



## RESEARCH ARTICLE

# Impact of *Mesorhizobium ciceri* Inoculation on Symbiotic Nitrogen Fixation of Various Chickpea (*Cicer arietinum* L.) Cultivars

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## ABSTRACT

In this study, conducted in pots under outdoor conditions, the aim was to determine the symbiotic performance of 10 registered chickpea cultivars (Akça, Aksu, Arda, Aslanbey, Bayram, Botan, Cevdetbey, Göktürk, Ilgaz and Yazıcı) inoculated with *Mesorhizobium ciceri*. Inoculation significantly increased the average nodule fresh weights, nodule dry weights, shoot height, shoot fresh weights, shoot dry weights, root fresh weights, root dry weights, chlorophyll content, N% and total N compared to the un-inoculated control. The shoot fresh weight, N% and total N significantly correlated with nodule weight. Additionally, chlorophyll content is highly correlated with N%. The magnitude of response to inoculation differed significantly among cultivars, except shoot height and root fresh weight. The amount of fixed N per plant by the cultivars varied significantly between 0 (Ilgaz) and 23.5 mg (Akça). Akça, Arda, Aslanbey, Cevdetbey, Göktürk and Yazıcı were the most compatible cultivars based on the amount of fixed N. These results show that inoculation efficiency and nitrogen fixation in chickpea can be increased by selecting compatible cultivars.

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## 1. Introduction

Nitrogen is the most limiting nutrient for crop production in many agricultural areas worldwide (Fageria & Baligar, 2005). The atmosphere, which contains about 78% nitrogen gas, is the main source of nitrogen on Earth. Although nitrogen is abundant in the atmosphere as a gas, it cannot be taken up and utilized directly by plants. Plants can only take nitrogen from the soil in the reduced form of ammonium and nitrate (Gul et al., 2014; Li et al., 2013). Today, most of the nitrogen is applied to cropping systems in the form of industrially produced nitrogen fertilizers, which causes various ecological problems worldwide (J. P. Verma et al., 2013). Increasing fertilizer costs and the negative effects of chemical fertilizers on the

environment have forced the search for alternative sources in terms of plant nutrition. In this context, biological nitrogen fixation, a process in which elemental atmospheric nitrogen is converted into organic forms by symbiotic and asymbiotic microorganisms possessing nitrogenase enzymes, has gained great importance as an alternative nitrogen source to chemical fertilizers (Elkoca et al., 2008). These microorganisms, which have nitrogenase enzymes, convert gaseous nitrogen into a form that can be taken up by plants and provide an important nitrogen gain biologically (Bohloul et al., 1992; Elkoca et al., 2010; Mohammadi & Sohrabi, 2012).

Chickpea is the second most produced edible legume plant in the world after dry beans with 14.8 million hectares of cultivation area and 18.1 million tonnes of production. In

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Türkiye, it ranks first among edible legumes with 456 thousand hectares of cultivation area and 580 thousand tonnes of production (FAO, 2022). Chickpea, a member of the legume family, can fix atmospheric nitrogen to the soil symbiotically through *Rhizobium* bacteria (Kantar et al., 2007). *Mesorhizobium ciceri* is the bacterium that forms nodules and fixes nitrogen in chickpea. This bacterium is specific to chickpea and does not show inoculation affinity with any member of the cross-inoculation groups (Kantar et al., 2007; Rupela & Saxena, 1987). Therefore, there is a need for inoculation with effective strains in soils where chickpea has not been previously grown or do not contain effective bacterial strains (El Hadi & Elsheikh, 1999; Gul et al., 2014; Somasegeran et al., 1988; D. P. S. Verma, 1984).

