

## Co-Inoculation of *Rhizobium* and *Azotobacter* on Growth of Faba bean under Water Deficit Conditions

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**Abstract:** Biofertilizers can be expected to decrease the use of chemical fertilizers. The effects of native *Rhizobium leguminosarum* bv *viciae* strain F46 and *Azotobacter chroococcom* strain AGO11 inoculation and nitrogen fertilizer was studied on growth and yield of faba bean (*Vicia faba* L.) in deficit irrigation. The trial was conducted as a split plot factorial experiment in Agricultural Research Station of Boroujerd. Co-inoculation of *Rhizobium* and *Azotobacter* increased total nitrogen content, nodulation, seed yield and biological yield under water deficit condition. Hence it can be suggested that co-inoculation of *Rhizobium Leguminosarum* and *Azotobacter chroococum* can improved some of growth indices under drought conditions.

**Key words:** Inoculation • *Rhizobium* • *Azotobacter* • Water deficit • Faba bean

### INTRODUCTION

Legumes are well known for its important roles in maintaining productivity in agricultural systems [1, 2]. Grain legumes have a nitrogen fixing symbiosis with soil root nodule bacteria [3]. Faba bean (*Vicia faba* L.) is an annual legume that is consumed as plant foods for human and animal nutrition, because it is rich in protein. Faba bean cultivation in Iran is about 100,000 ha and its dry seed yield is about 2100 kg/ha. Faba bean like other legumes has a nitrogen fixation symbiosis relation with *Rhizobium Leguminosarum* bv. *viciae*. Nitrogen is one of the most important elements for growth of plants. Nevertheless, there is 78000 tons of N<sub>2</sub> above on one hectare of the earth; nitrogen deficiency is one of the most important limited factors in agricultural production in Iran. Nitrogen is one of the most consumed chemical fertilizers in the world and Iran; in other hand these fertilizers are considered as a major environmental pollutant [4]. Drought is one of the main limiting factors in agricultural development in arid and semi-arid regions such as Iran. Inoculation with *Rhizobium* increased the

seed yield of faba bean in six areas in Australia [5] and the seed yield and weight of hundred seeds in Sudan [6]. Seed inoculation of some native *Rhizobium Leguminosarum* bv. *viciae* on faba bean in the south of Iran indicated that seed yield increased from 35% to 69% due to the inoculation [7]. *Azotobacter chroococum* is a free-living nitrogen fixing rhizobacteria that can promote the growth of various crops by some mechanisms such as production of gibberellic acid, indole-3 acetic acid (IAA) and cytokinin [8, 9]. Inoculation of *Rhizobium, pseudomonas putida*, *P. fluorescens* and *Bacillus cereus* increased significantly the plant growth and nodulation of faba bean [10]. Seed inoculation of faba bean with *Rhizobium leguminosarum* bv. *viciae* and five different *Azotobacter chroococum* under gnotobiotic conditions of culture resulted in significant effects on nodulation and plant growth at the flowering stage [11].

The main purpose of this study was evaluation of the effects of biofertilizers on growth and yield of faba bean for decreasing the effects of drought stress in Boroujerd. This region is located in the west of Iran which is one of the main areas under faba bean cultivation.

## MATERIALS AND METHODS

**Site Description and Weather Conditions:** The trial was conducted at the Agricultural Research Station in Boroujerd (Longitude: 48°55' E, latitude: 33°40' N, height: 1476 m) in November 2009. Climate of this area is characterized by moderate summers and cold and humid winter. The mean annual temperature is about 14°C, the average precipitation is about 400 mm and average evapotranspiration is about 1500 mm. The soil was a Typic Xerochrepts with silty loam texture, organic carbon contents of %0.85 in the A horizon and pH=7.8 in the topsoil. Irrigation was used immediately for all treatments after sowing because of very low precipitation. The amount of precipitation from the beginning of experiment until the first harvest was 347 mm and from the first harvest until the second harvest was 43 mm.

