

Identifying Different Effective Strains of Pseudomonas Bacteria (Growth Stimulants) on Yield and Yield Components of Barley in Farm Conditions

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ABSTRACT

In order to evaluate the effect of different strains of Pseudomonas bacteria on yield of Maku brand barley in farm condition, an experimental study was conducted in 2009 in completely randomized blocks in three replicates in Saeed Abad Agricultural Education Campus in Tabriz, Iran. The experimental treatments included inoculation of seeds with fifteen different strains of Pseudomonas bacteria and one treatment without inoculation as the control. The results showed that the effect of inoculation treatments with different strains of the Pseudomonas bacteria on weight of spike in five percent statistical significance level, and on weight of root, number of seeds per spike, weight of one thousand seeds, and yield in one percent statistical significance level were meaningful. According to the obtained results, the growth stimulating bacteria strains have enhanced all understudy traits of barley compared to the control. So, the application of different strains of Pseudomonas bacteria, especially strains 136 and 138 not only can lead to increased yield and performance in planting barely, but also lower utilization of chemical fertilizers.

KEYWORDS: bio-bacteria, Pseudomonas, yield, barley

INTRODUCTION

Human societies have been facing serious environmental and economical problems with the increased application of chemical fertilizers, and wide range of efforts have already been underway to find proper solutions to better the quality of agriculture soil, crops, and also eliminate pollutants. One of the biological ways of increasing crops in agriculture sector is taking effective advantage of microorganisms living in soil, which have the potential to increase plant yield and performance (Khavazi *et al.*, 2005).

Pseudomonas bacteria are generally aerobic and from view point of supplying energy and Shymyvarganotrov carbon are rod shaped. Furthermore, from the view point of staining, they are gram negative. Pseudomonas bacteria are abundantly found in rhizosphere. They can create colony around root rapidly, which can lead to growth stimulation of plant. So, they are considered as rhizosphere bacteria, which are growth stimulants (PGPR) of plants (Sindhu *et al.*, 1999). As the growth stimulating, the Pseudomonas bacteria are able to facilitate plant growth thru increased solubility of nutrients, production of growth stimulating material, production of anti-biotic material, production of lytic enzymes and secondary metabolites such as hydrogen cyanide to control pathogens. Some strains of the florescent Pseudomonas are also able to consolidate nitrogen. But nitrogen consolidation is not the most important mechanism in stimulating growth of growth stimulating bacteria. Among growth stimulating bacteria, the florescent Pseudomonas is very important due to its capability of producing wide range of growth regulators, combining iron and chelated iron uptake and absorption, production of organic acids such as succinic acid and lactic acid, and finally controlling plant pathogens (Zahir *et al.*, 2004).

Zahir *et al.* (1998) reported 19.8% increase in corn seed yield after its inoculation with Pseudomonas and Azotobacter. Glick *et al.* (1994) evaluated the capabilities of eleven strains of Pseudomonas in increasing the length of root of canola plant under gnotobiotic conditions. The lengths of root and stem of canola plant in dry and also wet conditions were also increased by inoculation with Pseudomonas putida GR12-2. Hall *et al.* (1996) also reported increased length of seedling roots of tomato, barley, wheat, and oatmeal thru their study by inoculation with wild

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strains. Eghbal and Hossenian (2013) reported increased yield and yield components due to inoculation with *Pseudomonas* in lab and also under natural conditions. It has been proved that the fluorescent *Pseudomonas* bacteria can directly and also indirectly provide growth stimulation of plants thru different means including plant growth regulators such as gibberellin, cytokinin, indole acetic acid (Shanmugan *et al.*, 2006). Yousefi and barzeghar (2014) reported that farmers can retain their normal wheat yield by utilization of *Azotobacter*, *Pseudomonas*, and half the normal amount of chemical fertilizers.

The present study aimed to determine the most suitable strain of *Pseudomonas* as a bio-fertilizer for increasing barley production in agricultural system with enough inputs in Saeed Abad Agricultural Education Campus in Tabriz, Iran.

