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GROWTH AND YIELD RESPONSE OF WHEAT TO INOCULATION WITH AUXIN PRODUCING PLANT GROWTH PROMOTING RHIZOBACTERIA

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Abstract

Effectiveness of rhizobacteria for promoting the growth and yield of different cultivars of wheat was evaluated by conducting Leonard jar and pot trials. Thirty one cultures of bacteria isolated from wheat rhizosphere soil were screened on the basis of their auxin producing ability *in vitro*. They were further tested for their growth promoting activity by conducting Leonard jar experiments on four cultivars of wheat under axenic conditions. Based upon the data recorded regarding auxin production *in vitro*, and screening in Leonard jar experiments, four isolates (W_9, W_{11}, W_{14}, and W_{29}) were selected to conduct pot experiment in the wire house under non-axenic conditions and considered as plant growth-promoting rhizobacteria (PGPR). Seeds of four wheat cultivars (Pasban-90, Inqlab-91, Watan-93, and Punjab-96) inoculated with these four PGPR isolates were sown in pots. Uninoculated control was kept for comparison in each cultivar. To eliminate any nutritional stress, nutrients were applied as NPK @ 120-75-50 kg ha^{-1}, respectively. Results showed that selected PGPR isolates significantly increased plant height (up to 9.9%), number of tillers (up to 32.3%), spike length (up to 6.8%), spikelets $spike^{-1}$ (up to 14.0%), straw and grain yields (up to 16.1 and 29.0%, respectively) in all the tested cultivars of wheat with different degree of efficacy. Among the various PGPR isolates tested, W_{11} was found the most effective in promoting growth and yield of different cultivars of wheat compared to control. Overall, the response to inoculation with various PGPR isolates varied with cultivars.

Introduction

Plant growth-promoting rhizobacteria (PGPR) are a natural source for improving the efficiency and vitality of crop plants. PGPR encompass all the bacteria that inhabit plant roots and exert a positive effect by mechanisms, ranging from direct influence e.g., increased solubilization and uptake of nutrients or production of plant growth regulators, to an indirect effect e.g., pathogen suppression such as biocontrol, production of siderophores or antibiotics (Kloepper *et al.*, 1989; Glick *et al.*, 1994). Beneficial rhizobacteria are usually referred to as PGPR (Kloepper *et al.*, 1989), or by Chinese as YIB the Yield increasing bacteria (Chen *et al.*, 1994).

A diverse array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Acetobacter, Bacillus, Klebsiella, Enterobacter, Serratia* and *Xanthomonas* have been shown to promote plant growth either through the action of direct or indirect mechanisms. Like other phytohormones, auxins are synthesized endogenously by plants and their hormonal effects have been elucidated largely by their exogenous application. There is also ample evidence that numerous soil microorganisms are actively involved in the synthesis of auxins in pure culture and in soil (Arshad & Frankenberger, 1998; Biswas *et al.*, 2000a, b). Generally, microorganisms isolated from the rhizosphere and rhizoplane of various crops have revealed more potential of auxin production than those from the root free soil (Sarwar & Kremer, 1995; Arshad & Frankenberger, 1998). L-Tryptophan (L-TRP), an amino acid, serves as a physiological precursor for biosynthesis

of auxins in plants and in microbes (Frankenberger & Arshad, 1995). Root exudates are the only natural source of TRP for rhizosphere microflora. Kravchenko *et al.*, (1991) and Martens & Frankenberger (1994) found detectable amounts of TRP in root exudates of some but not in all varieties of wheat. High performance liquid chromatography (HPLC) analysis provided unequivocal proof that auxins were produced in soil when incubated with L-TRP (Frankenberger & Brunner, 1983; Sarwar *et al.*, 1992).

Significant increases in growth and yield in wheat, maize and potato have been reported in response to inoculation with PGPR (Chen *et al.*, 1994; Javed & Arshad, 1999; Zahir *et al.*, 2000). Studies have also shown that the growth promoting ability of some bacteria may be highly specific to certain plant species, cultivars and geographical area. Chen *et al.*, (1994) found that PGPR used in geographical area outside the isolated source had variable efficacy with *Pseudomonas* species. Similarly, Poi & Kabi (1979) reported that inoculation with *Azotobacter* strains isolated from the rhizosphere soils of *Cucurbita maxima*, wheat and jute improved the grain yields but the strains were crop specific. This indicated that isolation of efficient plant growth promoting rhizobacteria and their reintroduction to the soil may strongly be responded by crops of same geographical area. Thus bacteria were isolated from various rhizosphere soils of wheat, screened on the basis of auxin production *in vitro*, and their growth and yield promoting ability was tested both under axenic and non-axenic conditions.

Materials and Methods

Isolation of rhizobacteria

Bacteria were isolated from the rhizosphere of different varieties of wheat crop grown at different locations of Punjab by using glucose peptone agar medium. Plants of wheat were uprooted alongwith good amount of non-rhizosphere soil, transferred into polythene bags, and brought to the laboratory. The non-rhizosphere soil was removed by gentle shaking leaving behind the rhizosphere soil only (strongly adhered to the roots). The rhizosphere soil was separated from roots by dipping and gentle shaking in sterilized water under aseptic conditions. The soil suspension obtained was used to isolate rhizobacteria by dilution plate technique. Thirty one bacterial colonies showing prolific growth were selected and numbered as W_1, W_2, ...W_{31}. The cultures were purified by further streaking on fresh plates, maintained on agar and incubated at 4°C. Selected isolates of wheat rhizobacteria (W_9, W_{11}, W_{14}, and W_{29}) were tentatively identified on the basis of morphological and biochemical characteristics (Table 1). These isolates most likely belonged to the following genera: *Pseudomonas* (W_9 and W_{29}), *Acinetobacter* (W_{11}), and *Azotobacter* (W_{14}).

