Effect of Rhizobium Bacteria (*Rhizobium leguminosarum*) and Nano-Iron Application on Yield and Yield Components of Different Pinto Beans Genotypes.

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ABSTRACT

Pinto Bean (*Phaseolus vulgaris* L.) is one of the major legumes cultivated in Iran and around the world. It is reported that Mycorrhizal inoculation increases the plant's tolerance to drought. The most accepted mechanism for this effect is the improvement of water-use efficiency of the Mycorrhized plant. Iron deficiency is a widespread agricultural problem in many crops, especially in calcareous soils. In these soils, total Fe is high but occurs in chemical forms not available to plant roots. This study was carried out to determine the effect of *Rhizobium leguminosarum* inoculation and Nano-iron foliar application on yield and yield components of pinto beans genotypes. The genotypes used in the experiments involve 'Talash', 'Khomein' and 'COS16'. The experiment was designed in a randomized complete block design in factorial arrangement with three replications. The results of analysis of variance (ANOVA) showed the significant effect of *Rhizobium leguminosarum* treatment on plant height, number of pods, number of seeds per pod, pod skin weight, number of seeds per plant, seed yield and significant effect of Nano-iron on skin weight of pod, seed length, and seed number per pod. For local varieties, results indicated that the highest plant (57.83 cm) and the maximum number of pod per plant (14.5) were obtained from control treatment (no bacterial inoculation). Results also indicated that the longest pod (9.75 cm) and the maximum seed per plant (71.83) and the maximum seed yield (18.57 g) were obtained from treatment of seed inoculation with *Rhizobium leguminosarum* in 'Khomein' variety.

Keywords: Bean, Inoculation, Legume, Nano particles, Nutrition.

INTRODUCTION

Grains with low protein content comprise the staple food of the developing world (Anonymous, 2008). Among the plant protein sources, the highest protein amount is found in the seeds legumes, while the highest protein contents belong to kidney bean with 17-35% protein contents and A, B and D vitamins (Piha and Munns, 1987). Alongside human nutrition, kidney beans also play an important role because they add nitrogen to the soil (Pena-Cabriales et al., 1993; Graham, Ramalli, 1997; Graham et al., 2003). Pinto bean (*Phaseolus vulgaris* L.) is one of the major grain legumes cultivated in Iran and around the world. It has been reported that mycorrhizal inoculation increases the plant’s tolerance to drought. The most accepted mechanism for this effect is the improvement of water-use efficiency of the mycorrhized plant (Ahmad Khan et al., 2003; Bolandnazar et al., 2007; Maiquetia et al., 2009).

Rhizobial inoculation significantly increased yield, tannin and protein contents in bean seeds. Moreover, the incidence of bean yellow mosaic virus infection has reportedly decreased (Babiker et al., 1995). Common bean inoculated with some native rhizobia strains resulted in increased dry weight of shoot and nitrogen percentage in shoot; besides, it increased absorption of nutrients. It was also determined that inoculation of L-75 increased the weight of nodules by 100% and nitrogen uptake by 70% compared to the control treatment; inoculation of L-54 compared to the control treatment and N200 kg/ha urea, increased nitrogen uptakes by 45% and 32% respectively (Yahya-Abadi et al., 2008).

Six micronutrients including Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants (Mortvedt et al., 1991). Iron deficiency is a widespread agricultural problem in many crops, especially in calcareous soils. In these soils, total Fe
is high, but it occurs in chemical forms not available to plant roots (Lindsay and Schwab, 1982). Plants respond to Fe limitation by inducing a series of physiological and morphological changes in the roots to facilitate the mobilization of sparingly soluble Fe compounds in the root environment. Acidification of the Rhizosphere (Romheld et al., 1984), enhanced root ferric chelate reductase (FC-R) activity (Bienfait et al., 1983), and increased Fe(II) uptake capacity (Eide et al., 1996; Zaharieva and Romheld, 2000) are characteristic responses of the Strategy I plants to Fe deficiency (Romheld, 1987). All living things, including plants, need food for growth. Soil is the supplier of the majority of nutrients needed by plants. In many acid soils around the world as well as in half of the cultivated lands with the potential to produce food, heavy metals are the main factor limiting the plant growth.

Iron is the first rare element recognized as necessary for plants and animals, playing an important role in biochemical and physiological processes. It works as a key enzyme co-factor that plays a role in plant hormone synthesis and is engaged in many electron transportation reactions (Kerkeb and Connolly, 2006). Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants (Havlin et al., 1999). Iron deficiency is common in citrus, deciduous fruits, groundnuts and many other crops (Tariq et al., 2004). A deficiency in the soil is rare, but iron can be unavailable for absorption if soil pH is not between about 5 and 6.5. A common problem is when the soil is too alkaline (the pH is above 6.5). Iron functions to accept and donate electrons and plays important roles in the electron transport chains of photosynthesis and respiration; so it plays a role in energy transfer within the plant. The aim of this study was to evaluate the effect of Rhizobium bacteria (Rhizobium leguminosarum) and Nano Fe particles on different genotypes of beans under hot and dry weather condition.

