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# EFFECT OF *RHIZOBIUM* STRAINS AND PHOSPHORUS ON GROWTH OF SOYBEAN (*GLYCINE MAX*) AND SURVIVAL OF *RHIZOBIUM* AND P SOLUBILIZING BACTERIA

**[*]ZARRIN FATIMA, MUHAMMAD ZIA AND [**]M FAYYAZ CHAUDHARY**

*Department Biological Sciences,*
*Quaid-i-Azam University, Islamabad, Pakistan*

**Abstract**

Pot experiments were conducted to evaluate the effect of *Rhizobium leguminosarum* strains (TAL-377, TAL-379 and TAL-102) alone and in combination with Phosphorus on soybean. The parameters studied were survival of *Rhizobium* at pod filling stage and after harvesting, and root/ shoot dry and fresh weight of soybean under natural condition. Surface stererilized soybean seeds var. NARC-4 were sown in earthen pots filled with soil and sand 1:3. Phosphorus (P) was applied as single super phosphate (SSP) at the time of sowing in the soil. Soybean seeds were inoculated with *Rhizobium* strains as seed coating just before sowing. The effect of growth was highly significant ($\alpha$ 0.05) with an increase in root/shoot dry and fresh weight in plants with mixture of *Rhizobium* inoculums with phosphorus on soybean. Among three strains TAL-102 performed well as compared to TAL-377 and 379 *Rhizobium* strains. The CFU count of *Rhizobium* and P solubilizing bacteria was found maximum both at pod filling and after harvesting stage when *Rhizobium* strains and P was applied in mixed culture. A mixture of effective strains with phosphorus is a promising way for enhancing the growth of legume crops.

## Introduction

Symbiotic nitrogen fixation, a key component in biological nitrogen fixation, has not been as successful in substituting for chemical fertilizer as initially expected. *Rhizobium* inoculants seem to be an attractive and cost effective source of N for legume cultivation in Pakistan and it requires little technical expertise. Phosphorus plays an important role in the plant's energy transfer system since phosphorus deficiency retards growth and tillering. In soil, phosphorus is quite abundant but it reacts readily with iron, aluminium and calcium to form insoluble compounds. These reactions results in very low phosphorus availability and low efficiency of phosphorus fertilizer used by plants (Jodie & Pete, 2000). Pakistani soil containing approximately 210 $mgkg^{-1}$ of phosphorus is considered to be deficient (Memon, 1996). P deficiency is wide spread in almost 90% of the soil and the application of phosphoric fertilizer is considered essential for crop production.

Soybean (*Glycine max*) is one of the most important oil seed crop in the world. It contain 18 to 22% oil, highly desirable in diet and have 40 to 42 % of good quality protein. Therefore it is the best source of protein and oil and truly claim the title of the meat/oil on plants. Generally, it is used in the food industry for flour, oil, cookies, candy, milk, vegetable cheese, lecithin and many other products. At present, USA has the largest area under its cultivation. Soybean is also grown in other parts of the world including Brazil, China, Argentina, Indonesia, Korea, Japan. In Pakistan soybean has suffered a set back and has therefore, not been able to attain a respectable position among the oil seed crops. Its cultivation remained limited to very small acreage and showed a declining trend (Pakissan.com Agric consultancies, Pakissan com; soybean, htm).

[*]*zarrinfatima75@yahoo.com* [**]*fayyazchaudhary@yahoo.com*

The present study was designed to evaluate the effect of various exotic *Rhizobium* strains alone and in combination with phosphorus on root/shoot biomass production and survival of *Rhizobium* at pod filling stage and after harvesting on soil. This study aimed to enhance soybean production among the small holder farmers in Pakistan through the use of *Rhizobium* inoculation and moderate applications of P and to sustain our soil nutrient level.

