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Phenological and morphological response of chickpea (*Cicer arietinum* L.) to symbiotic and mineral nitrogen fertilization

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Abstract

The effects of *Rhizobium* inoculation as biofertilizer and mineral nitrogen fertilization on development of phenological stages and some morphological traits of chickpea (*Cicer arietinum* L.) cv. ILC 482 were investigated in 2008, at the Experimental Farm of Mohaghegh Ardabili University. The trial was laid out in spilt plot design based on randomized complete block with four replications. Experimental factors were inorganic 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. Application of N fertilizer and *Rhizobium* inoculation affected significantly the morphological traits of chickpea. The highest values of morphological traits were recorded in the high levels of nitrogen usage and *Rhizobium* inoculation. Number of days from sowing to flowering, flowering to podding, podding to maturity and growth period increased significantly with increasing nitrogen application amount. Appearance of phenological stages and growth period accelerated significantly in inoculated plants. Moreover, the highest grain yield (1446.6 kg ha^{-1}) and biological yield (2853.2 kg ha^{-1}) were observed in the inoculated plants that were treated with 75 kg urea ha^{-1}. Results obtained from this study indicated that plants with longer growth period produced higher biological and grain yield compared to plants that had shorter growth period. It seems that, application of 75 kg urea ha^{-1} can be beneficial to improve growth and final yield of inoculated chickpea in studied area.

Key words: *Cicer arietinum*, grain yield, morphological traits, phenological stages, *Rhizobium* inoculation.

Introduction

Chickpea (*Cicer arietinum* L.) is the third most widely grown grain legume in the world after bean and soybean (Soltani et al., 2006). The Mediterranean origin of the crop imparts special significance to chickpea in the agriculture of this area, where it has multiple functions in the traditional farming systems. Besides being an important source of human and animal food, the crop also plays an important role in the maintenance of soil fertility, particularly in the dry and rainfed area. In addition, it is also widely used as green manure. Chickpea seeds contain about 20.6% protein, 61.2% carbohydrates and 2.2% fat (Saini et al., 2004; Werner, Newton, 2005; Togay et al., 2008).

Maintaining soil fertility and use of plant nutrient in sufficient and balanced amounts is one of the key components in increasing crop yield (Caliskan et al., 2008). Among various nutritional requirements for production, nitrogen is known to be an essential element for plant growth and development. Nitrogen deficiency limits cell division, chloroplast development, enzyme activity and reduces dry matter yields (Werner, Newton, 2005; Salvagiotti et al., 2008). McKenzie and Hill (1995) studied the effects of two levels of N applications (0 and 50 kg N ha^{-1}) on chickpea and reported that increasing of N rate from 0 to 50 kg N ha^{-1} significantly increased seed and biological yield, harvest index, number of pods per plant and 1000 seed weight. Walley et al. (2005) investigated chickpea response to starter N (0, 15, 30 and 45 kg N ha^{-1}) and stated that application of 45 kg N ha^{-1} enhanced seed yield by as much as 221 kg ha^{-1} over control. Amany (2007) reported that urea foliar application had significant effects on plant height, number of branches, pods and seeds per plant, 1000 seed weight, biological and seed yield and harvest index in chickpea.

As a legume, chickpea can obtain a significant portion (4–85%) of its N requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium* strains (Walley et al., 2005; Chemining wa, Vessey, 2006). The rest of N is obtained from soil inorganic N, mineralized organic matter, residual N from the previous crop and/or fertilizer applications (Caliskan et al., 2008; Salvagiotti et al., 2008). Chickpea and *Rhizobium leguminosarum* sub sp. *ciceri* association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain and environmental factors (Ogutcu et al., 2008). 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, Newton, 2005).

The inoculation of seeds with *Rhizobium* is known to increase nodulation, nitrogen uptake, growth and yield parameters of legume crops (Adgo, Schulze, 2002; Rudresh et al., 2005; Sogut, 2006; Stancheva et al., 2006). Albayrak et al. (2006) studied the effects of inoculation with *Rhizobium leguminosarum* on seed yield and some morphological traits of common vetch (*Vicia sativa* L.) and observed that inoculated common vetch cultivars gave higher biological yield (8.5%), seed yield (7.6%), straw yield (10.4%), pod length (25.5%), number of seeds per pod (16.2%), number of pods per plant (28.4%), main stem length (3.5%) and 1000 seed weight (5.5%) compared to non-inoculated cultivars. Malik et al. (2006) found that seed inoculation with *Rhizobium* significantly increased plant height, leaf area index, number of pods per plant, number of seeds per pod, 1000 seed weight, biological and seed yield and harvest index in soybean. Togay et al. (2008) reported that inoculation with *Rhizobium* significantly increased plant height, first pod height, number of branches, pods and seeds per plant, grain yield and biological yield in chickpea. Shrivastava et al. (2000) investigated the beneficial effect of soybean inoculation with *Rhizobium* and observed that this biofertilizer on average enhanced the yield by 8.6% over recommended fertilizer alone. Begum et al. (2001), Saini et al. (2004) and Ogutcu et al. (2008) reported the same results in chickpea.

