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Isolation and screening of rhizobia for auxin biosynthesis and growth promotion of mung bean (*Vigna radiata* L.) seedlings under axenic conditions

Muhammad Ashfaq Anjum[1], Zahir Ahmad Zahir[1], Muhammad Arshad[1] and Muhammad Ashraf[2]
[1]Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Pakistan
[2]Department of Botany, University of Agriculture Faisalabad, Pakistan

Abstract

A series of screening experiments to evaluate the effectiveness of rhizobia for producing auxins and improve growth and nodulation of mungbean (Vigna radiata L.) were carried out under axenic conditions. Forty four iolatess of rhizobia were isolated using standard procedures. Auxin biosynthesis by these rhizobial isolates was determined in the absence and presence of L-Trp, a physiological precursor of auxins. Rhizobial isolates varied widely in auxins biosynthesis capabilities. On the basis of auxins biosynthesis, a pouch experiment was conducted for screening thirty four efficient isolates of rhizobia for the growth promotion of mung bean. Results of pouch study showed that inoculation with selected rhizobial isolates increased the root /shoot length, fresh, and dry shoot weight of mung bean up to 33, 59, 71, 148, 107 and 188%, respectively, over untreated control. Further studies are needed under glasshouse and field conditions for confirmation of these results.

Key words: Rhizobia, auxins, screening, nodulation, mung bean, axenic

Introduction

Plant hormones play a vital role in controlling plant growth and development throughout ontogeny. Among the phytohormones, auxin (from the Greek term auxien, to increase) was the first plant hormone discovered by Darwin (1880) causing a bending in coleoptiles of the canary grass (*Phalaris canariensis*) coleoptiles. Went (1926, 1928), afterwards confirmed that the isolated active material from the coleoptiles was responsible for inducing phototrophic response in the coleoptiels. Haagen-Smit *et al*. (1942) isolated auxin (IAA) from a plant which was later confirmed by Berger and Avery (1944). Since then, auxin (Indole-3-acetic acid) has been isolated from several other plants. Despite the fact that plants are capable to synthesize auxins, yet they respond to exogenously applied auxins during certain growth phases (Frankenberger and Arshad, 1995).

Another potential and economic source of auxins is soil microbiota. Several microorganisms including rhizobia have also been reported to synthesize auxins (Frankenberger and Arshad, 1995; Zahir *et al*., 1997; Asghar *et al*., 2004; Zahir *et al*., 2005).

Rhizobia are the bacteria which fix atmospheric nitrogen (N_2) in legume plants through legume-rhizobium symbiosis by forming nodules on the roots/stems of these plants. They also produce plant hormones like auxins, gibberellins, cytokinins, abscisic acid and ethylene in addition to symbiotic nitrogen fixation (Frankenberger and Arshad, 1995). Use of rhizobial inoculants is wide spread in many countries of the world being economic, easy to apply and eco-friendly. They play a vital role in improving crop yields. Seed inoculation of legumes with these inoculants has been producing dramatic results in the developed as well as developing countries of the world since the beginning of the 20th century (Zahir *et al*., 2010).

In addition to symbiotic nitrogen fixation, rhizobia also produce plant growth regulators (PGRs) including auxins (Arshad and Frankenberger, 1991; Frankenberger and Arshad, 1995). Auxin biosynthesis by rhizobia is increased many folds in supplementation with suitable precursor (L-tryptophan) (Zahir *et al*., 2005, 2010).

Bhattacharyya and Basu (1997) reported that *Rhizobium sp*. isolated from the root nodules of *Desmodium gangeticum* produced a high amount of indole acetic acid (IAA) from L-tryptophan in culture. Ghosh and Basu (2006) reported that mature root nodules of *Phaseolus mungo* contained higher amount of indole acetic acid (IAA) than non-nodulated roots. The broth supplemented with precursor, L-tryptophan, produced high amount of IAA. Roy and Basu (2004) reported that *Rhizobium sp*. isolated from *Clitoria ternatea* L. produced a high amount of IAA (16.4 µg mL^{-1}) from tryptophan in an unsupplemented basal medium. Datta and Basu (2000) isolated a *Rhizobium sp*. from the root nodules of *Cajanus cajan* which produced high amounts (99.7 µg mL^{-1}) of IAA in L-tryptophan supplemented basal medium.

Hence a laboratory study dealing with *in-vitro* auxin biosynthesis by rhizobial strains in the absence and

*Email: ashfaq1038@gmail.com

presence of L-tryptophan and their application for increasing the growth of mung bean under axenic conditions was conducted.

Materials and Methods

Isolation of rhizobia and measurement of auxins biosynthesis

Mung bean (*Vigna radiata* L.) root samples were collected from different locations of Faisalabad i.e. University of Agriculture, (UAF), Ayub Agricultural Research Institute (AARI) and National Institute for Agriculture and Biology (NIAB). The root samples were saved in polythene bags and transferred to the laboratory. In the laboratory, the roots were washed gently with tap water to remove the soil. Then nodules were separated from the roots and placed in petri-plates. The collected nodules were surface sterilized by momentaring dipping in 95% ethanol solution followed by dipping in 0.2% $HgCl_2$ solution for 3-5 minutes and 6-7 times washings with sterilized water (Russel *et al*., 1982). The surface sterilized nodules were crushed in a minimal volume of sterilized water with the help of a sterilized glass rod to obtain a milky suspension. A loopful of the suspension was streaked out on yeast extract mannitol (YEM) agar medium [yeast, 0.5 g; mannitol, 10.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; distilled water, 1000 mL; pH 6.8] plates and incubated at 28 ± 1 ^{0}C (Vincent, 1970). Well isolated, colourless, single colonies, shiny in appearance, were picked and re-streaked on clean plates to obtain the pure cultures. In this way, forty four fast growing colonies of bacteria were selected, isolated and purified from the mung bean nodules. The purified rhizobial cultures were stored at 4±1 ^{0}C on slants and maintained for further experimentation.