Measurement of optical density

Optical density was measured to maintain uniform population of bacteria in the broth at the time of inoculation. For this purpose, 25.0 mL of glucose peptone medium were taken in 250 mL flasks and inoculated with wheat isolates. The control was without inoculation. All the flasks were incubated at 28 ± 1°C for four days with frequent shaking. The contents of the flasks were then filtered through Whatman filter paper No. 2 and filtrates were used for measuring optical density on spectrophotometer (ANA-720W, Tokyo Photo-electric Company Limited, Japan) at 550 nm wave length. Optical density of 0.5 was used to obtain uniform population of bacteria [10^7-10^8 colony forming units (cfu) mL^{-1}] in the broth at the time of inoculation in the subsequent experiments.

Table 1. Morphological, physiological, cultural and biochemical characteristics of selected PGPR isolates.

Test	W_9	W_{11}	W_{14}	W_{29}
Gram Stain	-ve	-ve	-ve	-ve
Shape of Bacteria	Rod	Rod (Pleomorphic)	Cocco-bacilli/oval (Pleomorphic)	Rod
Colony Colour on Nutrient Agar	Light green	White (Semi-transparent)	Off white	Light green
Colony Size/Shape on Nutrient Agar	Irregular size with swarming growth	Irregular size with rough surface	Regular size/circular	Irregular size with swarming growth
Colony Forming Unit mL^{-1}	$5.5x10^8$	$7.3x10^7$	$2.4x10^8$	$1.3x10^8$
Glucose Utilization	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Sucrose	+	+	+	+
Methyl Red	+	-	+	+
Voges Proskaur Reaction	-	-	-	-
Indole Production	+	+	+	+
Ammonia Production	+	-	+	+
Nitrate Reduction	+	-	-	-
H_2S Production	-	-	-	-
Urea Hydrolysis	+	+	+	+
Gelatin Liquefaction	-	-	-	-
Catalase Production	+	+	+	+
Oxidase Production	+	-	-	+

Auxin biosynthesis *in vitro*

Auxin production both in the presence and absence of L-TRP was determined by colorimetry. For this purpose, 25 mL of glucose peptone medium were added in 100 mL Erlenmeyer flasks, autoclaved and cooled. L-Tryptophan solution was sterilized by passing through 0.2 µm membrane filter under the suction force and added at desired concentration (@ 1.0 g L^{-1}) to the liquid medium. The flask contents were inoculated by adding 1.0 mL of 4 day old bacterial broth adjusted to optical density of 0.5 (10^7 to 10^8 colony forming units (cfu) mL^{-1}) measured at 550 nm by spectrophotometer. The flasks were plugged and incubated at 28 ± 1°C for 48 h. Non-inoculated/untreated control was kept for comparison. After incubation, the contents were filtered through Whatman filter paper No.2. Auxin compounds (IAA-equivalents) were determined by spectrophotometer using Salkowski colouring reagent as described by Sarwar *et al.*, (1992). While measuring IAA-equivalents, 3.0 mL of culture filtrate and 2.0 mL of Salkowski reagent (2.0 mL of 0.5*M* $FeCl_3$ + 98.0 ml of 35% $HClO_4$) were added to the test tubes. The contents in the test tubes were allowed to stand for half an hour for colour development.

Similarly, colour was also developed in standard solutions of IAA. The intensity of colour was measured at 535 nm by spectrophotometer (ANA-720W, Tokyo Photo-electric Company Limited, Japan). Standard curve was drawn for comparison to determine auxin production by rhizobacteria.

Leonard jar experiments (Shoot growth trials)

Leonard jar experiments were conducted in the growth room under controlled conditions to determine the effect of inoculation with rhizobacteria on shoot growth of four wheat cultivars viz., Pasban-90, Inqlab-91, Watan-93 and Punjab-96. The plastic glasses were filled with sand and Hoagland solution (Hoagland & Arnon, 1950) was applied from jars through a wick to provide nutrition to the plants. The whole apparatus was autoclaved prior to the transplantation of seedlings. Surface disinfected seeds were sown on sterilized filter sheet in a Petri plate and just after germination, the seedlings were transplanted to the glass containing sand under aseptic conditions. Five mL of 4-day-old inocula containing 10^7 to 10^8 cfu mL^{-1} were applied to the seedlings growing in sand, two days after transplanting. In case of control, seedlings were treated with the sterilized broth of glucose peptone medium containing no rhizobacteria. The jars were incubated in the growth room at 28 ± 2°C. Two weeks after transplanting, the plants were uprooted and length and weight of the seedling shoots were measured.

Seed inoculation and pot trial

Four isolates (W_9, W_{11}, W_{14}, and W_{29}) of plant growth promoting rhizobacteria (PGPR) showing best efficiency for auxin production and promoting shoot growth of seedlings of tested wheat cultivars in Leonard jar experiment were selected for pot experiment. Inoculum for pot trial was prepared by growing the selected PGPR in nutrient broth (glucose peptone medium) and incubated at 28 ± 1 °C with occasional shaking. Four-day-old inocula (10^7-10^8 cfu mL^{-1}) was injected into sterile peat @ 100 mL kg^{-1} peat. This was incubated for 24 hours at 28 ± 1 °C prior to seed inoculation. Seeds were inoculated by mixing with peat and 10% sugar solution while controls consisted of the seeds treated with peat having nutrient broth and sugar solution without PGPR. Treated seeds were dried under shade.

