Seed Inoculation and Inorganic Nitrogen Fertilization Effects on Some Physiological and Agronomical Traits of Chickpea (*Cicer arietinum* L.) in Irrigated Condition

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Abstract

The effects of seed inoculation with *Rhizobium* and inorganic nitrogen fertilization on some physiological and agronomical traits of chickpea (*Cicer arietinum* L.) cv. ILC 482, were investigated at the Experimental Farm of the Agriculture Faculty, University of Mohaghegh Ardabili. The trial was laid out in split plot design based on randomized complete block with four replications. Experimental factors were mineral nitrogen fertilizer at four levels (0, 50, 75 and 100 kg urea ha^{-1}) in the main plots, and two levels of inoculation with *Rhizobium* bacteria (with and without inoculation) as sub plots. N application and *Rh.* inoculation showed positive effects on physiological and agronomical traits of chickpea. The highest value of leaf RWC was recorded at 50 kg urea ha^{-1} what was statistically in par with 75 kg urea ha^{-1} application, while the usage of 75 kg urea ha^{-1} showed the maximum stem RWC. The maximum CMS was obtained from application of 75 kg urea ha^{-1}. Chlorophyll content, leaf area index and grains protein content showed their maximum values in the highest level of nitrogen usage (100 kg urea ha^{-1}). Moreover, inoculated plants had the highest values of all physiological traits. In the case of agronomical traits, the highest values of plant height, number of primary and secondary branches, number of pods per plant, number of grains per plant, grain and biological yield were obtained from the highest level of nitrogen fertilizer (100 kg urea ha^{-1}) and *Rh*. inoculation. Application of 75 kg urea ha^{-1} was statistically in par with 100 kg urea ha^{-1} in all of these traits. The results pointed out that some N fertilization (i.e. between 50 and 75 kg urea ha^{-1}) as starter can be beneficial to improve growth, development, physiological traits and total yield of inoculated chickpea.

Keywords: Cell membrane stability, Chickpea, Chlorophyll content, Nitrogen fertilizer, Relative water content, *Rhizobium* inoculation, Yield

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops throughout the world. It is cultivated on a large scale in arid and semi-arid environment, and has considerable importance as food, feed and fodder (Erman, et al., 2011). This annual legume is a significant contributor to agricultural sustainability through N_2-fixation and as a rotation crop allowing the diversification of agricultural production systems. World chickpea production has increased steadily in the past two decades (Ogutcu, et al., 2008; Namvar, et al., 2011).

Nitrogen (N) deficiency is frequently a major limiting factor for crops production in over the world (Fuzhong, et al., 2008; Salvagiotti, et al., 2008; Aminifard, et al., 2010). The most important role of nitrogen in the plant is its presence in the structure of protein and nucleic acids, which are the most important building and information substances of every cell. In addition, nitrogen is also found in chlorophyll that enables the plant to capture energy from sunlight by photosynthesis. Thus, nitrogen supply to a plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Moreover, it influences cell size, leaf area and photosynthetic activity (Caliskan, et al., 2008; Dordas and Sioulas, 2008; Ralcewicz, et al., 2009; Waraich, et al., 2011). Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crops.

Increasing and extending the role of biofertilizers such as *Rhizobium* can reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Werner and Newton, 2005; Chemining wa and Vessey, 2006; Albayrak, et al., 2006; Appunu, et al., 2008). As a legume, chickpea can obtain a significant portion (4-85%) of its N_2 requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium* strains (Saini, et al., 2004; Rudresh, et al., 2005). Chickpea and *Rhizobium leguminosarum* subsp. *ciceri* association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain and environmental factors (Ogutcu, et al., 2008). The inoculation of seeds with *Rhizobium* is known to increase nodulation, protein and chlorophyll content, nitrogen uptake, growth and yield parameters of legume crops (Sogut, 2006; Togay, et al., 2008; Erman, et al., 2011; Namvar, et al., 2011).

Crop production during the summer months in the semi-arid environment of the Mediterranean regions relies on irrigation. The limited water resources in these areas and the cost of pumping irrigation water are the most important factors that force many farmers to reduce irrigation in many arid and semi-arid regions (Manavalan, et al., 2009; Mansouri-far, et al., 2010). Evaluation of plant water status plays an important role in assessing plant growth, predicting potential yield and monitoring the general physiological status of vegetation stands (Waraich, et al., 2011). Relative water content (RWC) is considered to be a reliable parameter for quantifying plant water status and also is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues (Farooq and Azam, 2006; Manavalan, et al., 2009). Plant water status is intimately related to several physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration. It is defined that decrease of relative water content close stomata and also after blocking of stomata will reduce photosynthesis rate. Moreover, there are reports about decrease of chlorophyll in these conditions (Saneoka, et al., 2004; Mansouri-far, et al., 2010).

