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*Full Length Research Paper*

# Plant growth promoting capability of Azotobacter as mono and mix culture on *Vigna radiata*

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**In the present study mono and mixed culture bacterial combinations of Azotobacter were used to inculcate *Vigna radiata* seeds. In general, bacterial inoculations (mono and mixed cultures) promoted seed germination, early growth parameters, auxin content, soluble protein content, peroxidase and acid phosphatase activity relative to non-inoculated control seedlings. Increase of (23%) as well as decrease (6.8%) in the root length were observed with bacterial inoculations relative to non-inoculated seedlings. About 20.07% enhancements in fresh weight and 62% enhancement in dry weight was observed in case of bacterial inoculations. Among the monoculture Ab-4(A3) induced pronounced growth stimulator effects, mixed culture combinations B6, B7, and B10 showed pronounced synergistic effects relative to respective monocultures, while B2 exhibited negative in majority of the parameters being studied.**

**Key words:** Azotobacter, *Vigna radiata,* auxin, acid phosphatase, peroxidase.

## INTRODUCTION

A lot of work has been done on plant microbe interaction through which a number of mechanisms enhanced plant growth. Mechanisms implicated to plant growth stimulation include nitrogen fixation (Choudhury and Kennedy, 2004; Kloepper et al., 1989) suppression of plant pathogens (Calvo-Bado et al., 2006), mineralizaion of organic phosphorous or solubilization of inorganic phosphoric compounds, (Dobbelaere et al., 2003), phytohormone production (Tsavkelova et al., 2005), root colonization, antibiotics production, siderophore production and enhanced mineral uptake (Dobbelaere et al., 2003). Microbial inoculation of soil is required for a number of applications, such as plant growth promotion, inhibition of plant pathogens, and biodegradation of toxic compounds, soil structure improvement and microbial leaching of metals (Van Veen et al., 1997). In plant-microbe interaction, root colonization by beneficial bacteria is a fundamental requirement (Bashan and Holguin, 1994). Rhizobacteria may sense and respond to plant signals, exchange nutrients with plant cells, suffer damage due to plant defense responses and colonize or even invade root tissues forming symbiotic association (Miller and Wood, 1996). Increase in yield of vegetables, forage and grain crops with inoculation of diazotrophic rhizobacteria, has successfully been demonstrated (Bashan, 1998). Root lectins of leguminous plants are involved in the recognition and subsequent binding to rhizobia (Diaz et al., 1989). *Azotobcter vinelandii* produces two polymers: the extra cellular polysaccharide alginate and the intracellular polyester poly-ß-hydro butyrate (PHB) (Castaneda et al., 2000). Alginate is important for cyst formation in *A. vinelandii* as a coating protective polysaccharide material (Nunez et al., 1999). It was suggested that cyst formation and colonization pattern play roles in regulating nitrogenase activity of plants (Katupitiya et al., 1995). Plant growth promoting bacteria are becoming the attention of agronomist and microbiologist for their positive role in plant development (Defago and Hass, 1990). The objective of the present study was to evaluate the impact of mono and mixed

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culture inoculations of Azotobacter strains on the growth (length and weight) and biochemical parameters (auxin content, protein content, peroxides content and acid phosphate content) of *V. radiata* seedlings.

## MATERIALS AND METHODS

### Bacterial Strains and growth conditions

Five bacterial strains of Azotobacter (Ab-1, Ab-2, Ab-4, Ab-6) isolated by Aziz (2000) were used for the present work. Bacterial isolates were obtained from rhizosphere (Ab-5 of *Coronopus didyma* and Ab-6 of *Trifolium* sp.), rhizoplane (Ab-4 of *Rumex dentatus*) and histoplane (Ab-1 of *Chenopodium morale* and Ab-2 of *R. dentatus*) of different weeds/plants growing in S.S. Farms, Baedian Road, Lahore, Pakistan. Five mono and twenty-six mixed cultures of these five strains were used for inoculating *V. radiata* seeds. Bacterial strains were grown on L-agar (Tryptone 10.0 g l-l; Yeast extract 5 g l-l, Sodium chloride 5.0 g l-l and agar 12.0 g l-l for solid media (pH 7.0) at 37°C for 24 h.