## Materials and Methods

**Plant materials and growing conditions:** Seeds of soybean cv. NARC- 4 obtained from National Agricultural Research Centre (NARC) Islamabad, were surface sterilized in aqueous solution of mercuric chloride (0.1%) for 2 min., and thoroughly rinsed with distilled water. Thereafter, seeds were soaked in distilled water for six hour and then inoculated with *Rhizobium* strain TAL 377, TAL 378, TAL- 102 and mixture of these three strains just before sowing alone and in combination with phosphorus in earthen pots containing 7 kg soil and sand 1:3 ratio. The phosphorus was applied as single super phosphate 8 g in each pot, and mixed well in soil at the time of filling the pots. For inoculation with peat-based inoculums, surface sterilized seeds were moistened in sugar solution (48%) before application of inoculums to get a thin uniform coating of inoculums on seeds before sowing. Four plants pot$^{-1}$ were allowed to grow during mid July in natural environment. The experiment was laid out in Complete Randomized Design (CRD) with four replications. The treatments were as follows:

T1 = TAL377
T2 = TAL377+ phosphorus
T3 = TAL379
T4 = TAL379+Phosphorus
T5 = TAL102
T6 = TAL102+ Phosphorus
T7 = TAL377+379+102
T8 = TAL377+379+102+ Phosphorus
T9 = Phosphorus
T10 = Control

**Fresh/dry weight of shoots and roots (g):** At maturity, plants were harvested and samples of roots and shoots were collected to determine above ground biomass (weight of shoot), below ground biomass (root weight). Root and shoot dry weight plant$^{-1}$ in gram were taken after oven drying at 70$^{o}$C for three days.

**CFU count g$^{-1}$ soil:** CFU count (Colony forming unit) g$^{-1}$ of *Rhizobium* in 1g soil were determined by plating 0.1 ml of tenfold serial dilution on yeast mannitol agar (YMA) medium containing congo red. For phosphorus solubilizing bacteria, media containing $Ca_3(PO_4)_2$ 1.25g, Glucose 2.5g $(NH_4)SO_4$ 0.05g, $MgSO_4$ $7H_2O$ 0.025g, KCl 0.05g, yeast extract 0.025g, agar 4.5g, NaCl 0.09g, $FeSO_4$ and $MnSO_4$ (for trace element) in 250ml distilled water was used (Samasegaran & Hoben, 1985).

Number of bacteria/g soil was calculated from the colony forming units obtained on plates (James, 1987).

**Statistical analysis:** The data was analyzed statistically by analysis of variance and differences among the significant treatments were determined by least significant difference (LSD) test.

**Results and Discussion**

**Physicochemical properties of the experimental soil:** The experimental soil was loam in texture and available nitrogen was 2.73%, potassium 0.120 µg $Kg^{-1}$, phosphorus 3.0mg $Kg^{-1}$, and organic matter 0.75%. CFU count showed presence of *Rhizobium* population $1.08x10^6$ $g^{-1}$ soil and phosphorus solubilizing bacterial population $1.4x10^6 g^{-1}$ soil.

**Fresh/dry weight of shoot and root $plant^{-1}$ (g):** Biomass yield is an important measure of plant vigor and health. Maximum fresh and dry weight of root and shoot per plant (fresh weight of shoot 68.8%; dry weight of shoot 66.8% and fresh weight of root 83%; dry weight of root 60% respectively) were recorded when *Rhizobium* strains were applied to the plants in the form of mixture with phosphorus as well as without phosphorus (Table 1, Fig. 1). Among the three strains alone treatment, strain TAL102 performed well as compared to TAL377 and TAL379. The statistical analysis of the data revealed that the effect of phosphorus and *Rhizobium* strain on growth and yield of soybean crop was significant ($\alpha$ 0.05) so that, we should be aware that adequate supply of P with *Rhizobium* strains (mixture inoculation) plays an important role in physiological and developmental processes in plant life and the favorable effect of this important nutrient might accelerate the growth processes, which ultimately resulted in increased grain yield of the crop. Taiwo *et al*., (1999) also suggested that there is a high requirement of P by legumes as this element has a pronounced effect on root development, straw length, crop quality and yield of the crop. Therefore, the nitrogen biofertilizer can be used as an alternate or as supplement to nitrogen fertilizer to increase agricultural production with less input capital and energy. Generally, *Rhizobium* strain with P performed well. Asia *et al*., (2005) also observed that P application increased biomass yield by 20.7% greater than that of control. Root life span is important for sustained P uptake during reproduction, for nutrient translocation into developing seeds, and for whole plant carbon budget during reproduction. Similar results were also obtained by Amos *et al*., (2001). Lukiwatid & Simanungalid (2002) have previously reported that the mix inoculation improve the productivity of soybean as compared to control. In this study it was observed that efficiency of *Rhizobium* is influenced by the availability of P in the soil as it was directly involved in the nutrition of legume and also helps to improve the root as well as shoot growth of the crop. It can thus be used as an alternate or as supplement to the chemical nitrogen fertilizer to increase agriculture production with lesser capital input and energy.