MATERIAL AND METHOD

The present research study is undertaken to evaluate effect of fifteen different strains of *Pseudomonas* bacteria, which are growth stimulants and nitrogen consolidators, on Maku brand barley in farm conditions in Saeed Abad Agricultural Education Campus in Tabriz, Iran. This research study was carried out in 2009 in three completely randomized blocks with three replicates. The experimental treatments included inoculation of seeds with fifteen different strains of *Pseudomonas* bacteria and one non-inoculated treatment as the control. The bacterial inoculums mentioned above were all prepared by the biology division of the State Soil and Water Research Institute. The formulations of the inoculums were in the powder form during the experiment. In order to inoculate seeds of the Maku brand barley with different strains of the *Pseudomonas* bacteria, first 15 grams of seeds were placed in a plastic bag. Then one drop of 40% Arabic gum solution was added to the content of the plastic bag and the plastic bag was shaken vigorously. After that one gram of inoculum was added to the content of the plastic bag and then was shaken in a way that a uniform coating of the inoculum was present on seeds. In the planting phase in farm, in each plot of land slits with 20 centimeters distance and depth of 10 centimeters were made by hand gouging. Then seeds were placed inside the slits and covered with 5 centimeters of soil. The planting density was 350 seeds in each square meter. Based on the results of soil analysis, and also recommendations of the fertilizer producers, the necessary nitrogen, potassium, and phosphor were added to the irrigation water in the form of solution. It must also be mentioned that for weighing purpose of root, similar and simultaneous planting was done in vases. Harvesting was done at the physiological maturity stage and plant foliages were cut near the surface of soil. The areal section of the plants were harvested in each plot and placed in an oven with 70°C heat for 72 hours to get constant weight. Traits such as height, number of seeds per spike, weight of 1000 seeds for each vase were statistically analyzed by utilization of MSTATC software. Then grouping of means by utilization of the Least Meaningful Difference method on five percent statistical significance level was carried out and graphs were drawn using Excel software.

DISCUSSION OF RESULTS

The variance analyses for each one of the understudy traits reveal that there are meaningful differences on one percent and five statistical significance levels among different strains of *Pseudomonas* bacteria for most of the understudy traits in a way that of majority of the inoculated treatments with different strains of *Pseudomonas* bacteria led to meaningful differences on weight of spike, on five percent statistical significance level, on weight of root, number of seeds per spike, and weight of a thousand seeds, and yield per hectare, on one percent statistical significance level (table 1-1). These meaningful differences show the existence of diversity for these traits and the possibility of selection among different strains of *Pseudomonas* bacteria from view point of understudy traits.

Height of plant

The obtained results from the variance analysis (table 1) show that there is no significant difference from view point of height of plant among utilized strains of bacteria. The control treatment (non-inoculated treatment) had the highest height of 107 centimeters. This shows that different strains of the bacteria did not have any effect on height of barely plant. Yet, in another study entitled "Effect of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barley" Mehrvarz *et al.* (2008) reported that bacteria and its reciprocating reaction with different levels of phosphorus chemical fertilizer did not have any significant effect on height of barely plant. It seems that utilization of the bacteria have not had any role in height of barely plant.

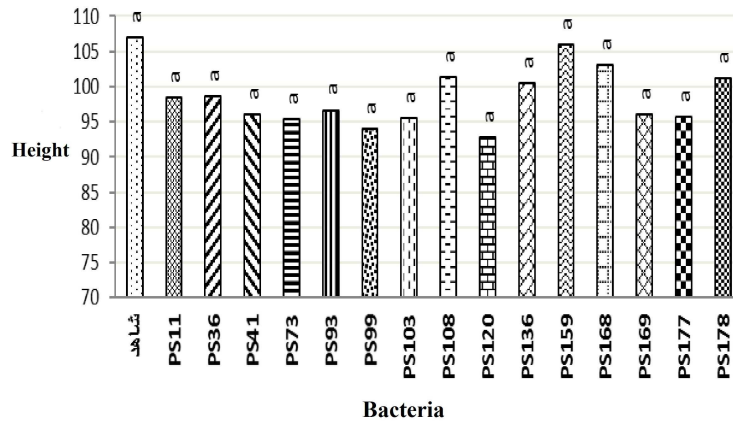


Figure 1: Effects of different strains of Pseudomonas bacteria on height of bare plant