### Germination experiments

Fresh cultures of each strain were resuspended in 10 ml of autoclaved distilled water and then the cell density of these (1.2 OD at 540 nm) bacterial cultures were adjusted to 108 cells ml-1 with the help of spectrophotometer. Healthy seeds of *V. radiata* var. NM-92 obtained from NARC Islamabad, Pakistan, were surface sterilized by soaking in 0.1% HgC12 solution for 5 min with continuous shaking. After that seeds were washed with sterilized distilled water thrice. Sterilized seeds were then soaked in bacterial suspensions (monoculture and mixed culture suspensions) with the help of sterilized forceps for about 15 to 20 min. 10 ml of autoclaved distilled water was poured in labeled petriplate (lined with double layer of Whattman filter paper No. 1 autoclaved and oven dried). With the help of sterilized forceps, seeds (control as well as inoculated) were spread in the respective labeled petri plate (15 seeds of *V. radiata* per Petri plate) uniformly.

Petri plates were kept in dark at 25 ± 1°C for germination. Germination was recorded daily. After germination, plates were shifted to light (10D lux and 16 h day length) at 25 ± 1°C after adding 10 ml of Hewitt nutrient solution in each plate (Hewitts, 1963). Seedlings were grown for 10 days after shifting to light. Experiment was repeated eight times. Seedlings were removed from the Petri plates. Different growth parameters (germination, root length, shoot length, seedling length, number of leaves and number of roots) were studied. Fresh weight, dry weight and dry weight per gram fresh weight of each treatment was taken in grams.

### Biochemical analysis

For biochemical analysis auxin (Mahadevan, 1984), soluble protein (Bhatti et al., 1993; Lowry et al., 1951), peroxidase (David and Murry, 1965) and acid phosphatases content (Iqbal and Rafique, 1987) were studied. Following Mahadevan (1984) auxin was extracted from shoots of plant material which was crushed in 2 ml of ethyl ether and centrifuge to get supernatant. The supernatant was mixed with 1 ml of 5% sodium bicarbonate, shaken and sodium bicarbonate layer was acidified to pH 3 with HCl (6 N). 1 ml of ethyl ether and 2 ml of Salkowski's reagent was added in each test tube. This material was kept in the dark at room temperature for 30 min for color development. Auxin content was estimated with spectrophotometer at 535 nm.

Bhatti et al. (1993) method was used for soluble proteins extraction. Plant material was crushed in phosphate buffer (0.1 M pH 7.0) at a ratio of 1: 4 (w/v). Then centrifuged (14,000 rpm for 10 min at 4°C) and the supernatant (0.4 ml) was mixed with 2 ml of Folin's mixture and put at room temperature for 15 min. Then, 0.2 ml of Folin and Ciocalteu's phenol reagent was added, mixed and placed for 45 min at room temperature for the color development. Protein content was estimated at 750 nm and was calculated using standard curve.

### Statistical analysis

Data obtained was analyzed statistically following Steel and Torrie (1981). Least significant difference was also calculated.

## RESULTS

Five bacterial strains of Azotobacter isolated by Aziz (2000) were used for plant microbes' interaction study. Besides these five monocultures, twenty six mixed cultures of all possible combinations of these strains were used to determine their role in stimulating the germination and early growth of *V. radiata*.

In majority of cases, bacterial inoculations provoked germination from 0.15% in C¬1 to 4.7 in A¬1, A¬2, A¬3, A¬4, A¬5, B¬7, C ¬3, C ¬5, and D¬5 as compared to non- inoculated seedlings. In few cases bacterial inoculations (D4, E1, C¬10, C¬2, B¬1, B¬9, and C¬4) had inhibitory effects on germination (Table 2). Shoot lengths were markedly increased with the inoculation of Azotobacter strains (monocultures) and their combinations as compared to the control (non-inoculated seedlings). Percentage increase varied between 6.748% in D4 (mixed culture combination) to 17.938% in A3 (monoculture) (Table 2). Root lengths of inoculated seedlings were markedly increased (except E1, D¬3 and C3 mixed cultures, which cause reduction in the root length to 6.835, 3.948 and 3.297%, respectively (Table 2). Seedling lengths of *V. radiata* were enhanced significantly with the bacterial inoculations except E1 as compared to control. Increase in seedling lengths varied from 4.037% in C3 (mixed culture combination inoculation) to 20.066% in A3 (monoculture) (Table 2). Number of leaves and the number of roots was not affected by the bacterial inoculations as compared to non-inoculated seedlings. Bacterial inoculations affected differently to weight parameter. Most of the inoculations caused decrease in dry weight per gram fresh weight of seedlings as compared to non-inoculated (Table 2). Increase was 11.167% in A2 and 0.941% in D1. Maximum reduction was manifested with the inoculation of A1 (41.279%) and minimum reduction was recorded with the inoculation of D5 (8.544%).