Nodule formation and nitrogen fixation in legumes are influenced by both bacterial and host plant genes (Buttery et al., 1997; Danso et al., 1987; Davis et al., 1985; Goyal et al., 2021; D. P. S. Verma, 1984). Therefore, even if the bacteria used in inoculation are effective and environmental conditions are suitable for nitrogen fixation, the amount of nitrogen fixed is limited if the bacteria and the host are not compatible. Genotypic variation among cultivars has a significant impact on nodulation and nitrogen fixation in chickpea as in many legume species (Bhuiyan et al., 2009; Pandey et al., 2018; Priyadarsini et al., 2017; Roy et al., 2018). Thus, it is crucial to identify chickpea cultivars with high nodulation and nitrogen fixation potential. While many chickpea cultivars have been developed in our country in recent years, there has been insufficient research on the response of these cultivars to bacterial inoculation and their nitrogen fixation potential. In light of this information, this study aims to determine the effect of *Mesorhizobium ciceri* inoculation on nodulation, nitrogen fixation and plant growth parameters of some chickpea cultivars.

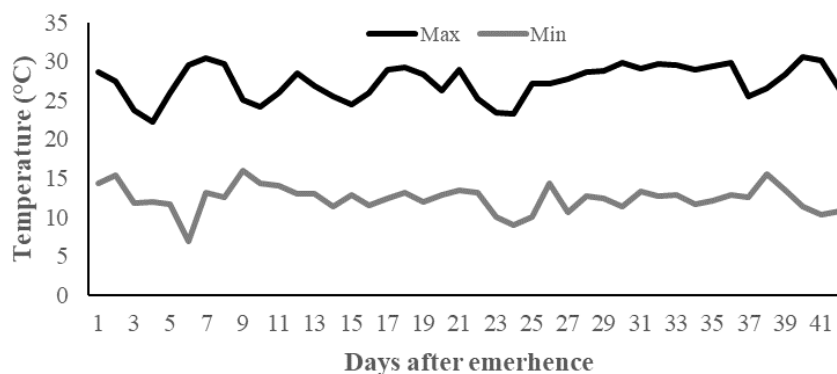
## 2. Materials and Methods

The experiment was conducted in 2-liter pots under outdoor conditions in the Field Crops Department of Atatürk University, Erzurum. The pots were filled with a mixture of sifted field soil, peat and sand in a ratio of 1:1:1. Some properties of the experimental soil are presented in Table 1. In the study, 10 registered chickpea cultivars (Akça, Aksu, Arda, Aslanbey, Bayram, Botan, Cevdetbey, Göktürk, Ilgaz, Yazıcı) were used as seed material. Bacterial culture (*Mesorhizobium ciceri*) in peat was obtained from the Central Research Institute of Soil, Fertilizer and Water Resources, Ankara.

**Table 1.** Characteristics of the experimental soil used in the study.

Contents	Value	Contents	Value
pH	7.27	Mg (cmol/kg)	11.99
EC ( $\mu\text{mhos/cm}$ )	81.45	Na (cmol/kg)	1.16
Organic matter (%)	0.78	B (ppm)	0.03
CaCO <sub>3</sub> (%)	1.99	Cu (ppm)	0.53
Total N (ppm)	5.14	Fe (ppm)	0.59
P (ppm)	33.55	Zn (ppm)	0.16
K (cmol/kg)	2.28	Mn (ppm)	0.21
Ca (cmol/kg)	14.05	Cl (ppm)	2.59

The research was conducted using a completely randomized design with 3 replications. The responses of chickpea cultivars to *Mesorhizobium ciceri* inoculation were compared to an un-inoculated control treatment. The un-inoculated control treatment was sown first to prevent bacterial contamination. In the inoculation treatment, bacterial culture in peat ( $1.1 \times 10^5$  cfu/g) was applied to seeds soaked in a 10% glucose solution. The inoculation was performed in the shade and the inoculated seeds were immediately sown. Five seeds were planted in each pot and three plants were retained per pot after emergence. Pots were irrigated with tap water as needed. Sowing took place on June 29, 2024 and plants were harvested 42 days after emergence. Daily minimum and maximum temperatures were recorded throughout the experimental period, ranging between 7 and 30.6°C (Figure 1).



**Figure 1.** The minimum and maximum air temperatures recorded during the experimental period.

## 2.1. Data Collection

A portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) was utilized to assess leaf greenness as an indicator of chlorophyll content of chickpea cultivars just before harvesting. SPAD measurements were conducted on 10 fully expanded leaves in each pot and averaged. The relative leaf chlorophyll content was represented in SPAD units with “1” indicated very light green pigmentation (chlorotic) and “50” indicated very dark green pigmentation.

