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THE EFFECT OF PHOSPHORUS SOLUBILISING BACTERIA ON SPRING AND AUTUMN CHICKPEA YIELD AND YIELD COMPONENTS UNDER SUPPLEMENTAL IRRIGATION CONDITION

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Abstract

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Phosphorus (P) is one of the most important but the least available mineral nutrients to plants in many cropping environments. The use of P solubilising bacteria (PSB) as seed or soil inoculants can enhance P uptake by the plant and thereby enhance crop yields. In this study, the effect of seed inoculation by the PSB (*Pseudomonas putida* and *Pantoea agglomerans*) on spring and autumn chickpea yield and yield components under supplemental irrigation condition was studied in Iran during the 2013 – 2014 growing seasons. Three levels of supplemental irrigation (no irrigation, one time irrigation and two time irrigation) were allocated to the main plots and cultivation season (spring and autumn) with two levels of PSB (no PSP inoculation and PSB inoculation) were applied to the subplots. Results showed that the highest biologic yield (2231.7 kg ha^{-1}), seed and pod weight (6.9 g per plant), 100 seeds weight (27.6 g), pods per plant (55.9), and seed yield (838.7 kg ha^{-1}) were achieved from the two time irrigation treatment. It was observed that the effects of cultivation season were significant on the number of pods per plant, number of seeds per plant, 100 seeds weight, seed yield, and biologic yield. PSB inoculation significantly affected the number of pods per plant, number of seeds per plant, 100 seeds weight, seed and pod weight, seed yield, and biologic yield, so that, compared to the no PSB inoculation treatment, the highest seed yield (857.14 kg ha^{-1}) was resulted from the inoculation with PSB. Also, the highest seed yield (911.4 kg ha^{-1}) and biologic yield (2296.8 kg ha^{-1}) was obtained in the autumn cultivation season. According to results of this research, the interaction among these three factors had a significant effect on the seed yield.

Key words: chickpea; supplemental irrigation; cultivation season; phosphorus solubilizing bacteria; seed yield

Introduction

Being a legume, chickpea contains around 12.4% -28.1% raw proteins, 78% of which is digestible. More ever, containing 50-60% of different carbohydrates, 6% oil, and a considerable amount of phosphorus, iron, calcium, and A, B_1, B_2, B_4, and C vitamins has made it an important component of the developed countries diets (Geeravani, 1991).

People from different countries, with various diets, use this legume to prepare their foods. From the beginning, they have been using it as a fresh vegetable, full seed, germinated seed, as well as flour. In addition to its numerous nutritive applications, chickpea has prominent therapeutic effects (Muehlbare and Tullu, 1997). Beside all these benefits, its plant fixes the atmospheric nitrogen molecules which supplies the plant with the needed nitrogen, and also plays an important role in the process of soil fertilization. Under appropriate conditions, 23-97 kg ha^{-1} of nitrogen is being fixed by chickpea. Therefore, it can play a terrific role in crop rotation (Wani et al., 2007).

During the last years, thanks to its favorable weather conditions and fertile soils, Ilam province has been among the

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top rating provinces in producing rain-fed chickpea. Increasing the yield per hectare has been proposed as an approach to increase agricultural crop production.

The gap between genetic potential and potential yield has been mainly attributed to factors beyond the environmental stresses. Drought is one of these factors which reduces chickpea yield in West Asia and North Africa. This decline depends on the severity of the stress and the tolerance of the variety (Canony, 1998). During the occurrence of drought stress, meeting the plant needs in drought vulnerable stages would help to achieve maximum yield. Podding and seed filling stages are the most drought vulnerable stages of this plant. Thus supplemental irrigation during growth critical stages could abate the loss and increase the yield (Baghery et al., 1997). Dahiya et al. (1993) suggested that two time irrigations during podding and branching stages result in the highest seed yield.