Auxin content of *V. radiata* seedlings was enhanced with the bacterial inoculations except B¬3, D¬4, E1, B¬6 and C4 inoculations which caused 33.571, 26.588, 9.396, 8.026 and 5.916% reductions, respectively. Mixed culture combinations C¬3, C¬8, C10 and D¬1 showed synergistic stimulation in the auxin content of *V. radiate* seedlings (Table 3). All the bacterial inoculations caused

**Table 1.** Bacterial composition of mono and mixed cultures used for inoculating seeds of *Vigna radiata*.

| Monocultures | | Mixed Cultures | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Symbol | Strains | Two strains culture compositions | | Three strains culture composition | | Four strains culture composition | | Five strains culture compositions | |
| | | Symbol | Strains | Symbol | Strains | Symbol | Strains | Symbol | Strains |
| $A_1$ | Ab-1 | $B_1$ | Ab-1, Ab-2 | $C_1$ | Ab-1, Ab-2 Ab-4 | $D_1$ | Ab-1, Ab-2 Ab-4 Ab-5 | $E_1$ | Ab-1, Ab-2 Ab-4 Ab-5 Ab-6 |
| $A_2$ | Ab-2 | $B_2$ | Ab-1, Ab-4 | $C_2$ | Ab-1, Ab-2 Ab-5 | $D_2$ | Ab-1, Ab-2 Ab-4 Ab-6 | | |
| $A_3$ | Ab-4 | $B_3$ | Ab-1, Ab-5 | $C_3$ | Ab-1, Ab-4 Ab-5 | $D_3$ | Ab-1, Ab-2 Ab-5 Ab-6 | | |
| $A_4$ | Ab-5 | $B_4$ | Ab-1, Ab-6 | $C_4$ | Ab-1, Ab-4 Ab-6 | $D_4$ | Ab-1, Ab-4 Ab-5 Ab-6 | | |
| $A_5$ | Ab-6 | $B_5$ | Ab-2, Ab-4 | $C_5$ | Ab-1, Ab-5 Ab-6 | $D_5$ | Ab-2, Ab-4 Ab-5 Ab-6 | | |
| | | $B_6$ | Ab-2, Ab-5 | $C_6$ | Ab-2, Ab-4 Ab-5 | | | | |
| | | $B_7$ | Ab-2, Ab-6 | $C_7$ | Ab-2, Ab-4 Ab-6 | | | | |
| | | $B_8$ | Ab-4, Ab-5 | $C_8$ | Ab-2, Ab-5 Ab-6 | | | | |
| | | $B_9$ | Ab-4, Ab-6 | $C_9$ | Ab-4, Ab-5 Ab-6 | | | | |
| | | $B_{10}$ | Ab-5<br>Ab-6 | $C_{10}$ | Ab-1<br>Ab-2<br>Ab-6 | | | | |

significant increase in the protein content of the seedlings. Maximum increase was observed with A¬3 (78.571%) inoculation among the monoculture inoculations whereas relatively more increase was observed with D5(137.50%) inoculation among the mixed culture inoculations when compared to the control (Table 3). In V. radiata, all the bacterial inoculations stimulated the activity of peroxidase significantly relative to non-inoculated seedlings. Maximum increase was observed was observed with B¬3 inoculation (520.437%) among the mixed culture inoculations and with A4 inoculation (241.73%) among the monoculture inoculations (Table 3). Acid phosphatase activity of inoculated seedlings Increased significantly (3.23 to 95.732%) with the exception of E1, C4, D5, B1, B¬10, D¬2 and B5 where 32.530, 26.680, 20.280, 14.217, 13.475, 12.792 and 3.290% decreases were recorded, respectively (Table 3).