Plants were harvested 42 days after emergence. The root and shoot parts were separated after washing the roots thoroughly with tap water. Nodules on roots were removed and the fresh weights of nodules, shoots and roots per plant were determined. Dry weight parameters were recorded after drying the root, shoot and nodule samples in an oven at 65°C for 24 hours. Plant nitrogen content (N%) was determined using the Kjeldahl method. Total N per plant (shoot dry weight x N%) and the fixed N (total N in inoculated plants – total N in uninoculated plants) were calculated (Elkoca et al., 2015; Ögütçü et al., 2008).

## 2.2. Statistical Analysis

The data was subjected to analysis of variance using the MSTATC Statistical Package (version 1.4, Michigan State University, MI, USA) and means were separated according to Duncan's multiple range test at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Effect of Inoculation

In this study, an average of 69.3 mg of fresh and 9.7 mg of dry nodule weight per plant was determined in the uninoculated control treatment, serving as evidence of the presence of native rhizobial population in the soil (Table 2). The success of inoculation depends on the ability of the introduced rhizobium bacteria to survive and colonize by competing with the native bacterial population in the soil (Bordeleau & Prevost, 1994). When inoculation is successful, significant increases in plant dry matter production and total nitrogen amounts are observed in chickpea (Ögütçü et al., 2008; Pandey et al., 2018; Solaiman et al., 2010). Therefore, increases in these parameters as a result of rhizobial inoculation are considered among the most important indicators of nitrogen fixation (Beck, 1992; Hardarson & Danso, 1993). In our study, *Mesorhizobium ciceri* inoculation provided significant increases in all parameters examined in chickpea as an average of the cultivars (Tables 2 and 3). These results demonstrate that the bacteria used in the inoculation could compete with the native bacterial population in the soil, indicating successful inoculation.

### 3.2. Effect of Cultivar

Infection of root hairs, nodule development and the resulting amount of nitrogen fixed are influenced by host plant genes (Dwivedi et al., 2015; Perret et al., 2000; D. P. S. Verma, 1984). Therefore, different genotypes and cultivars within the same legume species may react differently to inoculation. As a result of the genotypic effect, significant differences may occur between cultivars in terms of the parameters examined in bacterial inoculation studies (Di Bonito et al., 1990; Farid & Navabi, 2015; Pandey et al., 2018). In this study, significant differences were also detected among the cultivars in terms of nodulation, plant growth parameters, chlorophyll content, N% and total N (Tables 2 and 3).

Ilgaz had no nodule formation, while Aksu had the highest nodule fresh and dry weights (434.4 and 60.2 mg plant<sup>-1</sup>, respectively). On the other hand, Aslanbey, Bayram, Botan and Cevdetbey with nodule dry weight ranging between 27.8 and 37.1 mg plant<sup>-1</sup> were in the second highest group. The highest shoot fresh and dry weight values were recorded in Akça, Aslanbey, Cevdetbey and Aksu (Table 2). Meanwhile, Arda, Cevdetbey, Göktürk, Aslanbey and Ilgaz were the leading cultivars in root fresh weight, with the highest root dry weight determined in Akça and Aslanbey (Table 3). The chlorophyll contents of Göktürk, Cevdetbey and Aksu were higher than the other cultivars. Plant nitrogen content varied considerably between 2.07% (Ilgaz) and 2.53% (Aslanbey) according to cultivars. The highest total N per plant was determined in Akça (69.4 mg) and Aslanbey (73.7 mg), followed by Aksu, Botan, Cevdetbey and Yazıcı with amounts ranging between 56.2 mg and 60.8 mg (Table 3). In other inoculation studies, it was also reported that significant differences were found among genotypes in terms of nodulation, plant growth, chlorophyll and nitrogen content, indicating that the host factor is an important determinant of the parameters investigated (Bidlack et al., 2001; Hossain et al., 2018; Hungria & Bohrer, 2000; Keneni et al., 2012; Ndiaye et al., 2000).

