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Effectiveness of Phosphate Solubilizing Bacteria inoculation for Improving Phosphorus Absorption and Root Growth Indices

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ABSTRACT: The current investigation was conducted to explore the efficiency of using some phosphate solubilizing bacteria in phosphorus absorption, developing of root volume, and dry weight of potato root of Agria cultivar derived from tissue culture. In this experiment, minitubers of tissue culture were inoculated with two strains of Pseudomonas bacterium (Ps116 and Ps173) from species *Pseudomonas fluorescens* and two genera of bacillus bacterium (*Bacillus subtilis* and *Bacillus megaterium*) all along with control treatment as separately and in mixture with each other within factorial experiment based on completely randomized design and in three replications. The results indicated that inoculation with Pseudomonas had significant impact on rate of phosphorus absorption and vegetative parameters in potato root at probability level 1%. The rate of phosphorus absorption and volume of root as well as dry weight of root showed significant difference with inoculation of several specious of Bacillus at levels 1% and 5% respectively. Likewise, it was characterized that the interaction effect of Pseudomonas × bacillus was significant on rate of phosphorus absorption at probability level 5%. With respect to comparison among these traits, it was identified that the inoculation by Pseudomonas (Ps173) with 34.5% increase in contrast to control treatment and 16.4% compared to Ps116, had the maximum effect on rate of phosphorus absorption. This strain of Pseudomonas had maximum impact on rate of developing root volume. Also, dry weight of root under inoculation with Ps173 and in comparison with Ps116 and control treatment indicated the maximum value i.e. 25.9% and 38.4% respectively. *B. megaterium* caused increase in root dry weight 1.48 times in control treatment. In the dual-inoculation treatment with two specious of bacteria, the maximum rate of phosphorus absorption was observed in composed treatment of *B. subtilis* and Ps173 with more than 57% increase in the available phosphorus absorption for the plant.

Keywords: *Pseudomonas*, *Bacillus*, Phosphorus absorption, Potato minituber

INTRODUCTION

Today, application of bio-fertilizers is crucially important in rising yield of crop production and maintaining sustainable productivity of soil in sustainable agricultural ecosystems. Optimal exploitation from these sources not only affects on soil properties positively, but also useful in terms of economic, social, and environmental aspects and it may serve as a suitable substitute for chemical products (Dodd, 2000; Gosling *et al.,* 2006; Kennedy *et al.,* 2004).

The phosphate solubilizing bacteria are known as the factors for rising rate of phosphorus absorption that their use in form of bio-fertilizers may improve soil nutritional status, secretion of plant growth regulators, and control of soil-borne diseases and eventually it may lead to better growth and yield in farming plants (Alagawadi & Gaur, 1992; Gaur *et al.,* 2004; Henri *et al.,* 2008; Verma *et al.,* 2010; Vyas & Gulati, 2009). The results from studies done by many researchers indicate that these materials stimulate growth in plant by various mechanisms including production of plant hormones, increase in phosphorus absorption in plant, fixation of nitrogen, production of antibiotics, siderophores as well as secretion of enzymes, which regulate amount of ethylene in the plant (David *et al.,* 1993; Glick *et al.,* 2007; Hayat *et al.,* 2010; Khan *et al.,* 2008). The microorganisms colonizing plant roots generally include bacteria, algae, fungi, protozoa and actinomycetes. Enhancement of plant growth and development by application of these microbial populations is well evident (Bhattacharyya & Jha, 2012; Gray & Smith, 2005; Hayat *et al.,* 2010; s-Saharan & Nehra, 2011; Zahir & Arshad, 1996).