Phenology is the study of relationship between climatic factors and periodic phenomena in organisms. Pattern of phenological events is variously used for characterization of vegetation type (Yadav, Yadav, 2008). Soltani et al. (2006) stated that phenology (development) refers to ontological changes occurring at different distinct phases in a crop's life cycle. The study of plant phenology provides knowledge about the pattern of plant growth and development as well as the effects of environment and selective factors on phenological behavior. Yadav and Yadav (2008) suggested that climate change forced deviations in the length of growing period, and competition among species may change the resource use patterns in different species. Flowering time is an important stage in crops development because environmental conditions during the reproductive phase have a major impact on final yield. The onset of flowering often determines the entire crop duration (Thies et al., 1995; Haque et al., 2006; Soltani et al., 2006).

The time available for chickpea crops to produce adequate vegetative structures and then grain yield is often limited by hot or cold temperatures, rainfall distribution, or competition for use of land by other crops in rotation. To achieve good yield, crop duration (phenology) must closely match the available growing season (Soltani et al., 2006). The dynamics of chickpea phenology vary with cultivar, photoperiod, temperature, soil water and nutrient status. Changes in development and maturity time may determine the economic yield in chickpea (Soltani et al., 2006; Gan et al., 2009).

Gan et al. (2009) reported that increasing in N fertilizer rate increased the days from planting to maturity in chickpea plants. Same results reported with Thies et al. (1995) in soybean and brush bean and Haque et al. (2006) in rice. However, a few attempts have been made to evaluate the phenology of legume crops, i.e. chickpea under effects of organic and inorganic nitrogen fertilizer.

Determination of the morphological and phenological 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 little investigation about the combined effects of nitrogen fertilization and *Rhizobium* inoculation on morphological and phenological traits in some legume crops such as chickpea. Considering the above facts, the present study was undertaken to know the phenological and morphological response of chickpea to different levels of organic and inorganic nitrogen fertilizer.

Material and methods

Field experiments were conducted at the Experimental Farm of Mohaghegh Ardabili University, in 2008. The area is located at latitude 38º 15′ N and longitude 48º 15′ E at an altitude of 1350 m above the mean sea level. Climatically, the area is situated in the semi arid temperate zone with cold winter and moderate summer. Average rainfall is about 400 mm that most rainfall occurs in 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 spilt 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^{-1}. Sub plot treatments were two levels of inoculation with *Rhizobium* (inoculated and non-inoculated). In each level, nitrogen fertilizer was divided into three equal parts; the first part of the N was broadcasted by hand and incorporated immediately at planting time, second and third parts used at 6–8 leaves and flowering stages. Seeds of inoculation treatments were inoculated with *Rhizobium legominosarum* bv. *Ciceri* at the rate of approximately 1×10^8 colony forming units (CFU) ml^{-1} just before planting that was obtained from the Soil and Fertilizer Research Institute, Tehran.

The area was mouldboard-ploughed and disked before planting. Seeds of chickpea (*Cicer arietinum* L.) cv. ILC 482 were hand planted on 14 May in five row 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.

Crop phenology was monitored at 1–2 day intervals throughout the season and three stages of crop development were determined. These were: flowering (50% of the plants had one open flower at any node on the main stem in any plot); podding (50% of plants had at least one emerged green pod in any plot) and harvest maturity (95% of pods have obtained their mature colour in any plot). The time taken to complete each phenological phase, i.e. sowing to flowering (SF), flowering to podding (FP) and podding to maturity (PM) was recorded in days. Growth period (GP) was obtained from sum of all these periods for each plot (Soltani et al., 2006).

The plants were harvested at maturity and morphological traits such as plant height, number of primary and secondary branches per plant, total number of pods, filled and unfilled pods per plant, number of grains per pod, number of grains per plant and 100-grain weight were recorded on 15 randomly selected plants in each plot. Grain and biological yield was determined by harvesting the middle three rows of each plot.

Statistical analysis. Analysis of variance was done using SAS computer software package (SAS/STAT: user’s guide, 1998) and the mean values were compared with Duncan multiple range test (DMRT).

