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Research Article

Turk J Agric For
34 (2010) 361-371
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doi:10.3906/tar-0904-34

Effects of rhizobium strains isolated from wild chickpeas on the growth and symbiotic performance of chickpeas (*Cicer arietinum* L.) under salt stress

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Received: 27.04.2009

Abstract: This study was conducted in order to evaluate the symbiotic effectiveness of *Rhizobium leguminosarum* bv. *ciceri* strains isolated from perennial wild chickpeas (*Cicer anatolicum*) in comparison to standard bacterial culture, N application, and uninoculated control under NaCl salinity stress conditions. For this purpose, 4 strains (DN1, DN7, TN3, and TN4) were obtained from wild chickpeas. Chickpea *(Cicer arietinum* L.) seeds were inoculated with these strains and grown in pots containing sterile sand under different levels of NaCl (0, 50, and 100 mM) in a controlled plant growth cabinet. Dry weights of root and shoot, root-to-shoot ratio (RSR), number and dry weights of nodules, chlorophyll and N content of the plant, and amounts of total and fixed N decreased progressively with increasing salinity levels. In both non-saline and saline (50 and 100 mM NaCl) conditions, inoculations with *Rhizobium leguminosarum* bv. *ciceri* strains isolated from wild chickpeas significantly increased all the above parameters compared with the uninoculated control treatment, equal to or higher than standard culture and N application. However, chickpea rhizobia exhibited diversity in their salt tolerance. The plants inoculated with DN7, TN4, and standard culture produced more shoot mass, nodule dry weight, total N, and fixed N under saline conditions, especially at 50 mM NaCl, than the plants inoculated with DN1 and TN3. These results indicated that the ability of chickpea to grow and survive in saline conditions improved when it was inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains isolated from wild chickpeas, especially DN7 and TN4.

Key words: *Cicer arietinum,* inoculation, nitrogen fixation, *Rhizobium leguminosarum* bv. *ciceri*, salt stress, wild chickpea

Yabani nohut bitkilerinden izole edilen rhizobium suşlarının tuzlu koşullarda nohudun (*Cicer arietinum* L.) gelişimi ve simbiyotik performansı üzerindeki etkisi

Özet: Bu çalışmada, çok yıllık yabani nohut (*Cicer anatolicum*) bitkilerinden izole edilen *Rhizobium leguminosarum* bv. *ciceri* suşlarının simbiyotik etkinlikleri tuzlu şartlarda standart bakteri kültürü, azot uygulaması ve aşısız kontrolle kıyaslamalı olarak test edilmiştir. Bu amaçla, yabani nohut bitkilerinden dört suş (DN1, DN7, TN3 ve TN4) elde edilmiştir. Suşlarla aşılanmış nohut *(Cicer arietinum* L.) tohumları kontrollü bitki büyütme kabininde steril kum içeren

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saksılara ekilmiş ve farklı tuz seviyelerinde (0, 50 ve 100 mM NaCl) yetiştirilmişlerdir. Kök ve sürgün kuru ağırlığı, kök/sürgün oranı, nodül sayısı ve kuru ağırlığı, bitki klorofil ve azot içeriği, toplam ve fikse edilen azot miktarı tuzluluk seviyesindeki artışa bağlı olarak azalmıştır. Yabani nohut bitkilerinden izole edilen *Rhizobium leguminosarum* bv. *ciceri* suşları ile yapılan aşılamalar, hem tuzsuz hem de tuzlu şartlarda (50 ve 100 mM NaCl), incelenen bütün parametreleri aşısız kontrole oranla önemli seviyede artırmıştır. Ancak, bakteri suşları tuza tolerans bakımından farklılık göstermişlerdir. Tuzlu şartlarda, özellikle 50 mM NaCl uygulamasında, DN7, TN4 ve standart bakteri kültürü ile aşılanan bitkiler, DN1 ve TN3 ile aşılanan bitkilere nazaran daha yüksek sürgün kuru ağırlığı, nodül kuru ağırlığı, toplam ve fikse edilen azot miktarına sahip olmuşlardır. Bu sonuçlar, özellikle DN7 ve TN4 olmak üzere, yabani nohut bitkilerinden izole edilen *Rhizobium leguminosarum* bv. *ciceri* suşları ile yapılan aşılamaların nohudun tuzlu şartlardaki gelişim ve dayanıklılığını artırabileceğini göstermiştir.