Autumn and rain-fed plantings help chickpea to be phonologically adapted to desired temperature and moisture regimes. They also prolong the preflowering stage, so the plant has more opportunity for vegetative growth. This stage coincides with temperature and daytime reduction. In addition, the vegetative growth will be prolonged and, compared to spring planting, coincides with more appropriate thermal and moisture conditions (Pezeshkpour, 2004). The application of microbes as a biofertilizers is the most natural and desirable solution for keeping the soil system alive and active.

The PSB induced chemical changes in the rhizosphere enable the plant to enhance P uptake and crop yield by solubilisation of P fertilizers (Khan et al., 2009; Singh et al., 2011; Abou-el-Seoud and Abdel-Megeed, 2012). The PSB produce a number of organic acids to dissolve phosphates by means of anion exchange or chelating Ca, Fe or Al ions linked with the phosphates. These organic acids include citric, butyric, succinic, malic, acetic, glyconic, adipic, oxalic, malonic, lactic, gluconic, fumaric, and 2-ketogluconic acid. (Gyaneshwar et al., 2002; Chen et al., 2006; Yadav et al. 2011).

Materials and Methods

This experiment was conducted in Malekshahi town, Ilam province, 2013-2014. The mean precipitation of this region is 427 mm. This study was conducted in a split plot factorial design laid out in a randomized complete blocks with three replications. Three primary factors were addressed, including supplemental irrigation in the main plots (I1: no irrigation, I2: one time irrigation, and I3: two time irrigation), and cultivation season (S1: spring cultivation season and S2: autumn cultivation season) and PSP (*Pseudomonas putida* and *Pantoea agglomerans*) inoculation (P1: not applying the PSB and P2: applying the PSB) were allocated to the subplots in a factorial design.

The free variety of chickpea was studied in this research, since it has been suggested for autumn cultivation in Ilam region. A biofertilizer with a brand entitled No.2 fertile, one of phosphorus bacteria with a population up to 108 and from *Pseudomonas putida* and *Pantoea agglomerans* strain P5 and CFU/g, was chosen to be the phosphorus biofertilizer treatment. It was applied to the soil and in adjacent to the seeds in a soluble form and as *Pseudomonas putida* and *Pantoea agglomerans*. The field had been lied autumn now in the past few years.

Chickpea cultivation and experimental treatments application was conducted after the rain autumn and reaching field capacity moisture at 2013.12.11 and 2014.03.01, respectively. During the cultivation, chickpea seeds were inoculated with No.2 fertile through adding Arabian gum and were left to dry away from sunlight then were immediately cultivated. Cultivation density was determined to be 60 plants per m^2. Seeds were cultivated 7 cm below soil surface inside the furrows with 10 cm away from each other. During the cultivation season, weeds were removed two times mechanically and by hands.

In order to determine the effects of the treatments on yield and its components, the first two rows of each plot was considered as plot's margin. Ignoring the first and last 0.5m of each plot, 1 m^2 was randomly selected from the middle three rows and after counting the plants, its yield was evaluated. Data was analyzed using the MSTACT software package (to perform simple variance analysis and compare the means). More ever, Duncan's multiple range test was carried out to compare the means of the treatments at the 5% statistical level of significance.

Results and Discussions

The effect of supplemental irrigation on the number of pods per area was significant (Table 1). The highest number of pods per area was obtained from the two time supplemental irrigation and the control treatment (Table 2). Moisture stress causes the abscission of around 30-40% of flower buds. During flowering stage, the rise in temperature along with drought stress shortens this stage which in turn, decrease the number of flowers. It seems that the high number of pods per area in the supplemental irrigation treatments is due to vegetative growth prolongation and plant biomass increase which leads to allocating more photosynthetic materials for a longer period of time to plant pods. Aggrawal et al. (1984) reported the number of pods per plant as the most

Table 1
Results of variance analysis (mean square) of quantitative traits measured in different treatments

