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Growth Responses of *Triticum aestivum* to Plant Growth Promoting Rhizobacteria Used as a Biofertilizer*

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Abstract: Bacterial strains of *Pseudomonas* (Ps_1, Ps_2, Ps_3, Ps_4, Ps_5) *Azotobacter* (Ab_1, Ab_2, Ab_4, Ab_5, Ab_6) and *Azospirillum* (As_2, As_4, As_5, As_6, As_8) were used to inoculate the seeds of *Triticum aestivum* Var Inqalab-91. Inoculated and non-inoculated seeds were germinated and grown under field conditions until at maturity. All the bacterial inoculations provoked germination and growth parameters in *Triticum aestivum*, however *Azotobacter* strains exhibited more pronounced effect after 7 days of germination such as plant height (55.19%), number of leaves (50.01%) and *Pseudomonas* strains after 4 months of sowing such as plant height (82.60%), spike length (212.73%) and number of spikes (26.66%), when compared with that of control. All strains increased soluble protein content both after 7 days of germination and at maturity; As_6 (64.95%) exhibited maximum increase after 7 days and Ab_2 (791.79%) after four months of sowing. Auxin contents were also increased with the inoculation of bacterial strains when compared with that of control however, Ps_3 (162.96%) exhibited maximum increase after 7 days and As_2 (263.33%) after four months of sowing (at maturity). All strains increased peroxidase activity in *Triticum aestivum* As_6 (109.55%) manifested maximum increase after 7 days and Ps_1 (1436.09%) after four months of sowing. Acid phosphates activity was also enhanced with all the bacterial strains however As_2 (263.54%) showed maximum increase after 7 days, whereas after four months of sowing Ps_4 (146.95%) manifested maximum increase in this parameter. These results demonstrate that these strains can be used as biofertilzer for enhancing biomass as well as yield parameters.

Key words: PGPR, biofertilizer, *Triticum aestivum*

Introduction

During the 20th century, conventional breeding produced a vast number of varieties and hybrids that contributed immensely to higher grain yield, stability of harvests and farm income. Despite the successes of the Green Revolution, the battle to ensure food security for hundreds of millions miserable poor people is far from won. Mushrooming populations, changing demographics and inadequate poverty intervention programs hare eroded many of grains of the Green Revolution. This is not to say that the Green Revolution is over. However, for the genetic improvement of food crops to continue at a pace sufficient to meet the needs of the 8.5 billion peoples projected to be on this planet earth at the end of quarter century, both conventional technology and biotechnology are needed. Pakistan is an agriculture-based country about 60% of our population is currently related to the agriculture. Agriculture contributed 24.1% in the year 01-02 (Federal Bureau of Statistics of Pakistan, 2000-02). Important crops, which are cultivated in Pakistan, are wheat, Rice, Cotton, Sugar Cane etc. More then 90% of our population used wheat flour and other products of wheat. So the area under this important crop during 2000 was 8,033.9, (ha) production was 19,183.3 (tons) and yield ha^{-1} 2,388 kg

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(Federal Bureau of Statistics, 2000). Keeping the rapid growth rate of Pakistan it is estimated that after few years Pakistan have to faced the problem of wheat within the country which is due to decreased cultivated area, production and ultimately the yield of wheat, so Pakistan has to import wheat spend a lot of foreign exchange on it.