Anahtar sözcükler: *Cicer arietinum,* aşılama, azot fiksasyonu, *Rhizobium leguminosarum* bv. *ciceri*, tuz stresi, yabani nohut

Introduction

Rhizobium symbiosis with legume species is of special importance, producing 50% of 175 million tons of total biological N_2 fixation annually worldwide (Sarıoğlu et al. 1993). Chickpea (*Cicer arietinum* L.) and *Rhizobium leguminosarum* bv. *ciceri* association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain, and environmental factors (Rupela and Saxena 1987; Beck et al. 1991). However, chickpea is considered to be sensitive to salt stress and thus salinity is one of the most important environmental factors limiting production and biological N_2 fixation of chickpea in arid and semi-arid regions. Several studies carried out on chickpeas (Zurayk et al. 1998; Soussi et al. 1998; Welfare et al. 2002; Singla and Garg 2005; Garg and Singla 2009) have shown that NaCl salinity reduced plant growth, depressed N_2 fixation, reduced nodule numbers, and decreased percentage of tissue nitrogen.

Approximately 40% of the world's land surface is estimated to have potential salinity problems and most of these areas are confined to the tropics and Mediterranean regions (Zahran 1999; Bouhmouch et al. 2005). Saline conditions may limit the symbiosis by (i) affecting survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere, (ii) inhibiting the infection process, (iii) directly affecting root nodule function, or (iv) reducing plant growth, photosynthesis, and demand for nitrogen (Singleton et al. 1982; Singleton 1983). Several hypotheses have been suggested to explain the negative effect of salt on N_2 fixation in legumes, such as diminished photosynthate supply to the nodule (Bekki et al. 1987; Georgiev and Atkins 1993; Soussi et al. 1998), decreased supply of respiratory substrates to the bacteroids (Delgado et al. 1993, 1994), and alterations in the oxygen diffusion barrier (Serraj et al. 1994). Furthermore, salt stress can seriously change the photosynthetic carbon metabolism and leaf-chlorophyll content, as well as photosynthetic efficiency (Seeman et al. 1985; Soussi et al. 1998; Balibrea et al. 2003).

Genetic variability in salt tolerance exists within the rhizobia, and may significantly affect crop performance. There is also a wide variation among chickpea rhizobial strains in their ability to grow and survive under saline conditions (Zurayk et al. 1998). For instance, the use of rhizobia isolated from saline environment improved chickpea nodulation in saline soils (Ibrahim and Salih 1980) while sensitive *Rhizobium* strains produced poor nodulation and N_2 fixation under salinity (Saxena and Rewari 1992). Wild (naturally growing) leguminous plants in arid regions are subject to severe environmental conditions. Thus, symbiotic rhizobia of naturally growing legumes are more tolerant to some ecological conditions (salt, severe drought, elevated temperatures, etc.) than rhizobia from cultivated legumes (Zahran 2001). In fact, the salt-tolerant rhizobia isolated from wild legumes have specific characteristics that give them ecological significance and these rhizobia may be used as biofertilizers in salty soils (Zahran 2001). Therefore, this study attempted to evaluate the symbiotic effectiveness of *Rhizobium leguminosarum* bv. *ciceri* strains isolated from perennial wild chickpeas (*Cicer anatolicum*) under NaCl salinity stress conditions.

Materials and methods

Plant material

Chickpea (*Cicer arietinum* L.) cultivar Aziziye-94 was obtained from the Department of Agronomy, Faculty of Agriculture, Atatürk University, Erzurum, Turkey.

Bacterial strains

This study tested the effect of four *Rhizobium leguminosarum* bv. *ciceri* strains (Table 1) isolated from root nodules of wild chickpeas (*Cicer anatolicum*) collected from cold areas at high altitudes (2000-2500 m, 29°55′N and 41°16′E) in Erzurum province, Turkey. The standard culture in peat was obtained from the Soil and Fertilizer Research Institute, Ankara, Turkey.

Isolation, and morpho-physiological and biochemical characterization of rhizobia

The roots of wild chickpeas were transported to the laboratory in plastic bags. The roots of plants were thoroughly washed and nodules were severed and sterilized in 95% ethanol for 5 s and 3% H_2O_2 for 5 min. Each nodule was crushed and the content of the nodule was transferred onto a petri dish with yeast-extract mannitol agar (YEMA) (Vincent 1970; Somasegaran and Hoben 1985). Petri dishes were incubated at 28 °C until typical colonies of rhizobia appeared. Single colonies were marked and checked for purity by repeated streaking on YEMA medium (Vincent 1970) and verifying a single type of colony morphology, absorption of congo red (0.00125 mg kg^{-1}) and a uniform Gram-stain reaction. Colony morphology (color, mucosity, borders, transparency, and elevation) and acid/alkaline reaction were evaluated on YEMA containing bromothymol blue (0.00125 mg kg^{-1}) as an indicator (Alberton et al. 2006).

Table 1. Strains, sites, and altitudes from where *Rhizobium leguminosarum* bv. *ciceri* was collected.