### 3.3. Effect of Inoculation x Cultivar Interaction

In this study, chickpea cultivars generally performed better in the inoculated treatment than in the un-inoculated treatment. However, there was significant variation among cultivars in response to *Mesorhizobium ciceri* inoculation. Except for shoot height and root fresh weight, the inoculation-cultivar interaction had a significant effect on all parameters examined (Tables 2 and 3).

**Table 2.** The effects of *Mesorhizobium ciceri* inoculation on the nodulation, shoot height, shoot fresh and dry matter production of chickpea cultivars.

	Nodule fresh weight (mg plant <sup>-1</sup> )	Nodule dry weight (mg plant <sup>-1</sup> )	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
<b>Inoculation (I)</b>					
Un-inoculated (I-)	69.3 b	9.7 b	29.5 b	8.41 b	2.14 b
Inoculated (I+)	249.8 a	35.7 a	32.0 a	9.78 a	2.56 a
<b>Cultivar (C)</b>					
Akça	88.8 de	16.3 c	34.2 ab	10.62 a	2.94 a
Aksu	434.8 a	60.2 a	32.7 b	9.22 b	2.31 c
Arda	61.2 de	8.8 cd	24.1 d	7.35 d	1.79 e
Aslanbey	215.3 c	28.0 b	34.4 ab	10.67 a	2.90 a
Bayram	197.2 c	27.8 b	28.8 c	7.90 cd	2.04 d
Botan	283.3 b	37.1 b	28.4 c	9.83 ab	2.57 b
Cevdetbey	179.3 c	28.6 b	29.8 c	9.45 b	2.46 bc
Göktürk	40.3 ef	5.2 d	25.9 d	7.80 cd	1.92 de
Ilgaz	0.0 f	0.0 d	33.4 ab	8.77 bc	2.38 bc
Yazıcı	95.0 d	15.0 c	35.9 a	9.32 b	2.31 c
<b>I x C</b>					
Akça (I-)	51.3 f-i	6.7 hi	33.5	9.67 b-f	2.60 bc
Akça (I+)	126.3 ef	26.0 ef	34.9	11.57 a	3.27 a
Aksu (I-)	202.7 d	29.2 de	30.3	8.43 e-h	2.22 def
Aksu (I+)	667.0 a	91.1 a	35.1	10.00 a-e	2.41 b-e
Arda (I-)	10.0 hi	1.0 i	23.6	6.00 i	1.49 h
Arda (I+)	112.3 efg	16.7 e-h	24.7	8.70 d-h	2.09 ef
Aslanbey (I-)	110.0 efg	14.0 f-i	33.2	10.00 a-e	2.65 b
Aslanbey (I+)	320.7 c	42.0 cd	35.6	11.33 ab	3.15 a
Bayram (I-)	121.3 ef	15.3 fgh	27.5	7.57 ghi	1.98 fg
Bayram (I+)	273.0 c	40.3 cd	30.1	8.23 fgh	2.10 ef
Botan (I-)	122.7 ef	17.2 e-i	27.9	9.33 c-g	2.42 b-e
Botan (I+)	444.0 b	57.0 b	28.8	10.33 a-d	2.71 b
Cevdetbey (I-)	42.0 ghi	5.0 hi	26.6	8.67 d-h	2.24 def
Cevdetbey (I+)	316.7 c	52.3 bc	33.1	10.23 a-d	2.68 b
Göktürk (I-)	0.0 i	0.0 i	24.7	7.13 hi	1.71 gh
Göktürk (I+)	80.7 e-h	10.4 ghi	27.2	8.47 e-h	2.13 ef
Ilgaz (I-)	0.0 i	0.0 i	33.6	9.30 c-g	2.47 bcd
Ilgaz (I+)	0.0 i	0.0 i	33.2	8.23 fgh	2.29 c-f
Yazıcı (I-)	32.7 hi	8.7 ghi	34.4	7.97 fgh	2.02 fg
Yazıcı (I+)	157.3 de	21.3 efg	37.4	10.67 abc	2.60 bc
CV (%)	26.2	32.8	6.9	10.0	7.5
Source	F-test				
<i>I</i>	**	**	**	**	**
<i>C</i>	**	**	**	**	**
<i>I x C</i>	**	**	ns	*	*

\* and \*\* significant at 0.05 and 0.01 levels, respectively; ns, not significant.

