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The Determination of Symbiotic Effectiveness of *Rhizobium* Strains Isolated from Wild Chickpeas Collected from High Altitudes in Erzurum

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Abstract: This study was conducted in order to evaluate the symbiotic effectiveness of *Rhizobium leguminosarum* subsp. *ciceri* strains isolated from perennial wild chickpeas (*Cicer anatolicum*) collected from high altitudes (2000-2500 m) in mountains in Erzurum, Eastern Anatolia, Turkey. For this purpose, 21 isolates were obtained from wild chickpeas. Chickpea seeds were inoculated with these isolates and grown in pots containing sterile sand under both low (15 °C day/9 °C night) and normal (25 °C day/22 °C night) temperature conditions in a controlled plant growth cabinet. All strains investigated formed nodules under the normal temperature, but only 8 strains were able to produce nodules at the low temperature. In both normal and low temperature experiments, generally, all strains producing nodules provided N to the plant, as indicated by shoot dry weight, total N content, and N fixed. However, strains showed significant differences for almost all parameters measured. Among the strains tested, 7 strains at 25 °C day/22 °C night and 4 strains at 15 °C day/9 °C night demonstrated a good performance on chickpea in terms of their high shoot dry matter yields, total N content, N fixed, and symbiotic effectiveness. These results indicated that effective strains isolated from wild chickpeas had potential for use as inoculants on chickpea.

Key Words: Nitrogen fixation, *Cicer arietinum*, low temperature, *Rhizobium leguminosarum* subsp. *ciceri*, wild chickpea

Erzurum'da Yüksek Rakımlardan Toplanan Yabani Nohut Bitkilerinden İzole Edilen Rhizobium Suşlarının Simbiyotik Etkinliklerinin Belirlenmesi

Özet: Bu çalışma, Erzurum yöresinde düşük sıcaklığa sahip yüksek rakımlı (2000-2500 m) alanlardaki çok yıllık yabani nohut (*Cicer anatolicum*) bitkilerinden izole edilen, toplam 21 *Rhizobium leguminosarum* subsp. *ciceri* suşunun simbiyotik etkinliğinin belirlenmesi amacıyla yürütülmüştür. İzolatlarla aşılı nohut tohumları, içerisinde steril kum bulunan saksılara ekilmiş ve saksılar düşük (15 °C gündüz / 9 °C gece) ve normal (25 °C gündüz / 22 °C gece) sıcaklık koşullarına sahip kontrollü bitki büyütme kabinlerine yerleştirilmişlerdir. Normal sıcaklık koşullarında izolatların tamamı, düşük sıcaklıkta ise yalnızca sekiz tanesi nodül oluşturabilmiştir. Normal ve düşük sıcaklık şartlarında nodül oluşturan izolatlar, bitkiye azot sağladıklarının göstergesi olarak, genellikle bitki kuru ağırlığı ile toplam azot içeriğini arttırmışlardır. Ancak, izolatların incelenen parametreler üzerindeki etkinlikleri birbirinden önemli derecede farklılık göstermiştir. Bitki kuru ağırlığı, toplam azot içeriği, fikse edilen azot miktarı ve simbiyotik etkinlik dikkate alındığında, 25 °C gündüz / 22 °C gece sıcaklık koşullarında yedi, 15 °C gündüz / 9 °C gece sıcaklık koşullarında ise dört izolatın iyi bir performans gösterdiği ortaya konulmuştur. Bu sonuçlar, yabani nohut bitkilerinden elde edilen etkili izolatların, nohutta aşılama materyali olarak kullanılma potansiyeline sahip olduğunu göstermiştir.