Results and discussion

The effects of N fertilization and *Rhizobium* inoculation on some morphological and phenological traits and grain yield of chickpea are presented in Tables 1 and 2. The results obtained from the variance analysis of data indicated that nitrogen fertilization had significant effects on all of the studied traits except the number of grains per pod. Furthermore, inoculation with *Rhizobium* significantly affected studied traits except plant height, number of grains per pod and 100-grain weight. Moreover, interactions between N fertilization × *Rhizobium* inoculation were found significant in number of grains per plant, grain yield per plant and unit of area, biological yield and growth period.

Morphological traits. *Plant height (PH).* The plant height was significantly affected by nitrogen application while, inoculation with *Rhizobium* showed no significant effects on this trait (Table 1). The highest plant height was observed in the maximum rate of nitrogen application (100 kg urea ha^{-1}) that did not differ significantly with 75 kg urea ha^{-1}. The minimum plant height was recorded in the control. Application of 100 kg urea ha^{-1} increased plant height by 30.9%, compared to the control (Table 1). 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. Although variance analysis of data indicated that *Rhizobium* inoculation had no significant effects on plant height of chickpea, comparisons of means showed that the inoculated plants were taller than non-inoculated plants. Rudresh et al. (2005) also stated that plant height was not affected significantly with *Rhizobium* inoculation.

Number of primary branches (NPB) and secondary branches (NSB) 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^{-1} increased the number of primary and secondary branches per plant by 26.61 and 50.05%, respectively. The lowest and the highest values of these traits were recorded in 0 and 100 kg urea ha^{-1}, respectively (Table 1). Amany (2007) in chickpea and Caliskan et al. (2008) in soybean reported the same results.

Moreover, the greatest number of the primary and secondary branches per plant was recorded in inoculated plants. Inoculation with *Rhizobium* increased the number of primary and secondary branches by 8.50% and 14.70% respectively, compared with non-inoculated plants (Table 1). These results are in agreement with Rudresh et al. (2005) and Togay et al. (2008).

Table 1. Effects of nitrogen fertilization and *Rhizobium* inoculation on some morphological traits in chickpea (*Cicer arietinum* L.)

Treatments	PH cm	NPB per plant	NSB per plant	NP per plant	NFP per plant	NUFP per plant	NG per pod	NG per plant
Nitrogen rates (kg urea ha^{-1})								
0	33.47 **c**	1.93 **c**	4.67 **c**	17.85 **c**	11.76 **c**	6.09 **c**	1.03 **b**	11.42 **c**
50	37.43 **b**	2.31 **b**	6.61 **b**	19.42 **b**	13.03 **b**	6.39 **bc**	1.11 **ab**	14.12 **b**
75	44.37 **a**	2.60 **a**	8.51 **a**	20.61 **a**	13.92 **ab**	6.69 **ab**	1.18 **a**	16.41 **a**
100	45.97 **a**	2.63 **a**	9.35 **a**	21.30 **a**	14.32 **a**	6.98 **a**	1.17 **a**	16.63 **a**
Rhizobium inoculation								
non	41.25 **a**	2.26 **b**	6.80 **b**	18.97 **b**	12.66 **b**	6.30 **b**	1.08 **a**	13.38 **b**
with	39.37 **a**	2.47 **a**	7.77 **a**	20.62 **a**	13.85 **a**	6.77 **a**	1.15 **a**	15.91 **a**
Mean	**40.31**	**2.36**	**7.28**	**19.79**	**13.25**	**6.53**	**1.12**	**14.64**
Nitrogen (N)	**	**	**	**	**	*	ns	**
Rhizobium (*Rh.*) inoculation	ns	*	**	**	**	*	ns	**
N × *Rh.*	ns	ns	ns	ns	ns	ns	ns	*
CV %	**13.63**	**10.30**	**10.96**	**16.29**	**15.21**	**16.73**	**11.02**	**16.10**

Notes. PH – plant height, NPB – number of primary branches, NSB – number of secondary branches, NP – number of pods, NFP – number of filled pods, NUFP – number of unfilled pods, NG – number of grains. Mean values followed by the 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.

Total number of pods (NP), filled pods (NFP) and unfilled pods (NUFP) per plant. As shown in Table 1, total number of pods per plant showed significant response to nitrogen fertilization and *Rhizobium* inoculation. The highest total number of pods per plant was recorded in 100 kg urea ha^{-1} application that showed no significant difference with 75 kg urea ha^{-1}. The least rate of this trait was obtained from the control. Application of 100 kg urea ha^{-1} increased the number of pods per plant about 16.19% compared to the control in each plant (Table 1). 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^{-1}, but further increase in N dose (120 kg ha^{-1}) did not show any significant effect on this trait. Similar trends were reported by McKenzie and Hill (1995) and Amany (2007) in chickpea and Achakzai and Bangulzai (2006) in pea.

