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Nitrogen rates effects and seed inoculation with Rhizobium legominosarum and plant growth promoting rhizobacteria (PGPR) on yield and total dry matter of Chickpea (*Cicer arietinum* L.)

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ABSTRACT: In order to study nitrogen rates effects and seed inoculation with rhizobium legominosarum and plant growth promoting rhizobacteria (PGPR) on yield and total dry matter of Chickpea (Cicer arietinum L.), a factorial experiment based on randomized complete block design with three replications was conducted at the farm of Shahrivar village (30 km from Ardabil) in 2013. Factors were nitrogen rates at four levels (0, 25,50 and 75 kg urea/ha) as N_0, N_1, N_2 and N_3 respectively and five levels of inoculation seed with rhizobium legominuzarum and plant growth promoting rhizobacteria as (without inoculation seed as control, seed inoculation with Rhizobium legominuzarum, seed inoculation with rhizobium Legominuzarum+Azosprillum lipoferum strain OF, seed inoculation with Rhizobium legominuzarum+Psedomonas putida, seed inoculation Rhizobium legominuzarum+Azosprillum lipoferum strain OF+Psedomonas putida) as T_0, T_1, T_2, T_3 and T_4 respectively. The results of growth indices showed that in all of treatment compounds, total dry matter increased slowly until 25 days after sowing. From 25 till 105 days after sowing increased rapidly. From 105 till 115 days after sowing (harvest time), it decreased due to increasing aging of leaves and decreasing of crop growth rate. Maximum of total dry matter (374 gr/m^2) belonged to the higher nitrogen rates (75 kg urea/ha) and seed inoculation as T_4. While, the minimum of it was in without seed inoculation and nitrogen application. Plant height, number of pod per plant, grain 100 weight, grain yield, number and weight of nodules per plant were significantly affected by nitrogen rates and seed inoculation. Means comparison showed that maximum of grain yield (1276.78 kg/ha) and number of pod per plant (32.48) was obtained in the higher nitrogen rates. Increasing of nitrogen rates up 50 kg/ha increased number and weight of nodules per plant but it decreased in application of 75 kg N/ha. Minimum of these traits was obtained in without seed inoculation and nitrogen application. Seed inoculation with rhizobium and PGPR increased number and weight of nodules per plant. Maximum of these traits was obtained in seed inoculation with Rhizobium legominuzarum and Azosprillum+ Psedomonas.
Key words: Chickpea, Nitrogen rates, PGPR

INTRODUCTION

Chickpea (Cicer arietinum L.) is considered as one of the most important grain legumes in over the world (Amany, 2007; Togay et al., 2008). Chickpea seeds contain about 20.6% protein, 61.2% carbohydrates and 2.2% fat (Saini et al., 2004). The inoculation of seeds with Rhizobium is known to increase nodulation, growth and yield of legume crops (Sogut, 2006). Nitrogen fertilizer application and seed inoculation with PGPR and Rhizobium legominuzarum can increase yield of Chickpea (Peix et al, 2001). Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and promote growth when added to seeds, roots or tubers have been termed plant-growth-promoting rhizobacteria and increase plant growth and

yield (Kloepper et al,1980b ; Wu et al, 2005). Among them are strains from genera such as Azosprilium and Azotobacter (Rodriguez and Fraga, 1999). The mechanisms by which PGPRs promote plant growth are not fully understood. But, several mechanisms have been suggested by which PGPR can promote plant growth, including phytohormone production, enhancing stress resistance, N2 fixation, stimulation of nutrient uptake and biocontrol of pathogenic microorganisms (Rodriguez and Fraga,1999 ; Sindhu, et al,.1999), increasing the supply or availability of primery nutrients to the host plant (Wu et al., 2005), the synthesis of antibiotics, enzymes and fungicidal compounds (Ahmad et al, 2006 ; Bharathi et al, 2004; Jeun et al, 2004). Malik et al (2006) found that seed inoculation with Rhizobium increase significantly plant height, number of pods per plant, number of seeds per pod, 100 seed weight, biological and seed yield in soybean. Togay et al (2008) reported that inoculation with Rhizobium increase significantly plant height, pods and seeds per plant, grain yield and biological yield in chickpea. Yadegari and Asadi Rahmani (2010) have been shown that bean (Phaseolus vulgaris) yield increased in seed priming with PGPR. Similar results have also been reported by Lucas et al (2004) in Glycine max, Peix et al (2001) in chickpea and barley. The objectives of this study were to determine the effects seed inoculation with rhizobium legominosarum and plant growth promoting rhizobacteria (PGPR) on yield and total dry matter of Chickpea (Cicer arietinum L.) in condition of Ardabil Plain.