The isolates were transferred on fresh media when needed. Sterilized yeast mannitol broth (YMB) was inoculated with rhizobial isolates in the presence or absence of filter (0.2 µm)- sterilized L-Trp @ 1 g mL^{-1} of broth in glass tubes. These tubes were incubated at 28±1 ^{0}C for 48 hours in shaking incubator at 100 rpm. The contents were filtered through Whatman filter paper No. 2 before measuring auxin production in terms of Indole-3-acetic acid (IAA) equivalents. Auxins biosynthesis was determined (Table 1) colorimetrically by the method described by Sarwar *et al*. (1992). Rhizobial isolates giving higher auxin production were selected for further experiments.

Growth pouch experiment

A growth pouch experiment was conducted in the growth room for screening of the rhizobial isolates (selected on the bases auxins biosynthesis) under gnotobiotic conditions. Broth was prepared by using YEM medium. The test tubes containing 60 mL of medium were inoculated with selected rhizobial isolates and incubated at 28±1 ^{0}C for three days. An optical density of 0.5 recorded at a wavelength of 535 nm, was achieved by dilution to maintain uniform cell density (10^8-10^9 CFU mL^{-1}). Mung bean cultivars (NM-92, NM-98 and NM-2006) seeds were surface-sterilized by momentarily dipping in 95% ethanol solution followed by dipping in 0.2% $HgCl_2$ solution for 3-5 minutes and 6-7 thorough washings with sterilized water (Russel *et al*., 1982). Surface-sterilized mung bean seeds were inoculated by dipping for five minutes in the broth of selected (on the bases auxins biosynthesis) rhizobial isolates. Three inoculated seeds of each mung bean cultivar were placed in each sterilized (autoclaved) growth pouch. In case of control, seeds were dipped in sterilized YEM broth. Modified nitrogen free sterilized Hoagland solution was used for nutrients supply (Fahraeus, 1957). Each treatment was replicated three times. Growth pouches were placed in growth room at 28±2 ^{o}C adjusted to 10.0 hours light and 14.0 hours dark period. After fifteen days of sowing, plants were recovered from pouches along with roots and data regarding root length, shoot length, shoot and root fresh and dry weight were recorded. From the base of the seedling to the upper most tip of the seedling, shoot length and from the base to the longest root branch, root length were recorded. For fresh weights of root/shoot, half hour after harvesting the plant from pouch, roots were separated from shoots from the base and weighed separately. Dry weights of shoot/roots were taken after oven drying the plant at 70^o C until a constant weight is obtained.

Fifteen rhizobial strains performing best in the growth pouch experiment were selected on the basis of Principle Component Scores (Illian *et al.,* 2009) using minitab statistical software, for further experimentation.

Results

Auxins biosynthesis

Rhizobial isolates varied in their auxins producing capabilities with and without L-tryptophan supplementation and auxins biosynthesis increased many folds with the addition of L-tryptophan to the medium (Table 1). These isolates produced auxins *in vitro* in the range of 8.8 µg mL^{-1} IAA equivalents (N19) to 38.3 µg mL^{-1} IAA equivalents (N42) without L-tryptophan. Addition of the L-tryptophan to the media increased auxins biosynthesis by many folds (1.1 to 7.9 folds). Rhizobial isolates were classified as low, medium and high auxin producers in supplementation with L-tryptophan, based upon their auxin producing abilities i.e. low (below 40 µg mL^{-1} IAA equivalents), medium (41 to 70 µg mL^{-1} IAA equivalents) and high (above 70 µg mL^{-1} IAA equivalents). Maximum auxin biosynthesis was noted in case of rhizobial isolate (N42) which produced 126.4 µg mL^{-1} IAA equivalents in L-tryptophan supplemented media.

Table 1: Auxins biosynthesis by rhizobia in the absence and presence of L-tryptophan (L-Trp)

(Average of three repeats)