The study was conducted in the wire house, Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. Soil sample was collected, air-dried, sieved and analyzed for physico-chemical characteristics before filling the pots. The soil was clay loam having pH, 7.9; ECe, 1.6 dS m^{-1}; CEC, 6.8 cmol (+) kg^{-1} and organic matter, 0.72%. The inoculated and uninoculated seeds of four wheat cultivars (Pasban-90, Inqlab-91, Watan-93, and Punjab-96) were sown in soil filled pots (12 kg soil pot^{-1}) receiving nutrient inputs of NPK @ 120-75-50 kg ha^{-1} as urea, di-ammonium phosphate and muriate of potash, respectively. All of PK and half of N were mixed with soil at the time of sowing while remaining nitrogen was applied in solution form at tillering. Four seedlings were maintained in each pot after germination. The pots were arranged randomly having four replicates at ambient light and temperature. Canal water was used for irrigation. Data on plant height, number of tillers, spike length, spikelets per spike, straw and grain yields were recorded.

Statistical analysis

The data recorded in laboratory and pot trials were subjected to analysis of variance (Steel & Torrie, 1980) and their means were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results

Auxin biosynthesis *in vitro*

Results of colorimetric test of wheat isolates indicated that different isolates of rhizobacteria varied greatly in their efficiency for producing auxins in the broth medium, both in the presence and absence of L-TRP (Table 2). Among 31 isolates from the rhizosphere of wheat, 74% (23 isolates) produced auxins (ranging from 0.6 to 12.1 μg mL^{-1} IAA-equivalents) in the absence of L-TRP. Isolate W_9 was the most prolific producer of auxin (12.1 μg mL^{-1} IAA-equivalents) compared to all other isolates. Eight bacterial isolates (W_4, W_{15}, W_{16}, W_{17}, W_{18}, W_{27}, W_{28} and W_{30}) did not produce auxins in the absence of L-TRP.

In the presence of L-TRP, all the isolates produced auxins and bacterial efficiency for auxin synthesis was enhanced by several folds (ranging from 1.8 to 24.8 μg IAA-equivalents mL^{-1}) (Table 2). The isolate W_9 was most efficient in producing auxins from L-TRP and it was followed in descending order by W_{14} (21.3 μg mL^{-1}), W_{29} (19.7 μg mL^{-1}) and W_{11} (16.8 μg mL^{-1}). These strains were therefore used for plant growth experiment. All other isolates were also able to derive auxins from L-TRP, however, they were relatively less efficient in auxin biosynthesis and their IAA production ranged from 1.8 to 16.4 μg mL^{-1} growth medium.

Shoot growth trials on wheat (Leonard jar experiments)

Shoot length

Results showed that shoot length of three cultivars viz., Pasban-90, Inqlab-91, and Watan-93 significantly improved by bacterial inoculation, however, different isolates affected shoot length with different degree of efficacy (Table 3). A greater number of rhizobacterial isolates had a significant positive effect on the shoot length of wheat cv. Pasban-90 with maximum increase of 65.9% recorded in response to isolate W_9 as compared to uninoculated control. Nineteen other isolates also had significant increase in shoot length ranging from 10.6 to 55.8% while ten isolates viz., W_1, W_2, W_6, W_{12}, W_{17}, W_{19}, W_{22}, W_{23}, W_{28} and W_{30} gave negative results as compared to uninoculated control.

Maximum shoot length in the cv. Inqlab-91 was observed by inoculation with isolate W_{14}, which increased it by 77.2% over uninoculated control. Significant increases in shoot length were also recorded by W_7 (54.5%), W_9 (40.9%), W_{11} (65.9%), W_{15} (54.5%), W_{16} (70.5%), W_{22} (36.4%), W_{23} (65.9%), W_{26} (40.9%) and W_{27} (43.2%) over uninoculated control. The isolate W_{25} gave significant negative response (36.4% decrease in shoot length) compared with uninoculated control. However, rest of the isolates were statistically at par with control.

Table 2. *In vitro* auxin production by various isolates of wheat rhizobacteria. (Average of three repeats)

Rhizobacteria	IAA-equivalents (μg mL^{-1})	
	Without L-TRP	With L-TRP
W_1	6.2 cd	9.8 m
W_2	1.8 ij	2.7 t
W_3	1.1 jk	13.4 i
W_4	0.0 l	5.9 pq
W_5	4.1 g	15.1 g
W_6	1.8 ij	7.8 o
W_7	2.0 hij	12.2 j
W_8	1.2 jk	4.9 r
W_9	12.1 a	24.8 a
W_{10}	3.0 h	13.0 i
W_{11}	5.0 efg	16.8 de
W_{12}	5.0 efg	14.3 h
W_{13}	2.8 hi	10.6 l
W_{14}	10.7 b	21.3 b
W_{15}	0.0 l	1.8 u
W_{16}	0.0 l	3.7 s
W_{17}	0.0 l	5.5 qr
W_{18}	0.0 l	4.9 r
W_{19}	6.2 cd	16.4 def
W_{20}	6.1 cde	15.8 fg
W_{21}	6.2 cd	16.3 def
W_{22}	5.1 defg	13.8 hi
W_{23}	6.3 c	16.0 ef
W_{24}	4.3 fg	11.2 kl
W_{25}	5.2 cdefg	15.6 fg
W_{26}	5.0 efg	11.6 jk
W_{27}	0.0 l	6.5 p
W_{28}	0.0 l	8.4 no
W_{29}	5.4 cdef	19.7 c
W_{30}	0.0 l	2.9 t
W_{31}	0.6 kl	9.1 mn

Means sharing similar letter(s) do not differ significantly at $p < 0.05$.

Shoot length of cv. Watan-93 was significantly better in response to inoculation with the isolate W_{18} (36.2% increase over uninoculated control) followed by W_{11} and W_{24} (28.2 and 27.7% greater than control, respectively). Eight other isolates including W_2, W_8, W_9, W_{14}, W_{17}, W_{23}, W_{25} and W_{29} also increased shoot length significantly over uninoculated control. Two isolates (W_{20} and W_{26}) significantly reduced shoot length compared with control while rest of the isolates differed non-significantly compared with uninoculated control.

