The role of *Pseudomonas fluorescens* strains in growth and phosphate concentration of Rapeseed (*Brassica napus* L.)

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Abstract

In order to study the effect of *Pseudomonas fluorescens* on growth, morphological characteristics and phosphate uptake by rapeseed (*Brassica napus* L. Hyola 401), an experiment was performed in 2010. The study was carried out as a randomized complete blocks design with three replications. The treatments included plants inoculation by *Pseudomonas fluorescence* strain 11, strain 4, strain 169, the dual combinations of strains 11+4, 11+169, 4+169, combination of all three strains studied (11+169+4) and treatment without inoculation (control plant). The findings suggested that application of strain 169 increased plants height, number of leaves and pod; however, it was ineffective on dry and wet weight of root and shoot compared to control and other treatments. It also increased phosphate concentration of roots and shoots compared to the control plant. Results showed strain 169 had successful and important function on improving growth of rapeseed (*Brassica napus* L. Var Hyola 401). On the other hand, these bacteria caused an increase in phosphate uptake.

Keywords: morphological characteristics; rapeseed; phosphate concentration; *Pseudomonas fluorescence* strains

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Introduction

Plant Growth Promoting Bacteria (PGPR) are a group of soil microorganisms that have a positive effect on stimulating plant growth. The phosphate solubilizing bacteria are group of microorganisms that can convert insoluble phosphate in soil to form of solution phosphate (Sturz, 2003). Plant growth promoting bacteria can be useful in plant growth through two ways, namely, directly and indirectly. The indirect increase happens when effects of harmful bacteria such as pathogenic microorganisms are omitted by using one or several mechanisms. Indirect method requires producing an effective combination for plant growth stimulation and facilitation of nutrients uptake of the plant. Active root systems regularly release organic compounds into rhizosphere (Glick, 1995). This compound causes growth and increasing microbial community (Mandal, 2007). *Pseudomonas fluorescens* stimulates plant growth through different mechanisms of synthesis of antibiotics, the production of plant

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Table 1
Some physical and chemical properties of the soil

FC	EC (ds/m)	pH	TNV %	O.C %	Total N %	P (ava) ppm	K (ava) ppm	Clay %	Silt %	Sand %	Soil texture
15.8	2.9	7.7	13.7	1.3	0.1	65.5	315	14	16	70	Sandy loam

EC= Electrical Conductivity, O.C= Organic Crabon %, T.N.V= Total Neutralizing Value, FC=Field Capacity

Table 2
Variance analysis of effect of bacteria inoculation on morphological characteristics rapeseed

source	df	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Number of leaves	Number of pods	Height
block	2	3.63 ns	2.039ns	0.55 ns	29.73 **	0.0729 ns	208.53 ns	45.627 ns
bacteria	7	3.70*	4.058 ns	1.70 ns	0.44 ns	1.772 ns	840.99 **	113.50**
Error	14	1.993	2.658	1.35	1.32	1.234	232.090	33.57

*significant in statistical level 5%, ** significant in statistical level 1%, n.s: not significant

growth hormones such as Auxins, Cytokinins and Gibberellins, increasing phosphate absorption and other nutrients, nitrogen fixation, competition with the harmful species through root occupation, synthesis of enzymes that regulate the amount of ethylene in plants (Glick, 2004), creation of resistance systemic in plants and increasing resistance of plant against abiotic and biotic stresses (Abdul- G, 2007).

Rapeseed is an annual plant with several agronomic advantages. These include having both spring and autumn types, being an the ideal crop in rotation with cereals such as wheat and rice, high oil quality, high concentration of fatty acids particularly oleic and linoleic acids in oils and good agronomic characters such as resistance to cold, dehydration, salinity, optimum use of moisture and rain, lower cost, higher oil yield per unit area than other cultivated oilseeds and cultivation of this plant also is recommended in most parts of Iran (Aliyary and Shekari, 2000). Therefore in this study, the effects of *Pseudomonas fluorescent* strains was investigated on growth, morphological characters, content and concentration of phosphorus in rapeseed (cultivars Hyola 401).

Materials and Methods

This research was performed in 2010 at Agriculture College Research Farm of Islamic Azad University, Saveh Branch. Experiment was carried out as a randomized complete blocks design with three replications. The treatment included inoculation of plants with Plant Growth Promoting Rhizobacteria of *Pseudomonas fluorescence* strain 11, strain 4, strain 169 and strains combination of 11+4, 11+169, 4+169, 11+169+4 and the treatment without inoculation (control plant). Rapeseed (*Brassic*a *napus* L. Var Hyola 401) is an oil seed plant that does not require vernalization (Movahedi, 2008). The experiment was conducted as pot and the investigated soil characteristics are given in Table 1.

Rapeseed seeds were mixed with 20 ml of 20% sugar solution, for inoculation of seeds with microorganisms. Then 20 g inoculum was added to the sticky seeds. Seeds were planted in depth of 2 cm in each pot and the plants were harvested at pod formation stage. In this experiment stem height, root and shoot dry and wet weight, leaf number, pod number, phosphate concentration and content of roots and shoots were studied. Variance analyses were carried out by SAS software (Version 9.1), the means were compared by Duncan’s multiple range test (P<0.05), and the Figures were drawn by Excel.