Strain number	Locations of isolation	Altitude (m, a.s.l.)
DN1	Kapıkale	2350
DN7	Kapıkale	2350
TN3	Telsizler mountain	2100
TN4	Telsizler mountain	2100

Culture conditions, media, and treatment

Bacteria were grown in 250 mL Erlenmeyer flasks containing 100 mL of yeast extract mannitol broth at 28 °C for 6 days on a rotary shaker at 160 rpm (Prevost et al. 1987; Kantar et al. 2003). Chickpea seeds were surface-sterilized with 95% ethanol for 5 min, transferred into hydrogen peroxide solution of 3% for 5 min, and rinsed 6 times with sterile water (Atıcı et al. 2005). The seeds were left in water to imbibe for 4 h and aseptically germinated in petri dishes containing 20 mL of sterile deionized water and double filter papers for 5 days at 25 °C in a growth chamber. The germinated seeds with radicle emergence of 1 cm were treated with the bacterial cultures (2 mL) (10^8 cell mL^{-1}) (Öğütçü et al. 2008) and were aseptically transplanted to 1.5 L plastic pots (3 seeds per pot) containing sand (washed 10 times with sterile water and then autoclaved).

Controlled environment experiments

The experiment was conducted in a controlled plant growth chamber at 25 °C day/22 °C night and 16 h light/8 h dark periods (photoperiod of 16 h under light intensity of 550 Mmol m^{-2} s^{-1}, relative humidity of 55%-75%). The controlled environment experiments consisted of a completely randomized design with 3 replications. Treatments included the 4 *Rhizobium leguminosarum* bv. *ciceri* strains (Table 1) isolated from wild chickpeas, commercial peat culture (standard), N application (without inoculation), and uninoculated control (neither inoculation nor nitrogen). Pots received micro-nutrient solution as described by Broughton and Dilworth (1971) containing (μM): $CaCl_2$ (1000), KH_2PO_4 (500), $MgSO_4$ (250), K_2SO_4 (250), H_3BO_3 (2), $MnSO_4$ (1), $ZnSO_4$ (0.5), $CuSO_4$ (0.2), $CoSO_4$ (0.1), and Na_2MoO_4 (0.1). For the N application, 70 mg KNO_3 L^{-1} was added to the micro-nutrient solution (Beck et al. 1991; Kantar et al. 2003). Three concentrations of salt (0, 50, and 100 mM) in the form of NaCl were added to the growth medium after 10 days (Abdel-Wahab and Zahran 1981; Bouhmouch et al. 2005).

Data collection

Before harvesting, chlorophyll measurements were made in fresh leaves according to the following equation for quantification of the total chlorophyll content in an 80% acetone extract:

Total chlorophyll (mg g^{-1}) = 20.2 (A_{645}) + 8.02 (A_{663})

where A_{663} is the solution absorbance at 663 nm and A_{645} is the absorption at 645 nm (Arnon 1949).

Plants were harvested for 50 days after planting, root and shoot fractions were separated, and nodules were severed from the roots to dry at 65 °C for 24 h. After drying, dry weights of root, shoots, and nodules were determined and the root-to-shoot ratio (RSR) was calculated. N content was analyzed using the Kjeldahl method. Total N per plant (shoot dry weight × N content) and the fixed N (total N in inoculated plants – total N in uninoculated plants) were calculated (Öğütçü et al. 2008). N-fixation efficiency was calculated by comparing the strains with N applied control (total N in inoculated plants/total N in N application) × 100 (Beck et al. 1993; Öğütçü et al. 2008).

Statistical analysis

The data were analyzed using SPSS and mean values were separated according to Duncan's multiple range test at P = 0.05.

Results

Morpho-physiological and biochemical characterization of rhizobia

All strains isolated from wild chickpeas were found to have circular colonies with regular borders, flat in elevation, creamy in color, showing intermediate to high production of mucus (Table 2). Furthermore, all of the strains acidified the medium (as indicated by the bromothymol blue) and colony diameter ranging from 2 to 5 mm as stated in Bergey's Manual (Jordan 1984; Alberton et al. 2006).

Root and shoot dry weights

NaCl treatments significantly reduced the dry weights of both roots and shoots. As an average of the bacterial treatments, 50 and 100 mM NaCl treatments decreased root dry weight by 48.0% and 64.1% and decreased shoot dry weight by 23.0% and 38.3% over non-saline (0 mM NaCl) conditions, respectively (Figure 1). Bacterial inoculations significantly increased root and shoot dry weights compared to the uninoculated control under both non-saline and saline conditions. Interaction effect of "salinity × bacteria" was significant on root and shoot dry weights. Although the plants inoculated with standard culture had the highest root dry weight under both non-saline and 50mM NaCl conditions, the plants inoculated with DN1 and TN4 had significantly higher root dry weights (371.3 and 389.7 mg $plant^{-1}$, respectively) than those of the plants inoculated with standard culture (307.3 mg $plant^{-1}$) under 100 mM NaCl treatment (Figure 1). Similarly, the plants inoculated with standard culture and DN7 gave the highest shoot dry weights in both non-saline and 50 mM NaCl treatments, while standard culture (636.3 mg $plant^{-1}$) and TN4 (662.3 mg $plant^{-1}$) produced significantly higher shoot dry weights than those of the other bacterial strains in 100 mM NaCl treatment (Figure 1). Shoots appeared to be more sensitive to salinity than did roots. Therefore, 50 and 100 mM NaCl concentrations decreased RSR in all bacterial treatments (Table 3) and, except for TN4, decreases in RSR under salt treatments were considerably clear. As an average of control and bacterial treatments, RSR decreased from 0.89 to 0.52, when the root zone salinity increased from 0 mM to 100 mM (Table 3).