Of different microbial populations present in the rhizosphere, bacteria are the most abundant microorganisms (Kaymak, 2010).Various genera of bacteria, *Pseudomonas, Enterobacter, Bacillus, Variovorax, Klebsiella, Burkholderia, Azospirillum, Serratia* and Azotobacter cause a pronounced effect on plant growth and are termed as plant growth promoting rhizobacteria (PGPR). PGPR play a significant role in enhancing plant growth and development both under non-stress and stress conditions by a number of direct and indirect mechanisms (Glick et al, 2007; Nadeem *et al.,* 2010; Zahir *et al.,* 2004). The mechanisms that promote plant growth include: nitrogen fixation, phosphorus solubilization, production of siderophores, plant growth regulators and organic acids as well as protection by enzymes like ACC-deaminase, chitinase and glucanase (Berg, 2009; Glick *et al.,* 2007; Hayat *et al.,* 2010). This bacteria in which Pseudomonas and Bacillus are the paramount species, can convert insoluble phosphate into solubilizing forms and usable for the plants (Domey & Lipmann, 1988; Pagiri & Bezbaruah, 1990; Rao, 1992; Rokade & Patil, 1993; Whitlaw, 2000; Yadav *et al.,* 2010; Zhang *et al.,* 1997). Similarly, some of these bacteria may release significantly useful minerals like phosphorus, potassium, magnesium, manganese, iron, copper, and zinc and thus to change culture medium and plant growth (Puente *et al.,* 2004a; Puente *et al.,* 2004b). The efficiency of growth stimulating bacteria depends on potential of colonization in rhizome system and removal of hazardous microorganisms and or the plant pathogens as well as germination potential and regeneration in host plant. An active root system releases regularly organic compounds into plant rhizome environment (Pedra *et al.,* 2006). The role of such microorganisms in rising absorption of nutrients has been emphasized in many researches. Alagawadi & Gaur (1992) declared that composed inoculation of *Azotobacter* with *Pseudomonas* increased significantly the rate of phosphorus and nitrogen absorption in sorghum. The synergic effects under dual inoculation with multiple variety mixture from several mycorrhiza fungi and *P. putida* bacterium have been proved regarding phosphorus absorption in plants by Attia & Awad (2003). These researchers found that multiple-inoculation of tomato with mycorrhiza fungi and phosphate solubilizing bacteria increases phosphorus and nitrogen absorption in leaves. It has reported that the growth stimulating bacteria also provide improvement condition for some of growth parameters in shoot and root organs in addition to preparation of conditions for absorption of more nutrients. In this regard, Germida & Walley (1997) reported that inoculation of wheat and several straines of Pseudomonas might cause various impacts on growth parameters. So, despite of increase in cropping parameter, root biomass was decreased in some cases while some varieties increased root dry weight in depth 15cm. Similarly, the experiment done by Heba (2009) indicated that dual inoculation of potato with growth stimulating bacteria along with mycorrhiza fungi significantly increased dry weights of stem and root and plant as a whole compared to single inoculation with mycorrhiza fungus and or with Pseudomonas, Bacillus megaterium, *Azospirilium brasilensis* bacteria. Thus, preparation of favorable conditions for better absorption of elements in the inoculated plants with phosphate solubilizing bacteria, especially of genus Pseudomonas, along with improvement in plant photosynthetic potential as well as stimulation of synthesis of internal hormones like indoleacetic acid (IAA) may provide better condition for greater root formation and growth of shoot organ. This condition develops the relations among the origin and destination.

MATERIALS AND METHODS

A. Plantlets preparation

Stem cuttings as single nodes were taken from sterilized plant stems using Agria potato cultivars. They were transferred into a sterile MS culture media [24 and were exposed to 16 hour photoperiod 60 $\mu molm^{-2}s^{-1}$ Photosynthetic Photon Flux Density (PPFD) provided by cool white and red fluorescent lamps] and 22±2°C temperature regimes for 3 weeks. After 21 days, the single stem plantlets were sub-cultured and micropropagated on the same medium to produce sufficient numbers of plantlets for the experimental trials. These plantlets were transferred to the greenhouse and its minitubers were harvested 100 days after planting. Afterwards, the sufficient number of minituber was selected for implementation of this project.

B. Project implementation

This survey was carried out for study on the impact of application of several strains of phosphate solubilizing bacteria from species *Pseudomonas* and *Bacillus* on rate of phosphorus absorption, dry weight, and developing the volume of root in potato minituber of Agria cultivar. The location of conducting the present research was tissue culture laboratory and greenhouse of Isfahan Faradasht Krooch Company. In this experiment, inoculation was done with bacteria in greenhouse and during transferring the resultant minitubers from single nodules into pots. Factorial experimental with completely randomized design was used in the experimental project. This experiment included two factors respectively as follows: *Pseudomonas fluorescens* in two levels (strains 116 and 173) with control treatment and two specious of *Bacillus* in two levels (*B. subtilis* and *Bacillus megaterium*) along with control treatment, which was carried out with three replications.