## DISCUSSION

Growth promoting bacteria improved plant growth by decomposing mineral material and making availability of nutrients to plants (Dobbelaere et al., 2003), synthesizing and liberating growth hormones (Tsavkelova et al., 2005) and reducing the susceptibility to pathogens (Calvo-Bado et al., 2006). Growth stimulating bacteria especially *Rhizobia*, *Sinorhizobium meliloti* and *Azotobacter* species have been reported to increase the yield and nitrogen content of plants (Khalid et al., 2004). Growth improvement depends on plant bacterium association. Growth promoting bacteria induce direct plant promotion, biological control and systemic resistance in host plants (Zheng et al., 2000). In the present study bacterial strains showed significant stimulation in germinations and early growth parameters studied and thus improved the growth in majority of cases when compared to control. In general, A3 caused maximum enchantment in seedling growth and among the mixed cultures B10 stimulated seedling growth relative to control. Inoculations with bacterial cultures A1 (Ab-1) and B1 manifested maximum increase in fresh weights of *V. radiata* seedlings relative to non inoculated seedlings. Maximum enhancement in dry weight of seedlings was caused by the inoculations of A3 and D1 when compared with non inoculated treatment. Reduction in dry weight per gram fresh weight of seedlings occurred with all the bacterial inoculations except with D1 which enhanced this parameter when compared with control. According to Bashan and Levanony (1990) *Azospirillum* strains could increase the water status of plants. Increased dry weights and plant height are also reported by Bashan et al. (2000) when inoculated with mixtures of mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. Enhanced

**Table 2.** Effects of *Azotobacter* (mono and mixed cultures) inoculations on different growth parameters (% age germination, shoot length, root length, seedling length and dry weight per gram fresh weight) of *V. radiata*.