Table 2. Morphological and biochemical traits of strains isolated from wild chickpea.

Strain No.	Gram stain-reaction	Colony morphology	Colony color	Mucocity	Brom thymol blue with medium colony color	Congo red with medium colony color	Movement	Catalase test	Oxidase test
DN1	-	circular	creamy	+	yellow	white	+	+	+
DN7	-	circular	creamy	+	yellow	white	+	+	+
TN3	-	circular	creamy	+	yellow	white	+	+	+
TN4	-	circular	creamy	+	yellow	white	+	+	+

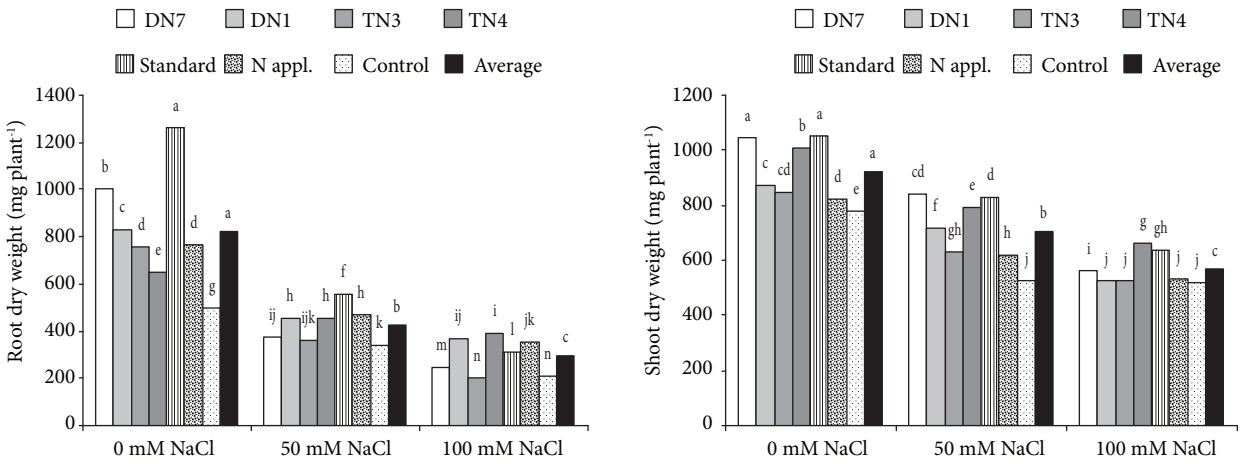

Figure 1. Root and shoot dry weights of chickpea inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains under varying levels of NaCl. Mean values with the same letter are not significantly different at $P \leq 0.05$.

Table 3. Root-to-shoot ratio (RSR) of chickpea inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains under varying levels of NaCl.

Treatments	NaCl Concentrations		
	0 mM	50 mM	100 mM
DN7	0.96	0.44	0.44
DN1	0.95	0.63	0.70
TN3	0.90	0.57	0.39
TN4	0.64	0.58	0.59
Standard	1.20	0.67	0.48
N appl.	0.93	0.75	0.67
Control	0.64	0.65	0.40
Average	**0.89**	**0.61**	**0.52**

Nodule number and nodule dry weight

The number and dry weight of nodule were also adversely affected by NaCl treatment. As an average of the bacterial treatments, 50 and 100 mM NaCl treatments reduced nodule number by 66.0% and 84.2% and reduced nodule dry weight by 40.1% and 98.0% over non-saline conditions, respectively (Figure 2). Nodule number generally was the highest in the plants inoculated with standard culture (37.7, 12.7, and 5.7 number plant^{-1}, respectively) and DN1 (32.7, 12.0, and 7.7 number plant^{-1}, respectively) under both non-saline and saline (50 and 100 mM NaCl) conditions (Figure 2). On the other hand, plants inoculated with standard culture (54.3 and 29.7 mg plant^{-1}, respectively), TN4 (52.0 and 26.3 mg plant^{-1}, respectively), and DN7 (40.3 and 27.0 mg plant^{-1}, respectively) produced the highest nodule dry weights in 0 and 50 mM NaCl treatments, whereas there were no significant nodule dry weight differences among bacterial treatments in 100 mM NaCl level (Figure 2). Less affected plants produced higher nodule number and dry weight; therefore, nodule dry weight showed a high correlation with shoot dry weight under 0 and 50 mM NaCl concentrations. These correlations were also positive but not significant at 100 mM NaCl (Figure 3).