Rhizobia	IAA equivalents (µg mL^{-1})		
	Without L-TRP	With L-TRP	Folds increase
Low auxin producer			
S43	27.3±0.07	31.3±0.24	1.1
A10	16.9±1.05	35.0±1.21	2.1
A19	11.4±0.26	25.0±0.84	2.2
N19	8.8±0.23	30.1±0.72	3.4
N20	19.6±1.85	24.3±1.34	1.2
N29	23.1±0.51	34.7±1.56	1.5
N37	20.0±0.67	38.0±0.69	1.9
N39	27.6±0.18	36.0±1.54	1.3
Medeium auxin producer			
S44	22.5±1.15	65.6±0.69	2.9
A4	17.4±1.14	60.8±1.47	3.5
A13	16.4±0.68	47.6±0.26	2.9
A16	22.3±0.45	69.3±0.45	3.1
A17	11.8±0.29	69.3±1.04	5.9
A21	19.5±0.06	40.3±2.64	2.1
A22	27.3±0.21	55.3±2.10	2.0
N5	24.9±0.79	69.0±0.71	2.8
N9	19.6±0.20	42.9±0.61	2.2
N13	15.9±0.64	41.0±0.64	2.6
N14	16.2±0.93	42.7±1.35	2.6
N16	20.7±0.35	64.7±1.59	3.1
N17	21.4±0.46	41.1±1.14	1.9
N21	13.1±0.08	43.3±1.17	3.3
N23	12.3±0.37	69.3±0.87	5.6
N24	9.1±0.68	44.8±1.02	4.9
N25	26.2±0.48	44.4±0.32	1.7
N43	28.6±2.12	44.3±1.64	1.5
N45	24.5±0.48	45.3±1.55	1.9
N49	23.2±0.40	56.1±0.69	2.4
High auxin producer			
S6	33.7±0.42	82.5±1.54	2.4
S9	28.5±0.48	85.8±0.18	3.0
S24	24.8±1.39	81.4±0.83	3.3
S25	22.6±0.96	85.8±2.59	3.8
A2	16.5±0.73	81.0±2.73	4.9
A3	19.5±0.21	86.9±0.80	4.5
A18	14.5±0.50	83.0±1.33	5.7
A23	23.4±0.08	105.3±2.16	4.5
N3	13.1±0.35	76.2±2.43	5.9
N8	21.6±0.26	77.8±2.70	3.6
N11	19.4±0.26	83.3±1.44	4.3
N12	24.9±1.15	96.2±1.94	3.9
N15	14.2±0.03	78.4±0.95	5.5
N18	10.8±0.67	85.2±0.68	7.9
N41	25.7±0.41	87.6±1.01	3.4
N42	38.3±0.36	126.4±1.03	3.3

LSD = 3.781, CV = 5.72

Low auxin producing rhizobial isolates S43, A10, A19, N19, N20, N29, N37 and N39 produced auxins from 8.8 to 27.6 µg mL^{-1} IAA equivalents in the absence of L-tryptophan while the addition of L-tryptophan to the broth medium, increased the auxin production 1.1-3.4 folds under *in vitro* conditions ranging between 24.3 to 38.0 µg mL^{-1} IAA equivalents. Maximum auxins in this group were produced by rhizobial isolate N37 (38.0 µg mL^{-1} IAA equivalents) which were 1.9 folds more than without L-tryptophan addition to the medium. Medium auxins producing rhizobial isolates produced auxins in the range between 9.1 to 28.6 µg mL^{-1} IAA equivalents without L-tryptophan while auxins biosynthesis increased 1.5 to 5.9 folds in L-tryptophan supplemented media. Maximum IAA equivalents produced by these isolates was 69.3 µg mL^{-1} IAA equivalents in the L-Trp supplemented broth by three rhizobial isolates i.e. A16, A17 and N23.These isolates produced 22.3, 11.8 and 12.3 µg mL^{-1} IAA equivalents, respectively, in the absence of L-tryptophan. High auxins producing rhizobia (S6, S9, S24, S25, A2, A3, A18, A23, N3, N8, N11, N12, N15, N18, N41 and N42) produced auxins in the range of 10.8-38.3 µg mL^{-1} IAA equivalents without L-tryptophan while auxins biosynthesis increased from 2.4 to 7.9 folds ranging between 76.2 to 126.4 µg mL^{-1} IAA equivalents. Maximum IAA equivalents were produced by rhizobial isolate N42 i.e. 38.3 µg mL^{-1} IAA equivalents without L-tryptophan supplementation while a 3.3 folds increase in IAA equivalents was noted in L-tryptophan supplemented medium.

Growth pouch experiments

Data regarding shoot length of mung bean (cv: NM-92) presented in Table 2 exhibited that 13 rhizobial isolates increased the shoot length by 25% or more than 25% compared with control and 20 isolates increased shoot length less than 25% while only one rhizobial isolate A16 showed a decrease in shoot length compared with un-inoculated control. Maximum increase in shoot length (33%) was given by isolate S25 while a decrease in shoot length (2%) was observed with isolate A16 inoculation over un-inoculated control. Data indicated that 12 rhizobial isolates increased shoot length of mung bean (NM-98) by 25% than their respective un-inoculated control, 20 isolates increased shoot length less than 25%, N5 isolate had no effect on shoot length while only one rhizobial isolate resulted in a decreased shoot length compared with control. Maximum shoot length (32% higher) was observed by S6 isolate while N45 resulted in a decreased shoot length by 7% than un-inoculated control. In case of mung bean variety NM-2006, only one rhizobial

Table 2: Effect of different rhizobial isolates on shoot /root length of three mung bean cultivars

(Averages of 3 replicates ± standard error)

