YYÜ TAR BİL DERG
(YYU J AGR SCI)
2010, 20(3): 194-200

Geliş Tarihi (Received): 11.03.2010
Kabul Tarihi (Accepted): 20.05.2010

Araştırma Makalesi/*Research Article (Original Paper)*

Low Night Temperature Tolerance Determining Traits Correlate with Paraquat Tolerance in Grapevine Genotypes

Nezahat TURFAN, Lale YILDIZ AKTAŞ*, Avni GÜVEN

Ege University, Faculty of Science, Department of Biology, 35100 Bornova İzmir-Turkey
*E-posta: lale.yildiz@ege.edu.tr, Tel: 90 (232) 3111782, Fax: 90 (232) 3881036

Abstract: The objective of this study was to reveal that low night temperature (LNT) tolerance concerning traits and relation with paraquat (PQ) tolerance in four grapevine (*Vitis vinifera* L.) genotypes. Grapevine genotypes were grown in greenhouse conditions and subjected to LNT at 5°C. Electrolyte leakage and lipid peroxidation of leaves indicates fundamental differences between the selected genotypes regarding their response to LNT stress. According to extent of damage, Dimrit and Razakı were assessed as tolerant, while Hatun Parmağı and Ata Sarısı were sensitive to LNT. Low night temperature tolerant genotypes, showed higher resistance to PQ as compared to sensitive genotypes. Photosytem II activity as an indicator of photodamage maintained almost stable in both tolerant and sensitive genotypes. In tolerant genotypes photosynthetic pigment contents was protected, whereas in sensitive genotypes, Hatun Parmağı and Ata Sarısı, significant losses occurred; in total chlorophyll (Chl) content about 48 and 49 % and in carotenoid (Car) content about 51 and 67 % when plants subjected to chilling. In sensitive genotypes, soluble protein content was remained unchanged, free proline content was sharply increased in a response of damage. The correlation analysis showed that lipid peroxidation level indicating membrane damage is having positive correlation with soluble protein content and negatively correlated with, PQ tolerance, Chl/Car ratio, chlorophyll and carotenoid content. This data confirmed the LNT tolerance of grapevine genotypes mainly based on better protection of photosynthetic apparatus and membranes from oxidative stress.

Key words: Chilling, Chlorophyll, Lipid peroxidation, Paraquat, Proline, *Vitis vinifera*

Asma Genotiplerinde Düşük Gece Sıcaklığına Toleransı Belirleyen Özellikler Paraquat Toleransı ile İlişkilidir

Özet: Bu çalışmada, dört asma (*Vitis vinifera* L.) genotipinde düşük gece sıcaklığına (DGS) toleransı belirleyen karakterler ile paraquat (PQ) toleransı arasındaki ilişki ortaya koyulmuştur. Asma genotipleri sera koşullarında yetiştirilerek, 5°C DGS'na maruz bırakılmıştır. Seçilen genotipler arasında DGS'na tepkide temel farklılıklar, yapraklardaki elektrolit sızıntısı ve lipid peroksidasyonu ile belirlenmiştir. Yapraktaki hasar oranına göre, Dimrit ve Razakı genotipleri DGS'na toleranslı, Hatun Parmağı ve Ata Sarısı ise duyarlı genotipler olarak bulunmuştur. DGS'na tolerant genotipler, duyarlı genotiplere göre PQ'a daha fazla tolerans göstermişlerdir. Tolerant ve duyarlı genotiplerin her ikisinde de, ışık hasarı indikatörü PSII aktivitesi sabit kalmıştır. Düşük sıcaklık uygulanan toleranslı genotiplerde fotosentetik pigment içerikleri korunurken, duyarlı genotiplerde, Hatun Parmağı ve Ata Sarısı, total klorofil (Kl) içeriğinde yaklaşık % 48 ve 49, karotenoid (Kar) içeriğinde ise yaklaşık % 51ve 67 önemli kayıp ölçülmüştür. Duyarlı genotiplerin çözünür protein içeriklerinde değişiklik olmazken, serbest prolin miktarları meydana gelen hasara tepki olarak hızla artmıştır. Sonuçların analizi, membran hasarının göstergesi olan lipid peroksidasyon düzeyi ile çözünür protein içeriği arasında pozitif bir korelasyon, PQ toleransı, Kl/Kar oranı, klorofil ve karotenoid içeriği arasında ise negatif korelasyon bulunduğunu göstermiştir. Bulgularımız, asma genotiplerinde DGS'na toleransın, fotosentetik mekanizma ve membranların oksidatif stresten daha iyi korunması temelinde olduğunu ortaya koymaktadır.