Chlorophyll content

Salinity had also a detrimental effect on plant chlorophyll content. As an average of the bacterial and the control treatments, 50 and 100 mM NaCl treatments diminished chlorophyll content by 58.6% and 82.8% over non-saline conditions, respectively (Figure 4). Under both non-saline and saline (50 and 100 mM NaCl) conditions, the highest and the lowest plant chlorophyll contents were observed in N application and noninoculation treatment, respectively. Plants inoculated with DN7 and TN3 had similar chlorophyll content to N applied plants under 50 mM NaCl conditions. However, only plants inoculated with TN4 were able to produce a similar amount of chlorophyll to N applied plants under 100 mM NaCl conditions (Figure 4).

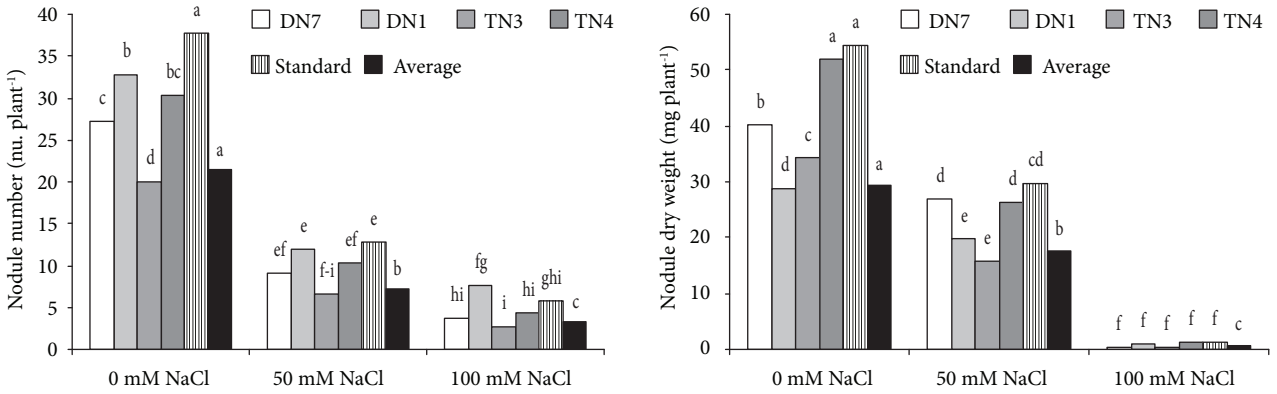

Figure 2. Nodule number and nodule dry weight of chickpea inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains under varying levels of NaCl. Mean values with the same letter are not significantly different at $P \leq 0.05$.

N content and total N

Plant N content and total N per plant were reduced by increasing NaCl in all inoculated and nitrogen given plants. On average, 50 and 100 mM NaCl levels reduced plant N content by 11.7% and 17.3%, and reduced total N per plant by 31.6% and 49.0% over non-saline conditions, respectively (Table 4). In all salinity levels (0, 50, and 100 mM NaCl), the highest and the lowest tissue nitrogen were determined in N applied and the uninoculated plants, respectively. Inoculated and N fertilized plants accumulated more total N in their tissues than uninoculated control plants in both non-saline and saline conditions. DN7 and TN4 produced significantly similar total N per plant to standard *Rhizobium* strain in both non-saline and saline conditions. DN1 and TN3 were not as effective as standard *Rhizobium* strain and nitrogen given plants (Table 4). Reducing effects were more severe on total N per plant than plant N contents due to reduced shoot biomass. As compared to the N applied plants, the plants inoculated with bacterial strains had generally higher shoot dry weight in both non-saline and saline conditions (Figure 1). Therefore, the plants inoculated with bacterial strains had higher or similar total N to the N applied plants, although N applied plants had the highest N content in both non-saline and saline conditions (Table 4).

Fixed N and N-fixation efficiency

Salinity treatments had a significant effect on fixed N. When compared to the inoculated plants, the inhibitory effect of 50 mM NaCl on the shoot dry weight and total N of uninoculated plants was more severe (Figure 1 and Table 4). Therefore, as an average of bacterial treatments, amount of fixed N was higher in the 50 mM NaCl treatment. However, 100 mM NaCl treatment reduced fixed N by 67.6% and N-fixation efficiency by 9.9% over non-saline conditions. In non-saline and 50 mM NaCl treatments, the plants inoculated with DN7 had significantly higher fixed N than those of the plants inoculated with standard culture, whereas TN4 produced the highest fixed N in 100 mM NaCl treatment. Thus, strains isolated from wild chickpeas, DN7 (140.0%) and TN4 (108.1%) had the highest N-fixation efficiency in 50 and 100 mM NaCl treatments, respectively.