Source of Variation	Degree of Freedom	Pods per Plant	Seeds per Pod	100 Seeds Weight	Weight of Seed and Single Bush Pod	Seed Yield
Replication	2	173.2^{ns}	202.1^{ns}	19.9^{ns}	5.92^{ns}	45410.3^{ns}
Factor a (Supplemental Irrigation)	2	897**	248.6^{ns}	2.86^{ns}	1.55^{ns}	101241.7**
Error a	4	33.9	69.8	2.67	0.51	3894.9
Factor b (cultivation season)	1	860.4*	1474.6**	12.39*	1.12^{ns}	893025**
a×b	2	105.1^{ns}	127.8^{ns}	4.81^{ns}	0.15^{ns}	3086.3^{ns}
Factor c (No.2 Fertile Phosphate)	1	655.4*	355.9*	148.3**	3.86*	385392.6**
a×c	2	233.6^{ns}	219.1*	2.86^{ns}	0.33^{ns}	12838^{ns}
b×c	1	621.7*	243.4*	2.2^{ns}	0.290^{ns}	39256.8*
a×b×c	2	64.3^{ns}	17.3^{ns}	13.8*	0.420^{ns}	31613.9*
Total Error	18	118.20	71.4	2.72	0.631	8360.63
Variation Coefficient	------	22.3	18.2	6.1	12.2	12.1

Ns, *, ** refer to not significant, significant at 5% and 1% significance level, respectively

Table 2
Comparison between supplemental irrigation and control treatment on seed yield and yield components of chickpea

Supplemental Irrigation	Pods per Plant	Seeds per Pod	100 Seeds Weight	Weight of Seed and Single Bush Pod	Seed Yield
I: Control	38.8^{c}	41.4^{b}	26.6^{a}	6.2^{b}	656.3^{c}
I2: One time irrigation	49.5^{b}	47.5^{ab}	27.1^{a}	6.3^{ab}	766.6^{b}
I3: Two time irrigation	55.9^{a}	50.3^{a}	27.6^{a}	6.9^{a}	838.8^{a}

Based on Duncan's multiple range test, those means which have at least a common letter, are not significantly different (at 5% significance level)

variable trait among yield components and suggested that the number of seeds per pod depends on the location of the given pod on the plant.

Furthermore, researchers (Goldani and Rezavani Moghadam, 2004) reported that environmental conditions significantly affect the yield and its components. Drought stress caused by dry farming, compared to supplemental irrigation treatment, increases the hollow pods. It seems that supplying more available moisture through supplemental irrigation expands the plant canopy, so more radiative energy is absorbed by the plant which in turn, increases yield components (including number of pods per area). Turk et al. (2003) suggested that moisture deficiency decreases the number of pods per plant.

Cultivation season significantly affected the number of pods per area (Table 1). The highest number of pods was obtained from autumn cultivation season (Table 3). The number of single seed pods per area depends on cultivation date, irrigation regime, cultivation intensity and other factors (Jalota et al., 2006). The amount of pods is among the most important yield components which deeply influences seed yield. The effect of cultivation date on producing single seed pods was very significant. It seems that due to prolonged vegetative growth stage in autumn cultivation, more stems are produced and vice versa as the reproductive growth stage prolongs, more pods are produced per stem.

Table 3
Comparison between cultivation season effects on seed yield and yield components of chickpea

cultivation season	Pods per Plant	Seeds per Pod	100 Seeds Weight	Weight of Seed and Single Bush Pod	Seed Yield
S1: Spring	43.2^{b}	40.1^{b}	26.4^{b}	6.3^{a}	596.3^{b}
S2: Autumn	53^{a}	52.8^{a}	27.6^{a}	6.7^{a}	911.4^{a}

Based on Duncan's multiple range test, those means which have at least a common letter, are not significantly different (at 5% significance level)

Some researchers (Goldani et al., 2000) have claimed that delay in cultivation date leads to less pods per plant. Apparently, more available moisture leads to expanded plant canopy which in turn, increases the amount of produced photosynthetic materials and nurtures bigger reproductive store. These results are consistent with those of Singh et al. (1997). It seems that supplying more available moisture through supplemental irrigation expands the plant canopy, so more radiative energy is absorbed by the plant which in turn, increases yield components (including number of pods per area). During flowering stage, the rise in temperature along with drought stress shortens this stage which in turn, decreases the number of flowers. The high number of pods per area in the supplemental irrigation treatments is due to vegetative growth prolongation and plant biomass increase which leads to allocating more photosynthetic materials for a longer period of time to plant pods (Goldani et al., 2000).