**Bacterial Strains and Cultural Conditions:** *Azotobacter chroococcum* strain AGO11 and *Rhizobium leguminosarum* strain F46 prepared from gene bank of soil and water research institute of Iran. For preparing of Inoculants, *Azotobacter chroococcum* was grown on winogradsky medium [12] and the *Rhizobium leguminosarum* bv. *viciae* was grown on yeast manitol Agar [13] for 48 hours in 28°C° in rotary shaker. The suspension of *Rhizobium* and *Azotobacter* thoroughly mixed with perlite as carrier separately and incubated in 28°C° for 72h. The weight of package *Rhizobium* and *Azotobacter* was considered 500gr and contained at least 10<sup>8</sup> viable cells of *Rhizobium* and *Azotobacter* per gram of inoculant. Before inoculation the seeds surface was mixed with 15% sugar completely for more adhesion of inoculums. Finally the seeds were inoculated and mixed thoroughly with *Azotobacter* and *Rhizobium* inoculums. Formerly some of plant growth promoting characteristics of *Rhizobium* and *Azotobacter* which used in this study was evaluated [14, 15].

**Irrigation:** All of the plots were irrigated immediately after sowing by furrow irrigation. Because of precipitation and low temperature the second irrigation started after four months. Based on the amount of transpiration for the other irrigation including 50 and 75mm collective evaporation from pan class A were applied with 0.5 m<sup>3</sup>/plot. Then the collection of effective rain was calculated in order to obtained water use efficiency.

Table 1: Measurement of nodulation [16].

Nodulation score	Distribution and number of effective nodules	
	Crown of root*	Elsewhere of root
0	0	0
0.5	0	1-4
1	0	5-9
1.5	0	>10
2	Few	0
2.5	Few	Few
3	Many	0
4	Many	Many
5	Many	Many

\* Consist of 7cm top of the root system

## Experimental Design and Statistical Methods:

The experiment was carried out as a split plot factorial experiment in a completely randomized block design with 3 replicates. The treatments included two irrigation levels after 50 mm=I<sub>1</sub> and 75 mm=I<sub>2</sub> collective evaporation from pan class A as main plots and *Rhizobium*, *Azotobacter* and nitrogen fertilizer each contain two levels as sub plot (2×2×2×2×3 = 48 plots). The size of each plot was 2.5 ×4 meters. Data were analysed by SAS/STAT software and the means were compared by Duncan's multiple range test.

**Plant Sampling:** The plants were harvested in two stages during the experiment according to growth and development of faba bean. In the first harvesting at middle of flowering stage, the nodulation and shoot dry weight were determined by harvesting in 1m×1m of each plot. In the final harvesting, total N content, number of pods/plants, number of seed/pod, weight of hundred seed, seed yield, biological yield, relative water content, water use efficiency and leaf area index were determined. Nodulation was measured in based of Table 1.

## RESULTS

*Azotobacter* inoculation increased total nitrogen content in drought stress (Fig.1f). The results showed that inoculation with *Azotobacter* increased significantly the biological yield up to 6474 kg.ha<sup>-1</sup> as well (Fig.1c and Table 2). Inoculation with *Azotobacter* had not significant effects on seed yield, weight hundred of seeds and nodulation. Inoculation with *Rhizobium* increased water use efficiency and leaf area index (Fig 1e). Also nitrogen fertilizer increased water use efficiency (Fig.1g). The highest mean of shoot dry weight belongs to co-inoculation of *Azotobacter* and *Rhizobium* (Fig. 1d).