Cell membranes are one of the first targets of many undesirable environmental factors and it is generally accepted that the maintenance of their integrity and stability is one of the major components of achieving high and acceptable yield. Cellular membrane dysfunction due to the various environmental conditions is well expressed in increased permeability and leakage of ions, which can be measured by the efflux of electrolytes. Hence, the estimation of membrane dysfunction by measuring cellular electrolyte leakage, lead to use of cell membrane stability (CMS) as an index to assess membrane status (Saneoka, et al., 2004; Farooq and Azam, 2006; Manavalan, et al., 2009).

Determination of the agronomical and physiological response of chickpea crop to N fertilization and *Rhizobium* inoculation is very important to maximize yield and economic profitability of chickpea production in a particular environment. Moreover, it seems that there is no investigation about the combined effects of nitrogen fertilization and *Rhizobium* inoculation on some physiological traits such as water status, cell membrane stability and chlorophyll content of legume crops i.e. chickpea in irrigated plants. But, it is important to elucidate the effects of different agronomical factors on these parameters under normal conditions in order to use of these findings to improve plants agronomic performances in stressed environment. Considering the above facts, the present study was undertaken to elucidate the effects of seed *Rhizobium* inoculation and inorganic nitrogen fertilization on some agronomical and physiological traits of chickpea.

MATERIALS AND METHODS

Field experiments were conducted at the Experimental Farm of Mohaghegh Ardabili University. The area is located at latitude 38˚15´ N and longitude 48˚15´ E at an altitude of 1350 m above the mean sea level. Climatically, the area placed in the semi arid temperate zone with cold winter and moderate summer. Average rainfall is about 400 mm and most of rainfall is concentrated between winter and spring. The soil was silty loam, with pH about 7.9 and Ec about 2.3 ds m^{-1}.

The experimental design was split plot in randomized complete block design with four replications. Main plot treatments consisted of four N fertilizer rates: 0, 50, 75 and 100 kg urea/ha. Sub plot treatments were the two levels of inoculation with *Rhizobium* (inoculated and non-inoculated).

Nitrogen fertilizer in each level was divided into three equal parts; the first part of the N was broadcasted by hand and incorporated immediately in planting time, and second and third parts were applied in the stages of 6-8 leaves and flowering. Seeds of inoculation treatments were inoculated with *Rhizobium legominosarum* bv. *Ciceri* just before planting. Inoculant was obtained from the Soil and Fertilizer Research Institute, Tehran.

The area was mold board-ploughed and disked before planting. Seeds of chickpea (*Cicer arietinum* L.) cv. ILC 482 were hand planted on 14 May in five rows plots, 5 m long with spacing of 0.5 m between rows. Two seeds were sown per hill. The field was immediately irrigated after planting to ensure uniform germination. After germination, the plants were thinned to one seedling per hill to obtain about 36 plants per m^{-2}. Weeds were controlled over the growth period with hand hoeing.

Relative water content (RWC) of leaf and stem was measured separately. Three plants were selected per plot. Then, leaves and stems of basal parts of plants were separated and weighed immediately to record fresh weight (FW). In order to determine the turgid weight (TW), which represents fully hydrated weight, the samples were floated in distilled water inside a closed Petri dish. At the end of the imbibition period, samples were placed in an oven at 70 ˚C for 24 h, so as to obtain dry weight (DW). The RWC were determined by the equation proposes by Mansouri-far et al. (2010):

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Cell membrane stability (CMS) was determined according to the method of Farooq and Azam (2006). For this purpose, 10 g of fully expanded leaves obtained from three plants that selected from each plot. These samples were submerged into distilled water contained in test tubes. The tubes were kept at 10 ˚C for 24 h, followed by warming at 25 ˚C and measuring the electrical conductivity of the contents. The leaf samples were then killed by autoclaving for 15 min and electrical conductivity of the medium measured again. CMS was determined by using the equation: $CMS = (1 - \frac{C_1 - C_W}{C_2 - C_W}) \times 100$

Where, C_1 and C_2 refer to electrical conductivity of the samples before and after autoclaving, respectively and C_w refer to electrical conductivity of the distilled water.