One of the best efforts in this way is the introduction of soil microbes in agriculture for seed and soil inoculation. The successful use of Plant-microbe interaction for improving agriculture production provides the excited example of this approach. There are lots of methods to overcome these problems, one of them is the use of PGPR and see the effect of these bacteria on *Triticum aestivum*. Modern agriculture aims to increase crop yield to satisfy the needs from a growing population, but to use sustainable approaches that should include the substitution of chemical inputs by a more effective use of natural resources. Biological nitrogen (N_2) fits well in this model, as it is a more environmentally clean way to satisfying plant N needs. Now a day's term PGPR is used for the bacteria's which enhanced the plant growth and productivity in one or more then one ways. The mechanism, which plant growth attributes to promotion, enhanced plant hormones concentration, (ii) a symbiotic nitrogen fixation, (iii) antagonizing against phytopathogenic microorganisms and (iv) solubilization of mineral phosphates and other nutrients (Burd *et al*., 2000). Inoculation must, therefore be rhizosphere competent and survive in the soil to promote plant growth. Bacteria are very important for the physical and the chemical characteristics of soil and play important roles in soil quality and plant productivity (Hill *et al*., 2000). *Acetobacter diazotoophicus*, *Herbaspirillum sereopedical*, *Azoarcus* sp. and *Azotobacter*. *Azospirillum* are predominantly surface colonizing bacteria while others are endophytic diazotrophs (Oda steenhoudt and Jos vanderleyden, 2000). In the free-living diazotrophs *Klebsiella pneumoniae* and *Azotobacter vinelandii*, activation of expression of genes involved in nitrogen fixation by the enhancer binding protein NIFA is controlled by the sensor protein NIFL in response to changes in levels of oxygen and fixed nitrogen *in vivo* (Money *et al*., 2001). The inhibitory activity of NIFL towards NIFA is stimulated by ADP binding to the C-terminal domain of NIFL, which bears significant homology to the histidine protein kinase transmitter domains (Money *et al*., 2001). In several diazotrophic species of *Proteobacteria*, P_{II} signal transduction proteins have been implicated in the regulation of nitrogen fixation in response to NH_4^+ by several mechanisms. In *Azotobacter vinelandii*, expression of *nifA*, encoding the *nif*-specific activator, is constitutive and thus, regulation of NifA activity by the flavoprotein NifL appears to be the primary level of nitrogen control (Rudnick *et al*., 2002). According to Raza *et al*. (2001) in all lupin cultivars plant dry weight, growth and biomass production enhanced with rhizobial inoculation. PGPR *Pseudomonas putinda* KT2440 strain colonizes the Rhizosphere of a number of agronomically important plants at high population densities (Espinosa-uregel *et al*., 2000; Turnbell *et al*., 2001). Yuming *et al*. (2003) three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 enhanced soybean nodulation and growth in greenhouse and field experiments. Coinoculation with non-*Bradyrhizobium* endophytic bacteria provided the largest and most consistent increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield. Dry and wet inoculation of alginate micro beads enhanced plant growth promotion in wheat and tomato seedling growing in unfertile soil and biodegraded within 15 days in moist soil (Bashan and Hernandez, 2002). Growth promoting bacteria induces systemic resistance in host plants (Zheng *et al*., 2000; Ramammorthy *et al*., 2000).

In this regard present studies was designed to evaluate the impact of mono culture inoculations of *Pseudomonas*, *Azotobacter* and *Azospirillum* isolated by Aziz (2000) on seed germination, early and late growth and yield of *Triticum aestivum var* Inqalab 91, was carried out. Inoculation effects on germination, growth and yield parameters were studied at two harvests in field along with biochemical analysis (auxin content, protein content, peroxidase content and acid phosphatase content) and data was statistically analyzed.

Materials and Methods

The present study was conducted in the Botanical Garden, University of The Punjab, Quaid-e-Azam Campus Lahore, Pakistan. The experimental site was located at longitude 74° East and latitude 31° north, which is at the altitude of 644.4 feet above sea level. The research work was initiated in last week of December 2001. Seeds of *Triticum aestivum* Inqalab-91 were taken from Punjab Seed Corporation Lahore, which is certified from Federal Seed Certification and Registration Department Islamabad. Fifteen bacterial strains five each of *Azotobacter*, Ab_1, Ab_2, Ab_4, Ab_5 and Ab_6, *Azospirillum*. As_2, As_4, As_5, As_6 and As_8 *Pseudomonas* i.e., Ps_1, Ps_2, Ps_3, Ps_4 and Ps_5 isolated by Aziz (2000) were used in this study. Bacterial pellets obtained from fresh cultures (24 h incubation) of each strains and the cell density of these bacterial cultures were adjusted to 10^8 cell mL^{-1} with the help of 2-D spectrophotometer. Healthy seeds of *Triticum aestivum* were surface sterilized by soaking in 0.1% $HgCl_2$ solutions for 5 min with continuous shaking. Then seeds were left soaked in sterilized distilled water for about 1 h. Sterilized seeds were then soaked in bacterial suspension with the help of sterilized forceps for about 15-20 min. For control treatment seeds were soaked in sterilized glass distilled water for same duration. Both inoculated and un-inoculated (control) seeds were then sowed in field. Germination was recorded daily for 8 days and after that thinning was done in each plot. Two harvests were taken one after 7 days of germination and second at maturity i.e., after 4 months of sowing. Growth parameters such, plant height, number of roots and of leaves with yield parameters i.e., Spike length, number of spikes and weight of 100 seeds were observed, after that crop was harvested at maturity when seeds become mature i.e., after 4 months of sowing. For biochemical analysis at both harvests activity of peroxidase (David and Murray, 1965), acid phosphatase (Iqbal and Rafique, 1956), soluble protein content (Bhatti *et al*., 1993) and auxin content were measured following Mahadevan (1984). Data obtained was analyzed statistically following method of Steel and Torrie (1981) Mean, Standard error of the mean, least significant difference and correlation were calculated.