Anahtar Sözcükler: Azot fiksasyonu, *Cicer arietinum*, düşük sıcaklık, *Rhizobium leguminosarum* subsp. *ciceri*, yabani nohut

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Introduction

Botanical, genetic, and archeological evidence points to chickpea (*Cicer arietinum* L.) originating within the Fertile Crescent, Turkey (Lev-Yadun et al., 2000). From there, it has spread to its present day range, principally concentrated between latitudes 20° and 40° and including west and central Asia, the Indian subcontinent, southern Europe, Africa, Latin America, and more recently North America and Australia (Croser et al., 2003). Widespread cultivation has resulted in chickpea being third in terms of world pulse production behind dry bean and field pea. Chickpea is the most widely produced pulse crop in Turkey. A nationwide project towards decreasing fallow areas supported chickpea-cereal rotation in the 1980s and consequently its hectarage increased from approximately 270,000 ha in the 1980s to 610,000 ha in 2006 (FAO, 2006), making Turkey a leading exporter of chickpea produce.

Nitrogen is known to be an essential nutrient for plant growth and development. Intensive farming practices that achieve high yields require chemical fertilizers, which are not only costly but may also create environmental problems. The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumers' health. Consequently, there has recently been a growing level of interest in environmentally friendly sustainable agricultural practices and organic farming systems (Rigby and Caceres, 2001; Lee and Song, 2007). Increasing and extending the role of biofertilizers such as *Rhizobium* would reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization is of great importance in alleviating environmental pollution and the deterioration of nature (Elkoca et al., 2008).

Rhizobium symbiosis with legumes species is of special importance, producing 50% of 175 million tonnes of total biological N fixation annually worldwide (Sarıoğlu et al., 1993). Chickpea and *Rhizobium leguminosarum* subsp. *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, *Rhizobium* species producing nodules in chickpea are specific only to this species and thus inoculation with effective strains is advised in soils with no or weak bacterial presence (Rupela and Saxena, 1987).

Temperate climates are characterized by short growing seasons, which are subjected to temperatures below the optimal for symbiotic nitrogen fixation. Chickpea is increasingly being sown in the autumn rather than the spring. However, freezing and chilling range temperatures are considered an important problem for autumn-sown chickpea in the countries surrounding the Mediterranean Sea, the tropical highlands, and temperate growing regions (Singh, 1993). Thus, autumn-sown chickpeas are often subjected to temperatures below the optimal range of 20 to 35 °C (Ellis et al., 1986). In spring planting areas, where the plant growing season is short, chickpeas are seeded into cool soils to maximize the length of the production season (Auld et al., 1988).

Cool soil temperatures in temperate climates affect the competitiveness of rhizobia for nodulation (Hardarson and Jones, 1979), retard the infection of root hairs, decrease nodulation (Fyson and Sprent, 1982; Bordeleau and Prevost, 1994), and depress nodule activity (Waughman, 1977). However, the strain of *Rhizobium* plays an important role in determining the efficiency of nitrogen fixation at low temperatures (Layzell et al., 1983; Pankurst and Layzell, 1984) and variation exists among *Rhizobium* strains in terms of the ability to nodulate under cool conditions (Rupela and Kumar Rao, 1987). Most of the bacteria isolated from cold environments are able to grow and show earlier nodulation and higher nitrogenase activity at low temperatures (Ek-Jander and Fahraeus, 1971; Nelson and Parkinson, 1978; Prevost et al., 1987a). Thus, this study attempted to evaluate the symbiotic effectiveness of *Rhizobium* strains isolated from perennial wild chickpea species (*Cicer anatolicum*) collected from cold environments at high altitudes of 2000-2500 m in mountains in Erzurum, Eastern Anatolia, on the temperate legume chickpea (*Cicer arietinum*) under both low and normal temperature conditions.

Materials and Methods

Plant material

Chickpea, *Cicer arietinum* L. cv. Aziziye-94 obtained from the Eastern Anatolia Agricultural Research Institute, Erzurum, was used as the plant material in the experiments.