Table1.

K available (mg/kg)	P available (mg/kg)	N total (%)	O.C (%)	Texture	Sand (%)	Loam (%)	Clay (%)	(%) SP	pH	Caco3 (%)	Depth of sampling (cm)
362	19	0.06	0.62	Silty-loam	26	68	5.5	45	7	16.8	0-30

MATIRIAL AND METHODS

A factorial experiment based on randomized complete block design with three replications was conducted in 2013 at the farm of Shahrivar village (30 km from Ardabil) in 2013 (Alt 1350 m). Climatically, the area placed in the semi-arid temperate zone with cold winter and hot summer. Average rainfall is about 342 mm that most rainfall concentrated between winter and spring. The table 1 shows physico-chemical properties of farm soil used in the experiment.

Table 1- physico-chemical properties of farm soil

Factors were nitrogen rates at four levels (0, 25, 50 and 75 kg urea/ha) as N_0, N_1, N_2 and N_3 respectively and five levels of inoculation seed with Rhizobium legominuzarum and plant growth promoting rhizobacteria as (without inoculation seed as control, seed inoculation with Rhizobium legominuzarum, seed inoculation with Rhizobium legominuzarum+Azosprillum lipoferum strain OF, seed inoculation with Rhizobium legominuzarum+Psedomonas putida, seed inoculation Rhizobium legominuzarum+Azosprillum lipoferum strain OF+ Psedomonas putida) as T_0, T_1, T_2, T_3 and T_4 respectively. For inoculation, seeds were coated with gum arabic as an adhesive and rolled into the suspension of bacteria until uniformly coated. The strains and cell densities of microorganisms used as PGPR in this experiment were 10^7 bacteri/gram. Row spacing was 50 cm and distances between plants in the rows were 5.72 cm for the appropriate final stand of 350000 plants ha^{-1}. Seeds were placed at 4-5 cm depth. Plots and blocks were separated by 1.5 m unplanted distances. The bacterial strains Psedomonas and Azosprilium were isolated from the rhizospheres of chickpea farms in Iran. The field was immediately irrigated after planting. Weeds were controlled manually. All other agronomic operations except those under study were kept normal and uniform for all treatments.For estimation of dry matter accumulation, from 0.075 m^2 in each plot was sampled randomly in each treatment compound and average for recording the change in dry weight in shoots (above ground). Sampling intervals were ten days at different stages of the corn growth (25, 35,45,55,65,75,85,95,105 and 115 days after sowing). For dry weight determination, samples were oven dried at 70 ±5˚ C to constant weight. The parameter of total dry matter (TDM) was determined with using of equation 1 according of method of Acuqaah (2002), Gupta and Gupta (2005).

$$TDM = e^{a+bt+ct^2+dt^3} \quad (1)$$

In this equation, t is the intervals of sampling and a, b and c are coefficient of equation. Yield components such as plant height, number of pod per plant, grain 100 weights, number and weight of nodules per plant was recorded on 10 randomly selected plants in each plot. Grain yield was determined by harvesting the middle three rows of each plot (0.5 m^2). Analysis of variance was done using SAS computer software package. The main effects and interactions were tested using the LSD test.

RESULTS AND DISCUSSION

Nitrogen rates and seed inoculation with Rhizobium legominosarum and plant growth promoting rhizobacteria (PGPR) had significant effects on yield and some agronomic traits of chickpea (Cicer arietinum L.) such as plant height, number of pod per plant, grain 100 weight, number and weight of nodules per plant (table 2).