Results

Harvest I

Germination and Growth Parameters

Percentage germination of *Triticum aestivum* was affected with the inoculation of bacterial strains. The germination was increased significantly, over control. Majority of strains exhibited significant stimulation on this parameter of *Triticum aestivum* (Table 1) and 0.82% (Ps_3) - 58.6% (Ab_6) increases in germination of seeds were recorded. Plant height of *Triticum aestivum* was enhanced significantly with all bacterial inoculations as compared to the control. Increases in this parameter ranged from 22.73% (Ps_1) to 55.54% , *Azotobacter* strains manifested more stimulation then *Azospirillum* and *Pseudomonas* strains (Table 1). Number of roots per seedling was markedly increased. Percentage increase in root numbers varied between 6.06% (As_5) to 96.96% (As_6), (Table 1). Among *Pseudomonas* strains maximum increase was observed with Ps_5 (75.75%) inoculation. In *Azotobacter* strains increases were ranged from 33.33% (Ab_1) to 75.7% (Ab_6) while in *Azospirillum* strains the increases over control varied between 6.06% (As_5) to 96.96% (As_6). Generally more increases were observed with *Azospirillum* strains relative to *Azotobacter* and *Pseudomonas* strains. Number of leaves per seedling was significantly increased with bacterial inoculation. Generally *Pseudomonas* strains exhibited more enhancements then *Azospirillum* and *Azotobacter* strains.

Harvest II

Growth and yield parameters

Plant height was increased significantly, with inoculation of bacterial strains. The maximum increase in the plant height was observed with the inoculation of Ps_1 (82.606%), while the increase in

plant height ranged between 9.23% (Ab_2) -82.60% (Ps_4), (Table 2). Spike length of plants was increased significantly with all bacterial inoculations. The increase in spike length with different inoculation ranged from 31.42% (Ps_2) to 218.09% (Ps_1) when compared with control, (Table 2). Number of spikes was affected by inoculation of bacterial strains. Majority of strains showed significant stimulation in this parameter, when compared with control. The percentage increases in this parameter, over non-inoculated control, ranged from 4.918% (Ps_5) to 29.508% (As_2), (Table 2). All the bacterial inoculations caused increase in weight of seeds in *Triticum aestivum*, except Ab_2, which caused 5.12% reduction in this parameter (Table 2), when compared with control. Increase in this parameter ranged from 4.64% (Ps_4) to 57.54% (Ab_4). However *Azotobacter* strains exhibited more pronounced results than *Pseudomonas* and *Azospirillum* strains.

Table 1: Effect of bacterial inoculation on growth and biochemical parameters of *Triticum aestivum* at harvest I