Leaves chlorophyll content was measured with a portable chlorophyll meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan) and Leaf area index was determined using the LI-COR model 3100 LI Area Meter. Seed protein content was recorded by seed analyzer device (Zeltex, ZX9500; Japan).

The plants were harvested at maturity on 8 September and yield components such as plant height, number of primary and secondary branches per plant, number of total pods, number of grains per pod and number of grains per plant was recorded on 15 randomly selected plants in each plot. Grain and biological yield was determined by harvesting the middle three rows of each plot. Analysis of variance was done using SAS computer software package and the mean values were compared with Duncan multiple range test (DMRT) at 0.05 probability level.

RESULTS AND DISCUSSION

Physiological traits

Leaf relative water content (L RWC) and stem relative water content (S RWC): Relative water content of leaf and stem showed significant response to studied experimental factors (Nitrogen application and *Rhizobium* inoculation). The highest value of leaf RWC recorded in 50 kg urea/ha (7.75% increasing over the control) that was statistically in par with 75 kg urea/ha application while, usage of 75 kg urea/ha showed the maximum stem RWC (6.26% increasing over the control). The highest rate of nitrogen application (100 kg urea/ha) reduced significantly both of the leaf and stem

RWC (Table 1). Moreover, inoculated plants showed more leaf and stem RWC than non-inoculated plants. *Rhizobium* inoculation increased the leaf and stem RWC by 3.30 and 4.28%, respectively compared to non-inoculated plants (Table 1). These results are in accordance with the findings of Saneoka et al. (2004), Fuzhong et al. (2008) and Gholinezhad et al. (2009). It has been showed that increasing nitrogen application will increase the protein synthesis, increase cell wall thickness and cause absorption of extra water by protoplasm and improve the relative water content (Saneoka, et al., 2004). Decreasing of RWC under higher levels of nitrogen application may be due to the increasing of plants leaf area and losing of more water in this condition.

Table1: Effects of nitrogen fertilization and *Rhizobium* inoculation on studied physiological traits in chickpea (*Cicer arietinum* L.)

Treatments	L RWC (%)	S RWC (%)	CMS (%)	Chlo (SPAD index)	LAI	Pro (%)
Nitrogen rates (kg urea/ha)						
0	69.67 **c**	76.25 **c**	65.16 **c**	37.09 **c**	2.41 **c**	15.74 **c**
50	75.07 **a**	79.49 **ab**	68.77 **ab**	39.64 **b**	2.77 **b**	17.86 **b**
75	73.24 **ab**	81.02 **a**	70.21 **a**	41.27 **ab**	3.06 **a**	19.52 **a**
100	71.95 **b**	78.33 **b**	67.32 **b**	42.38 **a**	3.03 **a**	20.41 **a**
***Rhizobium* inoculation**						
non	71.46 **b**	77.12 **b**	66.47 **b**	39.03 **b**	2.72 **b**	17.74 **b**
with	73.82 **a**	80.42 **a**	69.26 **a**	41.15 **a**	2.91 **a**	19.02 **a**
Mean	**72.64**	**78.77**	**67.86**	**40.09**	**2.81**	**18.38**
Nitrogen (N)	**	**	**	**	**	**
***Rhizobium* inoculation (*Rh.*)**	*	*	**	**	**	**
N × *Rh.*	ns	ns	ns	ns	*	ns
CV (%)	**11.36**	**10.87**	**11.54**	**13.68**	**12.69**	**10.01**

L RWC: Leaf Relative Water Content, **S RWC:** Stem Relative Water Content, **CMS:** Cell Membrane Stability, **Chlo:** Chlorophyll Content, **LAI:** Leaf Area Index, **Pro:** Protein Content. - Mean values followed by same letters in each column and treatment showed no significant difference by DMRT (P = 0.05). - *, ** and ns showed significant differences at 0.05, 0.01 probability levels and no significant, respectively.