Accordingly, this project had 27 experimental units. Three pots per unit were considered. To implement this project, 81 pots were considered totally each of them had two plants. These minitubers were inserted in pots with 25cm diameter and depth 25cm that was filled with mixture of perlite and sterile peat moss with ratios 1:1 to half-full. Only one minituber was kept ten days after insertion of minitubers in any vase. Inoculation was done in greenhouse and during transference. For inoculation of minitubers with bacteria after insertion in bacterium suspension solution with population rate of 1.6 ×109 and 1.8 ×109 alive and active bacteria per milliliter (CFU ml^{-1}), the minitubers have been treated respectively in flooding treatment for 10 minutes in single and composed bacterium solutions with respect to the given treatment and then they were transferred to the main culture medium. Tillage operation and care of minitubers was identically done in control treatments (without inoculum) and the inoculated treatments.

The minitubers were fed with Elite NPK fertilizer with composition of N.P.K and ratio (20:20:20) that included all micro-elements with concentration of 0.3 per liter and in soluble form and identical volume for every 12 days. The ambient conditions in greenhouse were automatically regulated in terms of light and temperature. Duration of growing was adjusted in 16 hours for the first two months of planting and before nodulation. During two last months of growth period, period of lenght day was determined as 12 hours and shorter.

The internal temperature of greenhouse was provided within range of 16-18°C during night and 24-26°C during day by regulation of ventilation system and central heating.

C. Measurements

After flowering, the rate of phosphorus absorption was measured and volume of root and its dry weight were evaluated. In order to measure the rate of phosphorus absorption in sampled leaves, some plants were randomly selected in greenhouse and after drying in oven and extraction by means of Dry Ashing technique, phosphorus was measured by spectrophotometer (Emami, 1996). After cropping, the roots were washed with distilled water.

To determine root dry weight of roots, the samples were inserted in oven at temperature 75°C for 48 hours and after reaching to a fixed weight, root dry weight was recorded. In order to determine volume of root, graduated cylinder, including certain volume of water was used. The statistical computations of the resultant data were done by SAS (9.01 version) software. To compare the mean values, duncan multi-range test was adopted at probability level 5%.

RESULTS AND DISCUSSION

The results indicated that inoculation with Pseudomonas had significantly affect on rate of phosphorus absorption and some vegetative parameters of potato root at probability level 1%. The rate of phosphorus absorption and root volume showed significant difference at probability level 1% and root dry weight at level 5% with inoculation of several varieties of bacillus bacteria. Similarly, it was identified that the interaction *Pseudomonas* × *Bacillus* was significant at probability level 5% on rate of phosphorus absorption (Table 1).

A. Influence of different inoculations by bacteria on Phosphorus absorption

With respect to the comparison between the mean data from dual impact of Pseudomonas ×Bacillus, the maximum rate of phosphorus absorption occurred in P173+B.subtilis composed inoculation treatment (Fig.1).

Table 1: ANOVA for phosphorus absorption, root volume, and dry weight of potato root affected by inoculation with phosphate solubilizing bacteria.

Sources of variances	Df	Sum of square		
		Phosphorus absorption (percent)	Root volume (cm^3)	Root dry weight (g)
Pseudomonas (Ps)	2	0.084 **	4.57 **	0.38 **
Bacillus	2	0.018 **	3.35 **	0.26 *
Pseudomonas × *Bacillus*	4	0.009 **	0.56 ^{ns}	0.06 ^{ns}
Experimental error	18	0.002	0.44	0.05
CV %	--	11.57	14.13	13.93

*, **, and ^{ns} denote respectively the significance at probability level 1% and 5% and without significant difference

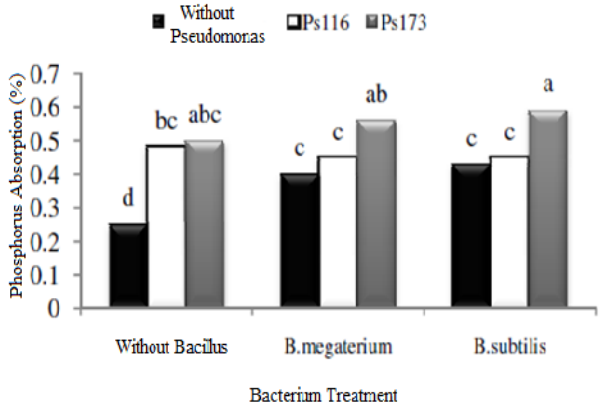

Fig.1. The comparison of mean rate of phosphorus absorption in interaction between *Pseudomonas* and *Bacillus.*