Inoculation with *Rhizobium* bacteria significantly increased the total number of pods per plant (Table 1). Plants that were inoculated with *Rhizobium* showed about 8.00% more pods per plant than non-inoculated plant. In a study conducted by Togay et al. (2008) related with chickpea, the number of pods per plant was statistically significantly affected with *Rhizobium* inoculation. These researchers noted that this trait increased from 11.50 pods per plant in non-inoculated plants to 12.35 pods per plant in inoculated plants. Malik et al. (2006) and Albayrak et al. (2006) reported the same results.

Different rates of nitrogen fertilizer and inoculation had significant effects on the number of filled and unfilled pods per plant. The maximum number of filled and unfilled pods per plant was recorded in application of 100 kg urea ha^{-1} while, the lowest values of these traits were obtained from the control (Table 1). Usage of 100 kg urea ha^{-1} increased the number of filled and unfilled pods per plant by 17.87% and 12.75% respectively, compared to the control. Results obtained from this study indicated that the highest level of nitrogen usage significantly increased the number of pods in each plant (Table 1). Increasing the number of unfilled pods per plant may be due to the less assimilation in chickpea plants for filling of whole pods at high levels of nitrogen application. *Rhizobium* inoculation significantly increased the number of filled pods (by 6.94%) and unfilled pods (by 6.08%) compared to the control in each plant (Table 1).

Number of grain per pod and number of grain (NG) per plant. Although variance analysis of data indicated that nitrogen fertilization and *Rhizobium* 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^{-1} application and *Rhizobium* inoculation (Table 1). In the study on the soybean it was shown that *Rhizobium* inoculation had no significant effect on number of grain per pod (Malik et al., 2006). Non significant effects of studied treatments on number of grains per pod may be due to more effects of genetic factors in control of this trait rather than environmental and management factors.

The significant main effect of nitrogen fertilization and *Rhizobium* inoculation, and significant nitrogen fertilization × *Rhizobium* inoculation interactions were obtained in chickpea for number of grains per plant (Table 1). The highest number of grains per plant was recorded in inoculated plants that were treated with 75 kg urea ha^{-1}. The least value of this trait was obtained from non-inoculated and non-fertilized plants (Fig. 1). Application of 75 kg urea ha^{-1} in inoculated plants increased the number of grains per plant by 40.82% compared to the control. Previous studies confirmed the positive effects of nitrogen application (Amany, 2007) and *Rhizobium* inoculation (Togay et al., 2008) on number of grains per plant.

Furthermore, as shown in Fig. 1 (A), inoculation with *Rhizobium* bacteria had the greatest effect on number of grains per plant in 75 kg urea ha^{-1} rather than other fertilizer levels which may be due to higher effectiveness of *Rhizobium* inoculation in this level compared to other levels of nitrogen usage. Inoculation increased the number of grains per plant about 27.87% in 75 kg urea ha^{1} compared with non-inoculated plants in the same fertilizer level.

100-grain weight (100-GW). 100-grain weight was affected only by nitrogen fertilization (Table 2). Increasing of nitrogen application rate significantly decreased the weight of 100-grain in chickpea. The highest 100-grain weight was recorded in the control while the lowest rate of this trait was obtained from 100 kg urea ha^{-1} (Table 2). Application of 100 kg urea ha^{-1} decreased the 100-grain weight by 7.51% compared to the control. The negative correlation between yield and 100-grain weight was reported in some studies (Walley et al., 2005; Achakzai, Bangulzai, 2006).