Cell membrane stability (CMS): The cell membrane stability was significantly affected by nitrogen application and inoculation with *Rhizobium*. The highest CMS was observed in the application of 75 kg urea/ha. This rate of nitrogen fertilization showed about 7.75% increasing over the control (0 kg urea/ha). Usage of 100 kg urea/ha decreased significantly CMS in chickpea plants (Table 1). Moreover, inoculated plants had statistically more CMS than non-inoculated plants. *Rh.* inoculation increased the CMS by 4.20% compared to plants that no inoculated with *Rh.* bacteria (Table 1). Saneoka et al. (2004) reported similar results. These researchers stated that higher N levels helped to increase CMS which suggest that N nutrition may play an important role in maintaining CMS under different conditions.

Chlorophyll content (Chlo) and Leaf area index (LAI): Nitrogen fertilization and *Rh.* inoculation had statistically significant effects on chlorophyll content and leaf area index in chickpea (Table 1). Increasing of nitrogen fertilizer amount increased significantly both of these traits. The highest values of chlorophyll content were recorded in the highest rate of nitrogen usage (100 kg urea/ha) while, no application of nitrogen fertilizer showed the lowest magnitudes of chlorophyll content (Table 1). In the same trend, the maximum and the minimum LAI of chickpea observed in the application of 100 and 0 kg urea/ha, respectively (Table 1). The increasing of LAI was attributed to the increase in leaf number and total leaf area/plant (Caliskan, et al., 2006). Application of 100 kg urea/ha increased chlorophyll content and LAI about 14.26 and 25.37%, respectively compared to application of 0 kg urea/ha.

Moreover, inoculated plants showed more chlorophyll content and LAI than non-inoculated plants. *Rhizobium* inoculation increased chlorophyll content and LAI by 5.43 and 6.99%, respectively compared to non-inoculated plants (Table 1).

LAI of Chickpea plants was affected significantly with the interactions between nitrogen application and *Rh*. inoculation (Table 1). The highest value of LAI was recorded in the plants treated with 75 kg urea/ha and inoculated with *Rhizobium*. The minimum values of this trait were observed in the lowest level of nitrogen application (0 kg urea/ha) and non-inoculated plants (Figure 1).

More nitrogen consumption resulted in increasing chlorophyll content and leaf area index and their preservation until the end of growth period. The results of this study about the chlorophyll content and leaf area agree well with the reports of Dordas and Sioulas, (2008), Gholinezhad et al. (2009), Aminifard et al. (2010) and Mansouri-far et al. (2010). Waraich et al. (2011) noted that nitrogen application, where light is not limiting, increases antioxidative defense mechanisms, resulting in reduced photooxidation of chloroplast pigments, and reduced leaf senescence. Moreover, the increase in chlorophyll content and LAI in the presence of bio-inoculant could be due to the effective symbiosis and positive effects of *Rhizobium* bacteria on growth and development of chickpea plants (Namvar, et al., 2011).

Protein content (Pro): As shown in Table 1, nitrogen application and *Rh.* inoculation had significant effects on protein content of chickpea grains. The highest protein content observed in the application of 100 kg urea/ha that was statistically in par with the usage of 75 kg urea/ha. The highest rate of nitrogen application increased grains protein content by 29.67% compared to the control. Moreover, plants treated with *Rh.* bacteria showed more protein content than control. *Rh.* inoculation increased in average grains protein content about 6.27% compared to non-inoculated plants (Table 1). These results are according to the reports of Adgo and Schulze, (2002) and Ralcewicz et al. (2009). It has been found that, when there is an adequate supply of N in the soil, leaf senescence is slower and the plant is able to supply its seeds with N and photoassimilate for a longer period, which results in higher protein and grain yield (Erman, et al., 2011).