Anahtar kelimeler: Klorofil, Lipid peroksidasyon, Paraquat, Prolin, Üşüme, *Vitis vinifera*

Introduction

Grape growing (with 483.000 ha growing area, 3.918.000 ton production and 1 253 varieties of grape) is of great significance in Turkey's horticulture (Sivritepe and Eriş 1999; Anonymus 2009). Vineyard spread 4/5 lands of the country with different climatic regions (Şensoy et al. 2009). The growth and development of grapevine are influenced by environmental factors, such as temperature. Chilling temperatures (below 10°C) is an environmental constraint that limit photosynthesis, growth and distribution of grapevine (*Vitis vinifera* L.) which is an economically important C_3 crop in many parts of the world (Flexas et al. 1999). Particularly, spring frosts at night may constrain grapevine physiology at the beginning of the growing season.

Photosynthesis is one of the first processes to be affected when chilling sensitive plants are exposed to low temperatures. Even though molecular mechanisms of chilling damage are still not clear, lipid composition affecting cell membrane stability and function is thought to be the main cause. Although the effects of LNT (low night temperature) stress on photosynthetic metabolism of grapevine are well documented (Flexas et al. 1999; Hendrickson et al. 2004; Bertamini et al. 2005), little is known about the changes of other physiological traits.

Some of the detoriorative effects of chilling may be due to increased production of reactive oxygen species (ROS) (Wise 1995). The formation of ROS in thylakoid membranes may cause a cascade of oxidative reactions of cellular components, and they have been implicated in photoinhibition (Hull et al. 1997) and cellular damage in chilling susceptible plants exposed to low temperatures (Tambussi et al. 2004). This effect of chilling stress on the physiology of plants is very similar to the stress caused by PQ (a bypridlium herbicide), which leads to the production of highly toxic free radicals generated by reaction of molecular oxygen with PQ radicals formed in the chloroplast during photosynthesis (Dodge 1971). Therefore, a close correlation may be expected between plant's tolerance to stress treated and PQ (Altinkut et al. 2001). Determining of LNT tolerance associated characteristics and establishment of the correlation between these traits and PQ tolerance would be of value for rapid selection of genotypes for different ecological conditions and breeding programs.

In the present study, PSII activity, photosynthetic pigment concentration, cell membrane stability and malondialdehyde (MDA) and proline content of young grapevine genotypes exposed to chilling stress (5°C) were investigated to provide LNT tolerance concerning traits and relation with PQ tolerance of four grapevine (*Vitis vinifera* L.) genotypes.

Materials and Methods

One-year-old cuttings of four *Vitis vinifera* L. genotypes (Dimrit, Razakı, Hatun Parmağı and Ata Sarısı) were planted in 3 L pots containing perlite, turf, soil, pine bark and organic manure (2:1:1:1:1) and grown in greenhouse for three months until the development of 10 functional leaves. The maximum irradiance available at the top of the plant was 1200 µmol m^{-2} s^{-1} on a clear day with a 12/12 h day/night photoperiod, and day / night temperature of 26/20 °C with relative humidity at 60 %.

The grapevine pot-plants seedlings were divided into two groups; first group was kept in greenhouse conditions as described above and served as control, while the other group of plants was subjected to LNT treatment (5°C) by transferring the plants in a cold room. After 12 h (18:00-06:00) LNT treatment (Tambussi et al. 2004; Bertamini et al. 2005), plants were transferred to a shaded area (150–200 µmol m^{-2} s^{-1}) for 30 min (22°C) and then exposed to direct sunlight for 60 min before sampling (1500 µmol m^{-2} s^{-1}, 25°C and 50 % relative humidity). *In vivo* chlorophyll fluorescence was measured using the plant efficiency analyzer (Hansatech UK) and the maximum quantum yield of photosystem II (*Fv/Fm*: variable and maximum fluorescence) was determined in dark-adapted (30 min) samples. Then fully-expanded leaves positioned fourth or fifth from the top were harvested and immediately used or frozen in liquid nitrogen before stored (-20°C for two weeks).

