

PARTITIONING OF DRY MATTER, PROLINE ACCUMULATION, CHLOROPHYLL CONTENT AND ANTIOXIDANT ACTIVITY OF CHICKPEA (*CICER ARIETINUM* L.) PLANTS UNDER CHILLING STRESS

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Abstract

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Chickpea (*Cicer arietinum* L.) which is one of the most common legume plants is generally grown as a winter crop in warm regions. Thus, low temperatures, which occur during growing period of the plants, can limit productivity. In the present study, six chickpea cultivars and lines were grown under controlled conditions to understand physiological responses of the plants to chilling stress. The highest total dry matter reduction in chilling conditions was found in genotype HH-2 (43 %) whereas the lowest one in Cevdetbey-98 (25.3 %). This can be due to the differential partitioning of dry matter in both genotypes under chilling conditions. The genotype HH-2 reduces dry matter content of roots and stems and not of leaves whereas the genotype Cevdetbey-98 diminishes the dry matter in leaves, the most cold-sensitive part of plants. After cold exposure, the leaf area in HH-2 was also less decreased than in Cevdetbey-98. Proline content and total antioxidant activity increased whereas total chlorophyll content decreased in all genotypes during chilling conditions. Lower relative increase in proline content of HH-2 and higher in Cevdetbey-98 suggests the possible protective role of proline accumulation in chickpea plants under chilling stress.

Key words: Chickpea, Chilling, Proline, Antioxidants, Chlorophyll

Introduction

Chickpea (*Cicer arietinum* L.) is known as an annual grain legume, which is used generally for human consumption. It is widely known as an important alternative source of nutrition in countries where proteins of animal origin are scarce and expensive (Akçin, 1998). Chickpea makes up more than 20% of world pulse production. Major producers include India, Pakistan, Mexico, Turkey, Canada and Australia (Margheim et al., 2004). Approximately 650 000 tones out of 7 000 000 tones total chickpea production of the world are produced in Turkey as it is of vital importance in nutrition of people in underdeveloped and developing countries

(Sepetoğlu, 2006). Chickpea is cultivated in winter season in warmer regions of Turkey, and the production area of this plant is expanding every year (Özdemir et al., 1999). Evolving as a winter crop, chickpea faces occasionally sublethal chilling temperatures (< 8°C) during the reproductive phase (Nayyar, 2005). It is stated that temperature within the chilling range can limit the growth of chickpea at all phenological stages but it is considered most damaging to yield at reproductive stage (Kumar et al., 2010). The reactions of different cold sensitive species to chilling stress are considered variable (Akman et al., 2001). The effects of chilling stress can vary according to temperature, duration, growth stage of the plant and some environmental factors as radia-

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tion, wind, water and nutrients (Saltveit and Morris, 1990). It is known that chilling stress can cause several dysfunctions at cellular level as damage to membranes, generation of reactive oxygen species, protein denaturation and accumulation of toxic products (Bowers, 1994) but metabolic mechanisms underlying chilling injury in chickpea are not well understood (Croser et al., 2003). In this work, physiological reactions of chickpea plants to chilling stress were studied by comparing sensitive and tolerant genotypes. Partitioning of dry matter, leaf area, alteration in proline accumulation, chlorophyll content and antioxidant activity were investigated.

Materials and Methods

The experiments were conducted in a fully controlled climate chamber in Plant Physiology Laboratory of Field Crops Department in Ege University. Chickpea cultivars Sari-98 and Cevdetbey-98 from Aegean Agricultural Research Institute, HH-2 and Nifa-95 cultivars from Pakistan, and chickpea lines VDI-5 and VDII-8 from Ege University, Department of Field Crops were used in the experiments. Pakistani chickpea cultivars used in this study can be characterized with their lower grain weight and brown pod color of seeds whereas Turkish genotypes have higher grain weight and cream pod color of seeds. The experiments were performed in a completely randomized design with 4 replications.