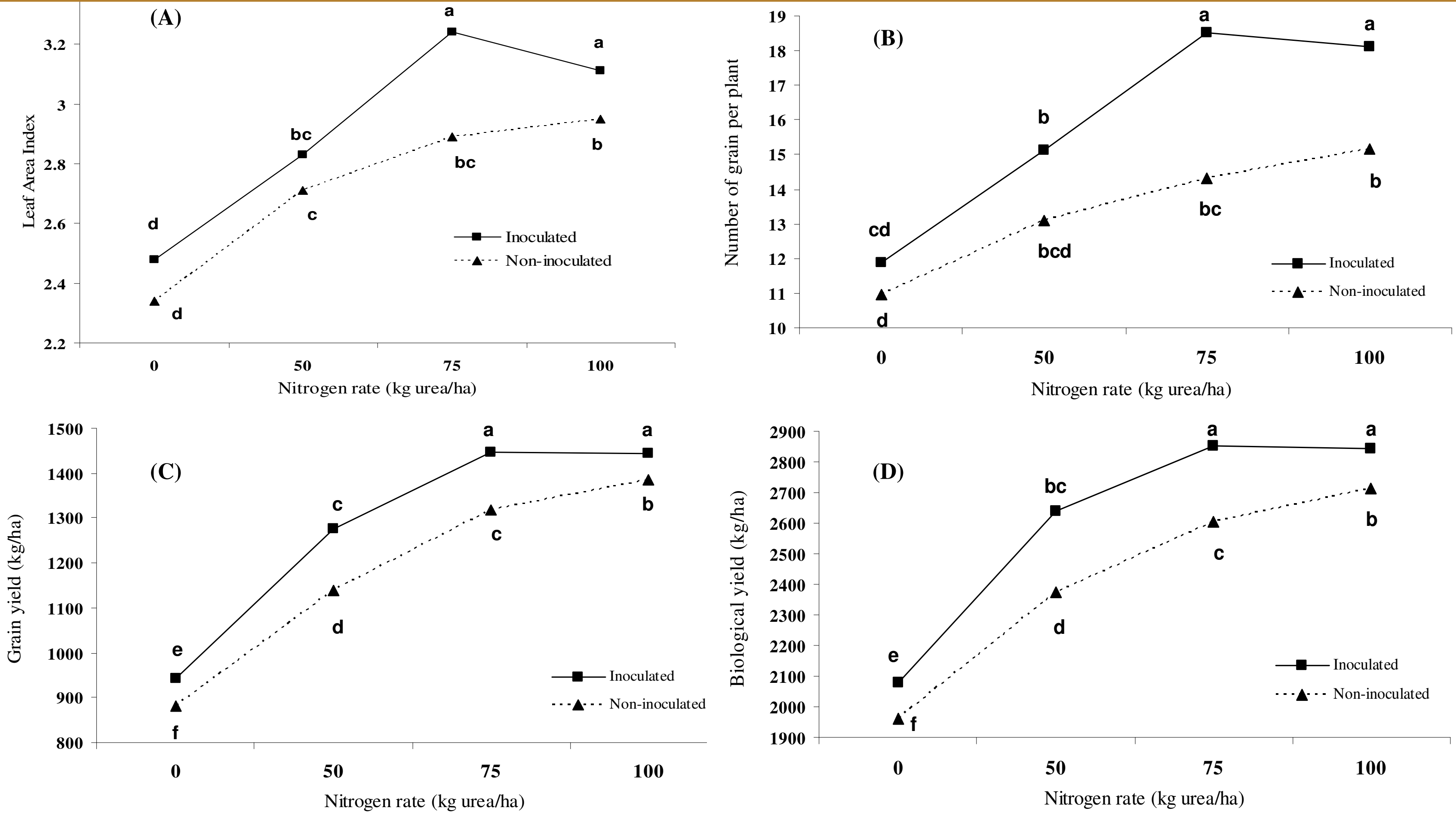

Figure 1. Effects of different levels of nitrogen application under *Rhizobium* inoculation and non-inoculation on leaf area index (A), number of grains per plant (B), grain yield per unit of area (C) and biological yield (D) in chickpea (*Cicer arietinum* L.). Values with same letters in each trait are not significantly different (DMRT at 5% level)

Yield and its components

Plant height (P H): The plant height was significantly affected by nitrogen application, while inoculation with *Rh*. showed no significant effects on this trait (Table 2). The highest plant height was observed in the maximum rate of nitrogen application (100 kg urea/ha) that not differed significantly from 75 kg urea/ha. The minimum plant height was recorded in control. Application of 100 kg urea/ha increased plant height by 37.30%, compared to control (Table 2). These results are in line with the findings of Amany (2007), Caliskan et al. (2008) and Aminifard et al. (2010) who reported that plant height was increased with application of nitrogen fertilizer. Although variance analysis of data indicated that *Rh.* inoculation had no significant effects on plant height of chickpea but, comparisons of means showed that the inoculated plants had greater height than non-inoculated ones. Rudresh et al. (2005) also stated that plant height was not affected significantly with *Rh*. inoculation.