Results of CFU $g^{-1}$ count of *Rhizobium* and Phosphorus solubilizing *Rhizobium* is presented in Table 2. The maximum CFU counts were observed in plants treated with mix strain with phosphorus both at pod filling stage and after harvesting stage because phosphorus plays a key role in improving the density of *Rhizobium* in the soil surrounding the root (Anon., 1999). This survival is due to sufficient nutrient, a condition in which bacteria in many environments, including soil, regularly find themselves (Foster & Spetor, 1995; Kjelleberg *et al.,* 1987; Roszak & Colwell. 1987; Van Elsas & Overbeek1993). The reason of this increase is also due to soil amendment, such as manure, lime and phosphate and by level of fertility because nutrient level affects rhizobial viability (Hirsch, 1996; Lowendorf, 1980). However, little is known about the physiological processes involved in the starvation survival of soil bacteria.

**Table 1. Effect of various *Rhizobium* strain alone and in combination with phosphorus on physicochemical data of soybean var. NARC-4.**

| Treatments | FW Shoot (g) $Plant^{-1}$ | | DW Shoot (g) $Plant^{-1}$ | | FW Root (g) $Plant^{-1}$ | | DW Root (g) $Plant^{-1}$ | |
|---|---|---|---|---|---|---|---|---|
| (T1)377 | $24.77^{F}$ | (9.8%)* | $7.98^{F}$ | (22.5%)* | $9.35^{F}$ | (42%)* | $4.23^{D}$ | (15%)* |
| (T2)377+P | $25.33^{F}$ | (11%)* | $8.13^{F}$ | (23.9%)* | $9.63^{EF}$ | (44%)* | $5.43^{C}$ | (18%)* |
| (T3)379 | $36.29^{E}$ | (38%)* | $10.47^{E}$ | (40.9%)* | $11.28^{DE}$ | (52%)* | $5.87^{C}$ | (39%)* |
| (T4)379+P | $47.63^{CD}$ | (53%)* | $11.17^{DE}$ | (44%)* | $12.92^{D}$ | (58%)* | $5.96^{C}$ | (39.9%)* |
| (T5)102 | $43.82^{D}$ | (49%)* | $11.86^{D}$ | (47%)* | $19.25^{C}$ | (72%)* | $6.39^{C}$ | (43.9%)* |
| (T6)102+P | $51.53^{C}$ | (56%)* | $13.37^{C}$ | (53.7%)* | $21.00^{C}$ | (74%)* | $7.46^{B}$ | (52%)* |
| (T7)377+379+102 | $71.23^{A}$ | (68%)* | $17.80^{A}$ | (65%)* | $23.59^{B}$ | (77%)* | $8.81^{A}$ | (59%)* |
| (T8)377+379+102+P | $71.70^{A}$ | (68.8%)* | $18.63^{A}$ | (66.8%)* | $32.07^{A}$ | (83%)* | $9.05^{A}$ | (60%)* |
| (T9)P | $56.33^{B}$ | (60%)* | $15.03^{B}$ | (58.8%)* | $11.11^{EF}$ | (51%)* | $4.06^{D}$ | (11.8%)* |
| (T10)CONTROL | $22.33^{F}$ | | $6.18^{G}$ | | $5.36^{G}$ | | $3.58^{D}$ | |
| LSD | 4.066 | | 1.066 | | 1.767 | | 0.9929 | |