Rhizobacterial inoculation had no significant effect on shoot length of cv. Punjab-96, however, positive response observed in shoot length was up to 22.0%, compared with uninoculated control; and the isolate W_{29} gave the highest value (20.5 cm) while isolate W_{17} caused maximum decrease in shoot length (23.2%) compared with uninoculated control.

Table 3. Effect of rhizobacteria on shoot length (cm) of four cultivars of wheat seedlings (Leonard jar experiments).

Rhizobacteria	(Average of six repeats)			
	Pasban-90	**Inqlab-91**	**Watan-93**	**Punjab-96**
Uninoculated Control	15.73 l	14.67 ghijk	19.83 ghi	16.80 NS*
W_1	15.40 lm	14.00 hijkl	21.16 defgh	17.22
W_2	13.60 n	15.67 fghij	22.17 cdef	15.65
W_3	17.40 k	12.33 jkl	20.16 fghi	14.90
W_4	18.40 jk	17.67 dfghi	21.66 defgh	17.71
W_5	19.47 ghij	10.00 kl	20.00 fghi	18.53
W_6	14.20 n	19.00 defg	20.67 efghi	20.22
W_7	22.23 c	22.67 abcd	20.66 efghi	13.81
W_8	20.60 def	18.33 defghi	23.00 cd	16.30
W_9	26.10 a	20.67 bcde	23.13 cd	19.50
W_{10}	18.83 hij	13.67 ijkl	22.00 cdefg	17.11
W_{11}	20.20 efg	24.33 abc	25.43 ab	18.80
W_{12}	12.23 o	10.33 kl	22.06 cdefg	16.74
W_{13}	15.80 l	17.66 efghi	20.50 fghi	17.61
W_{14}	21.40 cd	26.00 a	22.83 cde	19.40
W_{15}	22.37 c	22.66 abcd	20.56 fghi	18.90
W_{16}	24.40 b	25.00 ab	21.50 defgh	15.23
W_{17}	11.67 o	13.67 ijkl	23.20 cd	12.92
W_{18}	17.50 k	18.67 defgh	27.00 a	17.50
W_{19}	14.07 n	16.33 efghij	21.17 defgh	19.10
W_{20}	22.30 c	12.67 jkl	17.60 j	16.91
W_{21}	19.53 fghi	18.33 defghi	19.66 hi	20.14
W_{22}	11.30 o	20.00 cdef	20.53 fghi	14.80
W_{23}	14.47 mn	24.33 abc	23.20 cd	15.73
W_{24}	18.60 ij	18.33 defghi	25.33 ab	18.51
W_{25}	21.27 cde	9.33 l	24.00 bc	19.92
W_{26}	19.80 fgh	20.67 bcde	15.33 k	17.60
W_{27}	24.50 b	21.00 bcde	18.86 ij	14.42
W_{28}	12.27 o	10.33 kl	21.00 defghi	15.82
W_{29}	24.27 b	19.33 defg	24.16 bc	20.55
W_{30}	15.43 lm	18.67 defgh	22.00 cdefg	19.21
W_{31}	20.33 defg	17.67 efghi	22.10 cdefg	15.80

Means sharing similar letter(s) do not differ significantly at $p < 0.05$.

Shoot weight

Effects of inoculation with different rhizobacteria on shoot weight were variable for different cultivars of wheat, however, a majority of these rhizobacteria were found to affect the shoot weight positively (Table 4). In cultivar Pasban-90, the effects of 22 rhizobacterial isolates were positive as they increased shoot weight while other isolates

gave negative results compared with uninoculated control. The highest shoot weight (0.199 g plant^{-1}) was observed in isolate W_9 which was 76.1% greater than control and differed significantly from uninoculated control. Three isolates viz., W_{19}, W_{26}, W_{28} (statistically similar with each other) caused maximum decrease in shoot weight (up to 31%), and differed significantly from control.

Table 4. Effect of rhizobacteria on shoot weight (g plant^{-1}) of four cultivars of wheat seedlings (Leonard jar experiments).

	(Average of six repeats)			
Rhizobacteria	**Pasban-90**	**Inqlab-91**	**Watan-93**	**Punjab-96**
Uninoculated control	0.113 lmn	0.113 klm	0.150 efg	0.172 NS
W_1	0.096 no	0.123 jkl	0.210 ab	0.158
W_2	0.110 lmn	0.180 bcd	0.140 gh	0.198
W_3	0.133 ijk	0.067 s	0.133 hi	0.200
W_4	0.150 fghi	0.093 opq	0.177 c	0.169
W_5	0.174 bcde	0.097 nopq	0.167 cd	0.170
W_6	0.147 ghi	0.107 mno	0.203 b	0.178
W_7	0.147 ghi	0.170 de	0.143 fgh	0.160
W_8	0.138 hij	0.133 hij	0.177 c	0.173
W_9	0.199 a	0.153 fg	0.167 cd	0.189
W_{10}	0.148 ghi	0.097 nopq	0.157 def	0.182
W_{11}	0.176 bcd	0.180 bcd	0.220 a	0.192
W_{12}	0.084 op	0.100 mnop	0.200 b	0.170
W_{13}	0.116 klm	0.090 pq	0.180 c	0.188
W_{14}	0.174 bcde	0.200 a	0.180 c	0.195
W_{15}	0.124 jkl	0.193 ab	0.123 ij	0.189
W_{16}	0.162 defg	0.167 def	0.147 efgh	0.165
W_{17}	0.101 mno	0.090 pq	0.160 de	0.174
W_{18}	0.167 cdef	0.160 ef	0.220 a	0.193
W_{19}	0.078 p	0.143 gh	0.147 efgh	0.200
W_{20}	0.178 bcd	0.110 lmn	0.110 jk	0.179
W_{21}	0.112 lmn	0.140 ghi	0.180 c	0.180
W_{22}	0.101 mno	0.173 cde	0.100 k	0.164
W_{23}	0.156 efgh	0.197 a	0.213 ab	0.169
W_{24}	0.183 abc	0.187 abc	0.207 ab	0.177
W_{25}	0.191 ab	0.063 s	0.180 c	0.187
W_{26}	0.078 p	0.083 qr	0.107 k	0.178
W_{27}	0.176 bcd	0.140 ghi	0.140 gh	0.194
W_{28}	0.078 p	0.073 rs	0.150 efg	0.195
W_{29}	0.186 ab	0.180 bcd	0.180 c	0.210
W_{30}	0.116 lm	0.127 ijk	0.143 fgh	0.170
W_{31}	0.152 fghi	0.103 mnop	0.110 jk	0.162