Number of primary branches (N P B) and secondary branches (N S B) per plant: Number of primary and secondary branches per plant increased with increasing of nitrogen application rate. Increasing of nitrogen fertilizer from 0 to 100 kg urea/ha increased the number of primary and secondary branches per plant by 36.27 and 100.21%, respectively. The lowest and the highest values of these traits were recorded in 0 and 100 kg urea/ha, respectively (Table 2). Amany (2007) and Caliskan et al. (2008) reported similar results in chickpea and soybean, respectively.

Moreover, the greatest number of the primary and secondary branches per plant was recorded in inoculated plants. Inoculation with *Rh*. increased the number of primary and secondary branches by 9.29 and 14.26% respectively, than non-inoculated plants (Table 2). These results are in agreement with Rudresh et al. (2005) and Togay et al. (2008).

Table 2: Effects of nitrogen fertilization and *Rhizobium* inoculation on yield and it's components in chickpea (*Cicer arietinum* L.)

Treatments	P H (cm)	N P B (per plant)	N S B (per plant)	N P (per plant)	N G (per pod)	N G (per plant)	G Y (kg/ha)	B Y (kg/ha)
Nitrogen rates (kg urea/ha)								
0	33.47 **c**	1.93 **c**	4.67 **c**	17.85 **c**	1.03 **b**	11.42 **c**	911.5 **c**	2016.2 **c**
50	37.43 **b**	2.31 **b**	6.61 **b**	19.42 **b**	1.11 **ab**	14.12 **b**	1207.3 **b**	2506.1 **b**
75	44.37 **a**	2.60 **a**	8.51 **a**	20.61 **a**	1.18 **a**	16.41 **a**	1328.2 **a**	2729.3 **a**
100	45.97 **a**	2.63 **a**	9.35 **a**	21.30 **a**	1.17 **a**	16.63 **a**	1413.6 **a**	2778.2 **a**
***Rhizobium* inoculation**								
non	41.25 **a**	2.26 **b**	6.80 **b**	18.97 **b**	1.08 **a**	13.38 **b**	1166.5 **b**	2413.1 **b**
with	39.37 **a**	2.47 **a**	7.77 **a**	20.62 **a**	1.15 **a**	15.91 **a**	1263.7 **a**	2602.7 **a**
Mean	**40.31**	**2.36**	**7.28**	**19.79**	**1.12**	**14.64**	**1215.15**	**2507.45**
Nitrogen (N)	**	**	**	**	ns	**	**	**
***Rhizobium* (*Rh.*)**	ns	*	**	**	ns	**	**	**
N × *Rh.*	ns	ns	ns	ns	ns	*	*	*
CV (%)	**13.63**	**10.30**	**10.96**	**16.29**	**11.02**	**16.10**	**12.34**	**14.29**

- **P H:** Plant Height, **N P B:** Number of Primary Branches, **N S B:** Number of Secondary Branches, **N P:** Number of Pods, **N G:** Number of Grains, **G Y:** Grain Yield, **B Y:** Biological Yield
- Mean values followed by same letters in each column and treatment showed no significant difference by DMRT (P = 0.05). - *, ** and ns showed significant differences at 0.05, 0.01 probability levels and no significant, respectively.

Number of total pods (N P): As shown in Table 2, number of total pods per plant showed significant response to nitrogen fertilization and *Rhizobium* inoculation. The highest number of total pods per plant was recorded in application of 100 kg urea/ha with no significant difference from application of 75 kg urea/ha. The least number of pods was obtained in control. Application of 100 kg urea/ha increased the number of pods per plant about 19.32% compared to control in each plant (Table 2). Caliskan et al. (2008) investigated the effects of nitrogen and iron fertilization on growth and yield of soybean and reported that the number of pods per plant increased with N doses up to 80 kg/ha, but further increase in N dose (120 kg/ha) did not show a significant effect on this trait. Similar trends were reported by Adgo and Schulze (2002) and Amany (2007) in chickpea.

Inoculation with *Rh*. bacteria increased significantly the number of total pods per plant (Table 2). Plants inoculated with *Rh*. showed about 8.69% more pods per plant than non-inoculated plants. Togay et al. (2008) observed that number of pods per plant affected statistically significant with *Rh*. inoculation in chickpea. These researchers noted that this trait was increased from 11.50 pods per plant in non-inoculated plants to 12.35 pods per plant in inoculated plants. Albayrak et al. (2006) and Namvar et al. (2011) reported similar results.