Seeds were germinated in peat soil under controlled conditions at 25°C and 40% relative air humidity (Rh). Six days after sowing (DAS) 3-4 cm seedlings were transferred to PVC pots (38 cm x 16 cm x 11cm and 7.5 L volume) where the hydroponic system was provided. The half strength nutrient solution of hydroponic system contained milimolar concentrations of: 2.86 N (NH_4NO_3), 0.32 P (KH_2PO_4), 1.02 K (K_2PO_4), 1 Ca ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 1.67 Mg (MgSO_4), 9.10 Mn ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 0.52 Mo ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$), 0.15 Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 18 B (H_3BO_3), 0.156 Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 100 Fe (Fe-EDTA). The top of the pots was covered with an isolation material of 2 cm thick foam into which 64 holes with diameter of 2 cm were drilled. The seedlings were inserted through these holes having their roots in the nutrient solution and leaves above. The required oxygen for roots was supplied by air pumps im-

mersed into the pots. The half strength nutrient solution was replaced with absolute concentration at the fourth day of hydroponic practice. The conditions of 25°C and 40% RH were applied in control for 20 DAS whereas in chilling stress treatment the temperature was decreased to 7°C on the 12 DAS. Light was supplied from 8.00 am to 8.00 pm and light intensity at the plant level was at least $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. In both treatments, analyses were done at 20 DAS Fresh and dry weights of different parts of plants, relative water content, leaf area, total chlorophyll content, proline content and total antioxidant activity of leaves were determined.

Totally ten plants from each replication were separated into roots, stems and leaves after harvesting. The plant parts were rinsed in distilled water, blotted on filter paper and fresh weights were recorded. Dry weights were determined after drying at 105°C for 24 h. Water content of one unit dry matter was calculated as the ratio of dry matter amount to fresh weight value. Relative water content (RWC) of leaves was calculated according to the following formula^[1] considering fresh (FW), dry (DW) and saturated (S) weight of leaves.

$$[1] \text{ RWC} = (\text{FW} - \text{DW}) / (\text{S} - \text{DW})$$

Leaf area was determined by scanning of leaves after harvest and computing with software. A bulk of all leaves collected from ten plants was used for biochemical analyses.

Chlorophyll was extracted from the leaf blades of the seedlings with 80 % acetone and absorbance of the supernatant was read at 663 and 646 nm. Chlorophyll content of the plants was calculated according to Arnon (1949).

The proline content of the leaves was determined according to Bates et al. (1973). Approximately 0.5 g of plant material was ground, homogenized in 10 mL of sulfosalicylic acid and filtered through filter paper. Then 2 mL of the filtrate was incubated with 2 mL of acidic ninhydrine and 2 mL of glacial acetic acid for 1 h at 100°C. The reaction was stopped by transferring the samples into an ice bath. The reaction mixture was extracted with 4 mL of toluene and absorbance was read at 520 nm.

The total antioxidant activity was measured using the method described by Benzie and Strain (1999). An aliquot (50 μl) of an 80% (v/v) ethanolic extract was added to 1.5 ml of FRAP reagent (10 parts of 0.3 M sodium acetate buffer, 1 part of 0.01 M TPTZ (tripyrindyl triazine)

solution and 1 part of 0.02 M FeCl₃) and 0.15 ml distilled water. The reaction started by adding the extract and the absorbance was measured after 15 minutes at 593 nm.

The statistical evaluation of the results was performed using analysis of variance (ANOVA) and means of all data were compared by the LSD test at $P=0.05$.