Plant height

nitrogen rates significantly increased the plant height. Data regarding the effect of nitrogen rates on plant height are given in table 3. In general, the highest plant height (93.58 cm) were observed in the maximum rate of nitrogen application (75 kg urea/ha) and minimum of (69.37 cm) it was at no application nitrogen. These results are in line with the findings of Amany (2007) and Caliskan et al. (2008) who reported that plant height was increased with application of nitrogen fertilizer. variance analysis of data indicated that inoculation seed had significant effects on plant height of chickpea (table 2). Comparison of means showed that the inoculated plant had more height than non-inoculated plants. Maximum of plant height (96.07 cm) was obtained to seed inoculation with Rhizobium+Azosprillum+Psedomonas. Of course, Rudresh et al. (2005) suggested that plant height not affected significantly with seed inoculation.

Number of pod per plant

the results showed that the highest number of pod per plant recorded in 75 kg urea/ha application and the least number of this trait obtained from control (Table 3). Caliskan et al. (2008) in study of the effects of nitrogen and iron fertilization on growth and yield of soybean, reported that the number of pods per plant increased with N doses up to 80 kg ha^{-1}, but further increase in N dose (120 kg ha^{-1}) did not show a significant effect on this trait.Similar trends were reported by Mckenzie and Hill (1995) and Amany (2007) in chickpea. Seed inoculation increased significantly the number of total pods per plant (Table 3). Plants that inoculated with Rhizobium +Azosprillum +Psedomonas showed more pods per plant than non-inoculated plant. Togay et al (2008) suggested that number of pods per plant affected statistically at seed inoculation in chickpea. These researchers noted that this trait increased from 19.8 pods per plant in non-inoculated plants to 31.65 pods per plant in inoculated plants. Similar results reported by Malik et al. (2006) and Albayrak et al. (2006).

Grain 100 weight

grain 100 weight was affected by nitrogen rates and seed inoculation (Table 2). Increasing of nitrogen application rate decreased significantly the weight of 100-grains in chickpea. The highest 100-grains weight recorded in control while the lowest rate of this trait obtained from 75 kg urea/ha (Table 3). The negative correlation between yield and 100-grains weight was reported in some studies (Walley et al., 2005; Achakzai and Bangulzai, 2006).

Number and weight of nodules per plant

number and weight of nodules per plant showed significant response to nitrogen rates and seed inoculation. The highest number (17.33) and weight (16.35 mgr) of nodules per plant recorded in application of 50 kg urea/ha and the lowest of values (9.67 and 8.89 mgr respectively) of these traits observed in without nitrogen application (Table 3). The highest rate of nitrogen application (75 kg urea/ha) reduced the number and weight of nodules per plant compared to application of 50 kg urea/ha. In order to, increasing of nitrogen rates up 50 kg/ha increased number and weight of nodules per plant but it decreased in application of 75 kg N/ha. Rawsthorn et al. (1985) studied the effects of supplemental nitrate on chickpea and reported that nitrate at 0.71 and 1.43 mM stimulated early nodulation and nodule growth but lager concentrations of nitrate (2.86 mM) decreased significantly nodulation and symbiotic N_2 assimilation. In this study, the results indicated that application of nitrogen at low doses (50 kg urea/ha) had positive effects and higher amounts of nitrogen usage (75 kg urea/ha) can be very harmful for nodulation and biological nitrogen fixation in chickpea. Seed inoculation with rhizobium and PGPR had positive effects on number and weight of nodules per plant. Maximum of these traits (16.91 and 16.023 mgr respectively) wer obtained in seed inoculation Rhizobium+Azosprillum+Psedomonas and the minimum of it was at control treatment. These results concur with observations made by Begum et al., 2001; Adgo and Schulze, 2002; Rudresh et al., 2005; Stancheva et al., 2006 and Ogutcu et al., 2008.