Bacterial strain, culture conditions, media, and treatment

This study used 21 *Rhizobium leguminosarum* subsp. *ciceri* strains (Table 1) isolated from root nodules of wild chickpea (*Cicer anatolicum*) collected from regions at high altitudes (2000-2500 m) in Erzurum province, Turkey. The standard *Rhizobium* culture in peat was obtained from the Soil and Fertilizer Research Institute, Ankara. The inoculates were produced by growing the rhizobial strains in 250-ml Erlenmeyer flasks containing 100 ml of yeast extract mannitol broth and incubated at 28 °C for 6 days on a rotary shaker at 160 rpm (Prevost et al., 1987b). Chickpea seeds were treated with 95% ethanol for 5 min and then transferred into hydrogen peroxide solution of 3% for 5 min, and rinsed 6 times with sterile water. The seeds were then left in water to imbibe for 4 h and aseptically germinated in petri dishes at 25 °C for 48 h. The germinated seeds with radicle emergence of 1 cm were treated with the bacterial cultures (10^8 cells ml^{-1}) (Somasegaran and Hoben, 1985) and immediately sown in sterile sand in 1.5-l pots as 3 germinated seeds per pot.

Controlled environment experiments

Two experiments were conducted under controlled plant growth cabinet conditions in order to test the symbiotic effectiveness of the strains investigated at both low (15 °C day/9 °C night) and normal (25 °C day/22 °C night) temperatures under 16 h light/8 h dark period in 1998. The controlled environment experiments consisted of a completely randomized design with 3 replicates. Treatments included the 21 *Rhizobium leguminosarum* subsp. *ciceri* strains (Table 1) isolated from wild chickpea, commercial peat culture (standard), N application, and uninoculated control (no inoculation or nitrogen). Pots received micro-nutrient solution (Broughton and Dilworth, 1971). In N application, 70 mg KNO_3 l^{-1} was added to the micro-nutrient solution (Beck et al., 1993).

Data collection

Plants were harvested 50 days after emergence, root and shoot fractions were separated, and nodules were severed from the roots to dry at 65 °C for 24 h. N concentration was analyzed using the Kjeldahl method and N content (shoot dry weight x N concentration) per plant and the N fixed (plant N content in inoculated pots – plant N content in uninoculated pots) were calculated (Yaman and Cinsoy, 1996). Efficiency of symbiosis was calculated by comparing the strains with N applied control (plant N content in inoculated pots/plant N content in N application) × 100 (Beck et al., 1993).

Statistical analysis

The data were subjected to analysis of variance using the MSTATC Statistical Package (version 1.4, Michigan State University, MI, USA) and means were separated according to the least significant differences (LSD) test at P = 0.05.

Results

Under controlled environmental conditions at 25 °C day/22 °C night temperature, bacterial inoculations significantly (P = 0.05) increased all the parameters investigated compared with the uninoculated control treatment, equal to or higher than standard bacterial culture and N application depending on the strain (Table 2). HF 1 gave the highest shoot dry weight (2020.0 mg $plant^{-1}$), N content (31.1 mg $plant^{-1}$), N fixed (23.2 mg $plant^{-1}$), and efficiency of symbiosis (186.2%), although

Table 1. Isolates, sites and altitudes from where *Rhizobium leguminosarum* subsp. *ciceri* was collected.

Isolate number	Locations of isolation	Altitude (m a.s.l.)
HF 1, HF 3, and HF 5	Telsizler Mountain	2000
HF 10	Telsizler Mountain	2250
HF 170 and HF 175	Rabat Mountain	2350
HF 176, HF 177, and HF 178	Hasanbaba Mountain	2350
HF 262, HF 266, HF 269, HF 270, HF 271, HF 272, HF 274, HF 275, HF 277, HF 278, HF 286, and HF 289	Palandöken Mountains	2500

Table 2. The effect of inoculation with *Rhizobium leguminosarum* subsp. *ciceri* strains isolated from wild chickpea at high altitudes on investigated parameters of chickpea and symbiotic effectiveness of the strains under normal temperature (25 °C day/22 °C night) conditions.