Comparison among the treatments indicated that there is a significant difference among No.2 fertile phosphate inoculation different levels, since the number of pods per plant in the No.2 fertile phosphate treatment (Table 4), is about 20% more than the other treatment (not applying fertile phosphate).

In terms of No.2 fertile phosphate fertilizer effects on the number of reproductive pods, it should be mentioned that this may be due to increased level of phosphorus absorption and consequently its influence on improving the photosynthesis level and chickpea plant growth which in turn leads to more absorption of radioactive energy by the plant. All these would increase yield components, including the number of reproductive pods per plant. Comparing the interactive effects of two cultivation season on No.2 fertile phosphate inoculation also showed a significant difference. Thus, the number of pods per plant in the autumn cultivated plots which were inoculated by fertile phosphate was much more than spring cultivated plots without application of fertile phosphate. This result highlights the synergistic effects of the two treatments on each other.

In autumn cultivated plots, positive interaction between pseudomonas bacteria and Nitrogen fixing bacteria at the roots of chickpea plant, through increasing nitrogen fixing nodes, provides more nitrogen for the plant. Therefore, an influential factor in pod reproduction and seed production (i.e., supplying the plant with adequate nutrients) is being provided (Rokhzadi and Toashi, 2011).

According to results presented in Table 1 and Table 3, the effects of two factors (namely cultivation season and No.2 fertile phosphate inoculation) on the number of pods per plant were significant at 5% statistical level, respectively. Furthermore, the interactive effects of supplemental irrigation, cultivation season, and No.2 fertile phosphate inoculation on the number of seeds per plant were significant at 5% statistical level. Comparing the mean of the treatments showed that the number of seeds per plant in autumn cultivated plots (50.4) was about 21% more than that of control treatment (41.5). Also, it was observed that the number of seeds per plant in the inoculated treatment (49.6) was about 14.5% more than that of not inoculated treatment (43.3).

Considering the means of interaction between cultivation season and supplemental irrigation, it significantly affected the number of seeds per plant so that the highest number (53.2) was obtained from the two time irrigation in the autumn cultivated plot (Table 1). In terms of the interaction between cultivation season and No.2 fertile phosphate inoculation on the number of seeds per plant, it was significant and the highest number (58.2) was obtained from the inoculated autumn cultivated plot (Table 1).

It can be noticed that No.2 fertile phosphate inoculation, through providing adequate nutrients and increasing plant biomass, accelerates the flowering stage and propagates the number of pods per plant. This is consistent with the findings of Singh and Kapoor (1998).

Regarding the not irrigated treatment, the temperature rises in the pods, due to prolonged drought stress, resulting in a higher breathing rate and a decreased amount of available photosynthetic substrates to be transported to the growing seed, thus more pods would be lost.

It seems that during autumn cultivation season, available moisture increases which leads to a prolonged reproductive stage and a higher photosynthesis level. In turn, this would produce more flowers at the inflorescence.

As a result, more flowers would be inoculated, leading to a less number of lost flowers and a higher number of seeds per plant (Saxena, 1993).

Table 4
Comparison between the effects of No.2 fertile phosphate on seed yield and its components of chickpea

Fertile Phosphate	Pods per Plant	Seeds per Pod	100 Seeds Weight	Weight of Seed and Single Bush Pod	Seed Yield
P1: Not using	43.8[b]	43.3[b]	25[b]	6.1[b]	650.4[b]
P2: Use	52.4[a]	46.60[a]	29.1[a]	6.8 [a]	857.4 [a]

Based on Duncan's multiple range test, those means which have at least a common letter, are not significantly different (at 5% significance level)

Apparently, delayed cultivation date leads to a shorter reproductive stage, due to its coincidence with higher temperature, thus fewer flowers will be inoculated, and therefore less seeds would be obtained per plant. Saxena (1993) reported that in such a situation, higher temperature during pod growth stage increases breathing rate so there would be less photosynthetic substrate available for the growing seeds, hence more pods will be lost. It seems that the sufficiency of available moisture leads to a prolonged reproductive stage and a higher level photosynthesis which in turn, results in more flowers per inflorescence.