Strains	Germination (%)	Plant height (cm)	No. of roots	No. of leaves	Auxin content (μg g^{-1} fresh weight)	Protein content (μg g^{-1} fresh weight)	Peroxidase contents (μg g^{-1} fresh weight)	Acid phosphatase contents (μg g^{-1} fresh weight)
Control	29.0±2.12	28.025±0.9	8.2±0.89	12.0±0.79	2.7±0.91	505.0±38.89	2.4±0.22	5.2±0.75
PS_1	26.5±2.57	35.2±1.45	6.2±0.96	12.0±2.15	4.5±0.00	632.0±17.80	1.6±0.364	12.2±1.16
PS_2	22.5±1.76	34.3±2.35	7.7±0.96	10.7±2.01	7.0±0.17	730.0±50.6	4.3±0.37	08.8±1.0
PS_3	29.2±9.10	37.6±1.88	15.0±1.69	14.7±1.24	7.1±0.14	690.0±45.2	3.0±1.0	13.9±0.08
PS_4	43.5±5.65	39.9±1.63	10.2±1.19	16.5±2.19	4.6±0.07	610.0±8.48	2.8±0.00	08.5±0.94
PS_5	37.2±3.36	37.9±1.63	14.5±1.29	18.0±1.17	4.2±0.62	744.0±33.9	3.2±0.08	12.5±1.32
AB_1	28.0±5.76	40.4±1.43	11.0±1.27	14.5±0.96	5.2±0.38	789.0±28.9	3.8±0.29	09.6±0.07
AB_2	30.7±2.41	43.5±3.01	13.7±2.16	18.0±1.69	6.0±0.88	759.0±30.4	4.7±0.80	11.3±0.35
AB_4	41.5±5.66	34.7±1.47	7.25±0.89	6.7±0.96	3.9±0.60	611.0±00.7	2.6±0.27	12.3±0.84
AB_5	43.2±5.77	40.3±0.79	8.2±1.138	9.5±2.79	4.6±0.00	654.0±104.1	2.5±0.15	15.2±0.42
AB_6	46.0±3.46	39.7±1.27	14.5±1.34	18.7±0.96	6.1±0.84	564.0±46.6	3.8±0.41	13.7±0.15
AS_2	35.2±0.58	39.8±0.92	12.0±1.36	16.0±2.13	5.3±0.42	757.0±54.5	4.4±0.30	19.1±0.70
AS_4	42.2±1.35	40.9±0.71	13.2±2.01	14.2±2.21	4.5±0.00	814.0±08.4	2.6±0.05	18.0±0.35
AS_5	37.0±3.46	43.2±2.00	8.7±0.73	16.5±1.03	6.0±0.84	726.0±24.0	2.8±0.06	15.9±0.70
AS_6	32.7±6.35	39.4±1.04	16.2±2.24	15.2±1.13	4.0±0.91	833.0±00.7	5.0±0.37	17.2±0.10
AS_8	36.0±7.01	36.32±2.09	5.2±0.216	8.0±1.76	4.5±0.24	649.0±28.9	2.8±0.49	15.5±0.37
LSD at p = 0.05	5.056	2.695	2.199	2.760	0.810	47.70	0.773	0.918

Table 2: Effect of bacterial inoculation on growth and biochemical parameters of *Triticum aestivum* at harvest II

Strains	Plant height (Inches)	Spike length	No. of spikes	Weight of 100 seeds (g)	Auxin (μg g^{-1} fresh weight)	Protein (μg g^{-1} fresh weight)	Peroxidase (μg g^{-1} fresh weight)	Acid phosphatase (μg g^{-1} fresh weight)
Control	18.400±0.917	2.625±0.178	15.250±0.141	3.51±0.150	0.300±0.014	0.218±0.003	0.250±0.108	11.176±0.989
PS_1	33.600±2.218	8.350±0.875	19.750±2.381	5.28±0.209	0.590±0.077	0.978±0.008	3.967±1.382	14.252±3.059
PS_2	23.650±0.680	3.450±0.134	18.000±0.707	5.29±0.175	0.600±0.063	1.678±0.030	1.649±0.427	12.889±2.475
PS_3	28.200±1.936	3.925±0.120	16.250±0.216	3.87±0.199	0.500±0.067	1.705±0.038	1.000±0.386	11.803±0.610
PS_4	24.150±1.376	4.525±0.201	17.750±0.819	3.68±0.195	0.665±0.031	1.425±0.067	1.298±0.707	27.600±1.669
PS_5	26.500±0.314	4.125±0.272	16.000±0.353	3.96±0.094	0.495±0.003	1.587±0.016	0.914±0.037	24.059±3.254
AB_1	23.125±0.709	4.075±0.054	16.750±0.739	3.91±0.076	0.605±0.060	1.795±0.043	0.818±1.120	14.259±1.705
AB_2	20.100±0.911	3.600±1.270	18.250±0.414	3.33±0.169	0.730±0.007	1.944±0.086	1.120±0.485	21.784±2.805
AB_4	26.370±0.303	4.025±0.147	19.500±0.751	5.53±0.131	0.885±0.017	1.374±0.023	1.035±0.451	25.881±2.609
AB_5	24.535±0.573	3.750±0.130	16.000±0.935	5.08±0.044	0.790±0.084	1.394±0.033	0.900±0.590	20.744±1.516
AB_6	25.670±0.968	4.075±0.155	18.751±0.414	5.08±0.007	0.690±0.091	1.250±0.030	1.486±0.130	18.084±0.707
AS_2	24.770±0.884	3.875±0.089	16.000±0.355	4.23±0.046	0.109±0.002	1.272±0.031	0.713±0.155	24.958±4.301
AS_4	24.575±1.056	3.525±0.174	17.250±0.414	4.37±0.159	0.548±0.312	1.393±0.153	0.331±0.197	23.217±9.215
AS_5	23.350±0.791	4.050±0.055	17.250±0.649	4.12±0.039	0.689±0.191	1.637±0.018	0.422±0.034	21.821±4.950
AS_6	22.200±0.781	3.625±0.151	17.000±0.790	4.19±0.043	0.668±0.036	1.607±0.039	1.080±0.251	17.627±0.353
AS_8	25.520±0.896	4.075±0.174	19.250±0.414	4.06±0.044	0.680±0.120	1.446±0.035	1.007±0.374	17.321±1.768
LSD at p = 0.05	1.642	0.4224	1.233	0.0878	0.0136	0.1575	0.6642	2.0690