Mean values with the same letter in the column are not statistically different at  $p < 0.05$ .

**Table 3.** The effects of *Mesorhizobium ciceri* inoculation on the root fresh and dry matter production, chlorophyll, nitrogen content and total nitrogen amount of chickpea cultivars.

	Root fresh weight (g)	Root dry weight (mg)	Chlorophyll (SPAD)	Nitrogen content (%)	Total N (mg plant <sup>-1</sup> )
<b>Inoculation (I)</b>					
Un-inoculated (I-)	13.3 b	1.41 b	37.0 b	2.19 b	48.1 b
Inoculated (I+)	15.2 a	1.66 a	41.7 a	2.50 a	64.1 a
<b>Cultivar (C)</b>					
Akça	13.2 abc	1.75 ab	39.2 bc	2.35 bc	69.4 a
Aksu	12.5 bc	1.28 de	40.6 ab	2.42 ab	56.3 b
Arda	16.6 a	1.50 bcd	39.9 bc	2.39 abc	43.1 c
Aslanbey	15.7 ab	1.91 a	36.5 c	2.53 a	73.7 a
Bayram	11.0 c	1.23 e	39.8 bc	2.26 c	46.2 c
Botan	12.6 bc	1.40 cde	37.6 bc	2.36 bc	60.8 b
Cevdetbey	16.0 ab	1.66 abc	40.9 ab	2.42 ab	60.1 b
Göktürk	15.9 ab	1.52 bcd	43.3 a	2.40 abc	46.4 c
Ilgaz	15.4 ab	1.66 abc	36.9 c	2.07 d	49.1 c
Yazıcı	14.1 abc	1.46 cde	39.0 bc	2.42 ab	56.2 b
<b>I x C</b>					
Akça (I-)	12.0	1.34 cde	35.6 efg	2.22 def	57.6 c-f
Akça (I+)	14.5	2.16 a	42.7 abc	2.48 abc	81.1 a
Aksu (I-)	11.6	1.21 de	37.6 c-g	2.16 def	48.0 fg
Aksu (I+)	13.3	1.35 cde	43.7 a	2.67 a	64.5 bcd
Arda (I-)	15.1	1.39 b-e	37.2 d-g	2.24 def	33.4 i
Arda (I+)	18.0	1.61 bcd	42.6 abc	2.53 ab	52.8 efg
Aslanbey (I-)	13.9	1.65 bc	34.6 fg	2.35 bcd	62.4 b-e
Aslanbey (I+)	17.5	2.16 a	38.4 b-g	2.70 a	85.0 a
Bayram (I-)	10.4	1.10 e	37.0 d-g	2.23 def	44.2 gh
Bayram (I+)	11.6	1.37 cde	42.7 abc	2.29 cde	48.2 fg
Botan (I-)	12.2	1.30 cde	34.4 g	2.21 def	53.6 efg
Botan (I+)	13.0	1.49 b-e	40.7 a-d	2.51 ab	68.0 b
Cevdetbey (I-)	15.1	1.63 bc	42.2 abc	2.25 def	50.4 fg
Cevdetbey (I+)	16.9	1.68 bc	39.6 a-f	2.60 a	69.7 b
Göktürk (I-)	14.9	1.54 bcd	42.9 ab	2.14 def	36.3 hi
Göktürk (I+)	16.9	1.49 b-e	43.7 a	2.65 a	56.5 def
Ilgaz (I-)	14.2	1.52 bcd	33.5 g	2.04 f	50.4 fg
Ilgaz (I+)	16.5	1.79 b	35.2 efg	2.10 ef	47.8 fg
Yazıcı (I-)	13.6	1.40 b-e	35.3 efg	2.26 def	45.2 gh
Yazıcı (I+)	14.7	1.52 bcd	42.6 abc	2.58 a	67.3 bc
CV (%)	19.3	13.3	6.7	5.1	10.0
Source	F-test				
I	**	**	**	**	**
C	*	**	**	**	**
I x C	ns	*	*	*	**