Turk et al (2003) reported that delayed cultivation would decrease the number of seeds per area. They also suggested that the duration of growth period influences the number of seeds per pod, since as the growth period prolongs, so the duration of podding to seed filling stage would be prolonged, resulting in a higher number of seeds per pod and consequently higher seeds per plant.

Based on variance analysis results, it was determined that the effect of cultivation season and No.2 fertile phosphate inoculation were significant on 100 seeds weight at 5% and 1% significance level, respectively (Table 1). Comparison between the means showed that levels of cultivation season are significantly different and 100 seeds weight of autumn cultivated plot (27.7 g) was 5% more than that of spring cultivated plot (26.4 g). In autumn cultivated plot, more phloem sap is transported to growing seeds which results in a heavier seeds.

During flowering and podding stages, chickpea plant is very vulnerable to drought stress and any moisture tension would castrate the flowers and leads to immature seeds, therefore affects the 100 seeds weight and finally decreases the number of pods, seeds, harvest index an seed yield (Auld et al., 1980).

Higher 100 seeds weight could be due to prolonged seed filling stage or accelerated filling speed or both. In autumn cultivation, because of a prolonged growth season, both factors may contribute to higher seed weight. This is consistent with Ghorbanzadeh and Nasiri (2005) findings. Goldani et al. (2000) also reported that drought and high temperature shorten the seed filling stage of chickpea, leading to a decreased seed weight.

The interaction of the three factors also showed a significant difference, since autumn cultivated inoculated plots produced the highest 100 seeds weight. Thus, it is observed that the application of No.2 fertile phosphate in autumn cultivated plot would positively affect 100 seeds weight. In order to account for this observation, one can say that there is a synergistic and intensifying relationship between autumn cultivation and No.2 fertile phosphate which leads to the participation of soil microorganisms and increases their activity. Then, higher level of photosynthesis which is caused by more absorption of mineral elements, particularly phosphorus and nitrogen, increases 100 seeds weight of chickpea.

Based on the results of variance analysis (Table 1), the interactive effect of supplemental irrigation, cultivation season, and No.2 fertile phosphate inoculation was significant at 1% statistical level and the interaction between cultivation season and No.2 fertile phosphate inoculation and the interaction among supplemental irrigation, cultivation season, and No.2 fertile phosphate inoculation were significant at 5% statistical level.

The comparison of means indicated that various levels of supplemental irrigation are significantly different, so that seed yield at two time irrigation treatment (839 kg ha^{-1}) is about 9% more than that of one time irrigation treatment (767 kg ha^{-1}) and 28% more than that of not irrigated treatment (656 kg ha^{-1}). The high yield in the third level is due to relative reduction in drought stress during pod filling stage through prolongation of seed filling stage and consequently higher 100 seeds weight.

Toba Bicer et al (2004) asserted that the highest seed yield was obtained from a treatment irrigated during 50% flowering stage. The effect of cultivation season on seed yield was very significant (Table 1) and the highest seed yield was obtained from autumn cultivated plot. Delayed cultivation date and the presence of drought stress during seed filling stage decreased the seed yield. To account for this behavior of the plant, it seems that the rise in temperature prolongs seed filling stage and therefore decreases seed weight.

Research findings has shown that yield stability is deeply depends on climatic condition during the critical period, so that warm weather associated with drought stress decrease plant growth and leads to less pods and seeds per pod. Apparently, moisture availability results in plant higher persistence and yield stability, while drought stress causes more loss and leads to less podded branches.

Comparing the means of treatments, it is obvious that there is a significant difference between not inoculated plot (650 kg ha^{-1}) and inoculated one (857 kg ha^{-1}), which points to a 31% higher yield in the inoculated plot.