Electrolyte leakage of leaf disks measured using an electrical conductivity meter (Inolab-Cond 720, UK). A total of 10 (1cm Ø) discs per replication were excised from leaves and rinsed with distilled water (x4) to remove exogenous electrolytes. Leaf samples were placed in individual vials containing 10 ml of distilled

water. These samples were incubated at room temperature (25 °C) on a shaker (100 rpm) for 24 hours. The electrical conductivity of the bathing solution (EC1) was read after incubation. Samples were then placed in a thermostatic water bath at 95 °C for 15 minutes and the second reading (EC2) was determined after cooling the bathing solutions to room temperature. The procedure used was based on the method of Lutts et al. (1996).

The level of lipid peroxidation products was determined and expressed as MDA content according to Çakmak and Horst (1991). Proline content was determined according to the modified method of Bates et al. (1973).

Leaf photosynthetic pigments were extracted with 80% acetone (v/v) and the absorbance of supernatants were measured by spectrophotometrically. Total chlorophyll content was determined at wavelength 652 nm and carotenoids at 450 nm following the method of Linchtenthaler (1987). Paraquat tolerance of leaf disks (1cm Ø) were measured by immersing in 100 µM paraquat (1,1k-dimethyl-4,4k-bipyridylium dichloride, methyl viologen) for 12h under a light intensity of 12 000 lux according to the modified method of Altinkut et al. (2001). The total chlorophyll content were then analyzed according to the method of Linchtenthaler (1987).

The data presented are mean values taken from two independent experiments, each mean data with five replicates. All data were subjected to two-way ANOVA test with use of statistical software of SPSS 11.0, and means were compared by the protected least significant difference (LSD) test and Pearson Correlation test. Comparisons with *p* values less than 0.05 or 0.01 were considered significantly different.

Results and Discussion

Membranes are the most susceptible structures to chilling. In chilling stress irreversible permeability changes may occur when certain lipids aggregate to form an inverted structure with hexagonal packing symmetry, which disrupts the membrane bilayer causing an increased permeability of plasma membrane to water and solutes upon re-warming (Xin and Browse 2000). In thylakoids, membrane heterogeneity due to different lipid configuration domains also increases permeability (Webb and Green 1991). Leakage points may also result from damage of membrane components. The current results showed that electrolyte leakage (EL) of leaf disks increased sharply when grapevine plants were exposed to LNT (Fig. 1A). Moreover, there was a marked difference among *V. vinifera* genotypes; higher EL was observed in Hatun Parmağı and Ata Sarısı than in Dimrit and Razakı genotypes depending on the injury caused by stress.

The exposure of the plants to chilling stress resulted in significant increase in the MDA concentration of leaves in Hatun Parmağı and Ata Sarısı genotypes (Fig. 1C). However, Dimrit and Razakı had similar MDA concentration as those in the control. A higher susceptibility of these two cultivars to LNT was evidenced by the strong leakage increase and related with the MDA production during chilling (Fig.1A, C). It has been reported that cultivars with higher chilling tolerance have lower MDA content when subjected to the stress (Alonso et al. 1997; Queiroz et al. 1998; Feng and Cao, 2005). Better protection of membrane integrity in Dimrit and Razakı grapevine cultivars confirming that these genotypes were more tolerant to LNT treatment than other two cultivars (Zhang et al. 2006). Our results are in agreement with previous report of coffee cultivars with higher chilling tolerance have lower MDA content and less ion leakage when subjected to the cold stress (Campos et al. 2003). Determining Hatun Parmağı genotype as sensitive to chilling was supported the findings of Şensoy et al. (2009) indicating the genotype had high Effective Heat Summation requirement.