Number of grain per pod and number of grain per plant (NG): Although variance analysis of data indicated that nitrogen fertilization and *Rh*. inoculation had no statistically significant effects on number of grains per pod however, the highest magnitude of this trait was observed in 75 kg urea/ha application and *Rh*. inoculation (Table 2). In a study on the chickpea was shown that *Rh.* inoculation had no significant effect on number of grain per pod (Namvar, et al., 2011). Non significant effects of studied treatments on the number of grains per pod may be the consequence of strong genetic determination of this trait rather than environmental and management factors.

The significant main effect of nitrogen fertilization and *Rh*. inoculation, and significant nitrogen fertilization × *Rh.* inoculation interactions (Table 2) were obtained in chickpea for number of grains per plant. The highest number of grains per plant was recorded in inoculated plants treated with 75 kg urea/ha. The least value of this trait was obtained from non-inoculated and non-fertilized plants (Figure 1). Application of 75 kg urea/ha in inoculated plants increased the number of grains per plant by 68.97% compared to control. Previous studies showed the positive effects of nitrogen application (Amany, 2007) and *Rh*. inoculation (Togay, et al., 2008) on number of grains per plant.

Furthermore, as shown in Figure 1, inoculation with *Rh*. bacteria had the greatest effect on number of grains per plant in 75 kg urea/ha rather than other fertilizer levels that may be due to more effectiveness of *Rh*. inoculation in this level compared to other levels of nitrogen usage. Inoculation increased the number of grains per plant about 29.51% in 75 kg urea/ha than non-inoculated plants in same fertilizer level.

Grain yield (G Y): Data presented in table 2 showed that both of studied experimental factors (Nitrogen application and *Rhizobium* inoculation) had significant effects on grain yield of chickpea. The highest rate of nitrogen fertilizer (100 kg urea/ha) showed the greatest grain yield, however this rate of nitrogen fertilizer was in par with 75 kg urea/ha. Application of 100 kg urea/ha increased grain yield by 55.08% compared to the control. Furthermore, inoculated plants indicated higher grain yield rather than non-inoculated plants. Inoculation with *Rh*. increased grain yield about 8.33% above the control (Table 2).

Interaction effects of nitrogen fertilization and *Rh*. inoculation were found significant in grain yield of chickpea (Table 2). Grain yield continuously increased with increasing of N application in inoculated and non-inoculated plants. However, grain yield of chickpea increased until 75 kg urea/ha and further increase in N rate resulted in no significant grain yield increase (Figure 1). Moreover, grain yield of inoculated plants in all rates of nitrogen application was higher than in the non-inoculated plants in the same rate of nitrogen application (Figure 1). The highest grain yield was recorded in inoculated plants with 75 kg urea/ha application. The lowest rate of nitrogen application showed the lowest grain yield in non-inoculated plants (Figure 1). Study of the interactions between N application and inoculation showed that inoculation with *Rh*. bacteria had greater effect on grain yield in 75 kg urea/ha than other levels of fertilizer application (Figure 1).

Other researchers reported similar results about the effects of nitrogen application (Walley, et al., 2005; Amany, 2007; Caliskan, et al., 2008; Aminifard, et al., 2010; Mansouri-far, et al., 2010; Namvar, et al., 2011;) and *Rh.* inoculation (Albayrak, et al., 2006; Chemining wa and Vessey, 2006; Appunu, et al., 2008; Togay, et al., 2008; Ralcewicz, et al., 2009; Erman, et al., 2011) on grain yield in various crops.

Biological yield (B Y): Biological yield of chickpea also showed the same trend as grain yield. As shown in Table 2, the highest biomass obtained from the application of 100 kg urea/ha, however there was no significant difference among the application of 75 and 100 kg urea/ha in biological yield. Usage of 100 kg urea/ha increased the biological yield by 37.79%, compared to control.