Biochemical analysis: (At both harvests)

Auxin content of plants increased significantly, over control, with inoculation of all bacterial strains. Auxin contents of *Triticum aestivum* seedlings were enhanced 46.29% (Ab_4) to 162.96% (Ps_3), with the bacterial inoculations relative to non-inoculated seedlings. However maximum increase in Auxin content was observed with *Pseudomonas* strains as compared with *Azotobacter* and *Azospirillum* (Table 1). However after four months of sowing, the increases in auxin contents with the inoculation of bacterial strains ranged between 195% (Ps_5) to 263.33% (Ab_5) (Table 2) and maximum increase was observed with the inoculation of Ab_2 (263.33%), while the minimum increase was observed with the inoculation of Ab_4 (195%). All bacterial inoculations caused significant increase in protein contents of seedlings. Minimum increase in Ab_6 (11.66%) and maximum increase observed in As_6 (64.95%) was observed when compared with control treatment (Table 1). However *Azospirillum* strains showed significantly high increase when compared with *Pseudomonas* and *Azotobacter* strains. However after four month of sowing, the increases in soluble protein contents with the inoculation vary from 348.62% (Ps_1) to 791.74% (Ab_2) (Table 2) and *Azotobacter* strains manifested significantly better results then *Pseudomonas* and *Azospirillum* strains.

Enzymes are the biocatalysts, which play important roles in many biochemical reactions. Two enzymes acid phosphates and peroxidase were studied in this respect. The peroxidases activity was increased significantly, relative to control, with the inoculation of bacterial strains except Ps_1, which showed reduction in peroxidase contents to 32%. The percentage increases in the activity of this enzyme varied from 1.52-109.55% (Table 1). *Azospirillum* strains showed maximum stimulatory effect on this parameter relative to *Azotobacter* and *Pseudomonas* strains when compared with that of control. However after four month of sowing, the maximum increase in the peroxidases activity was observed with the inoculation of Ps_1 (1486%). The enhanced activity, over control, of this enzyme varied from 324.07% (As_4) to 1486.06% (Ps_1), with different inoculations (Table 2). The activity of acid phosphatase in *Triticum aestivum* increased with the inoculation of many bacterial strains. Percentage increases ranged from 61.66% (Ps_4) to 263.54 % (As2) (Table 1), with different strains. *Azospirillum* strains generally caused more enhancements in activity of this enzyme comparative to *Azotobacter* and *Pseudomonas* strains. However after four month of sowing, the increases in enzyme activity, over control, varied from 50.61% (Ps_3)-146.95% (Ps_4), with different inoculations (Table 2). The maximum increase in this parameter, over that of control, was observed with the inoculation of the Ab_2 (146.95%). Generally increases in this parameter were more pronounced with *Pseudomonas* strains relative to *Azotobacter* and *Azospirillum* strains.