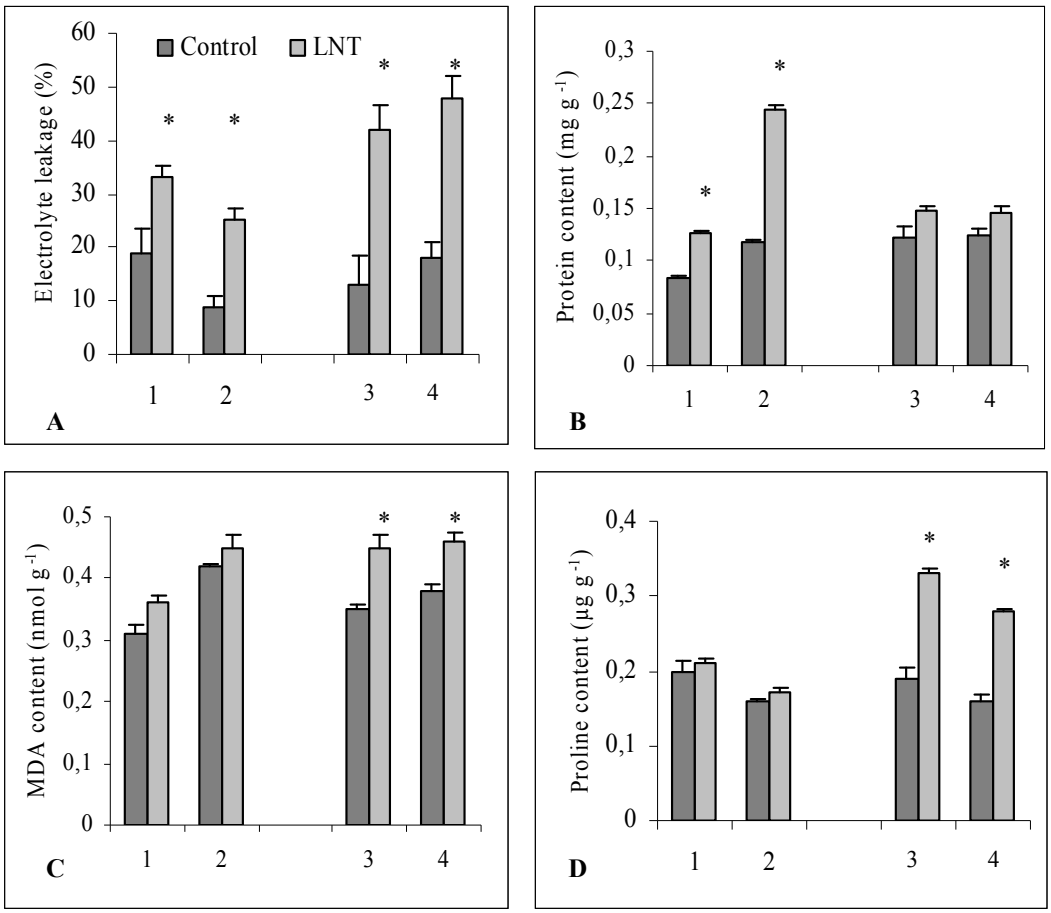

Figure 1. Effect of chilling stress on EL (%) (A), total soluble protein (mg g^{-1}) (B), MDA content (nmol g^{-1}) (C) and free proline content (µg g^{-1}) (D) in the leaves of different grapevine genotypes. 1, Dimrit; 2, Razakı; 3, Hatun Parmağı; 4, Ata Sarısı. * $P<0.01$

Free proline concentration in Hatun Parmağı and Ata Sarısı genotypes sharply enhanced when exposed to LNT treatment while tolerant genotypes Dimrit and Razakı showed no difference (Fig. 1D). The exposure of plants to low temperature induces many changes in physiological and biochemical parameters. Under chilling stress, production and accumulation of compatible organic solutes such as sucrose, organic acid and free amino acids in cytoplasm usually occurred (Renaut et al. 2005; Barka et al. 2006). Increase in proline content in susceptible genotypes may be considered as a step towards protection of proteins and scavenging free radicals caused by chilling stress, parallel to the findings in chilling sensitive chickpea (Nayyar et al. 2005). LNT stress did not affect leaf soluble protein contents in the susceptible grapevine cultivars, but significantly increased them in the leaves of tolerant ones (Fig. 1B). These results indicated that LNT caused an inhibition of protein synthesis in susceptible genotype (Bertamini et al. 2007).

Although the photosystems are the primary targets for chilling induced photoinactivation, 12 h LNT treatment was not affected PSII activity of all grapevine genotypes tested (Table 1). Our results are in accordance with Hendrickson et al. (2004) reported that grapevine leaves maintained high intrinsic quantum efficiencies of PSII (Fv/Fm) and very highly resistant to photo inactivation. Bertamini et al. (2005) also reported a good PSII activity was measured in the morning as the Fv/Fm ratio when grapevine plants exposed to one night chilling.