MATERIALS AND METHODS

Experiments Condition:
Experiments were carried out in 2013 in Northwest of Ilam, Iran. It is located on the geographic coordinates 33º57´16" latitude south, 46º 09´ 20" longitude west, and its altitude is 1114 meters. Here, soil with loam texture is the predominant pedogenetic unit. The climate is classified by the International Koppen system as arid climates with 535 mm mean annual precipitation.

Plant Material and Experimental Design:
The genotypes used in the experiments included ‘Talash’, ‘Khomain’ and ‘COS16’. The experiments were designed in a randomized complete block design with three replications. The plot size was 8.0 m² (4.0 x 2.0 m), each having four rows. The inter- and intra-row spacing was 50 cm and 10 cm respectively.

Treatments:
Treatment of seeds with and without Rhizobium leguminosarum bacteria and foliar application or no-use of Nano Fe particles were investigated as experimental factors. The Rhizobium leguminosarum bacteria were inoculated on the surface of seeds (100 kg seed, 1 kg inoculate), just before sowing. Weed control was done after each irrigation.

Statistical Analysis:
Means comparison was performed based on Duncan’s multiple range test (P≤0.05). All statistical analyses were performed using SAS (version 9.1) and SPSS (version 16) software.

RESULTS AND DISCUSSION

Results of variance analysis (ANOVA) showed a significant effect of seed inoculation with nitrogen-fixing bacteria (Rhizobium leguminosarum) on most studied characteristics such as plant height, number of pods per plant, number of seeds per plant, the seed diameter, weight of pod skin, number of seeds in pod and 1000 seeds weight were measured on ten plants selected randomly from each plots. The seed yield was obtained from mature seeds harvested and threshed from 4 m lengths of four rows and added 10 plant seed yield selected for measure.

Plant Height:
Analysis of variance showed that the effect of bacteria application and Bacteria×Nano-Iron Interaction on Plant height were significant (P≤0.05); also Nano-Iron non-significantly affects plant height in different varieties of bean (Table 1). Legumes have played a crucial role in agricultural production throughout history. This is obviously due to their capacity to fix nitrogen association with rhizobia using solar energy collected through the plant photosynthesis. Cost escalation of fossil fuel required for the manufacture of fertilizer nitrogen has helped to increase awareness about the importance of the Rhizobium-legume symbiosis (Rupela and Saxena, 1984). The tallest variety (‘Talesh’) was positively affected by bacterial application (61 cm) compared to the plot with no bacteria applied (32.57 cm) (Fig. 1).
Nitrogen increases the vegetative growth in plants (Celik and Fizyolojisi, 1998; Kacar and Katkat, 2007).

**Number of Pods per Plant:**

The results of variance analysis showed a significant effect (P≤0.01) of bacteria applications and Bacteria×Nano-Iron interaction on number of pods per plant (Table 1). Means comparison for bacteria applications indicated that the maximum number of pods per plant (71.83) was obtained from 'Khomein' variety also for this treatment. The minimum number of pods per plant (29) belonged to control treatment of bacteria (without grain inoculation) in local variety (Fig. 2). Karahan and Sehirali (1999) explained that both inoculation of bacteria and application of nitrogen increased the number of pod per plant. Elballa et al. (2004) revealed that microelements application enhanced the number of pods per plant, 1000 grain weight and grain yield. Custodio et al. (2013) declared that pod number and grain number are two major factors affecting soybean grain yield.

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**Table 1. Analysis of seed inoculation variance with *Rhizobium leguminosarum* and Nano-iron foliar application on some agronomic traits of Pinto Bean.**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Plant Height</th>
<th>Number of Pods per Plant</th>
<th>Number of Seeds per Pod.</th>
<th>1000 Grain Weight</th>
<th>Pod Length</th>
<th>Seed Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>513.788</td>
<td>11.361</td>
<td>0.250</td>
<td>1547.4</td>
<td>0.375</td>
<td>11.469</td>
</tr>
<tr>
<td>Bacteria</td>
<td>1</td>
<td>257.068</td>
<td>42.250</td>
<td>2.250</td>
<td>3952.2</td>
<td>2.402</td>
<td>0.005</td>
</tr>
<tr>
<td>Nano-Iron</td>
<td>1</td>
<td>3.869</td>
<td>12.250</td>
<td>0.694</td>
<td>0.234</td>
<td>26.003</td>
<td></td>
</tr>
<tr>
<td>Bacteria*Nano-Iron</td>
<td>1</td>
<td>2162.250</td>
<td>56.250</td>
<td>0.026</td>
<td>204.188</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Pinto Bean Varieties(PBV)</td>
<td>2</td>
<td>5624.041</td>
<td>167.194</td>
<td>0.333</td>
<td>1089.2</td>
<td>0.802</td>
<td></td>
</tr>
<tr>
<td>Bacteria*PBV</td>
<td>2</td>
<td>14.754</td>
<td>0.583</td>
<td>0.333</td>
<td>169.0</td>
<td>1.460</td>
<td>20.563</td>
</tr>
<tr>
<td>Nano-Iron *PBV</td>
<td>2</td>
<td>15.521</td>
<td>3.083</td>
<td>0.778</td>
<td>141.0</td>
<td>1.443</td>
<td></td>
</tr>
<tr>
<td>Nano-Iron *Bacteria *PBV</td>
<td>2</td>
<td>284.363</td>
<td>3.250</td>
<td>0.111</td>
<td>100.2</td>
<td>0.250</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>49.509</td>
<td>3.270</td>
<td>0.189</td>
<td>210.6</td>
<td>0.883</td>
<td>7.586</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>ns,*, and ** respectively represent non-significant, significant at 0.05 and 0.01 level of significance.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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![Fig. 1. Effect of Rhizobium bacteria treatments on plant height in pinto bean varieties.](image)