Discussion

Salinity significantly reduced the overall growth of plants and the results confirmed earlier reports that chickpea is highly sensitive to salinity. This was evident from the decline in the dry mass of roots and shoots with increasing stress (Figure 1). Under salt stress, decreases in root and shoot mass have been also reported by several researchers for chickpea (Zurayk et al. 1998; Elsheikh and Wood 1990a; Soussi et al. 1999, 2001; Welfare et al. 2002; Singla and Garg 2005). The root weights showed a greater decline than the shoot mass under salt stress conditions (Table 3). Similarly, many researchers also reported that roots were more sensitive to salt stress than shoots in chickpea (Rao and Sharma 1995; Soussi et al. 2001; Singla and Garg 2005) and other legumes such as faba bean (Cordovilla et al. 1999).

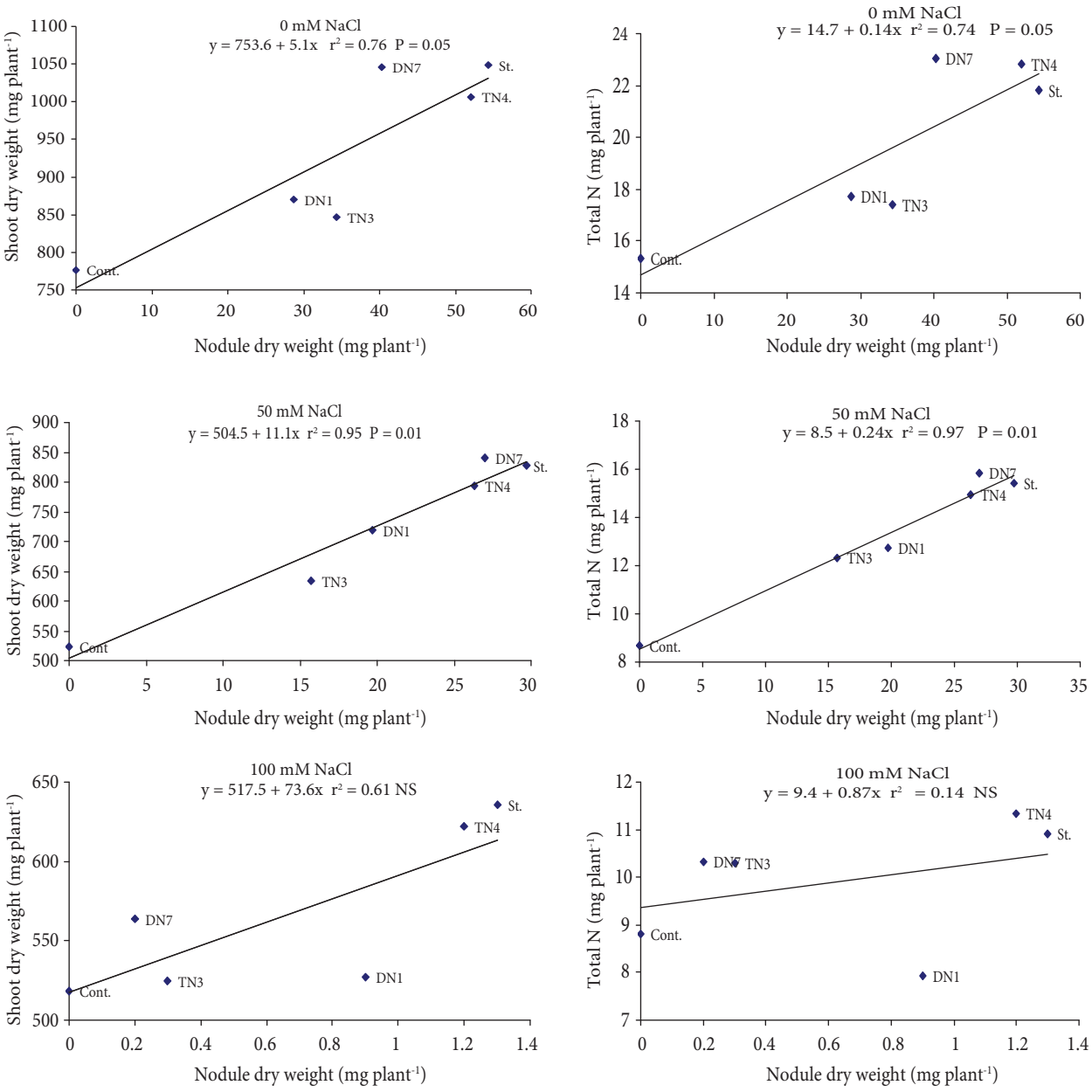

Figure 3. Relationships of nodule dry weight with shoot dry weight and total N under varying levels of NaCl. NS, non-significant.