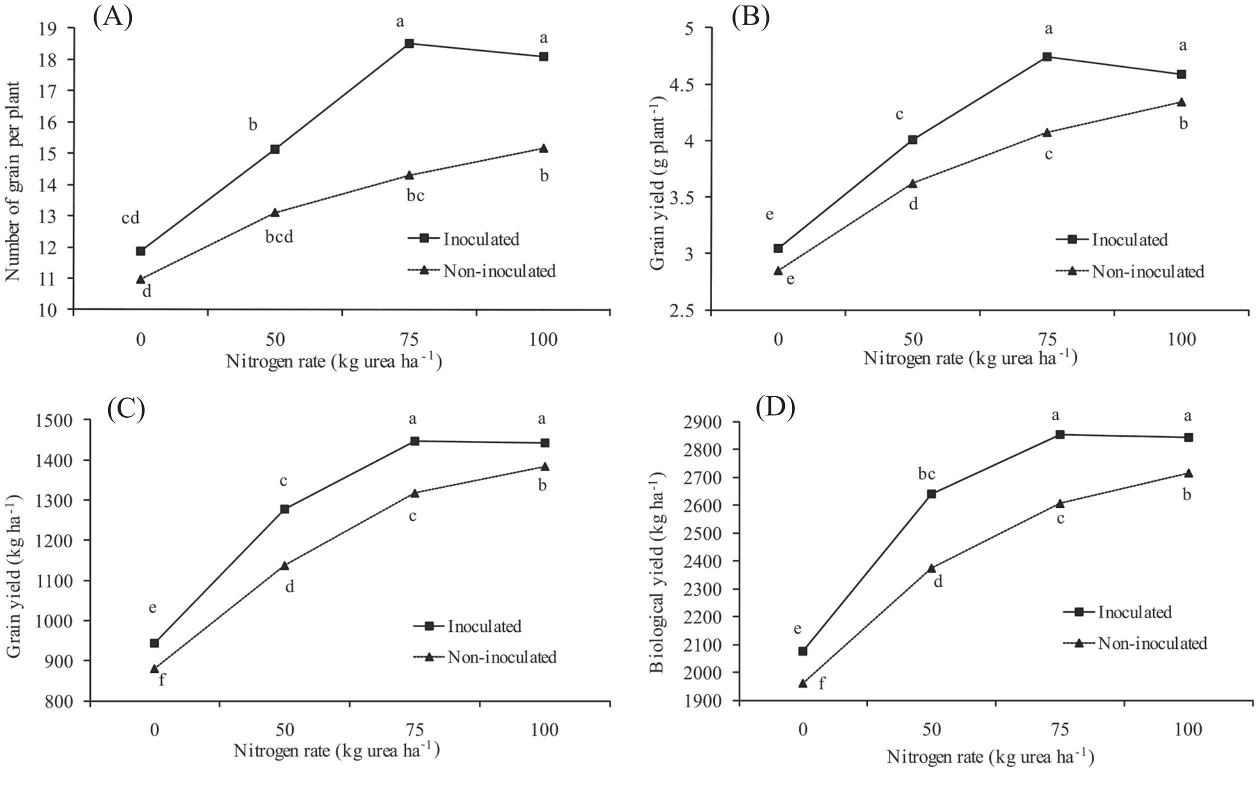
Table 2. Effects of nitrogen fertilization and *Rhizobium* inoculation on some studied traits and phenological stages in chickpea (*Cicer arietinum* L.)

Treatments	100-GW g	GY g plant^{-1}	GY kg ha^{-1}	BY kg ha^{-1}	SF day	FP day	PM day	GP day
Nitrogen rates (kg urea ha^{-1})								
0	28.47 **a**	2.94 **c**	911.5 **c**	2016.2 **c**	62.93 **b**	13.55 **c**	29.05 **b**	105.53 **c**
50	27.60 **ab**	3.81 **b**	1207.3 **b**	2506.1 **b**	63.00 **b**	14.07 **bc**	29.18 **b**	106.26 **c**
75	26.58 **b**	4.40 **a**	1328.2 **a**	2729.3 **a**	64.93 **a**	14.70 **ab**	29.48 **ab**	109.10 **b**
100	26.48 **b**	4.46 **a**	1413.6 **a**	2778.2 **a**	66.38 **a**	15.35 **a**	30.22 **a**	111.96 **a**
Rhizobium inoculation								
non	27.21 **a**	3.72 **b**	1166.5 **b**	2413.1 **b**	64.93 **a**	14.66 **a**	29.77 **a**	109.28 **a**
with	27.37 **a**	4.09 **a**	1263.7 **a**	2602.7 **a**	63.68 **b**	14.17 **b**	29.18 **b**	107.15 **b**
Mean	**27.28**	**3.90**	**1215.15**	**2507.45**	**64.31**	**14.41**	**29.47**	**108.21**
Nitrogen (N)	**	**	**	**	**	**	*	**
Rhizobium (*Rh.*) inoculation	ns	**	**	**	**	*	**	**
N × *Rh.*	ns	*	*	*	ns	ns	ns	*
CV %	**11.23**	**10.08**	**12.34**	**14.29**	**13.07**	**12.53**	**11.87**	**12.69**

Notes. 100-GW – 100-grain weight, GY – grain yield, BY – biological yield, SF – sowing to flowering, FP – flowering to podding, PM – podding to maturity, GP – growth period. Mean values followed by the 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.