Weight of root

The variance analysis table (table 1) shows that there is meaningful difference among different strains of the bacteria from view point of weight of root on one percent significance level. Figure 2 represents comparison of means with the Duncan test at five percent significance level. As it can be seen, inoculations with different strains of the Pseudomonas have meaningful effect on weight of root. The Pseudomonas putida strain 168 with 47.18 grams had the highest effect, which shows 46% increase compared to the control treatment, and Pseudomonas putida strain 41 with 44 grams had the second highest effect. Hall *et al.* (1996) reported increased length of root of seeds in an experiment studying the effect of inoculation of wild strains of Pseudomonas on the length of different plants roots such as tomato, barely, wheat, and oatmeal. Inoculation of species of canola called Brassica compestris with P Putida GR 12-2 led to increased length of root, dry weight of root in a sterile condition compared to the control treatment. The obtained results of this study prove that different strains of Pseudomonas Fluorescence are capable of increasing growth of canola plant even in the absence of plant pathogens. It has also been proved that thru different methods such as plant growth regulators such as gibberellin, cytokinin, indole acetic acid, Pseudomonas Fluorescence can directly and also indirectly help in growth of plants (Shanmugam *et al.*, 2006). Furthermore, Bouthiana *et al.* (2010) reported increased length and weight of root under the effect of biological fertilizers in their research experiments.

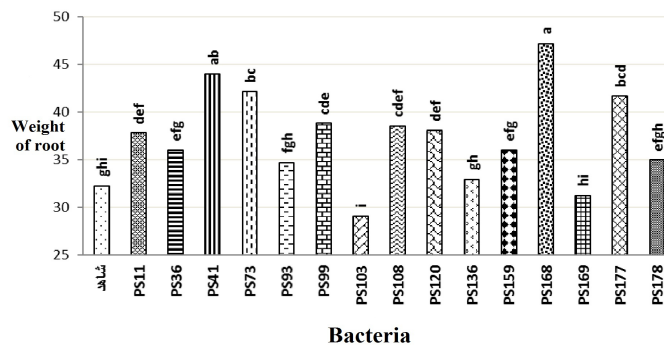


Figure2: Effects of different strains of Pseudomonas bacteria on weight of root of barely

Number of seeds per spike

The obtained results from comparing means reveal that inoculation of barely with different strains of Pseudomonas bacteria has led to a meaningful increase in the number of seeds per spike (table 2) and Pseudomonas putida 73, 168, and 136 led the highest number of seeds per spike, which represent sixteen percent increase compared to the control treatment. Furthermore, Pseudomonas putida 99, and 93 led to the lowest number of seeds per spike, which were, respectively, 19.5% and 14.8% less seeds per spike compared to the control treatment. Behzad (2008) reported that utilization of growth stimulating bacteria on maize had meaningful effect on total number of seeds on corn cub, corn yield, and single shrub corm plant yield. He also reported that addition of Pseudomonas fluorescence to other bacteria has caused increased effect of bacteria on the understudy traits of maize plant. In a research study, Hassan-zadeh *et al.* (2007) reported seventeen percent increase in number of seeds per

spike of barely due to the effect of growth stimulating bacteria. In a green house experiment, Reyhani-tabar (2000) proved that wheat inoculated with *Pseudomonas fluorescense* bacteria showed positive response in most of the growth related traits.

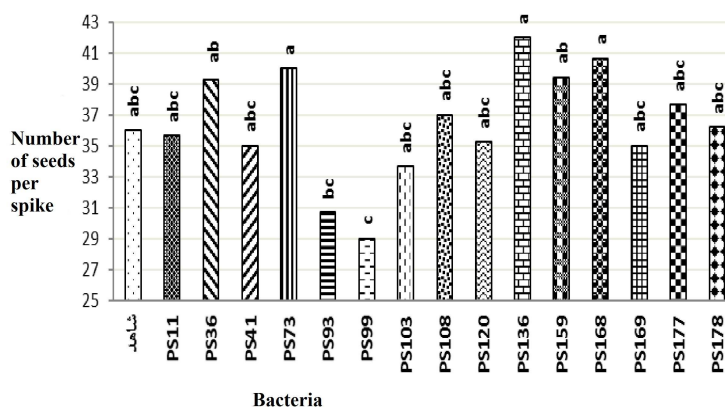


Figure 3: Effects of different strains of *Pseudomonas* bacteria on number of seeds per spike of barely