| Sr. No | Bacterial Strain/ Strains Combination (Symbol) | % germination | Shoot length (cm) | Root Length | Seedling length (cm) | Dry weight per gram fresh weight (mg/g) |
|---|---|---|---|---|---|---|
| 1 | C (Control) | 95.555±1.814 | 7.031±0.456 | 6.332±0.234 | 13.363±0.556 | 105.132±11.62 |
| 2 | $A_1$ | 100.000±0.000 | 8.096±0.361 | 7.386±0.536 | 15.482±0.632 | 61.734±3.08 |
| 3 | $A_2$ | 100.000±0.000 | 7.898±0.621 | 6.924±0.187 | 14.823±0.798 | 116.873±2.20 |
| 4 | $A_3$ | 100.000±0.000 | 8.292±0.469 | 7.752±±0.475 | 16.044±0.802 | 84.157±15.00 |
| 5 | $A_4$ | 100.000±0.000 | 7.725±0.452 | 7.584±0.597 | 15.310±0.638 | 90.674±0.16 |
| 6 | $A_5$ | 100.000±0.000 | 7.704±0.506 | 7.824±0.114 | 14.988±0.460 | 91.589±7.12 |
| 7 | $B_1$ | 93.333±5.443 | 7.895±0.410 | 7.140±0.506 | 15.036±0.864 | 76.558±8.75 |
| 8 | $B_2$ | 97.916±1.701 | 7.746±0.665 | 6.965±0.811 | 14.712±1.285 | 92.664±7.14 |
| 9 | $B_3$ | 95.833±3.402 | 7.900±0.5108 | 6.899±0.252 | 14.800±0.540 | 75.570±0.68 |
| 10 | $B_4$ | 96.266±.048 | 8.007±0.715 | 6.753±0.516 | 14.760±1.225 | 89.509±3.36 |
| 11 | $B_5$ | 98.0339±1.601 | 7.526±0.464 | 6.989±0.429 | 14.515±0.849 | 80.289±4.04 |
| 12 | $B_6$ | 97.777±1.814 | 8.002±0.527 | 6.824±0.312 | 14.827±0.334 | 78.252±0.12 |
| 13 | $B_7$ | 100.000±0.000 | 8.229±0.402 | 6.455±0.373 | 14.685±0.697 | 91.544±2.98 |
| 14 | $B_8$ | 95.553±3.630 | 8.160±0.416 | 7.136±0.489 | 15.296±0.417 | 89.338±0.64 |
| 15 | $B_9$ | 93.333±5.443 | 7.736±0.293 | 6.427±0.418 | 14.164±0.522 | 84.525±0.72 |
| 16 | $B_{10}$ | 97.916±1.701 | 8.151±0.426 | 7.577±0.513 | 15.728±0.790 | 85.885±1.90 |
| 17 | $C_1$ | 97.777±1.814 | 7.935±0.515 | 6.651±0.276 | 14.587±0.791 | 78.123±2.05 |
| 18 | $C_2$ | 91.770±1.448 | 8.029±0.529 | 6.939±0.765 | 14.969±1.279 | 73.489±1.80 |
| 19 | $C_3$ | 100.000±0.000 | 7.779±0.649 | 6.123±0.500 | 13.902±0.804 | 89.583±1.34 |
| 20 | $C_4$ | 93.750±5.103 | 7.769±0.400 | 7.249±0.318 | 15.218±0.650 | 83.247±5.16 |
| 21 | $C_5$ | 100.000±0.000 | 7.833±0.651 | 6.453±0.593 | 14.287±1.244 | 88.483±2.06 |
| 22 | $C_6$ | 96.266±3.0448 | 7.750±0.690 | 7.780±0.677 | 15.531±1.266 | 80.074±4.76 |
| 23 | $C_7$ | 95.693±1.761 | 8.148±0.412 | 6.647±0.660 | 14.795±0.897 | 84.766±7.88 |
| 24 | $C_8$ | 97.777±1.814 | 7.675±0.618 | 7.145±0.467 | 14.821±1.080 | 68.712±4.61 |
| 25 | $C_9$ | 95.833±3.402 | 8.191±0.443 | 7.278±0.110 | 15.469±0.549 | 80.633±2.95 |
| 26 | $C_{10}$ | 89.583±6.133 | 7.534±0.710 | 6.869±0.276 | 14.403±0.663 | 84.557±1.69 |
| 27 | $D_1$ | 95.833±3.402 | 7.645±0.318 | 6.415±0.308 | 14.060±0.597 | 106.122±10.91 |
| 28 | $D_2$ | 97.916±1.701 | 7.819±0.578 | 7.104±0341 | 14.924±0.675 | 77.668±0.36 |
| 29 | $D_3$ | 97.777±1.814 | 7.948±0.358 | 6.082±0.059 | 14.030±0.415 | 72.507±3.08 |
| 30 | $D_4$ | 85.903±5.864 | 8.011±0.398 | 7.017±0.637 | 15.028±.0.909 | 79.425±0.04 |
| 31 | $D_5$ | 100.000±0.000 | 7.505±0.615 | 7.037±0.448 | 14.543±0.883 | 96.149±12.09 |
| 32 | $E_1$ | 89.443±6.102 | 7.689±0.602 | 5.899±0.622 | 13.588±0.780 | 96.131±9.59 |
| | LSD at P 0.05 | 2.68 | 0.249 | 0.454 | 0.526 | 5.402 |

weights could be attributed to the accumulation of salts and nutrients, enhanced ion uptake and increased level of organic salts in cytoplasm (Sudhakar et al., 1993). Growth stimulation by bacterial inoculations is indicated by improved seeding length coupled with increased dry weight. Decrease in dry weight parameter might be due to unavailability or uptake of ions and formation of ligands or organic complexes, thus, restricting the bioavailability of these ions in the medium (Hughes and Poole, 1989). Alami et al. (2000) studied that inoculation effects of Rhizobium strain (isolated from rhizoplane of sunflower roots) on sunflower seeds, which caused increase in shoot and root dry mass under water stress and normal condition. Auxins constitute a class of phytohormones that play important roles in the coordination of plant growth and develop-ment. Bacterial inoculations increased auxin content of seedlings markedly relative to control. However, inocula-tions with B3, E1 caused reduction in the auxin content. According to Campbell (1985), the bacterial strains stimulate plant growth by synthesizing and liberating growth hormones. Although, IAA biosynthesis in these bacteria have been shown to occur through different biosynthetic pathways. Thus, the most important mechanism of direct growth promotion may be the production of plant growth regulators (Arshad and Frankenbergrer, 1998). Inoculations (mono and mixed culture treatments) promoted protein content of the seedlings significantly. Auxin and protein both are formed