The interaction of cultivation season and No.2 fertile phosphate inoculation also revealed a significant difference, since higher yield were obtained from the inoculated plots. The interaction of the three factors was also significantly different, because the application of all these factors together resulted in a higher yield (Figure 1). It is obvious that the two times irrigated autumn cultivated inoculated plot produced the highest yield (1103 kg ha^{-1}). Pseudomonas is one of the most important bacteria that increases plant growth, and in addition to biofixing nitrogen and solving soil phosphorus, greatly affects agricultural yield through producing significant nutrients and growth promoting hormone, particularly various auxins and gibberllines (Zahir et al., 2004).

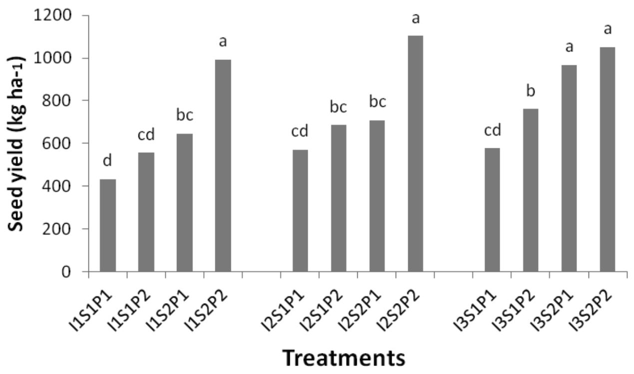

Fig. 1. Effect of supplemental irrigation (I1: without irrigation, I2: one time irrigation and I3: two time irrigation), cultivation season (S1: spring cultivation season and S2: autumn cultivation season) and No.2 fertile phosphate inoculation (P1: not applying the fertile phosphate and P2: applying the fertile phosphate) on yield of chickpea

Since seed yield is a process consisted of different traits such as reproductive pods number per plant, 100 seed yield and biologic yield, the coexistence of this plant and microorganisms would increase the seed yield.

Through affecting features such as plant elevation, number of pods per plant, and biologic yield, phosphate biofertilizer increases seed yield. By studying sugar cane plant, Sundara et al. (2002), concluded that applying *Bacillus megatherium*, a phosphate solving bacteria, with phosphate rocks, enhances the yield through increasing the activity of these microbes and increasing the solubility of phosphorus in the rhizosphere which leads to more tillering and stems. This would finally lead to higher stem yield.

Annamalai et al. (2004) showed that applying phosphate solving bacteria in a medicinal plant, namely *Phyllanthus amarus*, produced a higher yield compared to the control treatment. Another two researches which studied the application of phosphate solving microorganisms on wheat and green mung plants revealed that applying bacteria, namely *Bacillus circulans*, and a fungus, namely *Cladosporium harbarum*, along with phosphate rocks significantly increased the seed yield, compared to the control (Singh and Kapoor, 1998). In this research, high seed yield was attributed to the rise in nutrients absorption, particularly phosphorus, and the enhancement of plant photosynthesis.

Conclusions

Results showed that supplemental irrigation, cultivation season, and No.2 fertile phosphate inoculation applied singly or in combination significantly increased the number of pods per plant, number of seeds per plant, 100 seeds weight, seed and pod weight, seed yield, and biologic yield. However, the greatest increases were obtained from a combined application of all three factors. The highest seed yield (838.7 kg ha^{-1}) were achieved from the two time irrigation treatment, the highest seed yield (857.14 kg ha^{-1}) was resulted from the inoculation with No.2 fertile phosphate and Also, the highest seed yield (911.4 kg ha^{-1}) was obtained in the autumn cultivation season. According to results of this research, the interaction among these three factors had a significant effect on the seed yield (1103 kg ha^{-1}). There is a synergistic and intensifying relationship between autumn cultivation and No.2 fertile phosphate which leads to the participation of soil microorganisms and increases their activity with two time irrigation. For these reasons the application of the factors in combination should be encouraged in chickpea growing and increased the yield.

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