Results and Discussion

Limitation in productivity of different plant species and varieties under various stress conditions were reported in several studies (Nayyar et al., 2005; Tavakol and Pakniyat, 2007; Ilker et al., 2011; Zeng and Shannon, 2000). Dry matter production of chickpea genotypes, which were evaluated in this work, also decreased because of chilling stress (Figure 1). VDII-8 genotype had the highest total dry matter in both conditions whereas HH-2 had the lowest one. The responses of plants to

chilling conditions significantly varied. The highest total dry matter reduction was observed in the genotype HH-2 (43%) whereas the lowest one in Cevdetbey-98 (25.3%). Additionally, an apparent difference in partitioning of dry matter due to stress treatment was observed in all selected chickpea genotypes (Figure 1). Our data are in agreement with previous reports on variation of dry matter partitioning under chilling conditions (McKersie and Leshem, 1994). We established that the reduction of dry matter in chilling conditions was more pronounced in stems of plants (average 40.6% for the genotypes studied) (Figure 1). However relative reduction in stem and root dry weight did not significantly differ among genotypes while genotype-dependent variability in leaves was observed: the reduction of leaf dry weight was negligible in the genotype HH-2 in contrast to the more pronounced reduction in Cevdetbey-98 (Figure 1). Thus, the higher mobilization ca-