Means sharing similar letter(s) do not differ significantly at $p < 0.05$.

Wheat cultivar Inqlab-91 responded significantly to the bacterial inoculation and the highest shoot weight (77.0% greater than control) was obtained with isolate W_{14}. It was followed in descending order by W_{23}, W_{15}, W_{24}, W_{11}, W_{29}, W_2, W_{22}, W_7, W_{16}, W_{18}, W_9, W_{19}, W_{21}, W_{27} and W_8, and increases in shoot weight due to these isolates ranged from 17.7 to 74.3% over uninoculated control. Nine isolates viz., W_3, W_4, W_5, W_{10}, W_{13}, W_{17}, W_{25}, W_{26} and W_{28} reduced shoot weight significantly compared with control.

Shoot weight of cv. Watan-93 was also significantly influenced by inoculation and the effect varied from –33.3 to 46.7%, over uninoculated control. The highest shoot weight was found with W_{11} and W_{18}. Fourteen other isolates also showed significant positive effect on shoot weight as compared to control. Minimum shoot weight was produced by the isolate W_{22}. Six other isolates viz., W_3, W_{15}, W_{20}, W_{22}, W_{26} and W_{31} also gave significantly negative response over control. Rest of the isolates had non-significant effect on shoot weight.

Shoot weight of wheat cv. Punjab-96 produced in response to inoculation with isolate W_{29} was 22.1% higher than that produced in the uninoculated control. Statistically all the isolates had non-significant influence on shoot weight (ranging from –8.1 to 22.1%) compared with control.

Pot trial

Based upon *in vitro* auxin production and growth promoting activity observed in Leonard jar experiment conducted under axenic conditions, four PGPR isolates viz., W_9, W_{11}, W_{14}, and W_{29} were selected and used for pot trial under non-axenic natural conditions. Seed inoculation with selected PGPR isolates significantly affected the growth and yield of different wheat cultivars under wire house conditions, however, variation was found in the efficacy of PGPR isolates to promote growth and yield of these cultivars.

Plant height

Results showed that plant height increased significantly in response to PGPR inoculation only in cv. Inqlab-91 whereas the effect of inoculation was non-significant in other three cultivars (Fig. 1). Isolate W_{14} caused significant improvement in plant height (9.9% higher than the respective uninoculated control) of cv. Inqlab-91 while other isolates promoted plant height ranging from 0.6 to 5.5% compared to uninoculated control. The effect of PGPR inoculation on plant height in other three cultivars varied from –3.6 to 3.2% compared to uninoculated control, however, W_{11} was relatively the most effective isolate in increasing plant height in wheat cultivars Pasban-90, Watan-93 and Punjab-96.

Number of tillers plant^{-1}

PGPR inoculation significantly enhanced number of tillers of three cultivars while response was non-significant in case of one cultivar i.e. Inqlab-91 (Fig. 1). All PGPR isolates significantly enhanced tillering in cv. Punjab-96, ranging from 22.6 to 32.3%, compared with uninoculated control; and maximum tillers were recorded with W_{11}. Next to cv. Punjab-96, maximum number of tillers were recorded in cv. Pasban-90 in case of inoculation with isolate W_{29} (23.2% more than uninoculated control) and it was followed