Nodule dry weight is one of the most important parameters indicating effective symbiosis (Kantar et al. 2003; Öğütçü et al. 2008). In the present study, number and dry weight of nodules decreased progressively with increasing (0 to 100 mM NaCl) salinity level (Figure 2) and the nodule dry weight showed a high correlation with shoot dry weight and total N (Figure 3). Similar correlations have also been reported by other researchers (Daba and Haile 2000; Kantar et al. 2003). The nodulation of chickpea was reduced by more than 50% at 34.2 mM NaCl, and was completely depressed in plants that received a high saline treatment (Elsheikh and Wood 1990b). It has also been reported that significant reductions in nodule dry weight (59.8%) and N_2 fixation (63.5%) of chickpea were evident even at the lowest salinity level

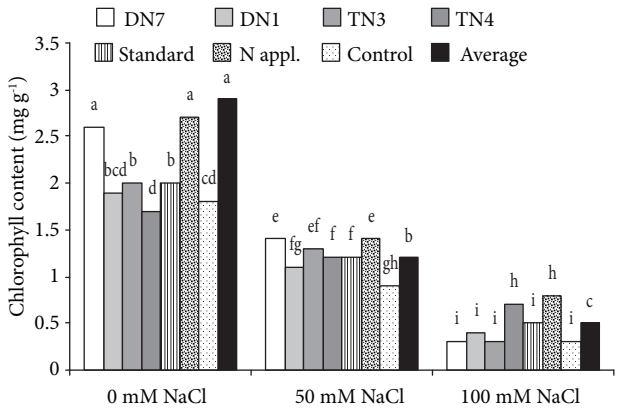

Figure 4. Chlorophyll content of chickpea inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains under varying levels of NaCl. Mean values with the same letter are not significantly different at $P \leq 0.05$.

of 30 mM (Zurayk et al. 1998). Many researchers have reported that detrimental concentrations of NaCl on chickpea nodulation and N_2 fixation ranged between 8.6 mM (Elsiddig and Elsheikh 1992) and 100 mM (Soussi et al. 1998). The establishment of efficient symbiosis involves a number of steps, beginning with rhizobia-host recognition and ending with biochemical N_2 fixation. The sharp decrease in both nodule number and dry weight in this experiment indicates that symbiosis breaks down at the early stages of symbiosis establishment. There are several possible reasons for this. Islam and Ghoulam (1981) showed that salinity affected root exudates, which altered chemotaxis ratios of the rhizobia, resulting in poor nodulation. Failure of nodulation has also been

Table 4. The effect of inoculation with *Rhizobium leguminosarum* bv. *ciceri* strains isolated from wild chickpea on N content, total N, and fixed N of chickpea and N-fixation efficiency of the strains under varying levels of NaCl.

	N content % NaCl Concentrations			Total N mg plant^{-1} NaCl Concentrations		
Treatments	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
DN7	2.20^{a-d}	1.89^{c-g}	1.83^{d-h}	23.02^{a}	15.86^{cde}	10.32^{hi}
DN1	2.03^{a-g}	1.77^{e-h}	1.51^{h}	17.70^{bc}	12.72^{fg}	7.94^{j}
TN3	2.06^{a-f}	1.95^{b-g}	1.96^{b-g}	17.42^{bcd}	12.32^{fgh}	10.30^{hi}
TN4	2.27^{ab}	1.89^{c-g}	1.71^{e-h}	22.84^{a}	14.97^{e}	11.33^{gh}
Standard	2.08^{a-e}	1.86^{c-h}	1.71^{e-h}	21.83^{a}	15.43^{de}	10.90^{ghi}
N appl.	2.32^{a}	2.24^{abc}	1.98^{b-g}	19.12^{b}	13.91^{ef}	10.48^{hi}
Control	1.97^{a-g}	1.66^{gh}	1.70^{fgh}	15.32^{de}	8.68^{ij}	8.80^{ij}
Average	**2.14^{a}**	**1.89^{b}**	**1.77^{c}**	**19.61^{a}**	**13.41^{b}**	**10.01^{c}**

	Fixed N mg plant^{-1} NaCl Concentrations			N-fixation efficiency % NaCl Concentrations		
Treatments	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
DN7	7.71^{a}	7.18^{c}	1.52^{l}	120.4	140.0	98.5
DN1	2.38^{j}	4.04^{g}	0.86^{m}	92.6	91.4	75.8
TN3	2.09^{k}	3.64^{h}	1.50^{l}	91.1	88.6	98.3
TN4	7.56^{b}	6.29^{f}	2.53^{i}	119.5	107.6	108.1
Standard	6.51^{e}	6.75^{d}	2.10^{k}	114.2	110.9	104.0
N appl.	-	-	-	100.0	100.0	100.0
Control	-	-	-	-	-	-
Average	**5.25^{b}**	**5.58^{a}**	**1.70^{c}**	**106.3**	**106.4**	**97.5**

Mean values with the same letter within parameters are not significantly different at $P \leq 0.05$.

attributed to damaged root hairs (Tu 1981; Elsheikh and Wood 1990a).