Discussion

All the strains used increased germination maximum increase in case of *Triticum aestivum* was manifested by Ab_6 (58.62%), which is related to the results of Afrasayab and Hasnain (2000a) under different stresses. The strains used in the present study increased both growth and yield parameters such as plant height, numbers of leaves, spike length, number of spike and weight of seeds etc Ab_2 exhibited maximum increase in shoot length (65.25%), root length (24.10%), plant height (55.19%) and number of leaves (50.01%), (Table 1). Whereas As_6 (96.96%) exhibited maximum increase in number of roots after 7 days of sowing, (Table 1). However after 4 months of sowing strain Ps_1 exhibited maximum increase in shoots length (54.66%), root length (256.61%) and plant height (82.60%), (Table 2) the possible way to this increase these parameters is a symbiotic nitrogen fixation, synthesis of growth regulators such as auxin, increase in soluble proteins and enzymes activity such as acid phosphatase and peroxidases. Rhizospheric bacteria *Pseudomonas* sp., *Azospirillum* sp., *Agrobacterium* sp., increased plant growth and the nutrient uptake of maize, wheat and legumes

(Bashan *et al*., 2004). As far as yield parameters in *Triticum aestivum* Ps_1 enhanced spike length (212.73%) and number of spikes (26.66%), when compared to non-inoculated plants, while Ab_4 (57.09%), caused increase in weight of 100 seeds (Table 2).

Burd *et al*. (2000) reported that plant growth promoting Rhizobacteria might enhance plant height and productivity by synthesizing phytochromes, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens and inducing systemic resistance in the plants to pathogens. *Acetobacter diazotoophicus*, *Herbaspirillum sereopedical*, *Azoarcus* sp. and *Azotobacter*. *Azospirillum* are predominantly surface colonizing bacteria while others are endophytic diazotrophs (Oda steenhoudt and Jos vanderleyden, 2000). Fixation of atmospheric nitrogen (N) by free-living soil microorganisms is considered a minor source of bioavailable nitrogen compared to systems such as the *Rhizobium*-legume and *Frankia*-alder symbioses (Kennedy and Islam, 2001). In the free-living diazotrophs *Klebsiella pneumoniae* and *Azotobacter vinelandii*, activation of expression of genes involved in nitrogen fixation by the enhancer binding protein NIFA is controlled by the sensor protein NIFL in response to changes in levels of oxygen and fixed nitrogen *in vivo* (Money *et al*., 2001). The inhibitory activity of NIFL towards NIFA is stimulated by ADP binding to the C-terminal domain of NIFL, which bears significant homology to the histidine protein kinase transmitter domains (Money *et al*., 2001). In several diazotrophic species of *Proteobacteria*, P_{II} signal transduction proteins have been implicated in the regulation of nitrogen fixation in response to NH_4^+ by several mechanisms. In *Azotobacter vinelandii*, expression of *nifA*, encoding the *nif*-specific activator, is constitutive and thus, regulation of NifA activity by the flavoprotein NifL appears to be the primary level of nitrogen control (Rudnick *et al*., 2002). According to Raza *et al*. (2001) in all lupin cultivars plant dry weight, growth and biomass production enhanced with rhizobial inoculation. PGPR *Pseudomonas putinda* KT2440 strain colonizes the Rhizosphere of a number of agronomically important plants at high population densities (Espinosa-uregel *et al*., 2000; Turnbell *et al*., 2001). Yuming *et al*. (2003) three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 enhanced soybean nodulation and growth in greenhouse and field experiments. Growth promoting bacteria induces systemic resistance in host plants (Zheng *et al*., 2000; Ramammorthy *et al*., 2000). Endophytic nature of plant makes the PGPR to colonize and persist in intercellular spaces of epidermal cell (Ramammorthy *et al*., 2000). Bascones *et al*. (2000) reported that nitrogenase dependent hydrogen production is one of the major factors that influence the efficiency of symbiotic nitrogen fixation. According to Luz *et al*. (2000) that Microalga *Chlorella vulgaris*, which following with PGPR may also exhibit, enhanced cell proliferation and increased biomass production by hormonal metabolism. Evidence of establishment of significant populations of these bacteria in the plant root surfaces in sparse, although many studies have demonstrated their occurrence in Rhizosphere soil surrounding the roots, (Rammamooarthy *et al*., 2000). Bashan *et al*. (2000) reported that inoculation with various PGPB improved seed N, protein and P contents of *Salicornia* sp., which is an oilseed sp. In present study increase in yield of crop might be due to induced systemic resistance against pathogens. Raza *et al*. (2001) reported that analyses of plant dry weight showed that rhizobial inoculation positively influenced growth and biomass production for all lupin cultivars tested compared to with non-inoculated plants. Bacterial strains increase auxin and soluble protein contents and also the enzymes activity in *Triticum aestivum* so increase in growth and yield parameters were due to these factors as well. In case of soluble protein contents maximum increase was manifested by As_6 (64.95%), (Table 1) after 7 days of germination and Ab_2 (791.79%), (Table 2), after four months of sowing. PGPR improved seed N, protein and P contents of *Salicornia* sp. when inoculated with PGPR (Bashan *et al*., 2000). Increase in N and protein contents of seeds 4-5 times with *Azospirillum halopraeterens* reported by Bashan *et al*. (2000). Auxin constitutes a class of phytochromes that play important role in the coordination of plant growth