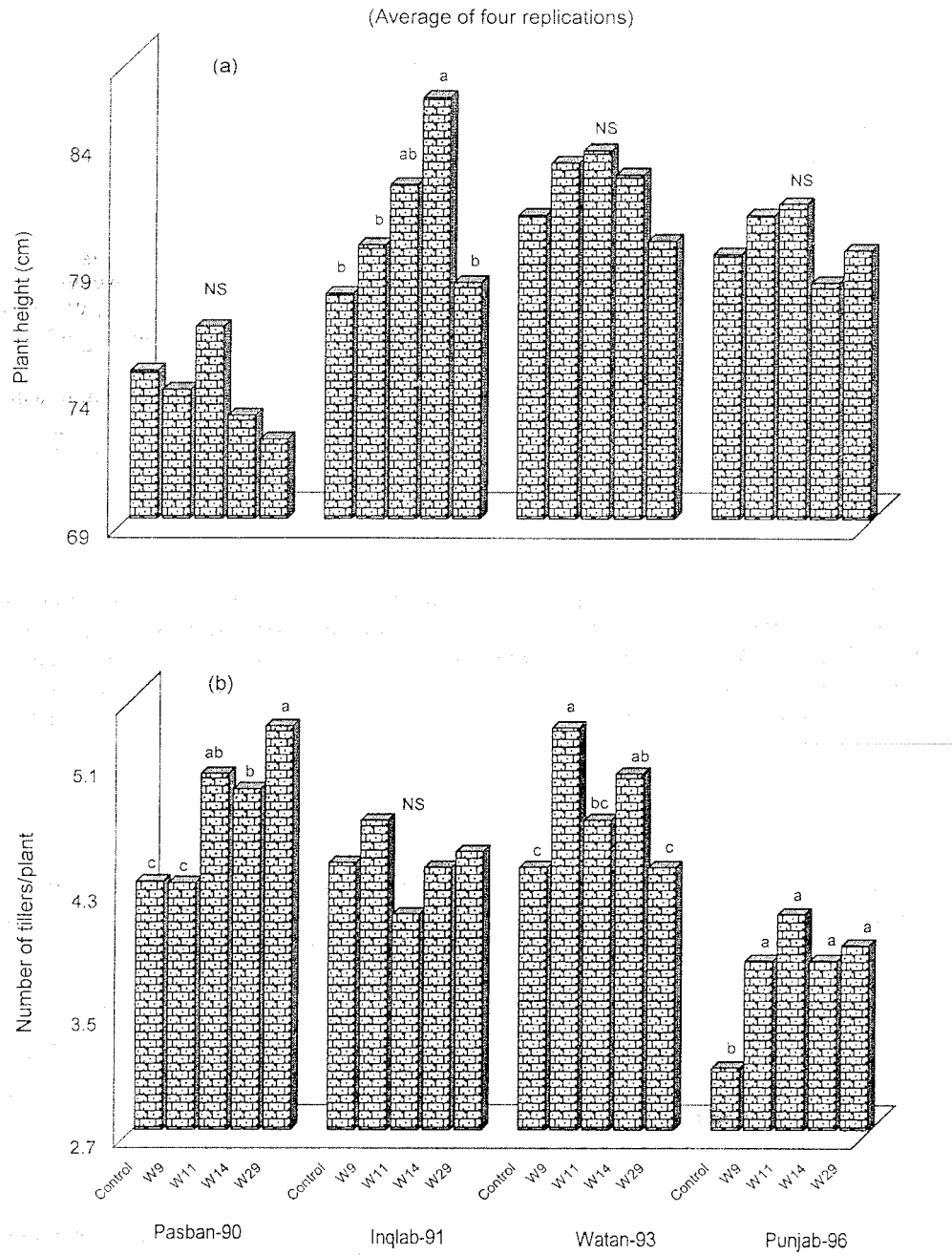

Fig. 1. Effect of PGPR (W_9, W_{11}, W_{14} and W_{29}) on plant height (a) and number of tillers (b)in four cultivars of wheat.

*Bars sharing similar letter(s) do not differ significantly at $p > 0.05$.

in descending order by W_{11}, W_{14}, uninoculated control and W_9. Cultivar Watan-93 also responded positively to PGPR inoculation and two isolates (W_9 and W_{14}) significantly increased the number of tillers, which were 20.5 and 13.6% higher than uninoculated control, respectively. In case of cv. Inqlab-91, PGPR isolates increased the tillers up to 6.1% over uninoculated control while W_{11} had suppressing effect on tillering (7.4%) compared to uninoculated control.

Spike length

All PGPR isolate had non-significant effect on spike length in all the cultivars tested (Fig. 2). Maximum increases in spike length were 7.1, 7.5, 6.8 and 5.7% over respective uninoculated control of cv. Pasban-90, Inqlab-91, Watan-93 and punjab-96, respectively.

Spikelets spike^{-1}

The effect of PGPR inoculation on spikelets spike^{-1} was significant in two wheat cultivars viz., Pasban-90 and Inqlab-91 (Fig. 2). In case of Pasban-90, all PGPR isolates significantly promoted spikelets which ranged from 9.2 to 13.9% over uninoculated control. Isolate W_9 showed highest promotory effect on spikelets. The other isolates also resulted in significantly higher spikelets (up to 10.8%) than the uninoculated control but statistically were at par with each other. Increase in spikelets was 14.0% in case of cv. Inqlab-91 with PGPR isolate W_{14} as compared to uninoculated control. Isolate W_{11} also significantly improved spikelets spike^{-1} (10.8%) compared to uninoculated control while other isolates had non-significant positive effect on it. The PGPR inoculation had non-significant effect on spikelets spike^{-1} in Watan-93 and Punjab-96 cultivars, which ranged from -2.6 to 5.2 % over uninoculated control.

Straw yield

Inoculation with PGPR caused significant increases in straw yields of three cultivars viz., Pasban-90, Inqlab-91, and Watan-93 compared with their respective uninoculated controls (Fig. 3). In case of cv. Pasban-90, isolate W_{29} produced most promising results and caused an increase of 12.4 % in straw yield compared with uninoculated control. In case of cv. Inqlab-91, effect on straw yield in response to inoculation varied from –0.5 to 10.1% compared to uninoculated control. Similarly in case of cv. Watan-93, increase in straw yield ranged from 5.3 to 16.1% compared to uninoculated control, and two isolates W_9 and W_{11} gave significantly higher straw yield. No significant increases in straw yields in response to inoculation were observed in cv. Punjab-96.