**Table 3.** Effects of *Azotobacter* (mono and mixed cultures) inoculations on different biochemical parameters (Auxin content, Soluble protein content, Peroxidase content and Acid phosphatase content) of *V. radiata*.

| Sr. No | Bacterial Strain/ Strains Combination (Symbol) | Auxin content (µg / g. f. wt.) | Soluble protein content (µg / g. f. wt.) | Peroxidase content (unit / g. f. wt.) | Acid phosphatase content (unit / g. f. wt.) |
|---|---|---|---|---|---|
| 1 | C (Control) | 0.1412±0.006 | 448.0±5.656 | 32.334±2.683 | 418.150±2.934 |
| 2 | $A_1$ | 0.873±0.161 | 688.0±11.313 | 63.117±0.049 | 818.454±1.735 |
| 3 | $A_2$ | 0.863±0.173 | 480.0±0.707 | 67.405±0.809 | 539.118±1.008 |
| 4 | $A_3$ | 0.899±0.179 | 800.0±28.284 | 46.019±0.122 | 594.461±8.159 |
| 5 | $A_4$ | 1.203±0.181 | 532.0±31.112 | 110.497±5.383 | 551.328±1.453 |
| 6 | $A_5$ | 1.145±0.068 | 648.0±5.656 | 73.253±2.008 | 541.510±4.331 |
| 7 | $B_1$ | 0.580±0.116 | 568.0±11.313 | 42.698±0.342 | 358.698±6.794 |
| 8 | $B_2$ | 0.713±0.069 | 756.0±25.456 | 55.581±0.05 | 459.041±1.765 |
| 9 | $B_3$ | 0.273±0.007 | 580.0±65.054 | 200.615±16.867 | 518.517±4.391 |
| 10 | $B_4$ | 0.533±0.065 | 720.0±11.313 | 61.291±0.651 | 485.748±2.106 |
| 11 | $B_5$ | 0.484±0.052 | 632.0±39.598 | 114.617±0.573 | 404.391±5.162 |
| 12 | $B_6$ | 0.397±0.173 | 742.0±4.242 | 143.094±6.297 | 533.621±1.038 |
| 13 | $B_7$ | 0.721±0.142 | 552.0±11.313 | 77.954±1.004 | 522.167±1.424 |
| 14 | $B_8$ | 0.689±0.094 | 598.0±7.071 | 62.900±1.188 | 452.727±1.928 |
| 15 | $B_9$ | 0.455±0.029 | 584.0±28.284 | 64.664±0.056 | 474.335±0.707 |
| 16 | $B_{10}$ | 0.691±0.013 | 584.0±22.627 | 52.253±1.204 | 361.803±1.661 |
| 17 | $C_1$ | 0.855±0.050 | 482.0±4.242 | 112.633±0.316 | 466.744±28.267 |
| 18 | $C_2$ | 0.691±0.111 | 618.0±21.213 | 60.783±2.274 | 484.195±1.661 |
| 19 | $C_3$ | 0.525±0.034 | 604.0±48.083 | 75.589±2.942 | 723.293±3.871 |
| 20 | $C_4$ | 0.388±0.005 | 520.0±11.313 | 51.001±0.559 | 306.587±3.500 |
| 21 | $C_5$ | 1.492±0.234 | 508.0±2.828 | 92.316±0.002 | 659.223±4.020 |
| 22 | $C_6$ | 0.985±0.012 | 568.0±11.313 | 97.464±1.360 | 468.251±2.136 |
| 23 | $C_7$ | 1.286±0.119 | 592.0±5.656 | 54.092±1.762 | 455.517±2.269 |
| 24 | $C_8$ | 1.204±0.194 | 524.0±2.828 | 84.348±0.157 | 431.664±2.848 |
| 25 | $C_9$ | 1.014±0.220 | 564.0±8.485 | 79.936±1.672 | 449.705±5.993 |
| 26 | $C_{10}$ | 2.272±0.233 | 640.0±33.941 | 123.767±0.778 | 590.18±1.542 |
| 27 | $D_1$ | 1.813±0.028 | 712.0±16.970 | 65.272±2.938 | 446.013±4.124 |
| 28 | $D_2$ | 0.974±0.134 | 568.0±5.656 | 67.452±0.241 | 364.657±2.907 |
| 29 | $D_3$ | 0.904±0.239 | 792.0±22.627 | 100.265±4.679 | 472.950±0.4105 |
| 30 | $D_4$ | 0.302±0.039 | 640.0±0.000 | 83.407±6.686 | 567.502±6.394 |
| 31 | $D_5$ | 0.487±0.007 | 1064.0±16.970 | 88.438±0.216 | 333.566±3.679 |
| 32 | $E_1$ | 0.373±0.112 | 720.0±11.313 | 105.308±0.874 | 282.125±5.310 |
| | LSD at P 0.05 | 0.114 | 21.642 | 3.787 | 6.252 |