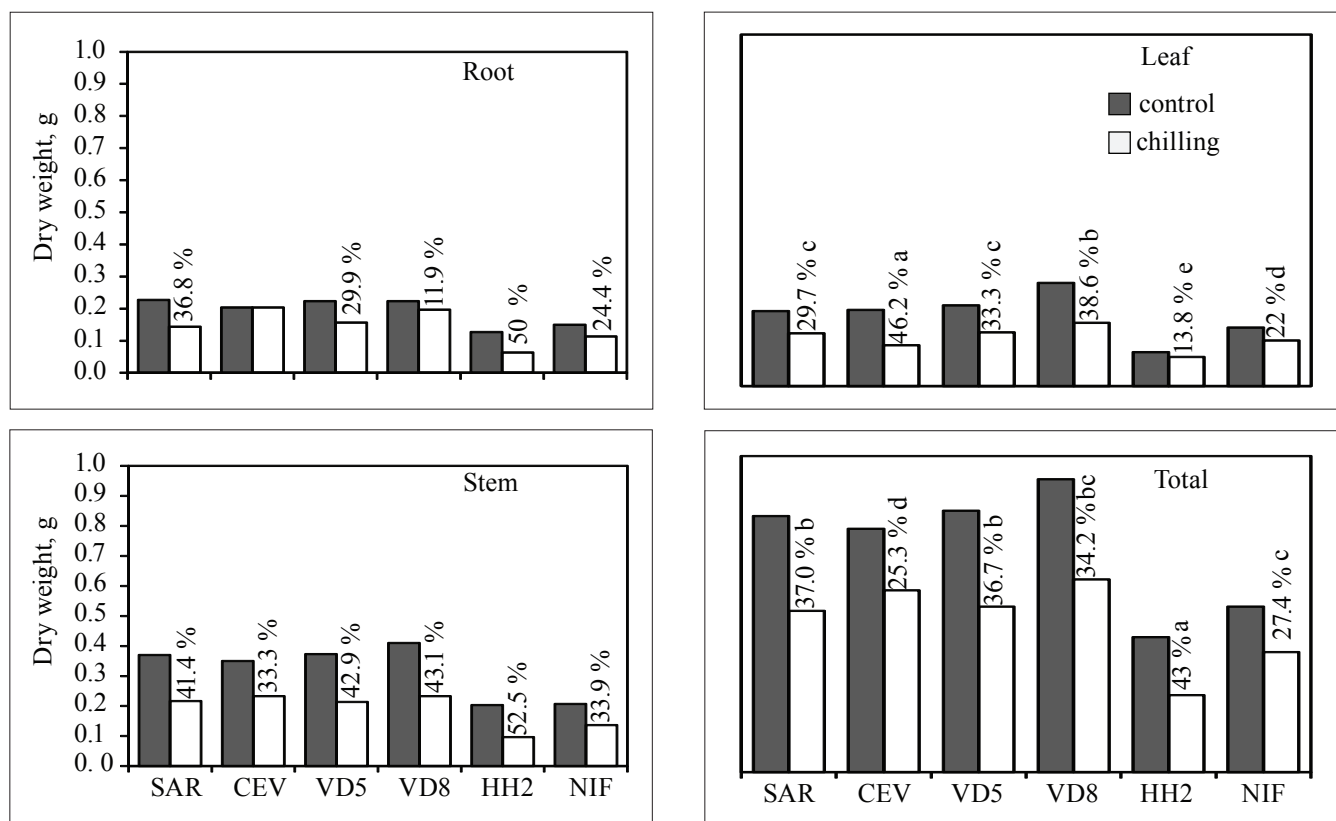


Fig. 1. Effect of chilling stress on partitioning of the dry matter of six chickpea genotypes (Sari-98, Cevdetbey-98, VDI-5, VDII-8, HH-2 and Nifa-95). Reduction of dry matter in chilling conditions (% of controls) and the result of LSD test ($P=0.05$) are indicated on the top of "chilling" column

capacity of Cevdetbey-98 allows diminishing the leaves, the most cold-sensitive plant part. Mahajan and Tuteja (2005) reported that reduction in leaf area, wilting and turning yellow of leaves are the main indicators of chilling effects on plants.

Leaf area of plants decreased (average 37.0 % for all genotypes studied) under chilling stress compared to the controls (Table 1). The significantly higher relative decrease of leaf area in chilling treatment was found in VDII-8 (46.9%) and Cevdetbey-98 (43.9%) whereas the reduction was substantially lower in HH-2 (20.5%). Our findings suggested that the lower decrease in leaf area and incapability to partitioning of assimilates to another organs of plants in HH-2 resulted in the highest reduction of total dry matter under low temperature. Conversely, in Cevdetbey-98 chilling stress caused higher relative decrease in area of the cold-sensitive leaves as well as a lower dry matter reduction. These results indicated that chilling tolerance level of chickpea plants might be regulated mostly by alteration in leaf dry weight and area.

The chilling stress did not result in discernible changes in relative water content (RWC) of leaves (Table 1). HH-2 had the highest RWC in leaf (92.2%) while in Sari-98 the lowest one (91.4%) was recorded. These findings are in agreement with results of Kadlecova et al. (2002) who observed no change in water status of leaves exposed to low temperature.

Proline is defined as an amino acid which is accumulated by plants under several stress conditions (Nayyar et al., 2005; Tatar and Gevrek, 2008; Demiral and Türkan, 2005). An elevation of proline content in the

stressed plants of all genotypes investigated was also observed in the present study (Figure 2). HH-2 which had the highest dry matter reduction under chilling stress accumulated lowest level of proline ($127.4 \mu\text{g g}^{-1}$), the relative increase (20.5%) in proline content being also the lowest in comparison to the other chickpea genotypes. Cevdetbey-98, which was more tolerant under chilling conditions (as judged by the lowest dry matter reduction), was in the group of genotypes which had significantly higher relative increase in proline content (247%). Contradictory results have been previously reported about whether proline plays a protective role in

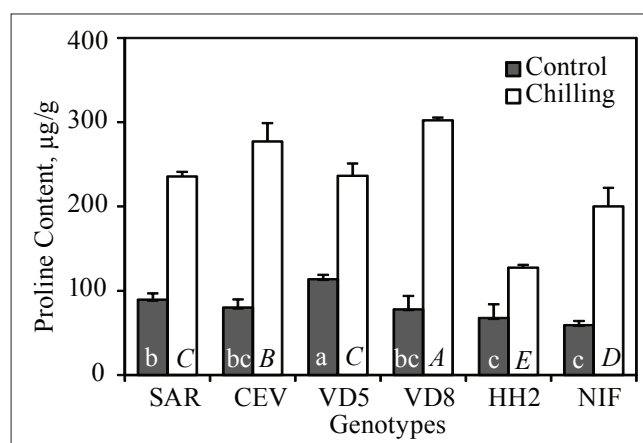


Fig. 2. Effect of chilling stress on proline content in the leaves of six chickpea genotypes (Sar: Sari-98, Cev: Cevdetbey-98, VD5: VDI-5, VD8: VDII-8, HH-2: HH2 and Nif: Nifa-95). Capital letters on bars indicate the result of LSD test ($P=0.05$) in chilling conditions and small letters refer to control conditions