Rhizobia	Shoot length (cm)			Root length (cm)		
	NM-92	NM-98	NM-2006	NM-92	NM-98	NM-2006
Control	14.8±0.14	15.0±0.72	16.3±0.36	13.3±0.27	14.7±0.27	14.3±0.49
S6*	19.5±0.62	22.0±0.83	17.3±0.72	18.0±0.41	15.5±0.24	20.7±0.98
S9*	19.2±0.49	17.5±0.47	19.3±0.27	16.7±0.36	18.0±0.71	16.2±0.14
S24*	18.2±0.68	17.5±0.36	19.0±0.47	20.2±0.83	17.8±0.36	18.8±0.36
S25*	19.7±0.72	20.0±0.24	19.2±0.36	16.3±0.83	18.3±0.27	19.0±0.24
S43	17.8±0.76	17.0±0.41	18.2±0.83	17.8±0.49	17.7±0.95	11.8±1.42
A2*	19.0±0.47	19.5±0.24	19.3±0.27	16.8±0.59	18.3±1.57	17.7±0.68
A3*	16.3±0.72	16.0±0.82	19.5±0.24	15.3±0.83	16.7±1.30	17.0±1.55
A4	16.7±0.54	16.0±0.72	17.3±0.27	13.3±0.72	16.7±1.19	16.3±0.98
A13	17.0±0.24	16.0±0.47	17.7±0.27	15.0±0.62	15.7±1.36	16.3±1.19
A16	14.5±0.62	18.0±0.62	18.0±0.47	13.0±0.85	13.8±0.59	14.7±0.98
A17*	18.5±0.24	19.5±0.36	19.0±0.47	19.0±0.85	17.7±0.49	18.8±0.36
A18*	19.0±0.47	17.0±1.25	17.3±0.27	18.3±0.83	17.0±1.25	17.0±0.47
A21	16.0±0.47	16.0±0.54	18.2±0.72	15.5±0.85	17.8±0.76	16.2±1.44
A22	16.5±0.62	16.5±1.55	15.8±0.76	13.0±0.85	15.5±0.62	15.3±1.38
A23*	19.5±0.41	16.0±0.72	19.2±0.49	19.0±0.47	18.8±0.89	18.3±1.11
N3	16.2±0.95	17.0±0.47	17.2±0.59	16.8±0.59	15.3±1.52	16.2±1.16
N5	16.3±0.72	15.0±0.85	17.3±1.44	13.3±0.72	13.8±0.14	16.0±0.47
N8*	18.0±0.24	19.0±0.41	17.8±1.36	15.8±0.49	16.7±0.76	17.3±0.59
N9	15.5±0.24	17.0±0.59	18.3±0.59	17.2±0.59	17.2±0.59	19.8±0.49
N11	17.0±0.47	19.0±0.54	17.2±0.89	16.7±0.72	17.3±0.98	16.5±1.55
N12*	19.3±0.27	21.0±0.98	17.5±0.62	17.3±0.98	19.0±0.24	15.2±0.76
N14	18.0±0.94	18.0±0.27	17.3±0.54	13.3±0.27	16.3±0.72	16.2±0.36
N15	16.8±0.36	17.0±0.72	16.3±0.27	15.5±0.62	14.3±0.54	15.5±0.24
N16	18.5±0.71	17.0±0.98	17.8±1.74	15.5±0.62	15.7±0.72	18.3±0.59
N18*	18.7±0.49	20.0±0.36	18.8±0.27	16.8±0.59	19.5±0.82	18.2±0.27
N21	16.3±0.27	18.0±0.27	17.0±0.47	17.7±0.27	17.7±1.19	17.8±0.68
N23	17.2±0.36	19.0±0.98	18.7±0.72	14.8±0.83	15.3±0.54	15.0±1.25
N24	18.5±0.62	17.0±0.94	17.3±1.06	11.5±0.62	17.0±0.82	16.0±1.31
N25*	16.5±0.62	17.5±1.19	18.5±0.95	17.5±0.14	16.8±0.47	18.3±0.36
N41	18.2±0.49	19.0±0.49	18.2±0.41	21.2±0.41	18.0±0.54	18.7±0.59
N42*	19.3±0.72	19.0±0.47	20.8±0.36	19.5±0.24	16.3±0.27	18.8±0.49
N43	18.7±0.54	20.0±0.47	18.3±0.54	15.0±0.82	11.8±0.36	15.5±0.62
N45	16.8±0.49	14.0±0.47	17.8±0.36	14.7±0.54	17.3±0.72	15.0±0.47
N49*	18.0±0.00	16.0±0.47	18.3±0.59	16.7±0.14	18.3±0.27	17.8±0.36
SED(Standard error of difference between two means)			= 1.102		= 1.411	
Error mean square			= 1.215		= 1.993	
P value at 5%			= 0.0001		= 0.0001	

*These were significantly better than rest of the rhizobial isolates according to General Linear Model and were selected for jar trial

isolate N42 increased shoot length by more than 25%, 31 rhizobial isolates indicated an increase of 4-20% in shoot length, N15 did not show any increase or decrease in growth parameter while A22 resulted in a decrease in shoot length (3%) compared with un-inoculated control.

Data regarding root length of mung bean (NM-92) indicated that 19 rhizobial isolates increased root length by more than 25%, 10 isolates increased from 11-23%, N14, A4 and N5 had no effect on root length while A16, A22 and N24 exhibited a negative effect (2-14%) on root length than un-inoculated control. Maximum root length was produced by N41 (59%) compared with un-inoculated control.

Data indicated that root growth of mung bean (NM-98) was positively affected producing 28 to 33% increase by 3 rhizobial isolates, 4 to 24% by 27 rhizobial isolates while 3-20% decrease in root length was observed by N15, A16, N5 and N43 than their respective un-inoculated control.