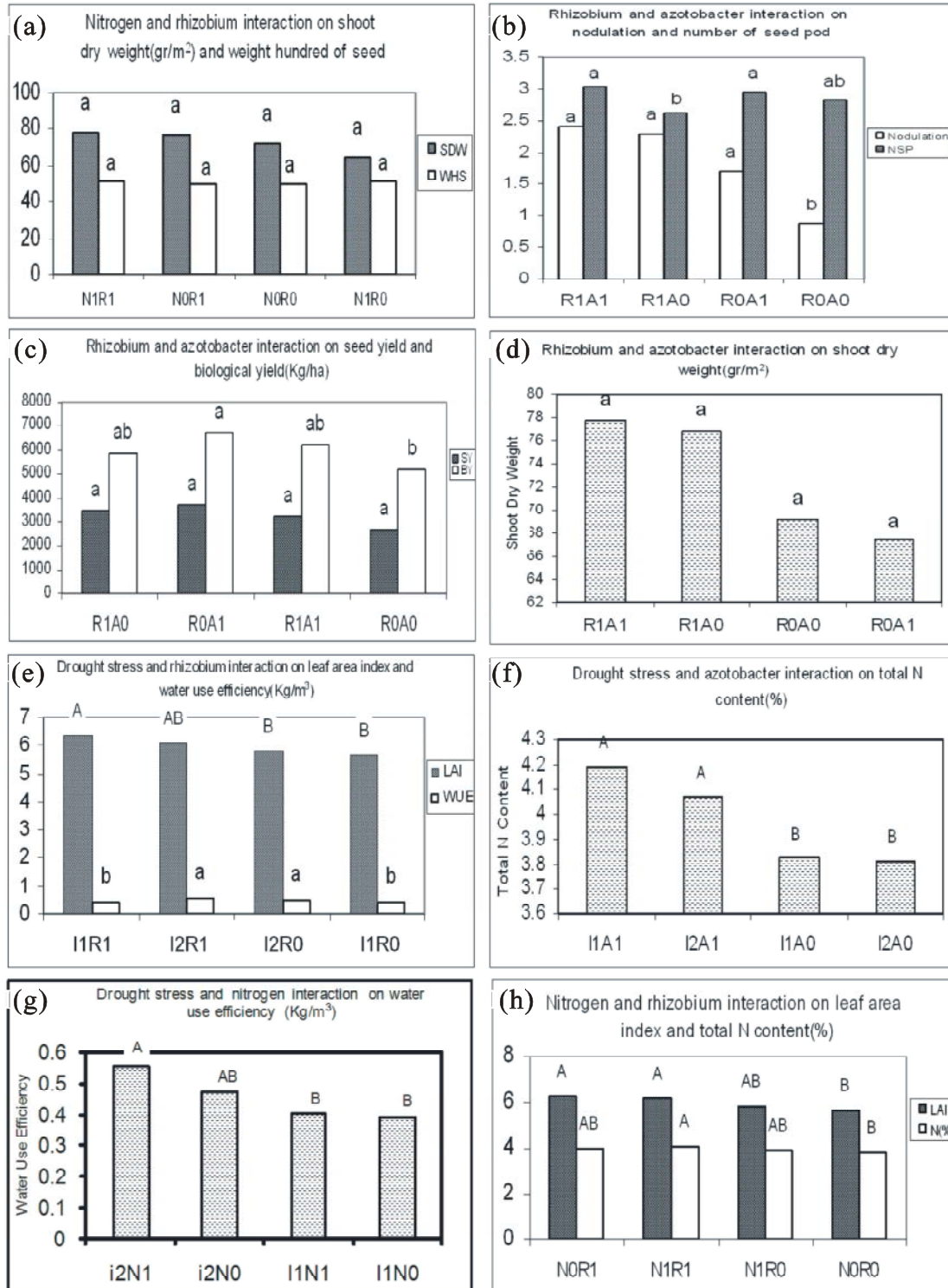


Fig. 1: Mean comparisons of double interactions for water use efficiency(WUE-e, g), leaf area index(LAI-e, h), total N content(N%-f, h), biological yield(BY-c) seed yield(SY-c), number of seed pod(NSP-a), weight hundred of seed(WHS-b), nodulation(N-a) and shoot dry weight(SDW-b, d) by Duncan's multiple range test. Means with the same letter are not significantly different