![Fig. 3. Effect of Nano-iron treatments on number of pods per plant in pinto bean varieties.](image)

**Number of Seeds per Pod:**

Variance analysis showed the significant effect of bacteria application (P≤0.01) and Nano-iron application (P≤0.05) on number of seeds per pod (Table 1). The maximum number of seeds per pod (4) was obtained from seed inoculation with *Rhizobium leguminosarum* in 'Khomein' variety (Fig. 4). The lowest number of seeds per pod (3) belonged to control treatments of Nano-iron (without Nano-iron) (Fig. 5). Drought occurrence in relation to anthesis stage made a drastic reduction in yield and yield components (Seghatoleslami et al., 2008) and the experiment was performed in southwest of Iran in which there is drought stress. Supporting evidence was...

1000 Grain Weight:

Variance analysis showed the significant effect of bacteria application and Nano-iron application (P≤0.01) on 1000 grain weight (Table 1). The maximum 1000 grain weight (25.837) was obtained from grain inoculation with *Rhizobium leguminosarum* in 'Khomein' variety (Fig. 6); the lowest 1000 grain weight (20.7 g) occurred in Nano-iron treatment from 'Talash' variety (Fig. 7).

Zeidan *et al.* (2006) found that the foliar spray of micronutrients considerably enhanced the number of pods per plant, 1000 grain weight and grain yield. When photosynthesis assimilates are translocated from vegetative organs to the other parts, grain weight enhances considerably (Kakiuchi and Kobata, 2006). It was shown that microelements affect leaf area and then lead to larger amounts of assimilate production in common bean. The results are in agreement with Arif *et al.* (2006) who found dramatic increase in 1000 grain weight by foliar application of iron in wheat crop.

Pod Length:

Results of variance analysis showed the significant effect (P≤0/01) of grain inoculation with bacteria on pod length (Table 1). Means comparison indicated that the longest pod (9.75 cm) was obtained from bacteria application treatment in 'Khomein' variety. The shortest pod (6.96 cm) belonged to the control treatment of bacteria application (without grain inoculation) in local variety (Fig. 8). Decrease in grain weight and increase in unfilled seeds were the main reasons for decreasing the grain yield. Decrease in seed yield due to yield components reduction, especially grain weight, has previously been reported by other researchers (Unger, 1992). Decrease in length of grain filling stage due to water stress is the main factor decreasing grain weight (Cantagallo *et al.*, 1997). Iron is necessary for protein synthesis (Kene *et al.*, 1990).

Grain Yield:

Results of variance analysis showed that the effect of grain inoculation with bacteria on grain yield in plant was significant (P≤0/01), but the effect of Nano-iron foliar application in all varieties was not significant on grain yield. The lowest yield of grain (5.49 g) was obtained from bacteria control treatment (without grain inoculation). The highest yield of grain (18.57 g/plant) belonged to the bacteria application treatment for 'Khomein' variety (Fig. 9).

In contrast, Mallarino *et al.* (2001) and Gerwing *et al.* (2003) showed that iron foliar application did not increase soybean yield. Iron foliar application enhanced soybean yield by influencing the number of seeds per plant and seed weight (Kobraee *et al.*, 2011). Kobraee *et al.* (2011)
claimed that zinc and iron application at the same time could lead to higher dry matter and grain yield compared to using them separately. Zinc and iron deficiency in soils could be a restricting factor of yield and extremely decrease crop yield quality (Salwa et al., 2011).

Drought is deleterious for plant growth, yield and mineral nutrition (Garg et al., 2004; Samarah et al., 2004).

Fig. 8. Effect of Rhizobium bacteria treatments on pod length in pinto bean varieties.

Fig. 9. Effect of Rhizobium bacteria treatments on seed yield.

Means comparison for local variety indicated that the highest plant (57.83 cm) and the maximum number of pod per plant (14.5) were obtained from control treatment of bacteria (without inoculation). Also Means comparison indicated that the longest pod (9.75 cm), the maximum seed per plant (71.83) and the maximum seed yield (18.57 g) were obtained from treatment of seed inoculation with Rhizobium leguminosarum in 'Khomein' variety.

REFERENCES