Grain yield

grain yield is the main target of crop production. Data presented in table 2 showed that both of studied experimental factors (nitrogen rates and seed inoculation with rhizobium and PGPR) had significant effects on

grain yield of chickpea. The highest rate of nitrogen fertilizer (75 kg urea/ha) showed the greatest grain yield (1276.78 kg/ha). Seed inoculation with rhizobium and PGPR significantly increased the grain yield. Means comparison showed that maximum of grain yield (1296.26 kg/ha) was obtained in seed inoculation with rhizobium and PGPR and minimum of it was in without seed inoculation. Similar results due to seed inoculation with PGPR have been reported by Dobbelaere et al (2003) and Cakmakı et al (2007). They have been reported that PGPR can increase yield. Yadegari and Asadi Rahmani (2010) have been shown that bean (Phaseolus vulgaris) yield increased in seed priming with PGPR. Similar results have also been reported by Lucas et al (2004) in Glycine max, Peix et al (2001) in chickpea and barley.

Table 2. Analysis of variance for the effects of Nitrogen rates and seed inoculation with PGPR and rhizobium on studied traits in chickpea

		MS					
S.O.V	DF	Plant height	number of pod per plant	grain 100 weight	number of nodules per plant	ight of nodules lant	Grain yield
Replication	2	82157.77 **	8658.13 **	9366.008 **	2541.98 **	2190.8 **	15543072.9 **
Nitrogen rates	4	1909.61 **	252.72 **	198.84 **	59.51 **	70.48 **	250851.6 **
Seed inoculation with PGPR and rhizobium	3	1525 **	319.82 **	29.15 **	184.53 **	198 **	353347.9 **
Seed inoculation with PGPR × Nitrogen rates	12	7.19 ns	9.23 ns	7.46 ns	0.91 ns	4.51 ns	842.3 ns
Error	38	131.3	22.21	10.33	8.56	9.93	26319.8

Dry matter accumulation

stages of dry matter accumulation at treatment compounds of nitrogen rates× seed inoculation with PGPR and rhizobium showed that in all treatments, total dry matter increased slowly until 25 days after sowing. From 25 till 105 days after sowing increased rapidly. From 105 till 115 days after sowing (harvest time), it decreased due to increasing aging of leaves and decreasing of crop growth rate (FIG 1). Maximum of total dry matter (374 gr/m^2) belonged to the higher nitrogen rates (75 kg urea/ha) and seed inoculation with rhizobium +Azosprillum +Psedomonas (fig 1-D) and minimum of it was in without of nitrogen application and seed inoculation (fig 1–A). Bashan et al (2004) and Cakmake et al (2002) reported that inoculation of seeds with Azospirillum could result in significant changes in various growth parameters, such as increase in total plant biomass. The increase in dry matter accumulation with seed priming with PGPR and rhizobium legominozarum indicates the favorable response of chickpea to seed priming. Similar observations were also made by Lucas et al (2004) in soybean, Peix et al (2001) in chickpea and barley.

Table3. Means comparison of effects of nitrogen rates and seed inoculation with PGPR and rhizobium on studied traits in chickpea

characteristics Treatments	Plant Height (cm)	number of pod per plant	grain 100 weight (gr)	number of nodules per plant	ight of nodules nt (mg)	Grain yield (kg/ha)
Nitrogen rates (kg/ha)						
Zero	c 69.37	c 21.59	29.28 a	9.67 c	c 8.89	931.34 c
25	b 80.63	24.86 bc	ab 28.14	16.82 a	16.3 a	1071.22 b
50	b 85.07	27.4 b	27.56a b	17.33 a	a 16.35	1210.22 a
75	a 93.58	32.48 a	25.93 b	b 14.001	b 12.038	1276.78 a
LSD	8.47	3.48	2.37	12.16	2.33	119.92
Seed inoculation with PGPR and rhizobium						
without inoculation seed as control	d 62.27	19.8 c	22.43 d	11.054 c	c 9.82	d 916.5
seed inoculation with rhizobium	c 78.46	b 24.74	25.91 c	b 13.75	bc 12.28	c 1075.41
seed inoculation with rhizobium +Azosprillum	bc 84.39	b27.8a	bc 27.74	14.86 ab	ab 14.069	cb 1138.76
seed inoculation with rhizobium +Psedomonas	89.12 ab	a 29.36	29.3 b	ab 15.7	ab 14.85	ab 1202.98
seed inoculation rhizobium +Azosprillum +Psedomonas	a 96.07	31.65 a	a 33.38	16.91 a	a 16.023	a 1296.26
LSD	9.47	3.89	2.65	2.41	2.6	134.08