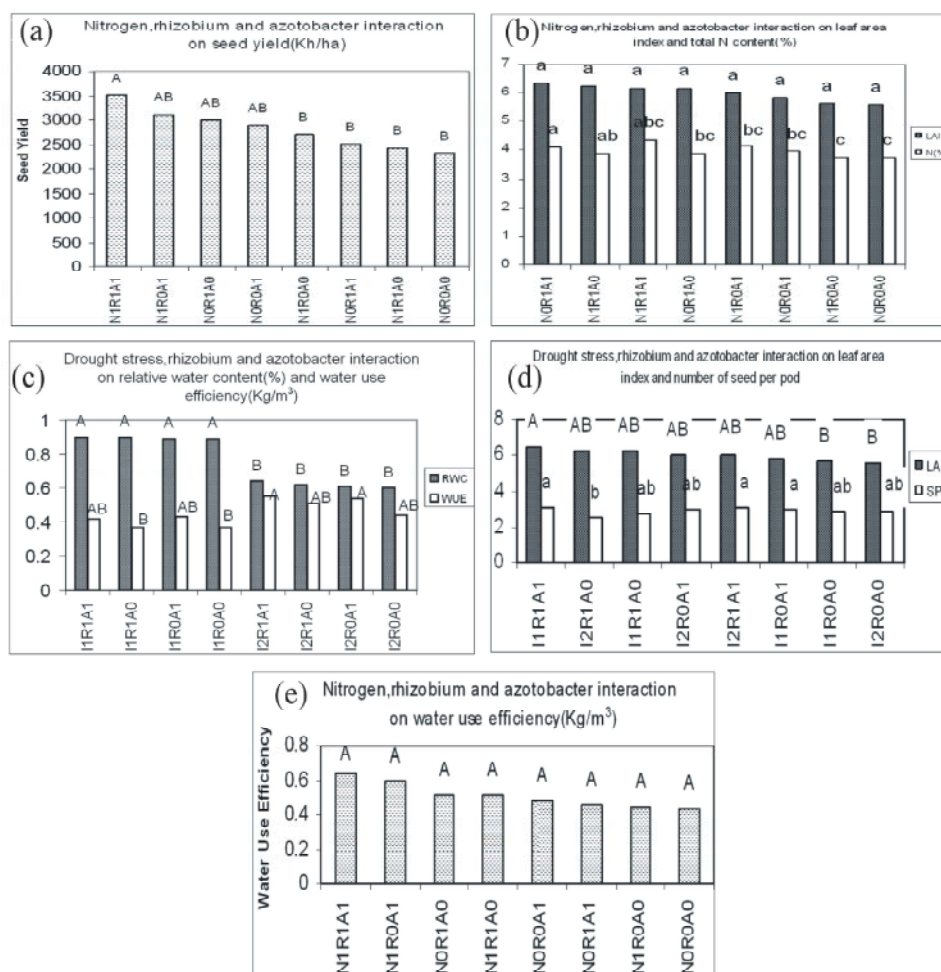


Fig. 2: Mean comparisons for triple interaction for water use efficiency (WUE-c, e), leaf area index (LAI-b, d) total N content (N%-b), relative water content (RWC-c), number of seed pod (NSP-d) seed yield (SY-a), by Duncan, s multiple range test. Means with the same letter are not significantly different.

Table 2: Mean comparisons of main effect of Irrigation, Nitrogen, Rhizobium and Azotobacter

Treatments	N%	StY (kg/ha)	SY (kg/ha)	BY (kg/ha)	NPP	SNP	WHS (g)	RWC (%)	N	LAI	WUE (kgm <sup>-3</sup> )
<b>Irrigation</b>											
I2	3.93	3066	2802.9	5868.6	31.87	2.82	49.08	0.62	1.75	5.94	0.51
I1	4.02	3205	2815.2	6141.6	35.45	2.9	51.79	0.90	1.89	6.3	0.40
<b>Nitrogen</b>											
-N	3.93	3218	2682	5901	33.70	2.93	50.80	0.76	1.91	5.96	0.43
+N	4.02	3356	2935	6109	35.50	2.79	50.79	0.75	1.72	6.02	0.48
<b>Rhizobium</b>											
-R	3.9	3206	2756.5	5962.9	33.64	2.89	50.12	0.75	1.29	5.7	0.44
+R	4.05	3317	2861.6	6047.3	33.68	2.83	50.79	0.77	2.35	6.22	0.46
<b>Azotobacter</b>											
-A	3.82	3320	2614.7	5536.1	30.77	2.73	50.59	0.75	1.58	5.9	0.42
+A	4.13	3471	3003.4	6474.1	36.56	2.99	50.28	0.76	2.06	6.07	0.49

\* (N %): Total nitrogen content (%); StY: Straw yield; SY: Seed yield; BY: Biological yield; NPP: number of pod plant; SNP: Number of seed pod; WHS: Weight of hundred seed; RWC: Relative water content; N: Nodulation; LAI: Leaf area index; WUE: Water use efficiency.