Results

The results of variance analysis (Table 2) showed inoculation treatments had significant effect on wet weight of shoots (P<0.05), plant height and the number of plant pods (P<0.01). Bacterial inoculation had no effect on

Table 3
Variance analysis of effect of bacteria inoculation on concentration and content of phosphate rapeseed organs

Source	df	Shoot phosphate concentration	Root phosphate concentration	Shoot phosphate content	Root phosphate content
Block	2	112/93**	144/23**	1358/39*	2646/88**
Bacteria	7	8/34 ns	8/99*	323/82*	203/94*
Error	14	8/544	11/45	278/17	234/45

*Significant in statistical level 5%, **Significant in statistical level 1%, n.s: Not significant

Table 4
Comparison of average effect of bacterial inoculation on fresh and dry weight shoot and root and characteristics another of rapeseed

Treatment	Shoot fresh weight (g)	Root fresh Weight (g)	Shoot dry Weight (g)	Root dry Weight (g)	Height	Pod number	Leaf number
Strain 169	6.0408a	6.366 ab	4.442a	3.355b	30.917a	37.75a	5.417a
Strain 11	6.501a	6.975 ab	4/49a	3.34b	29.25ab	22.583b	5.417a
Strain 4	6.722a	7.384 a	4.568a	3.682a	28.083bc	18.417c	5.25ab
Strains com. 169+11	6.726a	7.111a	4.549a	3.603a	26.208c	17.25c	5.083ab
Strains com. 169+4	5.036b	5.919b	3.449b	3.124c	24.833d	17.25c	5.083ab
Strains com. 11+4	6.111a	6.618ab	4.189ab	3.523a	24.667d	16.083c	4.667ab
Strains com. 11+4+169	6.112a	5.741b	4.064ab	3.274b	22.833e	10.417d	4.5b
Control	6.727a	6.256ab	4.4258a	3.599a	22.25e	6.75e	4.5b

Strain 169, 11, 4, 169+11, 169+4, 11+4, 11+4+169= Different Strains of *Pseudomonas fluorescence*

characteristics such as root wet weight, shoot and root dry weight and the number of leaves (Table 2).

There were no significant differences between different strains and only strains combination of 169 +11 showed the lowest shoots wet and dry weight (Table 4).

As Table 4 shows, strains 4 and strains combination 169+11 resulted in the highest root wet weight. By contrast, strains combinations 169+4 and 4+169+11 had the lowest root wet weight. The results of mean comparison showed strains combination 4+169 had the lowest root dry weight and the other strains and their combinations resulted in more root dry weight (Table 4). Table 4 also shows strain 169 had the greatest height and pods number. The highest leaves number per plants were observed in strains 169 and 11 (Table 4). Moreover, strains combination 4+11+169 and control had the least height. The lowest numbers of leaves were seen for strains combination 4+169+11 and control and the lowest number of pods per plant was observed for control plants.

The results of variance analysis (Table 3) showed the effect of bacterial inoculation was not significant on shoots phosphate concentration of rapeseed. Mean comparison of the effects of bacterial inoculation on shoots phosphate concentration showed that inoculation with different strains and their combination were effective and only control treatment had lower phosphate concentration (Table 5). The inoculation effects were significant ($P<0.05$) on root phosphate concentration and content of roots (Table 3). Strains combination 4+11+169 and control treatment had the lowest and strains combinations of 11+169 had the highest phosphate concentration of roots (Table 5). Comparison of the mean effect of bacterial inoculation on phosphorus content of root showed strain 169 had the most and strains combination 169+11+4 and control treatment had the lowest root phosphate contents (Table 5). Also as Table 3 is shows, the effect of bacterial inoculation was significant on phosphate content of shoots ($P<0.05$). Moreover, control plants and strains of 169 had the lowest and highest shoots phosphate content, respectively (Table 5).

The results of showed that *Pseudomonas fluorescence* strain 169 creating successful symbiosis with rapeseed had an important role in growth improvement of rapeseed and also in phosphate uptake.

Table 5
Comparison of average effect of bacteria inoculation on shoots and root phosphate condition

Treatment	Shoot phosphate concentration (mg/kg)	Root phosphate concentration (mg/kg)	Shoot phosphate content (mg in plant)	Root phosphate content (mg in plant)
Strain 169	11.852a	12.426a	53.95a	44a
Strain 11	11.352a	11.792a	48.64b	40.06b
Strain 4	10.986a	11.430ab	47.28b	38.84b
Strains com. 169+11	10.944a	10.75b	46.77bc	37.4b
Strains com. 169+4	10.519a	10.727b	45.66c	35.92b
Strains com. 11+4	10.167a	10.458bc	41.28d	33.95c
Strains com. 11+4+169	10.046a	10.329c	40.57d	32.28d
Control	9.194b	9.759c	37.67e	31.7d

Discussion

A successful relationship was established between *Pseudomonas fluorescent* strain 169 and rapeseed and this increased most of the traits under study. Hernandez et al. (1998) reported that inoculation of corn seeds with *Pseudomonas fluorescent* increased yield. Chabot et al. (1993) also observed that corn inoculation with bacteria increased wet weight by 33%. PGPR bacteria through production of growth hormones (Saharan and Nehra, 2011) and the ability of soluble phosphate (Abbaszadeh et al., 2007) affects plant growth and yield. Growth promoting bacteria through synthesizing a series of materials such as siderophore, phytohormones and Acc Di Amines enzymes facilitate nutrient uptake by plants and increase plant growth, therefore improving crop production per unit area (Glick, 1995). Pseudomonas bacteria also releases growth hormones such as Gibberellin and Auxin which increase the longitudinal growth of cells (stem internode) and cell division thus increases the plant height, stem diameter, number of leaves per plant and production (Koocheki et al., 2008). Cytokinin stimulates cell division process and it is effective on the enzyme activity, nutrient transport in plant and organelle appearance. Cytokinin also increases plant resistance against environmental stresses (Ines et al., 2005). The evidences show that the combination of *Pseudomonas fluorescens strains* probably competes with each other so that their favorable effects on plant growth are modified.

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