Data regarding root length in mung bean variety NM-2006 by rhizobial isolates indicated that 11 out of 34 rhizobial isolates, increased root length by 25% or more,

Table 3: Effect of different rhizobial isolates on fresh shoot /root weight of three mung bean cultivars

(Averages of 3 replicates ± standard error)

Rhizobia	Fresh shoot weight (g)			Fresh root weight (g)		
	NM-92	NM-98	NM-2006	NM-92	NM-98	NM-2006
Control	0.50±0.02	0.49±0.01	0.46±0.01	0.23±0.01	0.25±0.01	0.21±0.01
S6*	0.62±0.01	0.53±0.02	0.58±0.03	0.35±0.03	0.54±0.01	0.30±0.01
S9*	0.62±0.02	0.65±0.01	0.57±0.02	0.27±0.02	0.53±0.02	0.30±0.03
S24*	0.57±0.03	0.56±0.02	0.69±0.02	0.29±0.01	0.56±0.02	0.31±0.03
S25*	0.56±0.01	0.53±0.01	0.61±0.01	0.27±0.02	0.41±0.00	0.26±0.01
S43	0.68±0.01	0.61±0.00	0.63±0.01	0.34±0.02	0.42±0.01	0.22±0.02
A2*	0.55±0.02	0.58±0.01	0.59±0.01	0.27±0.03	0.45±0.01	0.32±0.01
A3*	0.57±0.01	0.65±0.01	0.57±0.03	0.28±0.01	0.36±0.01	0.28±0.01
A4	0.64±0.03	0.63±0.03	0.61±0.01	0.33±0.02	0.29±0.01	0.31±0.02
A13	0.67±0.02	0.58±0.02	0.52±0.02	0.28±0.02	0.28±0.02	0.37±0.01
A16	0.62±0.01	0.56±0.03	0.51±0.02	0.26±0.01	0.28±0.01	0.26±0.03
A17*	0.59±0.01	0.59±0.03	0.62±0.02	0.28±0.02	0.39±0.03	0.29±0.01
A18*	0.66±0.02	0.73±0.02	0.57±0.03	0.24±0.01	0.58±0.03	0.30±0.03
A21	0.49±0.03	0.67±0.01	0.54±0.02	0.30±0.03	0.28±0.01	0.31±0.01
A22	0.44±0.02	0.54±0.03	0.62±0.01	0.29±0.02	0.29±0.02	0.29±0.01
A23*	0.62±0.01	0.78±0.02	0.57±0.03	0.29±0.01	0.40±0.03	0.29±0.02
N3	0.64±0.01	0.58±0.03	0.66±0.02	0.26±0.02	0.32±0.03	0.32±0.01
N5	0.62±0.01	0.40±0.03	0.61±0.02	0.28±0.00	0.36±0.02	0.33±0.01
N8*	0.64±0.02	0.54±0.02	0.55±0.02	0.35±0.01	0.32±0.00	0.31±0.01
N9	0.78±0.02	0.41±0.02	0.53±0.02	0.23±0.01	0.24±0.00	0.28±0.02
N11	0.68±0.02	0.68±0.02	0.69±0.01	0.32±0.02	0.31±0.01	0.26±0.02
N12*	0.58±0.01	0.68±0.02	0.68±0.02	0.34±0.01	0.52±0.02	0.37±0.02
N14	0.71±0.01	0.69±0.01	0.68±0.01	0.34±0.01	0.48±0.02	0.33±0.03
N15	0.70±0.01	0.71±0.01	0.67±0.03	0.32±0.01	0.43±0.01	0.31±0.01
N16	0.66±0.02	0.65±0.01	0.68±0.01	0.33±0.03	0.39±0.02	0.31±0.01
N18*	0.69±0.03	0.59±0.01	0.56±0.02	0.35±0.01	0.30±0.01	0.27±0.01
N21	0.69±0.01	0.69±0.00	0.64±0.02	0.28±0.02	0.38±0.01	0.32±0.03
N23	0.68±0.02	0.69±0.02	0.68±0.01	0.33±0.02	0.49±0.01	0.32±0.02
N24	0.58±0.01	0.69±0.02	0.67±0.02	0.35±0.03	0.32±0.00	0.33±0.02
N25*	0.67±0.02	0.62±0.01	0.59±0.00	0.32±0.02	0.32±0.02	0.31±0.02
N41	0.68±0.03	0.78±0.02	0.69±0.01	0.32±0.02	0.42±0.02	0.47±0.02
N42*	0.73±0.03	0.53±0.00	0.76±0.00	0.40±0.01	0.34±0.01	0.52±0.02
N43	0.71±0.03	0.66±0.02	0.65±0.00	0.32±0.03	0.33±0.03	0.29±0.01
N45	0.62±0.02	0.56±0.01	0.71±0.01	0.31±0.00	0.47±0.01	0.28±0.01
N49*	0.78±0.01	0.84±0.00	0.71±0.02	0.36±0.01	0.54±0.02	0.36±0.02
SED(Standard error of difference between two means)			= 0.066		= 0.0711	
Error mean square			= 0.0043		= 0.0051	
P value at 5%			= 0.000		= 0.000	

*These were significantly better than rest of the rhizobial isolates according to General Linear Model and were selected for jar trial

the other 22 isolates increased root length from 3 to 24% than their respective un-inoculated control. Only one rhizobial isolate (S43) resulted in 17% decreased root length. Maximum root length (45%) was observed by S6 inoculation while A16 gave minimum increase (3%) over un-inoculated control.

Results regarding fresh shoot weight of mung bean var. NM-92 (Table 3) indicated that 19 rhizobial isolates increased fresh shoot weight by 25% or more, 13 rhizobial isolates less than 25% while only two rhizobial isolates i.e. A21, A22 produced 2 and 12%, respectively, less fresh shoot weight compared with control. Data depicted that fresh shoot weight of mung bean variety NM-98 was increased from 27 to 71% by 18 rhizobial isolates; the other 14 isolates resulted in an increased fresh shoot weight by less than 25% (8 to 24%) over control while two rhizobia N5 and N9 decreased fresh shoot weight by 18 and 16 %

compared with un-inoculated control. Maximum increase in fresh shoot weight (71%) was observed in case of N49 inoculation over control.