The chilling stress caused a significant reduction of chlorophyll concentration relative to the control in Hatun Parmağı and Ata Sarısı varieties, whereas the difference between the treatment and the control was not significant for the Dimrit and Razakı (Table 1). The loss of chlorophyll can be resulted from enhanced chlorophyllase activity (Bertamini et al. 2007) and damage of chloroplast membrane by chilling caused oxidative stress. Following chilling stress, significant reductions in carotenoid concentration were recorded only in susceptible genotypes (Table 1). The similar trend was observed in Chl/Car ratio in stressed genotypes. The results suggest that these two parameters could be used as an indicator of LNT stress tolerance of grapevine as previously observed in Riesling grapevine cultivar (Bertamini et al. 2007). The xantophyll-carotenoid pool is known for its possible function in the photoprotection of the photosynthetic apparatus (Koroleva et al, 1994). Similar results were described by Haldiman (1999) for *Zea mays*. In cold tolerant compared to cold sensitive maize higher carotenoid content was observed as the temperature decreased.

Table 1. Paraquat tolerance (Chl mg g^{-1} FW) and effect of chilling stress on PSII activity (Fv/Fm ratio), total chlorophyll (Chl) and carotenoids (Car) content and in the leaves of different grapevine genotypes. Con, Control, Chill; Chilling, PQ; Paraquat.

Genotype	Fv/Fm ratio		Chl (mg g^{-1} FW)		Car (mg g^{-1} FW)		Chl/Car ratio (%)		Chl (mg g^{-1} FW)	
	Con	Chill [a]	Con	Chill [a]	Con	Chill [a]	Con	Chill [a]	Con	PQ [a]
Dimrit	0.69	0.65 (94)	1.59	1.51 (95)	4.57	4.68 (102)	0.35	0.32 (93)	1.59	1.55 (97)
Razakı	0.75	0.72 (96)	1.44	1.2 (83)	4.37	3.65 (84)	0.33	0.33 (100)	1.44	1.43 (99)
HParmağı	0.61	0.55 (90)	1.71	0.82** (48)	5.12	2.63** (51)	0.33	0.31 (93)	1.71	1.43** (84)
Ata Sarısı	0.64	0.62 (97)	1.32	0.65** (49)	3.83	2.58** (67)	0.34	0.25 (73)**	1.32	0.53** (40)

[a] The value in parentheses is the percent of the treatment to the control. ** P<0.01

As an oxidative stress damage parameter, the PQ tolerance results obtained from Hatun Parmağı and Ata Sarısı grapevine cultivars corroborate the chlorophyll and carotenoid loss in these susceptible genotypes (Table 1). These results suggest that, in grapevines the main factor of LNT tolerance could be the better protection from chilling induced oxidative stress.

Table 2. Correlation between lipid peroxidation (MDA), Paraquat tolerance (PQ), and total chlorophyll (Chl) and carotenoids (Car) content, chlorophyll/carotenoid ratio and total soluble protein content in the leaves exposed to low night temperature.

	MDA	PQ tolerance
MDA	-	-0,499 **
PQ tolerance	-0,499 **	-
Total Chl	-0,612 **	0,811 **
Car	-0,490 **	0,640 **
Chl/Car ratio	-0,363 *	0,464 **
Protein content	0,377 *	-0,014

Correlation is significant at 0.01 level (**) 0.05 level (*).

Pearson correlation analysis showed that lipid peroxidation (MDA) level was significantly correlated with soluble protein content, and negatively correlated with chlorophyll and carotenoids concentration and Chl/Car ratio and PQ tolerance in leaf of plants exposed to chilling stress (Table 2). The tolerance criterion of varieties are associated with PQ tolerance which was significantly correlated with chlorophyll and carotenoid concentration and Chl/Car ratio and negatively correlated with MDA level. Correlation data indicates the existence of cross-tolerance, i.e. LNT treatment tolerant varieties are also tolerant to oxidative stress (PQ).

In conclusion, LNT damage is mainly associated with the changes in membrane stability which might be used as selection criterion of chilling sensitivity of grapevine genotypes in early growth stages, and may be employed for agricultural purposes.

Acknowledgments

We thank Dr. Cemal Ilgin, Manisa Viticulture Research Institute, Manisa-Turkey, for kindly providing us with the cuttings of grapevine genotypes.

References

Alonso A, Queiroz CS, Magalhaes AC (1997). Chilling stress leads to increased cell membrane rigidity in roots of coffee (*Coffea arabica* L.) seedlings. Biochimica et Biophysica Acta 1323: 75-84.

Altinkut A, Kazan K, Ipekci Z, Gozukirmizi N (2001). Tolerance to paraquat is correlated with the traits associated with water stress tolerance in segregating F_2 populations of barley and wheat. Euphytica 121: 81-86.

Anonymus (2009). Turkey in statistics, 2009. Turkish Statistical Institute Report, p. 53-54. Ankara, Turkey.