When compared to non-saline conditions, on average, the nodule dry weight showed a greater decline, which decreased by 40.1% and 98.0%, than the shoot mass, which decreased by 23.0% and 38.3% at 50 and 100 mM NaCl, respectively. Therefore, our results also indicated that nodulation is apparently more salt sensitive than is plant growth. Similarly, according to Elsheikh and Wood (1990a) and Soussi et al. (1998), nodulation of chickpea was reduced even by the lowest NaCl concentration (50 mM), while plant dry weight was affected only by 100 mM.

The depressive effect of salt stress on N_2 fixation by chickpea is directly related to the salt-induced decline in dry weight, N content and total N in the shoot (Elsiddig and Elsheikh 1992; Zurayk et al. 1998). In this experiment, on average, 50 and 100 mM NaCl levels also reduced plant N content by 11.7% and 17.3%, and total N per plant by 31.6% and 49.0% over non-saline conditions, respectively (Table 4). The reduction of N_2 fixing activity by salt stress is usually attributed to a reduction in respiration of the nodules (Ikeda et al. 1992; Soussi et al. 2001) and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules (Delgado et al. 1993, 1994). Chlorophyll damage and reduction in photosynthetic activity might also affect N_2 fixation by chickpea under salt stress (Soussi et al. 1999; Garg and Singla 2004). In the current study, saline stress led to the yellowing of leaves, and chlorophyll measurements also indicated that leaf chlorophyll content was reduced significantly under salinity conditions (Figure 4).

Symbiotic rhizobia of wild legumes are more tolerant to some ecological conditions (salt, severe drought, elevated temperatures, etc.) than rhizobia from cultivated legumes (Zahran 2001; Öğütçü et al. 2008). Some rhizobia isolated from wild legumes also successfully establish effective symbiosis under stress conditions (Zahran 2001; Öğütçü et al. 2008). Therefore, this study attempted to evaluate the symbiotic effectiveness of *Rhizobium leguminosarum* bv. *ciceri* strains isolated from perennial wild chickpeas (*Cicer anatolicum*) under NaCl salinity stress conditions.

In general, the plants inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains isolated from wild chickpea had similar shoot dry weights, chlorophyll, N percentage, and total N to the plants inoculated with the standard culture and N applied plants in both non-saline and saline (50 and 100 mM NaCl) conditions. Furthermore, the uninoculated control had the lowest values and generally bacterial inoculations significantly increased above parameters compared to the uninoculated control treatment under both non-saline and saline conditions (Figure 1, Figure 4, and Table 4). These results confirm that the ability of legume hosts to grow and survive in saline conditions is improved when they are inoculated with salt tolerant strains of rhizobia (Rao and Sharma 1995; Zou et al. 1995; Hashem et al. 1998; Shamseldin and Werner 2005). Moreover, significant increases in chlorophyll content, an indication of N_2 fixation (Hoque et al. 1999; Kantar et al. 2003; Öğütçü et al. 2008), N percentage, and total N due to bacterial inoculation supported the hypothesis that biological N_2 fixation by the *Rhizobium* could be responsible for the observed higher N uptake of inoculated plants under both non-saline and saline conditions (Kantar et al. 2003; Öğütçü et al. 2008).

The plants inoculated with DN7, TN4, and standard culture produced more shoot mass, nodule dry weight, total N, and fixed N under saline conditions, especially at 50 mM NaCl, than the plants inoculated with DN1 and TN3 (Figure 1, Figure 2, and Table 4). These results from our study confirmed earlier reports that chickpea rhizobia exhibited diversity in their salt tolerance (Zurayk et al. 1998; Elsheikh and Wood 1990b). It has been reported that growth of a number of rhizobia was inhibited by 100 mM NaCl (Zahran 2001). In our trial, when compared to the uninoculated plants, only plants inoculated with TN4 were able to produce significantly more total N at 100 mM NaCl. In addition, only TN4 fixed significantly more N than the standard culture at 100 mM NaCl (Table 4).

In the light of present results, discussed in detail above, it may be concluded that salinity levels used in present investigation negatively affected all parameters of growth such as root and shoot growth, root-to-shoot ratio, nodule number and dry weight, chlorophyll and N content, and total and fixed N in

chickpea plants. However, the ability of chickpea to grow and survive in saline conditions improved when it was inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains, especially DN7 and TN4, isolated from wild chickpeas. In fact, the best results for symbiotic N_2 fixation under salt stress are obtained if both symbiotic partners resist such stress. Therefore, further work is required to select chickpea genotypes that are tolerant to salt stress.

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