and development. IAA, the most abundant naturally occurring auxin, has been implicated in regulating a variety of developmental and cellular processes (Broek *et al*., 1999). All strains increase auxin contents in *Triticum aestivum* Ps_3 (162.96%) exhibited maximum increase after 7 days of germination, (Table 1) and As_2 (263.33%) after four months of sowing (at maturity), (Table 2). Enzymes are the biocatalysts, which play important roles in many biochemical reactions. Two enzymes acid phosphates and peroxidase were studied in this respect. Activity of enzymes, peroxidases was improved with inoculations of bacterial strains when compared with non-inoculated control, As_6 (109.55%) manifested maximum increase after 7 days of germination, (Table 1) and Ps_1 (1436.09%) after four months of sowing, (Table 2). Activity of enzyme acid phosphatase also improved with inoculation of bacterial strains. As_2 (263.54%), (Table 1) showed maximum increase after 7 days of germination and Ps_4 (146.95%) after four months of sowing (Table 2). Bashan *et al*. (2000) reported that inoculation of oil seed halophytes *Salicornia bigelovii* with *Azospirillum halopraeferens* increased N and protein contents 4-5 times. Increase in protein content with bacterial inoculations is also reported by Afrasayab and Hasnain (2000a, b). Two PGPR *Bacillus pumilus* and *B. licheniformis* produced high amounts of C1, - GAs, G_A1, GA_3, GA_4 and G_{20} and biological data suggested that the bacterial media have the potential to elongate the stem and shoots of *Alnus glutinosa* Gutierrez-Manero *et al*. (2001). Bashan *et al*. (2000) reported that inoculation of oil seed halophytes *Salicornia bigelovii* with *Azospirillum halopraeferens* increased N and protein contents 4-5 times. Increase in protein content with bacterial inoculations is also reported by Afrasayab and Hasnain (2000a, b). Aon *et al*. (2001) reported that enzymes have strong coorelation that enzymes activity play important role between physical, chemical and microbial soil properties, which ultimately enhanced plant growth. Two peroxidase and one Chitinase (35 k) isoforms have been induced in the PGPR treated plants inoculated with the rice sheath, blight pathogen Nandakumar *et al*. (1998). It has been reported by Chen (1999) that a novel acid phosphatase containing phosphotryrosyl phosphatase activity. Acid phosphatase from several bacterial species have been recognized as virulence factors that support intracellular survival by inhibiting the respiratory burst (Chhatwal *et al*., 1997; Reilly *et al*., 1996).

Conclusions

It is concluded from the above discussion that in *Triticum aestivum* all the bacterial strains stimulated significantly growth and yield parameters at both harvests in field conditions, when compared with that of control. Increase in growth parameters (shoot length, root length and plant height) as well as yield parameters (spike length, number of spikes and weight of seeds) at both stages was associated with increase in auxin and protein contents as well as peroxidase and acid phosphatase activities. *Azotobacter* and *Azospirillum* are non-symbiotic nitrogen fixer, It appears that these strains enhanced plant growth and yield parameters by applying nitrogen and synthesizing more auxin and proteins. Hence the strains used in the present work can be used as biofertilizer for the improvement of growth and yield parameters of commercially important cash crop i.e., *Triticum aestivum* var-Inqalab-91.

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