Table 1

Effect of chilling stress on leaf area (cm^2) and relative water content (RWC) of leaves (%) of six chickpea genotypes (Sari-98, Cevdetbey-98, VDI-5, VDII-8, HH-2 and Nifa-95). Means of the data are compared by the LSD test at $P=0.05$

Cultivars	Leaf area, cm^2			Leaf RWC		
	Control	Chilling	% Reduction	Control	Chilling	Mean
Sari-98	15.4b	10.5a	31.8	91.6	91.3	91.4c
Cevdetbey-98	16.4b	9.2a	43.9	91.8	91.9	91.9ab
VDI-5	17.3b	10.8a	37.6	91.5	91.6	91.5bc
VDII-8	20.9a	11.1a	46.9	92.1	91.3	91.7bc
HH-2	7.8c	6.2b	20.5	92.5	91.9	92.2ab
Nifa-95	15.7b	9.2a	41.4	91.8	91.6	91.7bc
Mean	15.6	9.5	37.0	91.9	91.6	91.7

stressed cells or is just a symptom of injury under stress conditions (Tatar et al., 2010; Misra and Gupta, 2005). In our findings, the lower increase in proline content in the most sensitive genotypes and higher increase in proline content in the most tolerant ones suggest a role of proline in protection of chickpea plants against chilling stress. The multi-functional performance of proline as an osmoregulator, membrane and protein stabilizer, antioxidant and signal transducer (Heuer, 2011) lends support to this assumption.

Musser et al. (1984) reported that low temperatures appear to damage the structure of chloroplasts and reduce the content of chlorophyll pigments. In our study, a reduction in total chlorophyll content of leaves was also observed under chilling conditions (Figure 3). The highest chlorophyll content in chilled leaves was reported in Sari-98 (2.54 mg/g) whereas the lowest one in VDI-5 (1.74 mg/g). However, relative reduction in chlorophyll content was considerably lower in VDII-8 (8.3%) which had the highest total dry weight under both control and chilling conditions.

It is known that almost all stress factors lead to an oxidative damage on plants as a primary reaction (Bolikhina et al., 2003). In the present study significant increase in total antioxidant activity in leaves of all cold-exposed chickpea genotypes was observed (Figure 4).

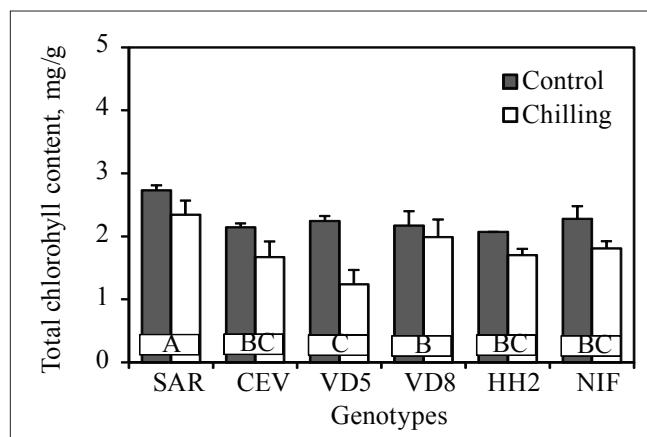


Fig. 3. Effect of chilling stress on total chlorophyll content in the leaves of six chickpea genotypes (Sar: Sari-98, Cev: Cevdetbey-98, VD5: VDI-5, VD8: VDII-8, HH-2: HH2 and Nif: Nifa-95). The mean values of genotypes were compared by the LSD test ($P=0.05$) and results are indicated as capital letters on the bars

Higher relative increase in antioxidant activity under stress treatment was found in VDII-8 (45.8%). It can be supposed that higher capacity to generate antioxidants under chilling conditions in this genotype lead to protect chlorophyll content.