Isolate No.	Shoot dry weight (mg plant^{-1})	Nodule dry weight (mg plant^{-1})	N concentration (%)	N content (mg plant^{-1})	N fixed (mg plant^{-1})	Efficiency of symbiosis (%)
HF 1	2020.0	54.7	1.54	31.1	23.2	186.2
HF 10	1573.7	57.4	1.32	20.8	12.9	124.6
HF 269	1400.5	110.7	1.63	22.8	14.9	136.5
HF 175	1300.7	56.4	1.67	21.7	13.8	129.9
HF 5	1276.4	49.7	1.46	18.6	10.7	111.4
HF 278	1219.3	68.6	1.30	15.8	7.9	94.6
HF 178	1204.1	49.2	1.32	15.9	8.0	95.2
HF 262	1152.7	87.4	1.44	16.6	8.7	99.4
HF 271	1136.9	242.8	1.38	15.7	7.8	94.0
HF 176	1093.3	52.2	1.63	17.8	9.9	106.6
HF 289	1060.9	55.2	1.68	17.8	9.9	106.6
HF 270	997.6	230.8	1.20	12.0	4.1	71.9
HF 275	964.4	251.0	1.31	12.6	4.7	75.4
HF 3	950.8	62.9	1.39	13.2	5.3	79.0
HF 177	907.8	261.6	1.11	10.1	2.2	60.5
HF 266	899.9	226.4	1.44	12.9	5.0	77.2
HF 170	893.4	242.5	1.21	10.8	2.9	64.7
HF 286	797.8	37.5	1.64	13.1	5.2	78.4
HF 277	749.4	235.2	1.68	12.6	4.7	75.4
HF 272	724.1	249.5	1.52	11.0	3.1	65.9
HF 274	594.6	23.9	1.67	9.9	2.0	59.3
Standard	1156.9	72.8	1.61	18.6	10.7	111.4
N application	991.3	0.0	1.68	16.7	-	100.0
Uninoculated	758.5	0.0	1.04	7.9	-	-
LSD (5%)	125.7	39.5	0.16	2.62	2.66	15.5

other strains tested also generally produced results better than the uninoculated control. The plants inoculated with HF 175, 176, 269, 274, 277, 286, and 289 had the highest N concentration, whereas other bacterial strains (except for HF 177 and HF 270) gave significantly higher N concentration values than the uninoculated control. HF 177 produced the highest nodule dry weight (261.6 mg) per plant, followed by HF 275 (251.0 mg), HF 272 (249.5 mg) HF 271 (242.8 mg), HF 170 (242.5 mg), HF 277 (235.2 mg) HF 270 (230.8 mg), and HF 266 (226.4 mg), with uninoculated control plants having no nodules (Table 2).

Under the low temperature conditions (15 °C day/9 °C night), only 8 *Rhizobium leguminosarum* subsp. *ciceri* strains were able to produce nodules and standard control had the highest nodule dry weight per plant (52.8 mg), followed by HF 175 (16.2 mg) and HF 262 (10.6 mg) (Table 3). Among the bacterial inoculations, HF 278 gave the highest shoot dry weight (835.6 mg plant^{-1}), N content (9.9 mg plant^{-1}), N fixed (3.7 mg plant^{-1}), and efficiency of symbiosis (79.2%), whereas uninoculated plants produced no nodules and had the lowest shoot dry weight, N concentration, and N content.

Table 3. The effect of inoculation with *Rhizobium leguminosarum* subsp. *ciceri* strains isolated from wild chickpea at high altitudes on investigated parameters of chickpea and symbiotic effectiveness of the strains under low temperature (15 °C day/9 °C night) conditions.

Isolate No.	Shoot dry weight (mg plant^{-1})	Nodule dry weight (mg plant^{-1})	N concentration (%)	N content (mg plant^{-1})	N fixed (mg plant^{-1})	Efficiency of symbiosis (%)
HF 278	835.6	2.7	1.19	9.9	3.7	79.2
HF 262	784.8	10.6	1.10	8.6	2.4	68.8
HF 177	694.7	3.4	1.25	8.7	2.5	69.6
HF 175	627.4	16.2	1.17	7.3	1.1	58.4
HF 10	616.8	1.4	1.40	8.6	2.4	68.8
HF 274	581.9	1.1	1.36	7.9	1.7	63.2
HF 170	555.8	1.4	1.49	8.3	2.1	66.4
HF 3	515.9	4.5	1.32	6.8	0.6	54.4
Standard	629.5	52.8	1.46	9.2	3.0	73.6
N application	854.4	0.0	1.46	12.5	-	100.0
Uninoculated	555.8	0.0	1.11	6.2	-	-
LSD (5%)	108.6	1.71	0.14	1.37	0.76	11.0