Compared to control treatment, this rate of increase might be computed higher than 57%. Among different species of phosphate solubilizing bacteria, strain 173 of Pseudomonas has the maximum effect on rising of absorption of this element in potato plant. If the efficiency of two varieties of Bacillus for phosphorus absorption is considered, according to the results of this study, one can express that in contrast to B.megaterium bacterium, B.subtilis bacterium had more impact. Doubtlessly, the intensification of synergy between two bacteria in conversion of insoluble and less available phosphate into solubilizing and accessible for the plant is one of the factors for incidence this rate of increase in phosphorus absorption in the composed treatment. Also, developing the volume of root due to application of these microorganisms may cause rising of more access for the root to further volume of soil and the bed surrounding of the root. Consequently with solution of more amounts of insoluble phosphate, the possibility is provided for increased absorption of this valuable element for growth and improving yield in the given plant. Phosphate solubilizing is one of the techniques of phosphorus absorption by growth stimulant bacteria, which practically cause increased access to nutrients in the host plants (Richarson, 2001). According to the studies, Serianth *et al.,* (2003) it was characterized that in comparison with inoculated minitubers, the phosphorus content was increased further in minitubers of *Ficus Benjamina* L. after inoculation with variety of *Bacillus coagulans*. Simultaneous inoculation of pea with rhizobium and one strain of Pseudomonas (*P. striata*) and or a variety of *Bacillus* (*B. polymyxa*) clearly increased plant growth parameters including nodulation, activity of nitrogenase enzyme, and phosphorus and nitrogen absorption(Gaur *et al*, 2004; Prasad & Chandra, 2003).

B. Effect of bacteria inoculations on developing roots

Using both varieties of bacterium caused increase in developing root volume in potato minitubers. The comparison of mean root volume after inoculation with Pseudomonas showed that strain 173 had the maximum effect on rising root volume in contrast to treatment without inoculation (control) up to 1.7 times ratio. As it observed in Fig. 2, of course, there was no significant difference between two varieties of bacterium in increase of root volume. With respect to comparison of mean developing rate of root volume in potato minitubers after inoculation with several varieties of *Bacillus* bacterium, it was revealed that the maximum rate of root volume resulted in by using *B. subtilis* up to 1.56 times in contrast with control treatment (without inoculation). No significant difference was seen between two varieties of bacterium in terms rising development of root volume (Fig. 3). According to results of this study, it can be mentioned that using phosphate solubilizing bacteria will act as factor for improving development of root volume in the host plant. One of the related theories about developing root volume in the inoculated potato with growth stimulant bacteria is the improvement in synergic effects in microorganisms and playing the favorable and efficient role in rising of root biomass so that it may contribute the area of root with stimulating lateral branches and on the other hand it causes developing the roots doubly with helping to absorption of more phosphorus and stimulation of assimilates synthesis (Joner & Jackobsen, 1995).

Increase in root volume denotes further development in root that makes possible increase in potential for further absorption water and nutrient elements from wider area of soil. As a result, it seems that potential and yield of water absorption and nutrients is improved in them by using growth stimulant bacteria in this experiment and rising of potato root volume and consequently their growth will be enhanced.

C. Effect of bacteria inoculations on dry root weight

In this study, dry weight of potato root showed significant increase after inoculation of its minitubers with several strains of *Pseudomonas* so that application of strain 173 of this bacterium increased root dry weight respectively up to 1.62 and 1.35 times compared to control treatment and also strain 116 (Fig. 4).

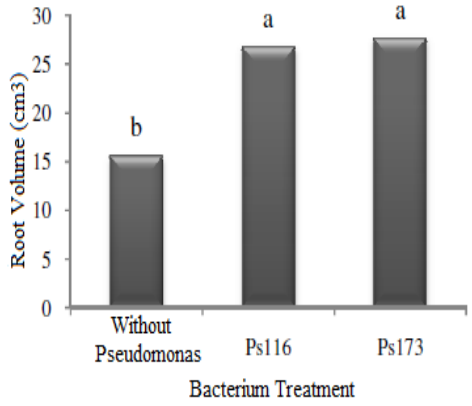

Fig. 2. Comparison of mean root volume in potato under inoculation with different strains of *Pseudomonas.*