Conclusion

Our findings indicated that HH-2 showing the highest reduction in dry matter under chilling conditions could be identified as a more cold-sensitive chickpea genotype. On the other hand, Cevdetbey-98 can be determined as a more tolerant one according to its lowest dry matter reduction. This can be explained by the inability of HH-2 to partitioning dry matter to stems and roots instead of leaves in contrast to the higher dry matter mobilization capacity of Cevdetbey-98 giving a chance to diminish the most sensitive part of plants, the leaves, when chilling conditions were imposed. Moreover, the lower decrease of leaf area in HH-2 and the more pronounced one in Cevdetbey-98 can also be important factors for distinct chilling tolerance of these genotypes. Proline content of leaves drastically increased in all genotypes under chilling conditions the response being significantly lower in the sensitive genotype HH-2 while increase was more remarkable in tol-

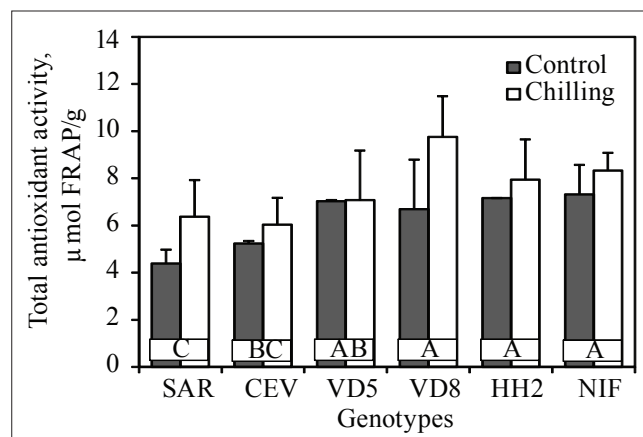


Fig. 4. Effect of chilling stress on total antioxidant activity in the leaves of six chickpea genotypes (Sar: Sari-98, Cev: Cevdetbey-98, VD5: VDI-5, VD8: VDII-8, HH-2: HH2 and Nif: Nifa-95). The mean values of genotypes were compared by the LSD test ($P=0.05$) and results are indicated as capital letters on the bars

erant Cevdetbey-98 and VDII-8. This allows surmising a positive relation between chilling tolerance level and relative increase in proline accumulation in chickpea plants. Antioxidant activity can also contribute to tolerance given the highest dry matter content of VDII-8 combined with lower chlorophyll reduction and higher antioxidant activity performed in chilling conditions.