Data regarding fresh shoot weight in mung bean variety NM-2006 indicated that 24 rhizobial isolates out of 34 resulted in an increase of shoot fresh weight by 25% or more, than their respective un-inoculated control while other 10 isolates increased fresh shoot weight by 11-24% over control. Maximum increase in shoot fresh weight was recorded in case of N42 inoculation (65%) while minimum increase was noted by A16 inoculation over un-inoculated control.

Data (Table 3) regarding mung bean variety NM-92 indicated that 22 rhizobia out of 34 rhizobial isolates increased fresh root weight by 25% or more, 11 rhizobial isolates resulted in an increase in fresh root weight (4 to 22%) over their respective un-inoculated control while N9 had no effect on fresh root biomass. Isolate N42 produced maximum fresh root weight by (74%) increase compared with control and A18 produced only 4% increase than un-inoculated control.

In mung bean variety NM-98, 26 rhizobial isolates increased fresh root weight by 28-132%, while 7 isolates gave an increase in fresh root weight which was less than 25% (12-24%) and only one isolate (N9) decreased root weight by 4% compare with itsrespectiveun-inoculated control.

Data represented that 30 out of 34 rhizobial isolates increased fresh root weight of mung bean (variety NM-2006) by 25% or more while 4 rhizobial isolates resulted in 5 to 24% increase in fresh root weight compared with un-inoculated control. Maximum fresh root weight was produced by N42 inoculation (148% increase over un-inoculated control).

Results (Table 4) depicted that 24 rhizobial isolates out of 34, increased shoot dry weight (NM-92) by more than 25%, while 5 isolates resulted in an increased shoot dry weight by less than 25% compared with control. However, 7 to 21% decrease in dry matter over control was observed with other 5 rhizobial isolates. Maximum increase in shoot dry weight (107%) over control was observed by A18 inoculation.

In case of mung bean variety NM-98, 27 rhizobia out of 34 increased shoot dry weight by more than 25%, 3 rhizobial isolates resulted in an increase in shoot dry weight by less than 25%, 2 rhizobial isolates had no effect on shoot dry weight while only 2 isolates of rhizobia decreased shoot dry weight by 21% than un-inoculated control. Maximum increase in shoot dry weight (93%) was observed by A23 inoculation while A21 and N21 gave minimum increase (21%) over control.

In mung bean variety NM-2006, out of 34 rhizobial isolates, 18 increased shoot dry weight by 25% or more, 12 produced less than 25% shoot dry weight, one isolate N9 had no effect on shoot dry weight while only 3 showed a decrease in shoot dry weight than un-inoculated control. Isolate A3 produced maximum dry weight of shoot (56%) over control while N11 exhibited a more negative effect on shoot dry weight (25% decrease) compared with control.

Data regarding root dry weight (Table 4) represented that 24 out of 34 rhizobial isolates resulted in increased dry root weight by 25% or more, 6 rhizobia increased root dry weight by less than 25% compared with un-inoculated control. Isolate N24 had no effect on root dry weight while 3 isolates decreased root dry weight of mung bean variety NM-92 than control.

It is evident from data that 18 rhizobial isolates out of 34, increased root dry weight of mung bean (variety NM-98) by more than 25% while 3 isolates produced root dry weight less than 25% compared with control. Isolates S25 and N21 did not affect dry root weight while 11 rhizobia produced 6 to 56% compared with un-inoculated control. A18 produced maximum root dry weight (88% isolate over control).

In mung bean cv. NM-2006, 33 rhizobial isolates increased dry root weight by more than 25% while only one rhizobia (S43) produced less than 25% increased dry root weight.

Selection of rhizobial isolates for further experiment

On the basis of all these six growth parameters i.e. shoot/ root length, fresh shoot/root weight, dry shoot/root weight, 15 rhizobial isolates i.e. S6, S9, S24, S25, A2, A3, A17, A18, A23, N8, N12, N18, N41, N42 and N49 were selected by analyzing these parameters using Principal Component Scoring method (Illian *et al*., 2009). These rhizobial isolates having maximum component scores were selected for further experimentation (Table 5).

Discussion

Rhizobial strains were isolated from the nodules of mung bean. These strains were screened for their auxins biosynthesis in the presence and absence of L-tryptophan. The selected strains (efficient auxins producers) were further evaluated for their ability to improve mung bean growth under axenic conditions in growth pouch trial.

In this study, all the rhizobial isolates produced auxins (expressed as IAA equivalents) in the presence and absence of L-tryptophan but with variable degrees of efficacy. However, the auxin production by all rhizobial isolates was more in the presence of L-tryptophan (an auxin precursor). Auxin biosynthesis by *Rhizobium sp.* has also been reported by Bhattacharyya and Pati (2000). They reported that *Rhizobium sp.* isolated from the root nodules of *Alysicarpus*

vaginalis produced higher amounts (107µg mL^{-1}) of auxin in the presence of L-TRP. Similarly, higher amount of auxins production by different bacterial strains in the presence of L-TRP has been reported by other researchers (Datta and Basu, 1998; Ghosh and Basu, 2002; De and Basu, 2007). It has been reported by Ghosh and Basu (1999) that mature stem nodules of *Aeschynomene aspera* L. contained high amount (2.54 µg g^{-1} fresh weight) of IAA which may be due to the production of higher quantities of auxin in L-TRP supplemented medium by the microsymbiont, *Azorhizobium caulinodans*. Similar results have also been reported by other scientists (Arshad and Frankenberger, 1992; Zahir *et al*., 2000; Asghar *et al*., 2002; Zahir *et al*., 2005; Ghosh and Basu, 2006).