Grain yield (GY). Data presented in Table 2 showed that both of the studied experimental factors (nitrogen application and *Rhizobium* inoculation) had significant effects on grain yield (plant^{-1} and ha^{-1}) of chickpea. The highest rate of nitrogen fertilizer (100 kg urea ha^{-1}) showed the greatest grain yield; however, this rate of nitrogen fertilizer was in par with 75 kg urea ha^{-1}. Application of 100 kg urea ha^{-1} increased grain yield per plant and per unit of area by 34.08% and 35.51% respectively, compared to the least application of nitrogen fertilizer (control). Furthermore, inoculated plants indicated higher grain yield (plant^{-1} and ha^{-1}) rather than non-inoculated plants. *Rhizobium* inoculation increased grain yield per plant and per unit of area about 9.04% and 7.01% respectively, compared with the control (Table 2).

Interaction effects of nitrogen fertilization and *Rhizobium* inoculation were found significant for grain yield (plant^{-1} and ha^{-1}) 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^{-1} and further increase in N rate resulted in no significant grain yield increase (Fig. 1). Moreover, grain yield (plant^{-1} and ha^{-1}) of inoculated plants at all rates of nitrogen application was higher than that of the non-inoculated plants at the same rate of nitrogen application (Fig. 1). The highest grain yield (plant^{-1} and ha^{-1}) was recorded in inoculated plants with 75 kg urea ha^{-1} application. The lowest rate of nitrogen application showed the lowest grain yield (plant^{-1} and ha^{-1}) in non-inoculated plants (Fig. 1). Study on the interactions between N application and inoculation showed that inoculation with *Rhizobium* bacteria had more effects on grain yield in 75 kg urea ha^{-1} than other levels of fertilizer application (Fig. 1).



Note. Values with same letters in each trait are not significantly different (DMRT at 5% level).

Figure 1. Effects of different levels of nitrogen application under *Rhizobium* inoculation and non-inoculation on number of grains per plant (A), grain yield per plant (B), grain yield per unit of area (C) and biological yield (D) in chickpea (*Cicer arietinum* L.)

Other researchers reported the same results about the effects of nitrogen application (McKenzie, Hill, 1995; Caliskan et al., 2008; Salvagiotti et al., 2008) and *Rhizobium* inoculation (Chemining wa, Vessey, 2006; Albayrak et al., 2006; Togay et al., 2008) on grain yield in different legume crops.

Biological yield (BY). Biological yield of chickpea also showed the same trend as grain yield. As shown in Table 2, the highest biomass was obtained from the application of 100 kg urea ha^{-1}, however, there was no significant difference in biological yield between the application of 75 and 100 kg urea ha^{-1}. Usage of 100 kg urea ha^{-1} increased the biological yield by 27.42%, compared to the control.

Nitrogen is known to be an essential nutrient for plant growth and development (Werner, Newton, 2005; Sogut, 2006; Salvagiotti et al., 2008) involved in vital plant functions such as photosynthesis, DNA synthesis, protein formation, respiration and N_2 fixation (Werner, Newton, 2005; Caliskan et al., 2008). The growth parameters such as leaf area index (LAI), biomass, and leaf photosynthesis significantly decreased due to unsatisfactory N availability (Chemining wa, Vessey, 2006; Malik et al., 2006; Caliskan et al., 2008). Results obtained from this study indicated that usage of N fertilization had positive effects on morphological traits and yield of chickpea. Adding N increases the production of total dry matter in plants (McKenzie, Hill, 1995; Caliskan et al., 2008; Salvagiotti et al., 2008) which can increase the potential of plant to produce more plant height, branches, pods, seeds, which ultimately results in high grain and biological yield. Nitrogen fertilization increases the total dry matter for a number of reasons: (i). Nitrogen can increase the LAI in plants (McKenzie, Hill, 1995; Malik et al., 2006; Caliskan et al., 2008). More LAI increases the interception of solar radiation by plants that resulted in the more accumulation in plants (Caliskan et al., 2008). (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 (Werner, Newton, 2005; Caliskan et al., 2008). In contrast, supplementation of adequate nitrogen for crops can increase their growth and development. In this condition, plants were able to produce more morphological traits that resulted in more grain yield.

Moreover, inoculated plants showed the more biomass than non-inoculated plants. Inoculation with *Rhizobium* bacteria increased the biological yield about 7.28% compared to the 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, Newton, 2005; Chemining wa, Vessey, 2006). The influence of *Rhizobium* bacteria on promoting legumes growth has been documented in many researches (Shrivastava et al., 2000; Rudresh et al., 2005; Malik et al., 2006). The observed benefits on chickpea by *Rhizobium* inoculation seem to be due to the supply of N to the crop (Chemining wa, Vessey, 2006; Togay et al., 2008). Moreover, growth promoting substances (phytohormones) are produced by these organisms. *Rhizobium* bacteria synthesize 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 *Rhizobium* activities enhance water and nutrient uptake (Werner, Newton, 2005).