Weight of one thousand seed

Inoculation of barely with different strains of *Pseudomonas* bacteria has led to an increase in the weight of one thousand seeds, and elevated this trait in a meaningful manner on one percent statistical significance level (table 1). There are significant differences among different strains of *Pseudomonas* bacteria. Strains of *Pseudomonas putida* 99, 168, and 136 with 31.33, 30.67, and 30 grams increase in the weight of one thousand seeds and with 25.32%, 22.68% and 20% increase compared to the control treatment led to the highest weight of one thousand seeds. The weight of one thousand seeds is considered as one of the components of yield, which is mostly related to genotype. Of course ambient conditions, especially conditions after seed formation have considerable effect on weight of one thousand seeds (Martinez *et al.*, 1984). With respect to the obtained results in this study and also results of similar studies, it can be concluded that by helping in absorption of water and nutrients, especially phosphor and transferring them to plant cells, growth stimulating bacteria lead to better growth and increased photosynthesis. As a result, in the development stage of seed, enough processed sap is transferred to seeds, and large seeds with acceptable weights are produced, which in turn the weight of one thousand seeds is increased. Reyhani-tabar (2002) showed in a greenhouse experiment that wheat showed positive response in majority of growth related characteristics to inoculation with different strains of *Pseudomonas fluorescense* bacteria. In a research study, Heydari *et al.* (2012) showed that inoculation of wheat seeds with *Pseudomonas* bacteria caused increase in weight of one thousand seeds. In a study to evaluate effect of *Azospirillum* and *Pseudomonas* on agro-technical traits of rice, Rahmati-khorshidi *et al.* (2010) reported that use of *Pseudomonas* bacteria by itself, and also in combinational treatment with *Azospirillum* was meaningful at one percent significance. Finally, Hamidi *et al.* (2006) reported that inoculation of maize with *Azospirillum* and *Pseudomonas* led to increase in weight of one thousand seeds.

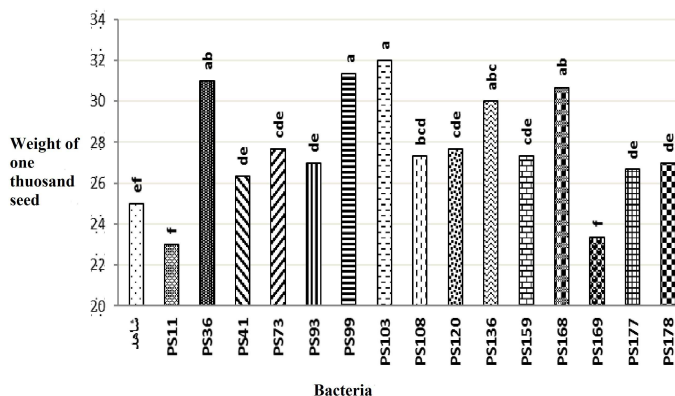


Figure 4: Effects of different strains of *Pseudomonas* bacteria on weight of one thousand seeds of barely
Yield per hectare

Yield per hectare was one of the growth indicators in this research study. As it can be seen in the table of variance analysis (table 1), inoculation with different strains of the bacteria was meaningful at one percent statistical significance level. The table representing comparison of means showed that there are meaningful differences among different strains of the bacteria (table 2). The strains of *Pseudomonas putida* 168 and 41 resulted in 6370 Kg and 5410 Kg yields, respectively, which are 40.7% and 19.5% higher compared to the yield of the control treatment (Figure 5). Zahir et al. (1988) reported 19.8% increase in yield of maize plant after inoculation with *Azotobacter* and *Pseudomonas* bacteria. It has also been proved that thru different ways such as plant growth regulators such as gibberellin, cytokinin, indole acetic acid, and *Pseudomonas* fluorescence can directly and also indirectly help in growth of plants (Shanmugam et al., 2006). Rahmati-khorshidi *et al.* (2010) reported that *Pseudomonas* had positive effect on yield of rice on one percent significance. Ramazan-pour (2010) reported that *Pseudomonas* bacteria are capable of dissolving Phosphor and this capability leads to increased yield. Furthermore, the capability of these bacteria in producing Auxin translated into higher yield of barely up to twenty percent. Studies undertaken by hamidi *et al.* (2006) on maize showed that inoculation with *Pseudomonas* and *Azospirillum* led to increased yield.