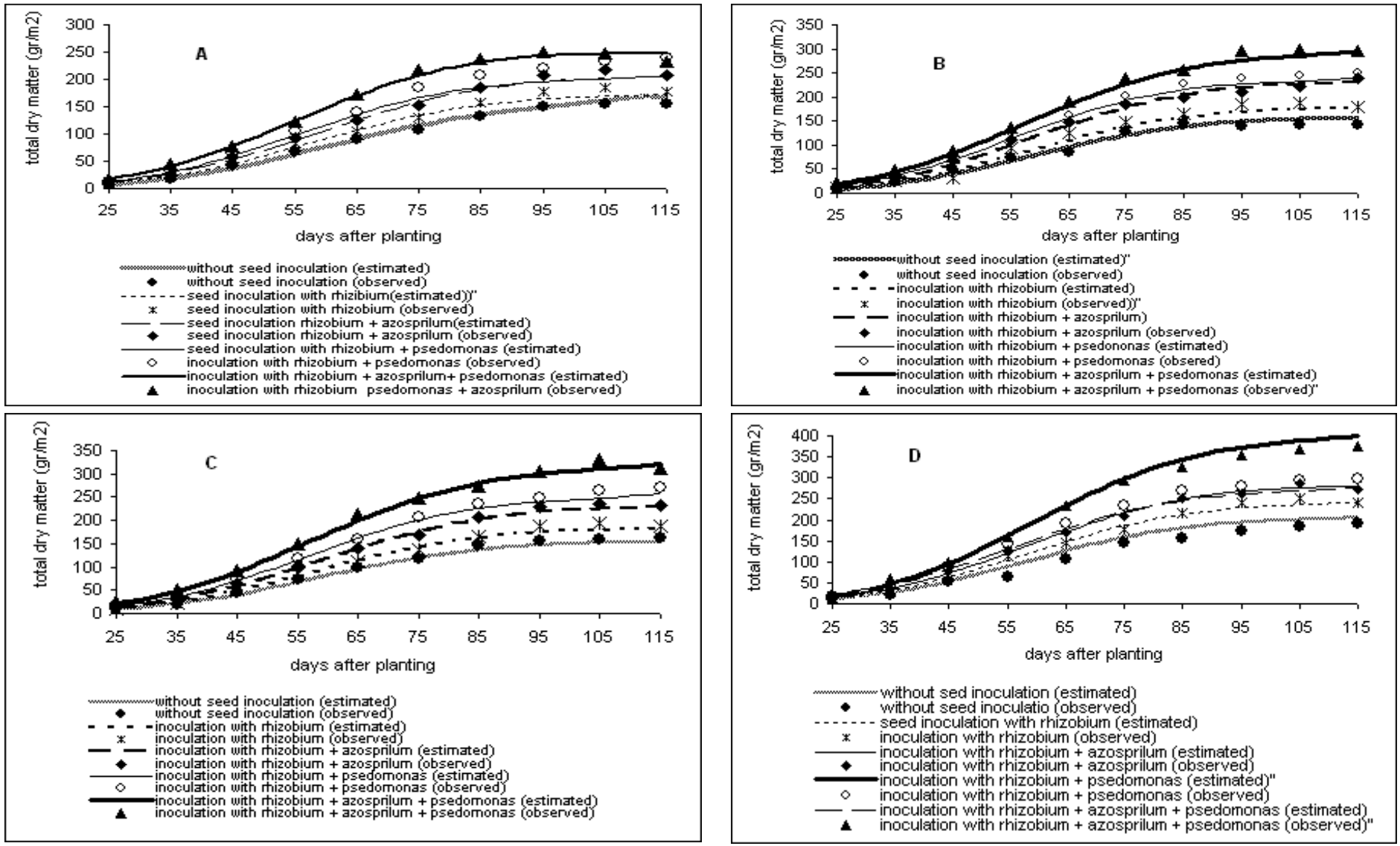

Figure 1. The effects of nitrogen rates and seed inoculation with PGPR and rhizobium on dry matter accumulation in without without of nitrogen application (A), application of 25 kg urea (B) , application of 50 kg urea (C), application of 75 kg urea (D)

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