Interactions between different levels of nitrogen and *Rhizobium* inoculation were found significant in biological yield (Table 2). Generally, inoculation with *Rhizobium* in all levels of nitrogen application increased biomass production rather than non-inoculated plants (Fig. 1). The highest biological yield was recorded at the plots of 75 kg urea ha^{-1} and *Rhizobium* inoculation. Control plots (non-fertilized and non-inoculated) had the lowest values of this trait (Fig. 1). These results are in accordance with the works of McKenzie and Hill (1995), Rudresh et al. (2005), Albayrak et al. (2006), Malik et al. (2006), Amany (2007), Togay et al. (2008). 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 compared to N fertilization.

Phenological stages. Significant variations were found among the different levels of nitrogen usage and *Rhizobium* inoculation for phenological periods in chickpea (Table 2). Increasing of nitrogen fertilizer amount increased significantly days from sowing to flowering (SF), days from flowering to podding (FP) and days from podding to maturity (PM). The highest number of days required for completion of these stages was recorded in the highest rate of nitrogen usage (100 kg urea ha^{-1}) while, no application of nitrogen fertilizer showed the shortest duration of the phenological periods (Table 2). In the same trend, the longest and the shortest growth periods (GP) of chickpea were observed in the application of 100 and 0 kg urea ha^{-1}, respectively.

Table 3 presents the effects of different levels of nitrogen application on increasing of phenological stages duration over the control. As shown in this table, appearance of phenological stages in chickpea was delayed with increasing of nitrogen fertilizer amount. Moreover, the days from flowering to podding showed more increasing in the high levels of nitrogen application than other phenological stages that maybe due to the greater sensitivity of this stage to environmental conditions and management factors. Increasing of nitrogen rate had the lowest effect on days from podding to maturity (Table 3). These results are in agreement with the findings of Thies et al. (1995), Haque et al. (2006) and Gan et al. (2009) who reported that appearance of phenological stages and growth period increased with increasing of nitrogen fertilizer rate. Haque et al. (2006) concluded that more and accelerated vegetative growth might be a factor for delaying of phenological stages appearance, i.e. flowering and crop maturity with the increase in the amount of nitrogen fertilizer.

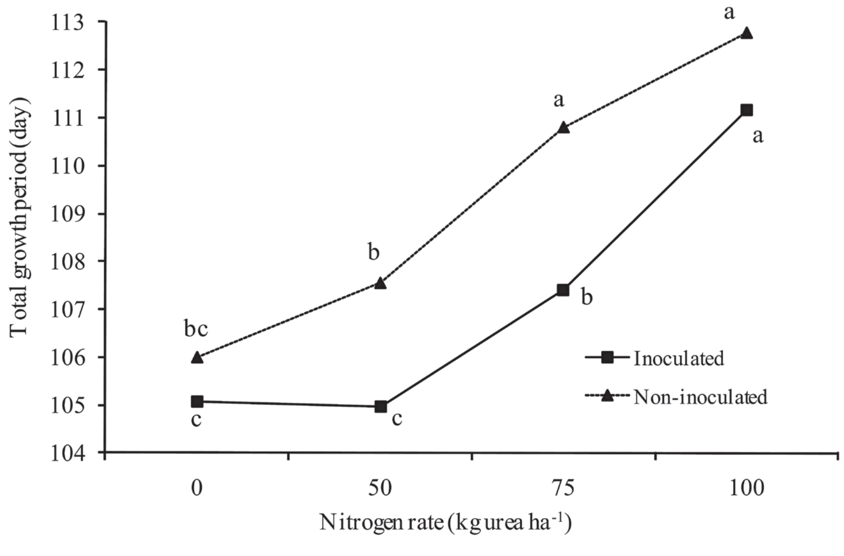
Table 3. Increasing of phenological periods in chickpea (*Cicer arietinum* L.) over the control as affected by different rates of nitrogen application

Nitrogen rates kg urea ha^{-1}	Phenological stage			
	SF	FP	PM	GP
50	0.11%	3.26%	0.44%	0.68%
75	3.08%	8.48%	1.39%	3.27%
100	5.19%	11.72%	3.87%	5.74%

Note. SF – sowing to flowering, FP – flowering to podding, PM – podding to maturity, GP – growth period.