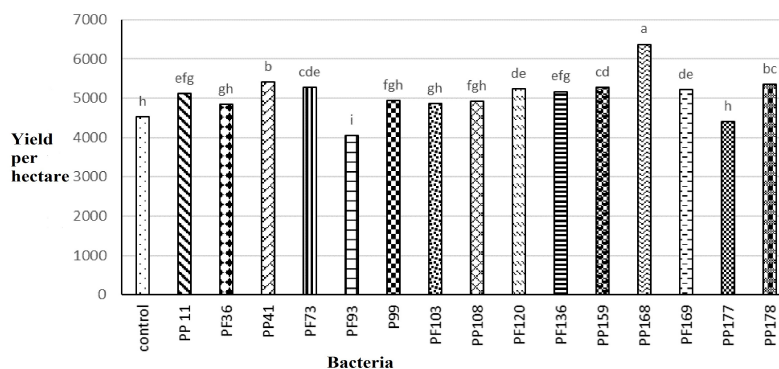


Figure 5: Effects of different strains of *Pseudomonas* bacteria on yield of barely

Table 1: Mean squares of understudy traits of barely treated with stimulating bacteria

Sources of change	Degrees of freedom	Plant height	Weight of root	Number of seeds per spike	Weight of 1000 seeds	Yield
Replication	2	40.93	89.56**	2050.4**	153.58**	118.86**
bacteria	15	52.48	70.7**	39.69**	56.55**	2637804.6**
error	30	9.75	428.5	1.988	1.286	274306.99
Change coefficient	--	8.5	11.41	15.24	6.3	3.87

* and **: statistical significance at 5% , and 1%, respectively

Table 2: Comparison of means of the understudy traits

Bacteria	Height of plant (cm)	Weight of root(gram per plant)	Number of seeds per spike	Weight of 1000 seeds (gram)	Yield (kg per hectare)
Control treatment	107a	32.3ghi	36abcd	25ef	4527fgh
<i>Pseudomonas putida</i> 11	98.37a	37.8def	36abc	23f	5121gh
flourescent <i>Pseudomonas</i> 36	98.57a	36efg	39ab	31ab	4843def
<i>Pseudomonas putida</i> 41	96a	44ab	35abc	26.33de	5410fgh
flourescent <i>Pseudomonas</i> 73	95.3a	42.1bc	30a	27.67cde	5272cd
flourescent <i>Pseudomonas</i> 93	96.6a	34.7fgh	31bc	27de	4048i
flourescent <i>Pseudomonas</i> 99	93.3a	38.8cde	29c	31.33a	4942cde
<i>Pseudomonas putida</i> 103	95.47a	29.1i	34abc	32a	4868bc
<i>Pseudomonas putida</i> 108	101.3a	38.6cde	37abc	27.33bcd	4921def
flourescent <i>Pseudomonas</i> 120	92.8a	38.1def	35abc	27.67cde	5243efg
flourescent <i>Pseudomonas</i> 136	100.5a	33gh	42a	30abc	5168ab
<i>Pseudomonas putida</i> 159	106a	36efg	40ab	27.33cde	5278def
<i>Pseudomonas putida</i> 168	103a	47.2a	41a	30.67ab	6370a
flourescent <i>Pseudomonas</i> 169	96a	31.3hi	35abc	23.33f	5211h
<i>Pseudomonas putida</i> 177	95.7a	41.7bcd	38abc	26.67de	4414h
<i>Pseudomonas putida</i> 178	101a	35efgh	36abc	27de	5354a

Cluster analysis

The cluster analysis of the different strains of utilized bacteria (diagram 1) shows that it is possible to classify these strains into three groups. The third group has two sub-groups. According to the cluster analysis the first group of the strains of bacteria have the most distance compared to other groups.