Grain yield

Different PGPR isolates upon inoculation showed variable effects on grain yield of different cultivars, however, inoculation promoted grain yield significantly in three cultivars except cv. Inqlab-91 (Fig. 3). In case of cv. Pasban-90, only W_{14} and W_{29} significantly increased the grain yield by 13.7 and 14.7%, respectively while the remaining isolates were at par with uninoculated control. Maximum grain yield observed in case of cv. Watan-93 with isolate W_{11} was 23.2% greater than control, which differed significantly from uninoculated control and rest of the isolates except W_{14}. Similarly,

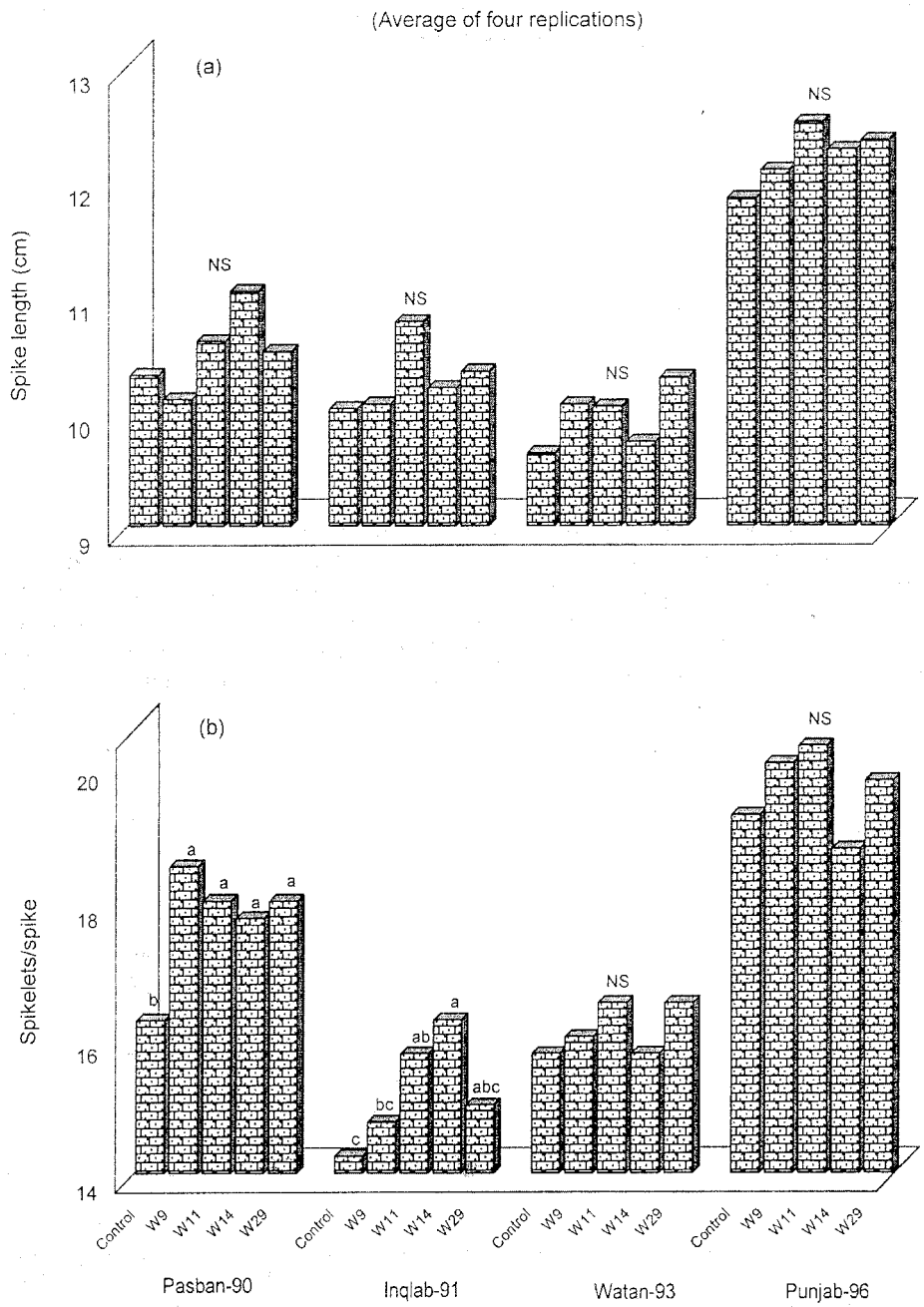

Fig. 2. Effect of PGPR (W_9, W_{11}, W_{14} and W_{29}) on spike length (a) and spikelets per spike (b) in four cultivars of wheat.

*Bars sharing similar letter(s) do not differ significantly at $p > 0.05$.