Response of phenological stages appearance of chickpea to *Rhizobium* inoculation was found significant. Plants that were inoculated with *Rhizobium* showed fewer days from sowing to flowering (1.96%), flowering to podding (2.03%) and podding to maturity (2.02%) than non-inoculated plant (Table 2). Furthermore, growth period of chickpea decreased significantly (1.98%) in inoculated plants (Table 2). The influence of *Rhizobium* inoculation on the timing of developmental stages (crop phenology) is not highly detailed in the literature. Production of phytohormones like auxin promoted seed germination and leaf extension (Werner, Newton, 2005) and might have caused faster development and shorter growth period in inoculated plants than non-inoculated plants.

Growth period of chickpea was significantly affected with the interaction effects of studied treatments (Table 2). Generally, growth period of inoculated plants in each level of nitrogen application was shorter than the non-inoculated plants in the same level of nitrogen application (Fig. 2). The longest growth period was recorded in the plants treated with 100 kg urea ha^{-1} and not inoculated with *Rhizobium* bacteria. The shortest growth period was observed in the application of 50 kg urea ha^{-1} and non-inoculation treatment that was statistically in par with the no N fertilization treatment in both levels of inoculation (Fig. 2).



Note. Values with the same letters are not significantly different (DMRT at 5% level)

Figure 2. Effects of different levels of nitrogen application under *Rhizobium* inoculation and non-inoculation on growth period duration in chickpea (*Cicer arietinum* L.)

Moreover, results obtained from this study indicated that plants with longer growth period produced higher biological and grain yield compared to plants that had shorter growth period (Table 2). Thies et al. (1995) stated that short growth duration in general, gives low yields as compared to medium and long growth duration and this is due to the fact that longer development period provides full use of available growth resources like water, nutrient and light for plants, which results in high crop yields.

Conclusions

1. Nitrogen fertilization and *Rhizobium* inoculation had significant effects on morphological traits of chickpea.

2. Despite the nitrogen effects, *Rhizobium* inoculation significantly decreased the appearance of phenological stages and growth period.

3. Chickpea plants that had long growth period produced higher yield compared to plants with short growth period.

4. It seems that, application of 75 kg urea ha^{-1} can be beneficial to improve growth and final yield of inoculated chickpea.

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Sėjamojo avinžirnio (*Cicer arietinum* L.) fenologinė ir morfologinė reakcija į tręšimą simbiotiniu bei mineraliniu azotu

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Santrauka

Inokuliavimo *Rhizobium* bakterijomis, kaip biotrąša, ir tręšimo mineraliniu azotu įtaka veislės ILC 482 avižirnio (*Cicer arietinum* L.) vystymuisi bei fenologiniams tarpsniams 2008 m. tirta Mohaghegh Ardabili universiteto eksperimentiniame ūkyje. Bandymas vykdytas išskaidytų laukelių metodu, keturiais pakartojimais. Tirti veiksniai – tręšimas neorganiniu azotu (4 lygiai – 0, 50, 75 bei 100 kg ha^{-1} karbamido) didesniuose laukeliuose ir 2 inokuliavimo *Rhizobium* bakterijomis lygiai (inoluliuota bei neinokuliuota) mažesniuose laukeliuose. Azoto trąšos ir inokuliavimas *Rhizobium* bakterijomis turėjo esminę įtaką avižirnio morfologiniams požymiams. Didžiausios morfologinių požymių vertės nustatytos patręšus didelėmis normomis azoto ir inokuliavus *Rhizobium* bakterijomis. Padidinus įterpiamo azoto kiekį, esmingai padidėjo dienų skaičius nuo sėjos iki žydėjimo, nuo žydėjimo iki ankštarų formavimo, nuo ankštarų formavimo iki brandos ir pailgėjo visas augimo laikotarpis, smarkiai paankstėjo inokuliuotų augalų fenologinių tarpsnių pradžia ir visas augimo laikotarpis. Didžiausią grūdų derlių (1446,6 kg ha^{-1}) ir biologinį derlių (2853,2 kg ha^{-1}) užaugino inokuliuoti augalai, patręšti 75 kg ha^{-1} karbamido. Tyrimų rezultatai parodė, kad didesnį biologinį ir grūdų derlių užaugino ilgiau vegetuojantys augalai, palyginti su trumpiau vegetuojančiais. Nustatyta, kad tręšimas 75 kg ha^{-1} karbamido gali būti efektyvus, siekiant pagerinti inokuliuotų avižirnių augimą ir galutinį derlių.

Reikšminiai žodžiai: *Cicer arietinum*, grūdų derlius, morfologiniai požymiai, fenologiniai tarpsniai, inokuliavimas *Rhizobium*.