Discussion

The first criterion for a *Rhizobium* strain to be used in legume inocula is that it must be highly effective in fixing nitrogen (Matos and Schröder, 1989). In both normal and low temperature experiments, generally, all strains tested provided N to the plant, as indicated by shoot dry weight, N content, and N fixed (Tables 2 and 3). Furthermore, strains showed significant differences for almost all parameters measured (Tables 2 and 3). Significant differences among rhizobial strains for various parameters such as nodule dry weight, shoot dry weight, N concentration, and N content were also reported in other studies. For example, significant differences among rhizobial strains were observed under growth room, greenhouse, and field conditions for lentil (Bremer et al., 1990), pigeon pea (Matos and Schröder, 1989), clover (Ferreira and Marques, 1992), and chickpea (Chandra and Pareek, 1985; Somasegaran et al., 1988; İçgen et al., 2002).

Average nodule dry weight per plant did not appear to account for differences in N content and N fixed under low or normal temperature conditions. For example, plants inoculated with HF 1, 5, 10, and 175 had low nodule dry weight, but high shoot N content and N fixed at the normal temperature (Table 2). In contrast, plants inoculated with HF 170, 177, 266, 270, 271, 272, 275, and 277 had high nodule dry weight and low shoot N content and N fixed. Similar results were also observed at low temperature. For instance, plants inoculated with HF 278 had high N content and N fixed, although HF 278 produced low nodule dry weight (Table 3). These results obtained from the low and normal temperature experiments suggest that other factors, such as nodule efficiency indicating specific activity, are more important than nodule weight in determining the amount of N fixed. Similar observations were reported by other researchers (Pereira and Bliss, 1989; Kipe-Nolt and Giller, 1993).

Since temperate climates are subjected to temperatures below the optimal for symbiotic nitrogen fixation (Waughman, 1977; Hardarson and Jones, 1979; Bordeleau and Prevost, 1994), tolerance to low temperature is a major characteristic to be considered in rhizobial selection programs for temperate climates (Pankurst and Layzell, 1984; Rupela and Kumar Rao, 1987). Most of the bacteria isolated from cold conditions are able to grow at low temperatures (Nelson and Parkinson, 1978). Thus, a number of studies have attempted to correlate environmental origins and growth in culture of rhizobial strains with their symbiotic effectiveness under low temperature stress. Ek-Jander and Fahraeus (1971) found that the performance of rhizobial strains at low temperatures was influenced by their geographical origin. Roughley (1970) also found that a strain isolated from a cold environment formed nodules and bacteroids under low temperatures, but a strain isolated from a warmer environment did not. In many studies, cold-adapted rhizobia isolated from low temperature conditions showed the capacity to improve symbiotic nitrogen fixation and yield of legumes under low temperature conditions (Prevost and Bromfield, 1991; Prevost et al., 1999). This study also made a parallel attempt to evaluate the symbiotic effectiveness of *Rhizobium* strains isolated from perennial wild chickpea species collected from cold environments at high altitudes between 2000 and 2500 m in mountains under low temperature conditions.

Temperatures below 15 °C generally reduce nodulation, and depress nodule activity and N fixation (Gibson, 1967, 1971; Waughman, 1977; Herdin and Silsbury, 1989). In our trial, the low temperature (15 °C day/9 °C night) also reduced nodule dry weight, total N, and N fixed per plant (Table 3) when compared with normal temperature (25 °C day/22 °C night) (Table 2).