Table 3: Grouping of different strains of Pseudomonas bacteria

Group number	Bacteria strains
1	168
2	41, 99, 159, 178, 108, 169, 11
3	A: 93, the control treatment B: 136, 36, 73, 120, 177, 103

(1: the control treatment, 2: Pseudomonas putida 11, 3: florescent Pseudomonas 36, 4: Pseudomonas putida 41, 5: florescent Pseudomonas 73, 6: florescent Pseudomonas 93, 7: florescent Pseudomonas 99, 8: Pseudomonas putida 103, 9: Pseudomonas putida 108, 10: florescent Pseudomonas 120, 11: florescent Pseudomonas 136, 12: Pseudomonas putida 159, 13: Pseudomonas putida 168, 14: florescent Pseudomonas 169, 15: Pseudomonas putida 177, 16: Pseudomonas putida 178)

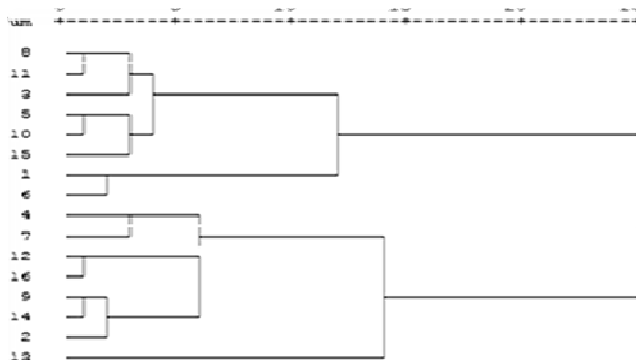


Diagram 1: Results from the cluster analysis of different strains of Pseudomonas bacteria

By referring to table 3, it is possible to interpret the status of different strains of the utilized bacteria. Based on this table, the first group includes only Pseudomonas putida 168, which has the most distance compared to the rest of groups. Again, with respect to this table (table 3), this group has led to the most yield (mean yield of 6370 kg per hectare), height and root weight of barely plant. In general, this group has better traits. The second group includes seven strains of Pseudomonas 11, 169, 108, 159, 99, and 44. The height, weight of one thousand seeds, and weight of root produced by utilization of these strains of bacteria are average. Of course in this group, Pseudomonas 41 ranks second with obtained root weight of 44 grams, and yield of 5410 kg per hectare compared to Pseudomonas 168. In general performance of this group of bacteria can be considered average. The strains listed in the third group; which is divided into two sub-groups, have similar traits and have weaker performance compared to other strains. The first sub-group in the third group includes the control treatment and Pseudomonas 93, which are placed at the lowest position from view point of height, weight of root, and yield. This group is ranked as the fourth from view point of yield. The second sub-group of the third group includes six Pseudomonas bacteria numbered 177, 120, 73, 36, 136, and 103, which are ranked as the third from view point of understudy traits. By studying density and population of florescent Pseudomonas bacteria on wheat rhizosphere, Rasouly-sadaghiani *et al.* (2005) reported that majority of the rhizosphere Pseudomonas were putida, and the reason for being so can be its high competitiveness and effective colonization in the wheat rhizosphere compared to other species. Glick *et al.* (1997) reported that the critical period of performance of the ACC Deaminase enzyme is the preliminary stage of the plant growth, and if the bacteria can be located in suitable place on the seed, its positive effect will be increased. So, one of the more important factors in increasing effectiveness of strains of Pseudomonas putida 41 and 168 in getting better yield can be attributed to effective colonization of these strains in preliminary stages of barely plant growth.

Table 4: Mean traits of the groups of the cluster analysis for different strains of *Pseudomonas*

Traits	Group 1	Group 2	Group 3	
			Sub-group 1	Sub-group 2
Height	108.73	99.3	95.8	96.46
Weight of root	47.18	37.36	33.49	36.67
Number of seeds per spike	42	35.762	30.83	38.28
Weight of 1000 seeds	33.05	27.26	26.09	28.96
Yield	6370.056	5348.19	4287.56	4968.15

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