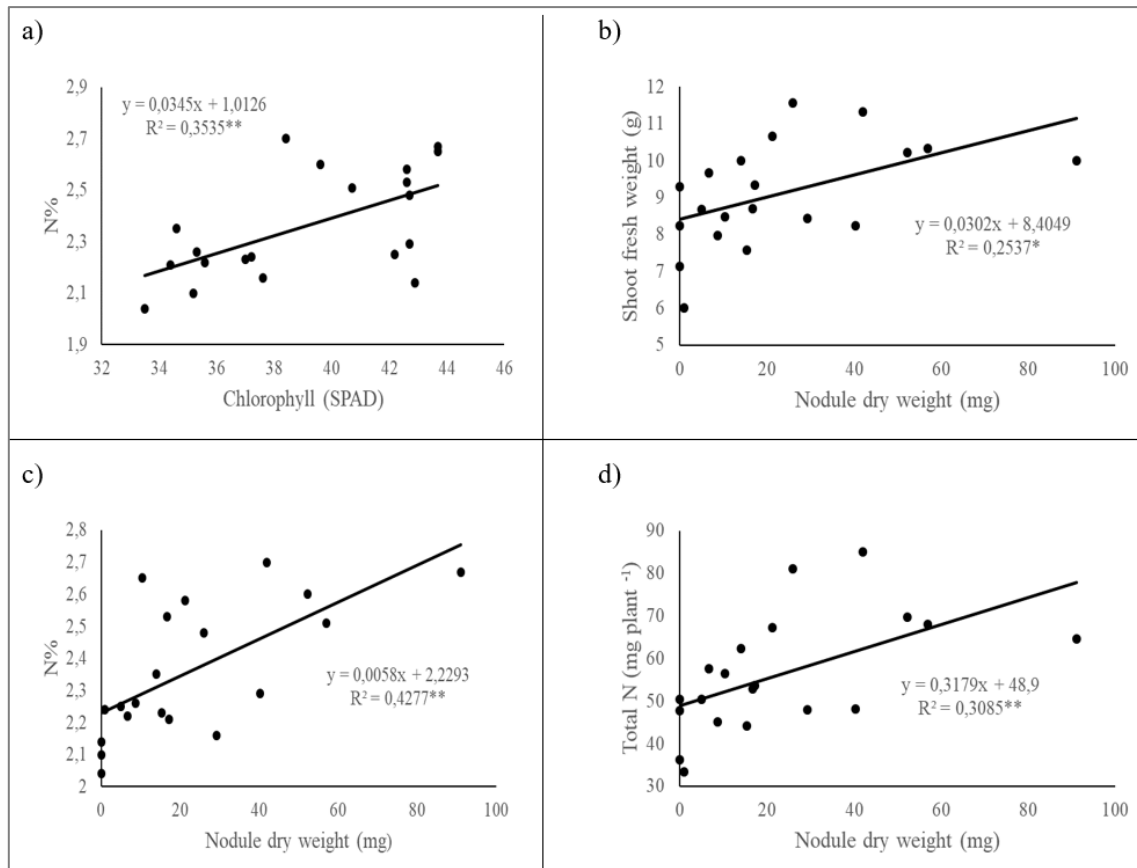
\* and \*\* significant at 0.05 and 0.01 levels, respectively; ns, not significant.

Mean values with the same letter in the column are not statistically different at  $p < 0.05$ .