Total N accumulation in legume plants is one of the best parameters to measure N fixation under experimental conditions (Danielle et al., 1987; Atıcı et al., 2005). In our low temperature experiment, except for HF 3 and HF 175, plants inoculated with *Rhizobium leguminosarum* subsp. *ciceri* strains isolated from wild chickpea had significantly higher N content than uninoculated control plants. The fact that inoculated plants had a N content higher than that of the uninoculated control indicates that an active N fixing system depending of the strains is probably present. Under the low temperature conditions, several isolates (HF 10, 177, 262, and 278) fixed as much as or more N than the standard control officially recommended for use in commercial chickpea inoculants. Except for HF 3 and HF 175, bacterial strains isolated from wild chickpea had efficiency of symbiosis similar to the standard control (Table 3).

The wide range of effectiveness obtained from the normal and low temperature experiments (Tables 2 and 3) and the indication that a potential exists for developing legume inoculants from *Rhizobium* strains isolated from cold environments are in agreement with the results of other studies. Clover rhizobia isolated from the sub-arctic region in Scandinavia showed a better adaptation to low temperatures and grew faster at 10 °C than isolates from southern areas (Ek-Jander and Fahraeus, 1971). Prevost et al. (1987b), working with arctic isolates for sainfoin, found some hopeful strains that were as effective as temperate standard strains or gave significantly higher yields than temperate standard strains. Effective arctic rhizobia had a potential for use as inoculants on sainfoin. In another study, Prevost and Bromfield (1991) also evaluated symbiotic effectiveness and competitive nodulation of the temperate forage legume sainfoin by 2 strains of arctic rhizobia and 2 strains of temperate rhizobia at low temperatures (9, 12, or 15 °C). On the basis of the data obtained from the research they suggested that the use of selected, cold-adapted rhizobia in sainfoin inoculants may be beneficial in temperate regions where low soil temperatures occur early in the growing season.

In conclusion, the data showed that inoculation with bacterial strains isolated from perennial wild chickpeas (*Cicer anatolicum*) collected from cold environments at high altitudes significantly increased all parameters investigated under both low and normal temperature conditions compared with uninoculated control. Generally, all strains producing nodules provided N to the plant, as indicated by shoot dry weight, N content, and N fixed. Furthermore, strains showed significant differences for almost all parameters measured. Among the strains tested, under the normal temperature conditions, 7 strains (HF 1, 5, 10, 175, 176, 269, and 289) and, under the low temperature conditions, 4 strains (HF 10, 177, 262, and 278) demonstrated a good potential on chickpea because of their high shoot dry matter yields, N content, N fixed, and symbiotic effectiveness, which may have potential for strain development for cold conditions.

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References

Atıcı, Ö., H. Öğütçü and Ö.F. Algur. 2005. Effect of putrescine on inducing symbiosis in chickpea and vetch inoculated with commercial or indigenous strains of *Rhizobium*. Symbiosis. 38: 163-174.

Auld, D.L., B.L. Bettis, J.E. Crock and K.D. Kephart. 1988. Planting date and temperature effects on germination, emergence, and seed yield of chickpea. Agronomy Journal. 80: 909-914.

Beck, D.P., J. Wery, M.C. Saxena and A. Ayadi. 1991. Dinitrogen fixation and nitrogen balance in cool-season food legumes. Agronomy Journal. 83: 334-341.

Beck, D.P., L.A. Materon and F. Afandi. 1993. Practical *Rhizobium*-legume technology manual, Technical Manual No: 19. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, pp. 1-54.

Bordeleau, L.M. and D. Prevost. 1994. Nodulation and nitrogen fixation in extreme environments. Plant and Soil. 161: 115-125.

Bremer, E., C. Van Kessel, L. Nelson, R.J. Rennie and D.A. Rennie. 1990. Selection of *Rhizobium leguminosarum* strains for lentil (*Lens culinaris*) under growth room and field conditions. Plant and Soil. 121: 47-56.

Broughton, W.J. and M.J. Dilworth. 1971. Control of leghemoglobin synthesis in snake beans. Biochemical Journal. 125: 1075-1080.