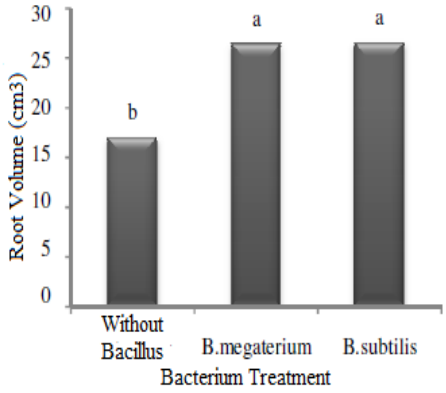

Fig. 3. Comparison of mean root volume in potato under inoculation with two varieties of *Bacillus*.

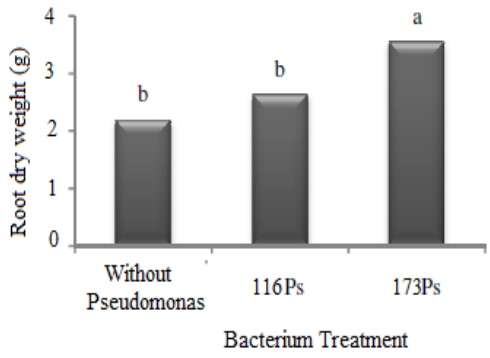

Fig. 4. Comparison of mean root dry weight in potato after inoculation with different strains of *Pseudomonas.*

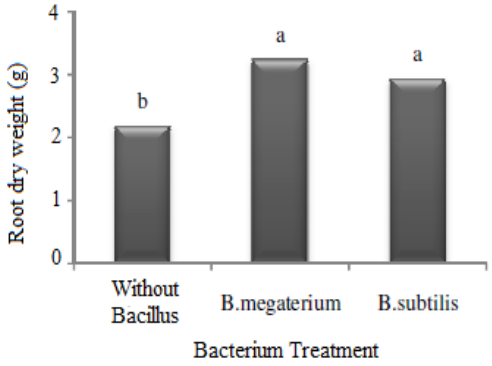

Fig. 5. Comparison of mean root dry weight in potato after inoculation with different varieties of *Bacillus.*

The latter strain had no significant effect on improving root dry weight compared to treatment without inoculation. The comparison of root mean dry weight in the inoculated treatment with two varieties of Bacillus bacterium showed that the maximum rate of increase in root dry weight belonged to *B. megaterium* treatment and with 1.48 times greater than control treatment. It is observed in Fig. 5 that there was no significant difference between two genus bacteria and compared to non-inoculated treatment, both varieties increased root dry weight in the host plant. The probable reason for incremental effect of growth stimulant bacteria in dry weight of plant root may be due to impact of bacteria on further growth in minor and lateral roots and growth of capillary roots so that finally it had more significant impact on root dry weight in host plants. It has been referred to increase of root weight of host plants including microorganisms in numerous scientific reports, which are consistent with the results of this study as well. In experiment of Heba (2009) the inoculated mycorrhiza potato with Pseudomonas had greater root dry weight compared to other treatments.

David *et al.*, reported that inoculation of potato with *Pseudomonas* and or *Azosperiliuim* caused increase in root dry weight in contrast to other treatments. Howei and Echandi (1983) observed that some strains of Pseudomonas might increase dry weight of potato root up to 1.5 times. In an exploration into dual impact of phosphate solubilizing bacteria and *Bradyrhizobium japonicum* in soybean, Rasipoor & Aliashgharzadeh (2007) declared that phosphate solubilizing bacteria caused increase in dry weight of shoot organ as well as wet and dry weights of root nodules.

CONCLUSION

It was identified from the results of this study that inoculation of potato tissue cultured minitubers with phosphate solubilizing bacteria may remarkably affect on rising of root volume and more access to wider area from its surrounding environment in order to facilitate absorption process and solution of insoluble and less-soluble nutrient elements including phosphorus and improving nutrient absorption.

These factors increase potential for efficient and better use of nutrient sources as well as water and optical conditions of light ambient conditions in the plant and cause rising yield of producing minitubers. This significant increase in improvement of reproduction of minitubers is remarkably noticed as a new finding in optimal application of bio-fertilizers technology and it may provide the reduced consumption of chemical products, improvement of quality and health of the produced nutrients and lowering production costs.

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