The nodulation process is tightly regulated by both host and rhizobia genetics (Goyal et al., 2021; Wang et al., 2018). Significant increases in nodulation are observed when compatible bacteria x cultivar associations are achieved (Rupela & Saxena, 1987). This indicates that specific expression of both plant and bacterial genes is necessary for successful nodulation and nitrogen fixation. The inoculation x cultivar interaction was also significant for nodule weight in this study. The highest nodule fresh and dry weights per plant (667.0 and 91.1 mg, respectively) were determined in the inoculated Aksu, significantly higher than the other inoculated cultivars. On the other hand, Ilgaz could not produce nodules in either the un-inoculated or inoculated treatment. Compared to the un-inoculated control, the inoculation treatment significantly increased the nodule dry weight of cultivars except Göktürk, Ilgaz and Yazıcı (Table 2). The highest and lowest shoot fresh and dry weights were recorded in inoculated Akça and un-inoculated Arda, respectively. The response of cultivars to inoculation in terms of shoot dry weight was higher in Aslanbey, Cevdetbey, Göktürk, Akça, Yazıcı and Arda. These cultivars showed 18.9%, 19.6%, 24.6%, 25.8%, 28.7% and

40.3% increase in shoot dry weight compared to the un-inoculated control, respectively. In contrast, the inoculation treatment did not increase shoot dry weight in Ilgaz (Table 2).

The highest root dry weight (2.16 mg plant<sup>-1</sup>) was found in inoculated Akça and Aslanbey, which was significantly higher than all other inoculated and un-inoculated cultivars. The lowest root dry weight (1.1 mg plant<sup>-1</sup>) was recorded in the un-inoculated Bayram (Table 3). Similar shoot dry weight, significant differences were observed in root dry weight among cultivars in terms of their response to inoculation. Inoculation did not increase root dry weight in Cevdetbey and Göktürk compared to the un-inoculated control. Aslanbey and Akça exhibited the most significant response to inoculation, increasing root dry weight by 30.9% and 61.2%, respectively, compared to the un-inoculated control (Table 3). Consistent with our findings, other studies conducted on chickpea, have also shown that the interaction between inoculation and cultivar has a significant impact on nodulation and plant dry matter production (Bhuiyan et al., 2009; Di Bonito et al., 1990; Eusuf Zai et al., 1999).



**Figure 2.** Relationships of chlorophyll and nodule dry weight with shoot fresh weight, N% and total N. \* and \*\* significant at 0.05 and 0.01 levels, respectively; n= 20.

Nitrogen is an essential component of chlorophyll production in plants (Mendoza-Tafolla et al., 2019). In case of nitrogen deficiency, plants become light green, while the

amount of chlorophyll increases and plant color becomes darker depending on the increase in the amount of nitrogen supplied to the plant (Kumawat et al., 2000; Odabaş & Gülümser, 2001).

Therefore, chlorophyll content is considered one of the indicators of plant nitrogen content and nitrogen fixation (Hoque et al., 1999; Wood et al., 1992). In this study, the average chlorophyll content of the cultivars significantly increased from 37.0 SPAD in the un-inoculated control to 41.7 SPAD in the inoculation treatment, indicating that inoculation provides nitrogen to the plant (Table 3). The high correlation coefficient ( $r= 0.59^{**}$ ) between chlorophyll content and N% confirmed this phenomenon (Figure 2a). The highest and lowest chlorophyll contents were measured in inoculated Aksu (43.7 SPAD) and un-inoculated Ilgaz (33.5 SPAD), respectively. However, cultivars exhibited different responses to the inoculation treatment in terms of chlorophyll content. The inoculation treatment significantly increased the amount of chlorophyll in Akça, Aksu, Arda, Bayram, Botan and Yazıcı compared to the un-inoculated control. In other cultivars, the increases in chlorophyll content in relation to inoculation were not statistically significant (Table 3).