Biological nitrogen fixation plays a vital role in increasing the growth of legumes by developing a symbiotic relationship between rhizobia and plants. Our study conducted under gnotobiotic conditions revealed that most of the rhizobial cultures increased the mung bean growth but with different degrees of efficacy. This variability in their growth promotion might be due to production of auxins. Variable response of rhizobial inoculation for improving growth of lentil, chickpea and pea has also been reported in previous studies (Yanni, 1992; Huang and Erickson, 2007; Zafar-ul-Hye *et al*., 2007; Etesami *et al*., 2009). Similarly, increase in root dry weight and shoot dry weight due to rhizobial inoculation has also been reported by Pal *et al*. (2000). Increase in plant growth and mineral nutrient uptake due to auxin production has also been reported by Etesami *et al*. (2009).

It was observed in the present study that some of the rhizobial isolates did not improve growth of mung bean seedlings under gnotobiotic conditions. Failure in growth promotion activity might be due to lack of efficiency by *Rhizobium* strains to colonize the root surface (Jadhav *et al*., 1994). Non-significant improvement in growth parameters has also been reported by van Rhijn *et al*. (2001) in pea (*Pisum sativum*) and alfalfa (*Medicago sativa* L.) plants in response to *Rhizobium* inoculants. This may be due to deficiency of exopolysaccharides to rhizobial strains due to mutation or some unknown reasons.

In our study, some of the rhizobial isolates reduced or negatively affected the growth of mung been seedling compared with un-inoculated control. It might be due to the production of toxic substances like phytotoxins and/or competition for nutrients (Antoun and Kloepper, 2001; Kloepper, 2003). In addition, there might be some unspecified type of antagonism that prevented the isolates to colonize the root surface (Jadhav *et al*., 1994). So, screening of effective rhizobial strains under controlled conditions on the basis of auxin production and growth promotion could be a useful strategy for the selection of efficient isolates.

Table 5: Principle component scores of rhizobial isolates on the basis of plant growth

Rhizobial isolate	Principle Component Score
Control	-3.60
S6*	0.52
S9*	0.23
S24*	0.54
S25*	0.23
S43	-0.36
A2*	1.09
A3*	0.84
A4	-0.80
A13	-1.21
A16	-2.20
A17*	0.61
A18*	0.49
A21	-1.44
A22	-2.21
A23*	1.59
N3	-1.03
N5	-1.86
N8*	0.32
N9	-1.56
N11	-0.28
N12*	2.30
N14	0.10
N15	0.05
N16	0.21
N18*	0.94
N21	-0.01
N23	-0.73
N24	-0.29
N25*	2.87
N41	-0.60
N42*	3.40
N43	-0.27
N45	-0.50
N49*	2.61

*These rhizobial isolates were selected for further experimentation

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References

Antoun, H. and J.W. Kloepper. 2001. Plant growth-promoting rhizobacteria (PGPR). p. 1477-1480. *In:* Encyclopedia of Genetics. S. Brenner and J.H. Miller (Eds.), Academic Press, NY.

Arshad, M. and W.T. Frankenberger, Jr. 1991. Microbial production of plant hormones. Plant Soil 133: 1-8.

Arshad, M. and W.T. Frankenberger, Jr. 1992. Microbial production of plant growth regulators. p. 307-347. *In:*

Soil Microbial Ecology B. Metting (ed.) Marcel Dekker, Inc., New York.

Asghar, H.N., Z.A. Zahir, M. Arshad and A. Khaliq. 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils* 35: 231-237.

Asghar, H.N., Z.A. Zahir, M. Arshad and A. Khaliq. 2004. Relationship between in-vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils* 35: 231-237.

Berger, J. and G.S. Avery, Jr. 1944. Isolation of an auxin precursor and an auxin (indole acetic acid) from maize. *American Journal of Botany* 31: 199-203.

Bhattacharyya, R.N. and B.R. Pati. 2000. Growth behaviour and indole-acetic acid (IAA) production by a rhizobium isolated from root nodules of *Alysicarpus vaginalis* DC. *Acta Microbiologica et Immunologica Hungarica* 47(1): 41-51.

Bhattacharyya, R.N. and P.S. Basu. 1997. Bioproduction of indole-acetic acid by a *rhizobium* sp. from the root nodules of *Desmodium gangeticum* DC. *Acta Microbiologica et Immunologica Hungarica* 44(2): 109-118.

Darwin, C.R. 1880. The Power of Movement in Plants. London. Murray.

Datta, C. and P.S. Basu. 1998. Content of indole-acetic acid and its metabolism in root nodules of *Melilotus alba*. *Folia Microbiologica* 43: 427-430.

Datta, C. and P.S. Basu. 2000. Indole-acetic acid production by a rhizobium species from root nodules of a leguminous shrub, *Cajanus cajan*. *Research in Microbiology* 155(2): 123-7.

De, P. S. and P. S. Basu. 2007. Content of different phytohormones and indole acetic acid metabolism in root nodules of *Derris scandens*. *Journal of Basic Microbiology* 36: 299-304.

Etesami, H., H.A. Alikhani and A.A. Akbari. 2009. Evaluation of plant growth hormones production (IAA) ability by Iranian soils Rhizobial strains and effects of superior strains application on wheat growth indexes. *World Journal of Applied Sciences* 6(11): 1576-1584.

Fahraeus, G. 1957. The infection of white clover root hair by nodule bacteria studied by a simple slide technique. *Journal of General Microbiology* 16: 374-381.

