for enhanced growth of plants in future.

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