Chandra, R. and R.P. Pareek. 1985. Role of host genotype in effectiveness and competitiveness of chickpea (*Cicer arietinum* L.) *Rhizobium*. Tropical Agriculture. 62: 90-94.

Croser, J.S., H.J. Clarke, K.H.M. Siddique and T.N. Khan. 2003. Low-temperature stress: Implications for chickpea (*Cicer arietinum* L.) improvement. Critical Reviews in Plant Sciences. 22: 185-219.

Danielle, P., L.M. Bordeleau and H. Antoun. 1987. Symbiotic effectiveness of indigenous arctic rhizobia on a temperate forage legume: sainfoin (*Onobrychis viciifolia*). Plant and Soil. 104: 63-69.

Ek-Jander, J. and G. Fahraeus. 1971. Adaptation of rhizobia to subarctic environment in Scandinavia. Plant and Soil. Special Volume: 129-137.

Elkoca, E., F. Kantar and F. Şahin. 2008. Influence of Nitrogen Fixing and Phosphorus Solubilizing Bacteria on the Nodulation, Plant Growth, and Yield of Chickpea. Journal of Plant Nutrition. 31: 157-171.

Ellis, R.H., S. Covell, E.H. Roberts and R.J. Summerfield. 1986. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. Journal of Experimental Botany. 37: 1503-1515.

FAO. 2006. http://faostat.fao.org/site/339/default.aspx

Ferreira, E.M. and J.F. Marques. 1992. Selection of Portuguese *Rhizobium leguminosarum* bv. *trifolii* strains for production of legume inoculants. Plant and Soil. 147: 151-158.

Fyson, A. and J.I. Sprent. 1982. The development of primary root nodules on *Vicia faba* L. grown at two temperatures. Annals of Botany. 50: 681-692.

Gibson, A.H. 1967. Physical environment and symbiotic nitrogen fixation. IV. Factors affecting the early stages of nodulation. Australian Journal of Biological Sciences. 20: 1087-1104.

Gibson, A.H. 1971. Factors in the physical and biological environment affecting nodulation and nitrogen fixation by legumes. Plant and Soil. Special Volume: 139-152.

Hardarson, G. and D.G. Jones. 1979. Effect of temperature on competition amongst strains of *Rhizobium trifolii* for nodulation of two white clover varieties. Annals of Applied Biology. 92: 229-236.

Herdin, A. and J.H. Silsbury. 1989. Nodulation and early growth of faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) as affected by strain of *Rhizobium*, NO_3- supply, and growth temperature. Australian Journal of Agricultural Research. 40: 991-1001.

İçgen, B., G. Özcengiz and N.G. Alaeddinoğlu. 2002. Evaluation of symbiotic effectiveness of various *Rhizobium cicer* strains. Research in Microbiology. 153: 369-372.

Kipe-Nolt, J.A. and K.E. Giller. 1993. A field evaluation using the ^{15}N isotope dilution method of lines of *Phaseolus vulgaris* L. bred for increased nitrogen fixation. Plant and Soil. 152: 107-114.

Layzell, D.B., P. Rochman and D.T. Canvin. 1983. Low root temperatures and nitrogenase activity in soybean. Canadian Journal of Botany. 62: 965-971.

Lee, J.Y. and S.H. Song. 2007. Evaluation of groundwater quality in coastal areas: implications for sustainable agriculture. Environmental Geology. 52: 1231-1242.

Lev-Yadun, S., A. Gopher and S. Abbo. 2000. The cradle of agriculture. Science. 288: 1602-1603.

Matos, I. and C. Schröder. 1989. Strain selection for pigeon pea *Rhizobium* under greenhouse conditions. Plant and Soil. 116: 19-22.

Nelson, L.M. and D. Parkinson. 1978. Growth characteristics of three bacterial isolates from an arctic soil. Canadian Journal of Microbiology. 24: 909-914.

Pankurst, C.E. and D.B. Layzell. 1984. The effect of bacterial strain and temperature changes on the nitrogenase activity of *Lotus pedunculatus* root nodules. Physiologia Plantarum. 62: 404-409.