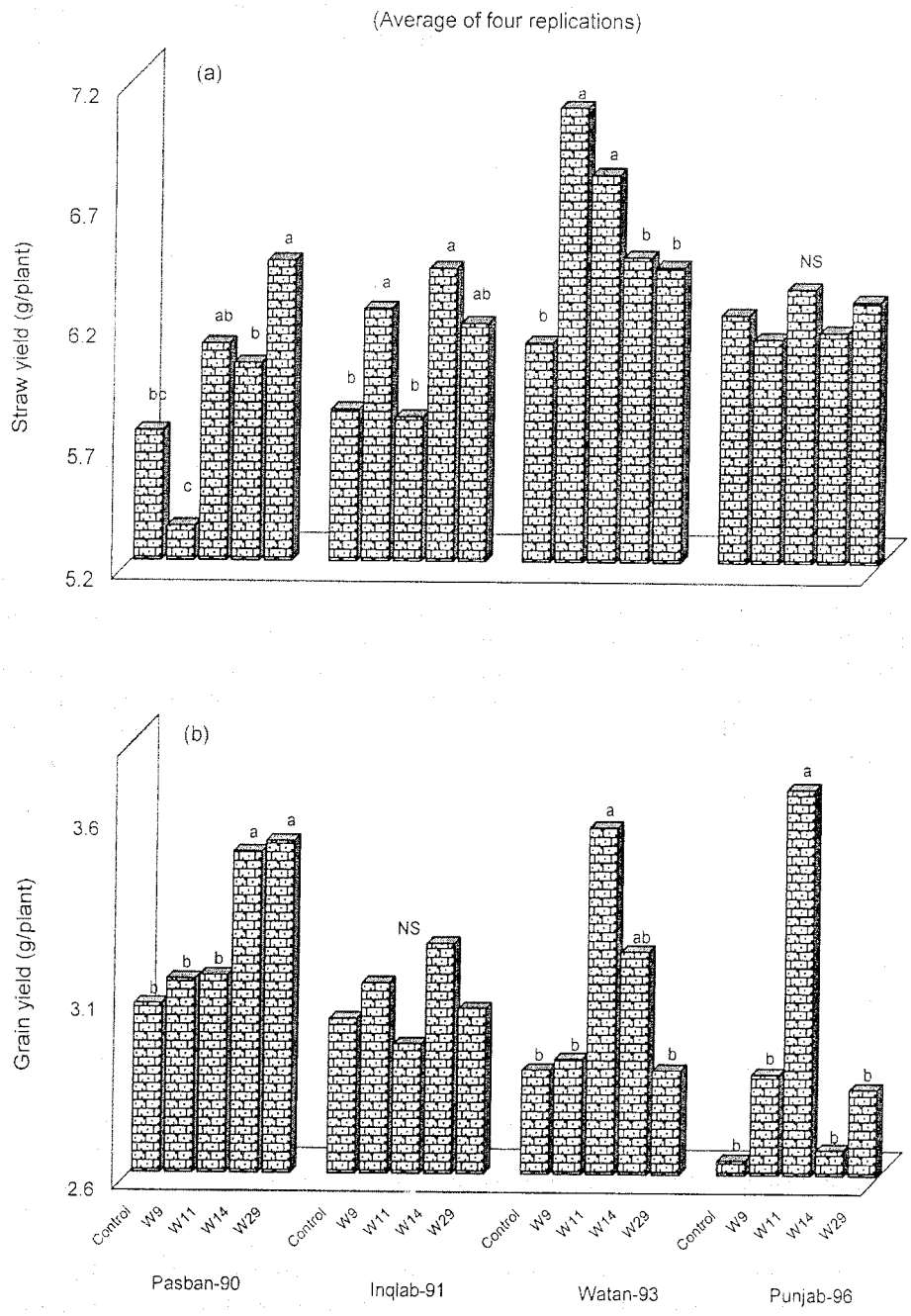

Fig. 3. Effect of PGPR (W_9, W_{11}, W_{14} and W_{29}) on straw (a) and grain yields (b) of four cultivars in wheat.

*Bars sharing similar letter(s) do not differ significantly at $p > 0.05$.

same isolate (W_{11}) caused maximum increase in grain yield of cv. Punjab-96 (29.0% over uninoculated control). None of the isolates had significant effect on grain yield in case of cv. Inqlab-91 and PGPR W_{14} was the only isolate which enhanced grain yield by 6.9% greater than uninoculated control.

Discussion

These studies revealed that a greater number (74%) of bacterial isolates of wheat rhizosphere were capable of producing auxins *in vitro* ranging from 0.6 to 12.1 μg IAA-equivalents mL^{-1}. This differential ability of rhizobacteria to produce auxins could be attributed to their different genetic makeup, growth kinetics and enzymatic activities involved in auxin synthesis under given cultural conditions. Results are in conformity to the findings of many workers (Barea *et al.*, 1976; Pal *et al.*, 2000; Padua *et al.*, 2000; Khalid *et al.*, 2001).

The addition of L-TRP to the medium inoculated with various rhizobacteria stimulated auxin (IAA-equivalents) production by several fold compared to unamended (no TRP) medium inoculated with same rhizobacteria. The L-TRP is believed to be the primary precursor for IAA synthesis and thus its addition to the medium could promote L-TRP-derived auxin production. Similar results have been reported by Zahir *et al.*, (2000) and Asghar *et al.*, (2000). Martens & Frankenberger (1991) also reported large amounts of auxin and its derivatives produced by fluorescent *Pseudomonas* sp., in the minimal medium when supplied with an exogenous source of TRP.

Results of Leonard jar and pot trials suggested that some rhizobacterial isolates were highly effective in promoting growth parameters and yield of wheat. In general, it was observed that more efficient auxin producing isolates have relatively more positive effect on the inoculated plant seedlings. This may imply that the ability of inocula to produce biologically active substances such as auxins could at least partly be responsible for evoking the physiological response in the inoculated plants. Similar results have also been reported by several workers viz., Xia *et al.*, (1990), Chen *et al.*, (1994), Khalid *et al.*, (1997) and Zahir *et al.*, (1998).

Present study also reveals that different PGPR isolates behaved differently when used for inoculating various cultivars. This might be due to different genetic make up of the different varieties or cultivars. These differences may also be attributed to crop/cultivar specific effects of PGPR isolates and production of different types of root exudates by different crops and varieties (Vancura, 1967; Vancura *et al.*, 1977) which may support the activity of inocula and/or serve as substrate for the formation of biologically active substances by the inocula (Frankenberger & Arshad, 1995).

Beneficial effects of PGPR inoculants on plant growth of wheat could be due to the result of multifarious mechanisms such as production of siderophores, antibiotics, extracellular metabolites and induced systemic resistance, in addition to direct influence such as production of plant growth regulators (Kloepper, 1994; Kloepper *et al.*, 1989; Arshad & Frankenberger, 1998). However, the production of plant growth regulators (such as auxins) may be the most plausible mechanism for increasing growth and yield of plants. Production of such compounds in the rhizosphere may directly affect root growth, if taken up by the plant, ultimately resulting in increased plant growth and yield.

Present study emphasizes to focus future research on the isolation of efficient auxin producing rhizobacterial strains having wide range of effectiveness for different cultivars.

Strain identification and its genetic stability are also important for quality control and product development besides getting more reliable results from different crops and/or varieties in response to biotreatment.

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