Nitrogen is known to be an essential nutrient for plant growth and development (Werner and Newton, 2005; Sogut, 2006) that involved in vital plant function such as photosynthesis, DNA synthesis, protein formation, respiration and N_2 fixation (Werner and Newton, 2005; Caliskan, et al., 2008; Salvagiotti, et al., 2008). The growth parameters such as LAI, biomass, and leaf photosynthesis significantly decrease due to unsatisfactory N availability (Adgo and Schulze, 2002; Chemining wa and Vessey, 2006; Appunu, et al., 2008; Dordas and Sioulas, 2008). Results obtained from this study indicated that usage of N fertilization had positive effects on chickpea physiological and agronomical traits. Adding N increases the production of total dry matter in plants (Fuzhong, et al., 2008; Salvagiotti, et al., 2008; Mansouri-far, et al., 2010) which can increase the potential of plant to produce more plant height, branches, pods and seeds that ultimately resulted in high grain and biological yield. Nitrogen fertilization increases the total dry matter for number of reasons: (i). Nitrogen can increase the LAI in plants (Caliskan, et al., 2008; Gholinezhad, et al., 2009, Aminifard, et al., 2010). More LAI increases the interception of solar radiation by plants that resulted in the more accumulation in plants (Waraich, et al., 2011). (ii). Nitrogen can increase the photosynthesis rate in plants. Increasing photosynthetic rate with N fertilization can be attributed to increasing amount of chlorophyll pigments, since N is one of the main components of chlorophyll (Caliskan, et al., 2008; Mansouri-far, et al., 2010). In contrast, supplementation of adequate nitrogen to crops can increase their growth and development. In this condition, plants are able to produce higher values of yield components that result in higher grain yield.

Moreover, inoculated plants showed more biomass than non-inoculated plants. Inoculation with *Rh*. bacteria increased the biological yield about 7.85% compared to control (Table 2). Inoculation of legumes with rhizobia, for the purpose of enhancing N_2 fixation and yield in legume crops, is possibly the oldest and most common method of voluntary release of microbes into the environment (Werner and Newton, 2005). The influence of *Rh*. bacteria on promoting legumes growth was documented in many researches (Saini, et al., 2004; Albayrak, et al., 2006; Appunu, et al., 2008). The observed benefits on chickpea by *Rh*. inoculation seem to be due to the supply of N to the crop (Chemining wa and Vessey, 2006; Togay, et al., 2008). Moreover, there could be released growth promoting substances (phytohormones) produced by these organisms. *Rhizobium* bacteria synthesized phytohormones like auxin as secondary metabolites in inoculated plants. Phytohormones are known to play a key role in plant growth regulation. They promote seed germination, root elongation and stimulation of leaf expansion. In addition, great root development and proliferation of plants in response to *Rh*. activities enhance water and nutrient uptake (Werner and Newton, 2005) that result in a better cell membrane stability and relative water content.

Interactions between different levels of nitrogen and *Rh*. inoculation were found significant in biological yield (Table 2). Generally, inoculation with *Rh*. in all levels of nitrogen application increased biomass production rather than non-inoculated plants (Figure 1). The highest biological yield recorded at the plots of 75 kg urea/ha and *Rh.* inoculation. Control plots (non-fertilized and non-inoculated) had the lowest values of this trait (Figure 1). These results are in accordance with the works of Adgo and Schulze (2002), Saini et al. (2004), Togay et al. (2008), Appunu et al. (2008) and Namvar et al. (2011). Sogut (2006) stated that supremacy of symbiotic N versus combined N is explained as: symbiotic N is already in the organic reduced form and hence, more readily available for plant metabolism. In contrast, in the absence of symbiotic N, plant must spend a lot of energy to take up nitrates and reduce them to the level of NH_3. Thus, inoculation resulted in greater dry matter yield compared to N fertilization.

CONCLUSION

Results obtained from this study clearly indicated that nitrogen application and *Rhizobium* inoculation had significant effects on physiological and agronomical traits of chickpea (*Cicer arietinum* L.) cv. ILC 482. The highest value of leaf RWC was recorded in 50 kg urea/ha that was statistically in par with 75 kg urea/ha application while, usage of 75 kg urea/ha showed the maximum stem RWC. The maximum CMS was obtained form application of 75 kg urea/ha. Chlorophyll content, leaf area index and grains protein content showed their maximum values in the highest level of nitrogen fertilization (100 kg urea/ha). Moreover, inoculated plants had the highest values of all physiological traits. In the case of agronomical traits, the highest values of plant height, number of primary and secondary branches, number of pods per plant, number of grains per plant, grain and biological yield were obtained from the highest level of nitrogen fertilizer (100 kg urea/ha) and *Rh*. inoculation. Application of 75 kg urea/ha was statistically in par with 100 kg urea/ha in all of these traits. The results pointed out that *Rh.* seed inoculation and some N fertilization (i.e. between 50 and 75 kg urea/ha) as a starter can be beneficial to improve growth, development, physiological traits and total yield of inoculated chickpea.

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