Barka EA, Nowak J, Clement C (2006). Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth promoting *Rhizobacterium, Burkholderia phytofirmans* strain PsJN. Applied and Environmental Microbiology 72: 7246-7252.

Bates LS, Waldern RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.

Bertamini M, Muthuchelian K, Rubinigg M, Zorer R, Nedunchezhian N (2005). Photoinhibition of photosynthesis in leaves of grapevine (*Vitis vinifera* L. cv. Riesling). Effect of chilling nights. Photosynthetica 43: 551-557.

Bertamini M, Zulini K, Muthuchelian K, Nedunchezhian N (2007). Low-night temperature effects on photosynthetic performance on two grapevine genotypes. Biologia Plantarum 51: 381-385.

Campos PS, Quartin V, Ramalho JC, Nunes MA (2003). Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. Journal of Plant Physiology 160: 283-292.

Çakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum 83: 463-468.

Dodge AD (1971). The mode of action of the dibyridylium herbicides, paraquat and diquat. Endeavour 30: 130-135.

Feng YL, Cao KF (2005). Photosynthesis and photoinhibition after night chilling in seedlings of two tropical tree species grown under three irradiances. Photosynthetica 43: 567-574.

Flexas J, Badger MR, Chow WS, Medrano H, Osmond CB (1999). Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperature and/or water stress. Plant Physiology 121: 675-684.

Haldiman P (1997). Chilling-induced changes to carotenoid composition, photosynthesis and maximum quantum yield of photosystem II photochemistry in two maize genotypes differing in tolerance to low temperature. Journal of Plant Physiology 151: 610-619.

Hendrickson L, Ball MC, Wood JT, Chow WS, Furbank RT (2004). Low temperature effects on photosynthesis and growth of grapevine. Plant, Cell and Environment 27: 795-809.

Hull MR, Long SP, Jahnke LS (1997). Instantaneous and developmental effects of low temperature on the catalytic properties of antioxidant enzymes in two *Zea* species. Australian Journal of Plant Physiology 24: 337-343.

Koroleva OY, Brüggeman W, Krause GH (1994). Photoinhibition, xanthophylls cycle and in vivo chlorophyll fuorescence quenching of chilling-tolerant *Oxyria digna* and chilling-sensitive *Zea mays*. Physiologia Plantarum 92: 577-584.

Lichtenthaler HK (1987). Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350-382.

Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivar differing in salinity resistance. Annals Botany 78: 389-398.

Nayyar H, Bains TS, Kumar S (2005). Chilling stressed chickpea seedlings: effect of cold acclimation, calcium and abscisic acid on cryoprotective solutes and oxidative damage. Environmental and Experimental Botany 54: 275-285.

Queiroz CG, Alonso A, Mares-Guia M, Magalhaes AC (1998). Chilling induced changes in membrane fluidity and antioxidant enzyme activities in *Coffea arabica* L. roots. Biologia Plantarum, 41: 403-413.

Renaut J, Hoffmann L, Hausman JF (2005). Biochemical and physiological mechanisms related to cold acclimation and enhancing freezing tolerance in poplar plantlets. Physiologia Plantarum 125: 82-94.

Sivritepe N, Eriş A (1999). Determination of salt tolerance in some grapevine cultivars (*Vitis vinifera* L.) under *invitro* conditions. Turkish Journal of Biology 23: 473-485.

Şensoy RİG, Balta F, Cangi R (2009). Bazı sofralık üzüm çeşitlerinin Van ekolojik koşullarındaki etkili sıcaklık toplamı değerlerinin belirlenmesi. Harran Üniversitesi Ziraat Fakültesi Dergisi 13: 49-59.

Tambussi EA, Bartoli CG, Guiamet JJ, Beltrano J, Araus JL (2004). Oxidative stress and photodamage at low temperatures in soybean (Glycine max L. Merr.) leaves. Plant Science 167: 19-26.

Webb MS, Green BR (1991). Biochemical and biophysical properties of thylakoid acyl lipids. Biochemica et Biophysica Acta 1060: 133-158.

Wise RR (1995). Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. Photosynthesis Research 45: 79-97.

Xin Z, Browse J (2000). Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell and Environment 23: 893-902.

Zhang JH, Liu YP, Pan QH, Zhan JC, Wang XQ, Huang WD (2006). Changes in membrane-associated H^+-ATPase activities and amounts in young grape plants during the cross adaptation to temperature stresses. Plant Science 170: 768-777.