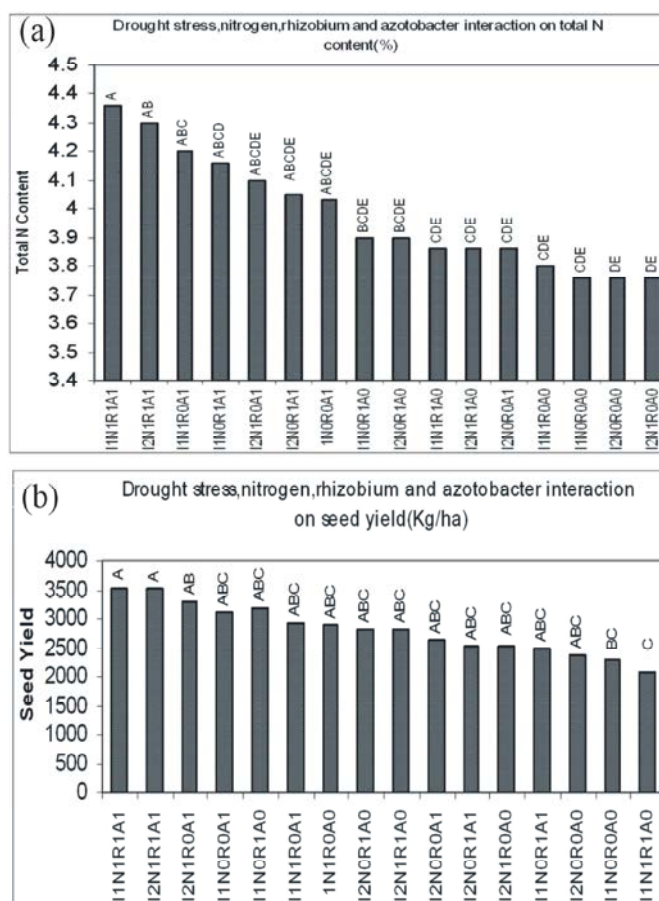


Fig. 3: Mean comparisons for quadruple interaction for, total N content (N%-a), seed yield (SY-b) by Duncan's multiple range test.

The interaction between *Rhizobium* and nitrogen was significant on leaf area index and total nitrogen content (Fig.1h).

The maximum seed yield was 3510 kg.h<sup>-1</sup> that belongs to triple interaction of *Azotobacter*, *Rhizobium* and nitrogen fertilizer (Fig.2a and b).

Effect of quadruple of irrigation, *Rhizobium*, *Azotobacter* and nitrogen fertilizer revealed that the best treatment about total nitrogen content and seed yield belongs to N<sub>1</sub>R<sub>1</sub>I<sub>1</sub>A<sub>1</sub> (Fig.3a and b).

The means comparison of the main effects of irrigation, nitrogen, *Rhizobium* and *Azotobacter* are shown in Table 2.

## DISCUSSION

Co-inoculation of *Rhizobium* and *Azotobacter* significantly increased some of the growth indices such as total N content, nodulation, seed yield and biological yield. Mixed inoculation of faba bean with *Rhizobium* /

*Azospirillum* and *Rizobium* / *Azotobacter* combinations led to changes in total content of K, P, Ca, Mg, Fe, B, Mn, Zn and Cu [17]. Seed inoculation of faba bean with *Rhizobium Leguminosarum* bv.viceae and five different *Azotobacter chroococcum* under gnotobiotic conditions of culture resulted in significant effects on nodulation and plant growth at the flowering stage [11]. Prior to this research reported that inoculation of faba bean with indigenous *Rhizobium Leguminosarum* bv. viciae in the south and north of Iran increased significantly the seed yield [7 and 18]. Full irrigation increased most of the growth parameters, as well as co-inoculation with *Rhizobium* and *Azotobacter* increased water and nutrient uptake under the deficit-irrigation because it alleviated effect of shortage of water. Previously some of the plant growth promoting characteristics of *Rhizobium* strain was evaluated which used in this study such as production of auxin and ACC deaminase enzyme [14]. Formerly *Azotobacter chroococcum* strain AGO11 had significant effects on growth and yield of wheat in field study in

some parts of Iran [15]. Therefore application of *Rhizobium leguminosarum* bv. *viciae* strain F46 and *Azotobacter chroococcum* strain AGO11 on faba bean under deficit-irrigation in Boroujerd region is recommended for further investigation.

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