α level 0.05, * percentage differences over the control are given in parenthesis

**Table 2. Effect of various *Rhizobium* strains alone and in combination with phosphorus on population of *Rhizobium* (CFU count $g^{-1}$) at pod filling stage as well as after harvesting soybean var. NARC-4.**

| Treatments | CFU $g^{-1}$ count of *Rhizobium* | | CFU $g^{-1}$ count of Phosphorus Solublizing *Rhizobium* | |
|---|---|---|---|---|
| | Pod filling stage | After harvesting | Pod filling stage | After harvesting |
| (T1)377 | $1.56x10^{4}$ | $2.34x10^{4}$ | $1.99x10^{6}$ | $2.10x10^{6}$ |
| (T2)377+P | $1.76x10^{4}$ | $3.04x10^{4}$ | $2.2x10^{6}$ | $2.00x10^{6}$ |
| (T3)379 | $2.14x10^{4}$ | $2.40x10^{4}$ | $2.2x10^{6}$ | $2.10x10^{6}$ |
| (T4)379+P | $2.17x10^{4}$ | $3.55x10^{4}$ | $2.6x10^{6}$ | $2.60X10^{6}$ |
| (T5)102 | $2.26x10^{4}$ | $3.68x10^{4}$ | $2.98x10^{6}$ | $2.46x10^{6}$ |
| (T6)102+P | $2.54x10^{4}$ | $3.75x10^{4}$ | $2.25x10^{6}$ | $2.60x10^{6}$ |
| (T7)377+379+102 | $4.02x10^{4}$ | $6.6x10^{4}$ | $3.99x10^{6}$ | $3.00x10^{6}$ |
| (T8)377+379+102+P | $4.98x10^{4}$ | $7.36x10^{4}$ | $4.5x10^{6}$ | $3.95x10^{6}$ |
| (T9)P | $1.05x10^{4}$ | $2.36x10^{4}$ | $1.65x10^{6}$ | $1.30x10^{6}$ |
| (T10)CONTROL | $1.01x10^{4}$ | $1.36x10^{4}$ | $1.5x10^{6}$ | $1.25x10^{6}$ |

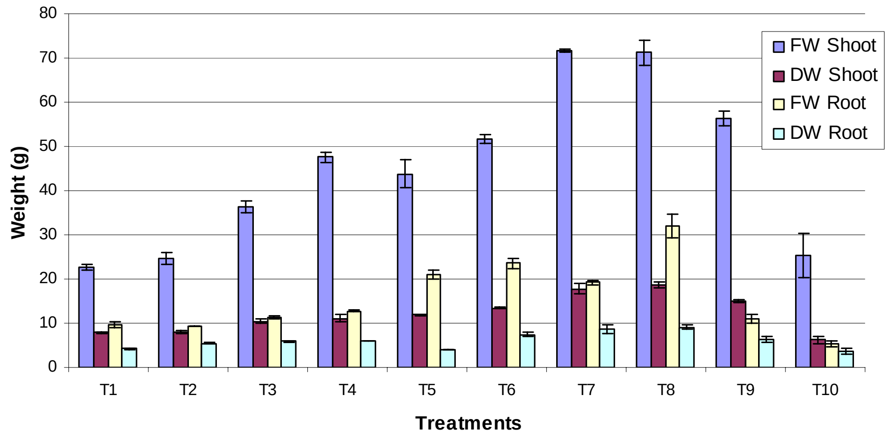


Fig.1. Response of different *Rhizobium* strains alone and combination with phosphorus on fresh/dry weight of shoots and roots plant$^{-1}$ (g) on *Glycine max* cv NARC-4.

The present study would suggest that root shoot biomass production of soybean seem to be favorably affected by mix culture of inoculums treatment whether used alone or in combination with phosphorus. Its performance in survival of *Rhizobium* as well as phosphorus solubilizing bacteria was better than other treatment over the control. Among three strains of *Rhizobium* application 102 perform well as compared to 377 and 379. A strategy of combined *Rhizobium* inoculation with phosphorus for the maximization biomass production of soybean and for maintenance of *Rhizobium* population in soil in our agriculture is needed.

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