from tryptophan molecules with different arrangements. Hence, the amount of protein content is directly related with auxin content as auxin increase the rate of metabolism thus, increasing the amount of protein content.

Bacterial inoculations also promoted peroxidase content of seedlings relative to control. Maximum increase was observed with the inoculation of B3 and A4. Two enzymes, acid phosphatase and peroxidase, were studied in this respect. Catlase and peroxidase, both enzymic systems, protect the cells from free oxy radicals. Catalase mediates the cleavage of $H_2O_2$ evolving $O_2$ (Scandalios, 1993) and peroxidase reduces $H_2O_2$ to $H_2O$ using several reductants available to the cells (Foyer et al., 1994). Plant peroxidases, a ubiquitous class of protein, are involved in several different physiological functions including wound healing, biosynthesis of cell walls and growth regulation (Zheng and Van Hyystee, 1992). The phosphatases are a diverse class of enzymes. The enzyme activity increases with increasing cell number and cell content in early stages and decreases with maturation (Ching et al., 1984). Generally, monoculture inoculations provoked germinations and early growth parameters along with auxin, protein, peroxidase and acid phosphatases contents more effectively than their mixed culture inoculations. One reason may be that bacteria from different sources interfere in the efficiency of one another. Whereas in some cases show positive interaction and enhance the

activity of one another, thus their association is beneficial for plant growth.

## REFERENCES


Alami Y, Achouak W, Marol C, Heulin T (2000). Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl. Environ. Microbiol., 66: 3393-3398.

Arshad M, Frankenbergrer Jr WT (1989). Plant growth substances in the rhizosphere: microbial production and functions. Adv. Agron. 62: 146-151.

Aziz F (2000). Growth promoting *Pseudomonas, Azospirillum* and *Azotobacter* strains isolated from roots of different weeds. M.Sc. Thesis, University of the Punjab, Lahore, Pakistan.

Bashan Y (1998). *Azospirillum* plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton and wheat. Can. J. Microbiol., 44: 168-174.

Bashan Y, Holguin G (1994). Root to root travel of the beneficial *Azospirillum brasilens*. Can. J. Microbiol., 6: 2120-2131.

Bashan Y, Levanony H (1990). Current status of *Azospirillum* inoculation technology: *Azospirillum* as challenge for agriculture. Can. J. Microbiol., 36: 591-608.

Bashan Y, Moreno M, Toroyo E (2000). Growth promotion of the sea water Irrigated oil seed halophyte *Saliocornia Bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* Spp. Biol. Fertil. Soil., 32: 265-272.

Bhatti GA, Qureshi N, Qureshi A, Sultana K (1993). Studies on heat shock response of wheat seedlings using *E. coli* GroEL antibodies. Pakphyton, 5: 157-166.

Calvo-Bado LA, Petch G, Parsons NR, Morgan JAW, Pettitt TR, Whipps JM (2006). Microbial community responses associated with the development of oomycete plant pathogens on tomato roots in soilless growing systems. J. Appl. Microbiol., 100: 1194-1207.

Campbell R (1985). Mirobiology of roots. In: Plant Microbiology. (Arnold, E., ed.), pp. 106-152. Edward Arnold, London.