Pereira, P.A.A. and F.A. Bliss. 1989. Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorus under field and environmentally-controlled conditions. Plant and Soil. 115: 75-82.

Prevost, D., L.M. Bordeleau, S. Caudry-Reznick, H.M. Schulman and H. Antoun. 1987a. Characteristics of rhizobia isolated from three legume indigenous to the Canadian high arctic *Astragalus alpinus, Oxytropis maydelliana* and *Oxytropis arctobia*. Plant and Soil. 98: 313-324.

Prevost, D., L.M. Bordeleau and H. Antoun. 1987b. Symbiotic effectiveness of indigenous arctic rhizobia on a temperate forage legume: Sainfoin (*Onobrychis viciifolia*). Plant and Soil. 104: 63-69.

Prevost, D. and E.S.P. Bromfield. 1991. Effect of low root temperature on symbiotic nitrogen fixation and competitive nodulation of *Onobrychis viciifolia* (sainfoin) by strains of arctic and temperate rhizobia. Biology and Fertility of Soils. 12: 161-164.

Prevost, D., P. Drouin and H. Antoun. 1999. The potential use of cold-adapted rhizobia to improve symbiotic nitrogen fixation in legumes cultivated in temperate regions. In: Fundamentals and Application of Cold-adapted Organisms (Eds.: R. Margesin and F. Schninner). Springer, Heidelberg, pp. 161-176.

Rigby, D. and D. Caceres. 2001. Organic farming and the sustainability of agricultural systems. Agricultural Systems. 68: 21-40.

Roughley, R.J. 1970. The influence of root temperature, *Rhizobium* strain and host selection on the structure and nitrogen fixing efficiency of the root nodules of *Trifolium subterraneum*. Annals of Botany. 34: 631-646.

Rupela, O.P. and J.V.D.K. Kumar Rao. 1987. Effects of drought, temperature and salinity on symbiotic nitrogen fixation in legumes with emphasis on chickpea and pigeonpea. In: Proceedings of The Consultants Workshop, 19-21 December 1984, ICRISAT Center, Patancheru, India, pp. 124-129.

Rupela, O.P. and M.C. Saxena. 1987. Nodulation and nitrogen fixation in chickpea. In: The Chickpea (Eds.: M.C. Saxena and K.B. Singh). CAB International, Wallingford, Oxon, pp. 191-206.

Sarıoğlu, G., S. Özçelik and S. Kaymaz. 1993. Selection of effective nodosity bacteria (*Rhizobium leguminosarum biovar. viceae*) from lentil grown in Elazığ. Turkish Journal of Agriculture and Forestry. 17: 569-573.

Singh, K.B. 1993. Problems and prospects of stress resistance breeding in chickpea. In: Breeding for Stress Tolerance in Cool-season Food Legumes (Eds.: K.B. Singh and M.C. Saxena). John Wiley & Sons, Chichester, pp. 17-37.

Somasegaran, P. and H.J. Hoben. 1985. General microbiology of *Rhizobium*. In: Methods in Legume *Rhizobium* Technology (Eds.: P. Somasegaran and H.J. Hoben). University of Hawaii, Department of Agronomy and Soil Science, Hawaii Institute of Tropical Agriculture and Human Resources, College of Tropical Agriculture and Human Resources (USAID Contract No. DAN–0613–C–00–2064-00, Library of Congress No. 87-106109), Hawaii, pp. 1-52.

Somasegaran, P., H.J. Hoben and V. Gurgun. 1988. Effects of inoculation rate, rhizobial strain competition, and nitrogen fixation in chickpea. Agronomy Journal. 80: 68-73.

Waughman, E.J. 1977. The effect of temperature on nitrogenase activity. Journal of Experimental Botany. 28: 949-960.

Yaman, M. and A.S. Cinsoy. 1996. Determination of the most effective *Rhizobium* strain (*Rhizobium japonicum* L.) in soybean. Journal of Aegean Agricultural Research Institute. 6: 84-96.