In rhizobium inoculation studies, increases in plant nitrogen percentage and total nitrogen content are the most important indicators of nitrogen fixation. Rhizobia-host compatibility is crucial in increasing plant nitrogen content and thus ensuring effective nitrogen fixation (Kantar et al., 2007; Santalla et al., 2001; Solaiman et al., 2007). This study observed significant variation in the effect of bacterial inoculation on nitrogen percentage, total and fixed nitrogen depending on the cultivars. The nitrogen percentage ranged from 2.04% (un-inoculated Ilgaz) to 2.70% (inoculated Aslanbey) and total N per plant ranged from 33.4 mg (un-inoculated Arda) to 85.0 mg (inoculated Aslanbey). The impact of inoculation on nitrogen percentage and total nitrogen varied significantly among the cultivars, similar to chlorophyll content. Except for Bayram and Ilgaz, the inoculation treatment significantly increased nitrogen percentage and total nitrogen in all cultivars compared to the un-inoculated control (Table 3). Akça, Arda, Aslanbey, Cevdetbey, Göktürk and Yazıcı, which fixed between 19.3 and 23.5 mg nitrogen per plant, exhibited the highest response to rhizobial inoculation. In contrast, Ilgaz did not fix any nitrogen, while Bayram fixed a considerably low amount of nitrogen (4

mg plant<sup>-1</sup>) (Table 4). Other researchers have also reported that the interaction between cultivar and bacteria significantly affects the amount of nitrogen fixed in chickpea (Beck, 1992; Elkoca et al., 2015; Solaiman et al., 2007).

**Table 4.** The amount of fixed N by the chickpea cultivars inoculated with *Mesorhizobium ciceri*.

Cultivars	Fixed N (mg plant <sup>-1</sup> )
Akça	23.5 a
Aksu	16.5 bc
Arda	19.4 abc
Aslanbey	22.6 ab
Bayram	4.0 d
Botan	14.4 c
Cevdetbey	19.3 abc
Göktürk	20.2 abc
Ilgaz	0.0 d
Yazıcı	22.1 ab
CV (%)	21.6

Mean values with the same letter are not statistically different at  $p < 0.05$ .

Correlation coefficients revealed significantly relationship between shoot fresh weight, N% and total N with nodule weight (Table 5 and Figure 2 b,c,d). Many researchers, including Bhuiyan et al. (2009), Ögütçü et al. (2010) and Solaiman et al. (2010) have reported similar positive correlations between nodulation and the parameters examined when effective nodules are formed. However, the amount of nitrogen fixed did not show a correlation with nodule weight (Table 5). For instance, Bayram with 40.3 mg nodule dry weight per plant only fixed 4 mg of nitrogen, while Akça and Göktürk with lower nodule dry weight per plant (26.0 and 10.4 mg, respectively), fixed larger amounts of nitrogen (23.5 and 20.2 mg, respectively) (Tables 2 and 4). These results indicate that nodule efficiency is more crucial than nodule weight in nitrogen fixation. Similar findings have been reported by other researchers such as Elkoca et al. (2015), Kipe-Nolt and Giller (1993) and Ögütçü et al. (2008).

**Table 5.** Correlation coefficients between nodulation and examined parameters.

	SH	SFW	SDW	RFW	RDW	Chlor.	N%	Total N	Fixed N
NFW	0.30 <sup>ns</sup>	0.48*	0.37 <sup>ns</sup>	-0.12 <sup>ns</sup>	-0.01 <sup>ns</sup>	0.39 <sup>ns</sup>	0.64**	0.53*	0.11 <sup>ns</sup>
NDW	0.34 <sup>ns</sup>	0.50*	0.40 <sup>ns</sup>	-0.10 <sup>ns</sup>	0.03 <sup>ns</sup>	0.41 <sup>ns</sup>	0.65**	0.56**	0.14 <sup>ns</sup>

\* and \*\* significant at 0.05 and 0.01 levels, respectively; ns: Not significant; n = 20.

SH: Shoot height; SFW: Shoot fresh weight; SDW: Shoot dry weight; RFW: Root fresh weight; RDW: Root dry weight.

#### 4. Conclusion

The results of this study showed that the response of chickpea to *rhizobium* inoculation varied significantly among cultivars and that symbiotic nitrogen gain in chickpea can be increased by selecting compatible cultivars. Akça, Arda,

Aslanbey, Cevdetbey, Göktürk and Yazıcı are the most compatible cultivars, considering the amount of nitrogen fixed. It would be useful to confirm the nitrogen fixation efficiency of chickpea cultivars used in this research conducted under pot conditions with field trials in future studies.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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