Frankenberger, W.T. Jr. and M. Arshad. 1995. Phytohormones in soil: Microbial production and function. New York, Marcel Dekker.

Ghosh, A.C. and P.S. Basu. 1999. Indole-acetic acid and its metabolism in the stem nodules of a leguminous emergent hydrophyte, *Aeschynomene aspera*. *Microbiology Research* 153: 337-340.

Ghosh, A.C. and P.S. Basu. 2002. Growth behaviour and bioproduction of indole-acetic acid by a *Rhizobium sp*. isolated from root nodules of a leguminous tree *Dalbergia lanceolaria*. Biology 40: 796-801.

Ghosh, S. and P.S. Basu. 2006. Production and metabolism of indole-acetic acid in roots and root nodules of *Phaseolus mungo*. *Research in Microbiology* 161(4): 362-366.

Haagen-Smit, A.J., W.D. Leach and W.R. Bergern. 1942. The estimation, isolation and identification of auxins in plant materials. *American Journal of Botany* 29: 500-506.

Huang, H.C. and R.S. Erickson. 2007. Effect of seed treatment with Rhizobium leguminosarum on pythium damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. *Journal of Phytopathology* 155: 31-37.

Illian, J.B., J.I. Prosser, K.L. Baker, and J.I. Rangel-Castro. 2009. Functional principle component data analysis: A new method for analysing microbial community fingerprints. *Journal of Microbiological Methods* 79(1):89-90.

Jadhav, R.S., N.V. Thaker and A. Desai. 1994. Involvement of the siderophore of cowpea *Rhizobium* in the iron nutrition of the peanu. *World Journal of Microbiology and Biotechnology* 10: 360-61.

Khalid, A., S. Tahir, M. Arshad and Z.A. Zahir. 2004. Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. *Australian Journal of Soil Research* 42(8): 921-926.

Kloepper, J.W. 2003. A review of mechanisms for plant growth promotion by PGPR. p. 81-92. *In:* Abstracts and short papers 6th International PGPR workshop. M.S. Reddy, M. Anandaraj, S.J. Eapen, Y.R. Sarma and J.W. Kloepper (eds.). October 5-10, 2003. Indian Institute of Spices Research, Calicut, India.

Pal, K. K., R. Dey, D. M. Bhattand S. M. Chauhan. 2000. Plant growth promoting fluorescent pseudomonads enhanced peanut growth, yield and nutrient uptake. Auburn University Web Site, Available at: http:/www.ag.auburn,cdu/pdtmaiiuscripts/pal.pdf. [Accessed on 7/01/2001].

Roy, M. and P.S. Basu. 2004. Studies on root nodules of leguminous plants bioproduction of indole acetic acid by a *Rhizobium sp*. from a twiner *Clitoria ternatea* L. *Acta Biotechnology* 12(6): 453-460.

Russell, A.D., W.B. Hugo and G. A. J. Ayliffo. 1982. Principles and practices of disinfection, preservation and sterilization. Black Wall Scientific, London.

Sarwar, M., M. Arshad, D.A. Martens and W.T. Frankenberger, Jr. 1992. Tryptophan dependent biosynthesis of auxins in soil. *Plant Soil* 147:207-215.

Van Rhijn, P., N.A. Fujishige, P.O. Lim and A.M. Hirsch. 2001. Sugar-binding activity of pea lectin enhances

heterologous infection of transgenic alfalfa plants by *Rhizobium leguminosarum* biovar *viciae*. *Plant physiology* 126: 133-44.

Vincent, J.M. 1970. A manual for the Practical Study of Root Nodule bacteria. Oxford Blackwell Scientific.

Went, F.W. 1926. On growth-accelerating substances in the coleoptiles of *Avena sativa*. Proc. Kon. Akad. Wetensch. Amsterdam 30: 10-19.

Went, F.W. 1928. Wuchsstoff und Wachstum. Rec. Trav. Bot. Neerland. 24:1-116.

Yanni, Y.G. 1992. Performance of chickpea, lentil and lupine nodulated with indigenous or inoculated Rhizobia micro-partners under nitrogen, boron, cobalt and molybdenum fertilization schedules. *World Journal of Microbiology and Biotechnology* 8: 607-613.

Zafar-ul-Hye, M., Z.A. Zahir, S.M. Shahzad, U. Irshad and M. Arshad. 2007. Isolation and screening of rhizobia for improving growth and nodulation of lentil (*Lens culinaris* Medic) seedlings under axenic conditions. *Soil and Environment* 26(1): 81-91, 2007.

Zahir, Z.A., M. Arshad, M. Azam and A. Hussain. 1997. Effect of an auxin precursor L-tryptophan and *Azotobacter* inoculation on yield and chemical composition of potato under fertilized conditions. *Journal of Plant Nutrition* 20: 745-752.

Zahir, Z.A., S.A. Abbas, M. Khalid and M. Arshad. 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. *Pakistan Journal of Biological Sciences* 3(2): 289-29.

Zahir, Z.A., H.N. Asghar, M.J. Akhtar and M. Arshad. 2005. Precursor (L-tryptophan)-inoculum (*Azotobacter*) interactions for improving yields and nitrogen uptake of maize. *Journal of Plant Nutrition* 28: 805-817.

Zahir, Z.A., M.K. Shah, M. Naveed and M.J. Akhtar. 2010. Substrate dependent auxin production by *Rhizobium phaseoli* improve the growth and yield of *Vigna radiate* L. under salt stress conditions. *Journal of Microbiology and Biotechnology* 20(9): 1288-1294