Castaneda M, Guzman J, Moreno S, Espin G (2000). The GacS sensor kinase regulates alginate and poly-ß-hydro butyrate production in *Azotobacter vine landii*. J. Bacteriol., 182: 2624-2628.

Ching TM, Thompson DM, Mertzger RJ (1984). Acid Phosphatases and seed shrivelling in triticales. Plant Physiol., 76: 478-482.

Choudhury A, Kennedy I (2004). Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol. Fert. Soils, 39: 219-227.

David R, Murry E (1965). Protein synthesis in dark-grown bean leaves. Can. J. Bot., 43: 817-824.

Defago G, Hass D (1990). In: Soil Biochemistry (Bollag JM, Stotzky G eds), Deker, New York, 6: 249.

Diaz LC, Melchers LS, Hooykass PJJ, Lutenberg BBJJ, Kijne JW (1989). Root lectin as a determinant of host plant specificity in the *Rhizobium* and legume symbiosis. Nature, 338: 579-851 .

Dobbelaere S, Vanderleyden J, Okon Y (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci., 22: 107-149.

Foyer CH, Lelandais M, Kunert KJ (1994). Phytooxidative stress in plants. Plant Physiol,. 92: 696-717.

Hewitts EJ (1963). Mineral nutrition of plants in culture media. In: Steward FC (eds) Plant physiology. Academic Press, New York, pp. 97-133.

Hughes MN, Poole RK (1989). Metals and microorganisms. Chapmann and Hall, London, pp. 280-285.

Iqbal J, Rafique N (1987). Toxic effects of $BaCl_2$ on germination, early seedling growth, soluble proteins and acid phosphatase in *Zea mays* L. Pak. J. Bot., 19: 1-8.

Katupitiya S, Miller J, Vesk M, Viccars L, Zeman A, Lidung Z, Elmerich C, Kennedy IR (1995). A mutant of *Azospirillum brasilense* Sp 7 impaired in flocculation with a modified colonization pattern and superior nitrogen fixation in association with wheat. Appl. Environ. Mcrobiol., 61: 1987-1995.

Khalid A, Arshad M, Zahir ZA (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol., 96: 473-480.

Kloepper JW, Lifshitz R, Zablotowics RM (1989) Free living bacterial inocula for enhancing cope productivity. Trends Biotechnol., 7: 39-44.

Lowry O, Rosebrough N, Farr A, Randall R (1951). Protein measurements with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mahadevan A (1984). In: Growth regulators, microorganisms and diseased plants. Oxford and IBH Publishing company, India, pp. 31.

Miller KJ, Wood JM (1996). Osmoadaptation by rhizobacteria. Ann. Rev. Microbiol., 50: 102-136.

Nunez C, Moreno S, Soberon-Chavez G, Aspin G (1999). The *Azotobacetr vine landii* resonse regulator AlgR is essential for cyst formation. J. Bacteriol., 181: 141-148.

Scandalios JG (1993). Oxygen stress and superoxide dismutases. Plant Physiol., 101: 7-12.

Steel RGD, Torrie JH (1981). In: Principles and procedures of statistics, a biometrical approach (eds), McGraw Hill International Book Company, p. 172-192

Sudhakar C, Redly PS, Varranjanecynlu K (1993). Effect if salt stress on enzyme of praline synthesis and Oxidation in green gram (*Phaseolus aureus* Roxb.) seedlings. J. Plant Physiol., 141: 621-623.

Tsavkelova E, Cherdyntseva T, Netrusov A (2005). Auxin production by bacteria associated with orchid roots. Microbiology, 74: 46-53.

Van Veen JA, Overbeek LS, Van Elas JD (1997). Fate and activity of microorganisms introduction into soil. Microbiol. Mol. Biol. Rev., 61: 121-135.

Zheng X, Van Hyystee RB (1992). Peroxidase-regulated elongation of segments from peanut hypocotyls. Plant Sci., 81: 47-56.

Zheng Z, Zeng W, Huang Y, Yang Z, Li J, Cai H, Su W (2000). Detection of antitumor of and anitmicrobial activities in marine organism associated actinomycetes isolated from the Taiwan strait, China. FEMS Microbiol. Lett., 188: 87-91.