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References

- Akçin, A.**, 1998. Legume Plants (in Turkish). Suleyman Demirel University, Faculty of Agriculture, Pub. No: 8, p.377, Konya.
- Akman, Y., M. Küçüködük, S. Düzenli and G.N. Tuğ**, 2001. Pant Physiology. Palme, ISBN: 975-97436-0-4, Ankara. (Tr).
- Arnon, D. I.**, 1949. Copper enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris*. *Plant. Phys.*, **24**:1-15.
- Bates, L. S., R. P. Waldren and I. D. Teare**, 1973. Rapid determination of free proline for water- stress studies. *Plant Soil*, **39**: 205-207.
- Benzie, I. F. F. and J. J. Strain**, 1999. The ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: *Methods in Enzymology*. **299**: 15-27.
- Blokhina, O., E. Virolainen and K.V. Fagerstedt**, 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.*, **91**: 179-194.
- Bowers, M. C.**, 1994. Environmental effects of cold on plants. In: Wilkinson RE (Ed.), *Plant-Environment Interactions*, Marcel Dekker, New York, pp. 391-411.
- 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. *Critic. Rev. Pl. Sci.*, **22**: 185-219.
- Demiral, T. and I. Türkan**, 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot.*, **53**: 247-257.
- Heuer, B.**, 2011. Role of proline in plant response to drought and salinity. In: M Pesarakli, ed. *Handbook of Plant and Crop Stress III* ed. Pp 213 -238. *CRC Press*, Boca Raton, NY.
- Ilker, E., O. Tatar, F. Aykut Tonk and M. Tosun**, 2011. Determination of tolerance level of some wheat genotypes to post-anthesis drought. *Turk. J Field Crops*, **16**: 59-63.
- Kadlecova, Z., M. Faltus and I. Prasil**, 2002. Comparison of barley response to short-term cold or dehydration. *Biol. Plantarum*, **45**: 637-639.
- Kumar, S., H. Nayyar, R. K. Bhanwara and H. D. Upadhyaya**, 2010. Chilling stress effects on reproductive biology of chickpea. *SATe Journal*, **8**: 1-14.
- Mahajan, S. and N. Tuteja**, 2005. Cold, salinity and drought stress: an overview. *Arch Biochem. Biophys.*, **444**: 139-158.
- Margheim, J. F., D. D. Baltensperger, R. G. Wilson, D. J. Lyon, G. L. Hein, R. M. Harveson, P., A. Burgener, J. M. Krall, J. T. Cecil, J. R. Rickertsen, A. P. Merrihan, M. Watson and B. J. Hansen**, 2004. Chickpea Production in the High Plains. The Board of Regents of the University of Nebraska on behalf of the University of Nebraska-Lincoln Extension.
- McKersie, B. D. and Y. Y. Leshem**, 1994. Chilling Stress. In: *Stress and Stress Coping in Cultivated Plants*, *Kluwer Academic Publisher*, The Netherlands, pp. 79-103
- Misra, N. and A. K. Gupta**, 2005. Effect of salt stress metabolism in two high yielding genotypes of green gram. *Plant Sci.*, **169**: 331-339.
- Musser, R. L., S. A. Thomas, R. R. Wise and T. C. Peeler**, 1984. Chloroplast ultrastructure, chlorophyll fluorescence and pigment composition in chilling- stressed soybeans. *Plant Physiol.*, **74**: 749-754.
- Nayyar, H.**, 2005. Putrescine increases floral retention, pod set and seed yield in cold stressed chickpea. *J. Agron. Crop Sci.*, **191**: 340-345.
- Nayyar, H., T. S. Bains and S. Kumar**, 2005. Chilling stressed chickpea seedlings: effect of cold acclimation, calcium and abscisic acid on cryoprotective solutes and oxidative damage. *Environ. Exp. Bot.*, **54**: 275-285.
- Özdemir, S., U. Karadavut and C. Erdoğan**, 1999. Stability analysis of some winter sown cultivars in east mediterranean region. *Turk. J. Agric. For.*, **23** (1): 201-205.
- Saltveit, M. E. and L. L. Morris**, 1990. Overview of chilling injury of horticultural crops. In: Ed. C.Y. Wang. *Chilling injury of horticultural crops*, *CRC Press*, Boca Raton, pp. 3-15.
- Sepeoğlu, H.**, 2006. *Field Crops I*. Ege University Faculty of Agriculture. Pub. no: 569, ISBN: 975-483-688-4, Izmir. (Tr).
- Tatar, O. and M. N. Gevrek**, 2008. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. *Asian J. Plant Sci.*, **7**: 409-412.
- Tatar, O., H. Brueck, M. N. Gevrek and F. Asch**, 2010. Physiological responses of two Turkish rice (*Oryza sativa* L.) varieties to salinity. *Turk. J. Agric. For.*, **34**: 451-459.
- Tavakol, E. and H. Pakniyat**, 2007. Evaluation of some drought resistance criteria at seedling stage in wheat (*Triticum aestivum* L.) cultivars. *Pak. J Biol. Sci.*, **10**: 1113-1117.
- Zeng, L. and M. C. Shannon**, 2000. Salinity effects on rice seedling and yield components. *Crop Sci.*, **40**: 996-1003.