Turkish Journal of Biology

Volume 23 | Number 4 Article 10

1-1-1999

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Tr. J. of Biology
23 (1999) 473–485

Determination of Salt Tolerance in Some Grapevine Cultivars (*Vitis vinifera* L.) Under *in vitro* Conditions

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Received: 20.08.1997

Abstract: Salinity tests were conducted on Çavuş, Müşküle and Sultani Çekirdeksiz grapevine cultivars under *in vitro* conditions. Plant materials used in the salinity test were propagated using the axillary bud culture method. Single-node shoots were subjected to five different NaCl concentrations (0.00, 0.25, 0.50, 0.75 and 1.00%) in MS+5 µM BA medium for two periods (4 and 8 weeks). Proliferation, growth, total chlorophyll content and viability of explants decreased due to the increase in NaCl concentration and length of treatment period. Moreover, it was determined that salt treatment caused necroses in explants and the severity of this injury varied depending on the cultivar, NaCl concentration and the treatment period. The most tolerant grapevine cultivar to salt treatment was Çavuş, and this was followed by Sultani Çekirdeksiz and Müşküle. It was determined that salt tolerant cultivars relatively maintained their growth rates and could avoid metabolic disordes such as chlorophyll deficiency.

Key Words: NaCl, selection, salt tolerance, *in vitro,* grapevine.

In Vitro Koşullarda Bazı Asma Çeşitlerinde (*Vitis vinifera* L.) Tuza Toleransın Belirlenmesi

Özet: Tuz testleri *in vitro* koşullar altında Çavuş, Müşküle ve Sultani Çekirdeksiz üzüm çeşitlerinde yapılmıştır. Tuz testlerinde kullanılan bitkisel materyal aktif tomurcuk kültürü yöntemi ile çoğaltılmıştır. Tek boğumlu sürgünler MS+5 µM BA ortamında, iki farklı zaman periyodunda (4 ve 8 hafta), 5 farklı NaCl konsantrasyonuna (%0.00, 0.25, 0.50, 0.75 ve 1.00) maruz bırakılmışlardır. Artan NaCl konsantrasyonu ve uygulama süresine bağlı olarak eksplantlarda, çoğalma oranı, büyüme, toplam klorofil miktarı ve canlılığın azaldığı tespit edilmiştir. Ayrıca tuz uygulamalarının eksplantlarda nekrozlara sebep olduğu ve bu zararlanmanın derecesinin çeşide, NaCl konsantrasyonu ve uygulama periyoduna bağlı olarak değiştiği belirlenmiştir. Tuz uygulamalarına en yüksek toleransı Çavuş üzüm çeşidi göstermiş, bunu Sultani Çekirdeksiz ve Müşküle üzüm çeşitleri izlemiştir. Tuza dayanıklı olduğu tespit edilen üzüm çeşitlerinin tuzlu ortamlarda büyüme oranlarını nispeten koruyabildikleri ve klorofil noksanlığı gibi metabolik bozukluklardan sakınabildikleri belirlenmiştir.

Anahtar Sözcükler: NaCl, seleksiyon, tuza tolerans, *in vitro,* asma.

Introduction

Grape growing (with 567,000 ha growing area, 3,450,000 ton production and 1,253 varieties of grape) is of great significance in Turkey's horticulture. Although vineyards are spread throughout the country, the leading regions are Aegean, Central Anatolia and Mediterranean, accounting for over 80% of total production (1, 2).

In Turkey, salinity has not yet been recognized by the growers as a factor limiting agricultural productivity. However, 4 million ha of land are salt-affected and this area is about 20% of the irrigable land in the country (3). The Tarsus, Çukurova, Menemen, Söke, Salihli, Manisa, Çumra and Iğdır valleys, where grape growing is common, are included in this salt affected land. The cause of the salinity problem in these areas is the rise of the salty water table close to the soil surface because of poor drainage. The use of the furrow irrigation method by grape growers leads to an increase in the severity of the salinity problem.

Recent studies have shown that salinity has osmotic (4, 5, 6), toxic (7, 8) and nutritional (9, 10) effects on grapes. Although grapevines are moderately salt tolerant, it has been determined that salinity causes several kinds of damage such as growth inhibition, metabolic disturbances, necrosis and yield and quality loss (4, 5, 6, 9-19). However, the severity of salt damage has been found to be dependent on the salinity level of the environment, the growth stage of the plant and the cultivars in terms of scion and rootstock (7, 10, 15, 20-24).

The availability of grapevine cultivars in Turkey offers an excellent opportunity for ascertaining the suitability of some cultivars to saline conditions or determining salt tolerant grape cultivars for genetic improvement. Therefore, the aim of this sutdy was to screen some Turkish grapevine cultivars for response to salinity and to develop an early diagnostic method depending on reliable parameters using *in vitro* techniques.

Materials and Methods

Salinity tests were conducted on Çavuş, Müşküle and Sultani Çekirdeksiz grapevine cultivars under *in vitro* conditions. Plant materials used in the salinity test were propagated using the axillary bud culture method as described by Eriş ve Sivritepe (25). At the end of the third subculture, single-node shoots were excised from proliferating cultures and subjected to five different NaCl concentrations (0.00, 0.25, 0.50, 0.75 and 1.00%) in Murashige and Skoog (M-5519, Sigma Chemical Co.) medium, supplemented with 5 μM Benzyladenine, 3% Sucrose and 0.7% Difco Bacto Agar, for two periods (4 and 8 weeks). The pH of the medium was adjusted to 5.8 before being autoclaved at 121°C and 105 Pascal for 15 minutes. The cultures were maintained at 25±2°C under a 16 hour photoperiod fluorescent light.

Experiments were set up using a Randomized Block Design and repeated twice. There were 5 explants in each 100 ml jar (containing 30 ml medium), 2 jars in each replicate and 3 replicates in each cultivar.

Subsequent to the 4 or 8 week salinity treatments, explants were removed from their media and evaluated for their response to salinity. The parameters used to determine the effects of NaCl treatments on grapevine cultivars were; proliferation ratio (total number of new shoots and buds/explant), fresh weight, shoot lenght, number of shoots and number of three-node shoots, number of leaves per shoot, total chlorophyll content (26) and viability. Explants were also scored for visible symptoms of salt injury on a 0-3 scale as follows: 0, no injury; 1, damage on shoot-tips and leaf edges; 2, necroses on the whole leaf and/or on a part of the stem; 3, dead. Then, degrees of injuries were represented as percentages.

In order to determine salt tolerance in cultivars and differences between the cultivars the following parameters were used.

Tolerance Index (TI): It was used to summarize the general effect on the cultivars of 5 different NaCl concentrations and to compare grapevine cultivars on the basis of reactions to salt treatment, eliminating growth differences according to cultivar. TI was calculated on the basis of fresh weight, which was used to designate growth, and total chlorophyll content, which was used to designate metabolic disturbance, as follows, according to LaRosa *et al* (27):

$$TI=100+\sum^{n}[x(T_x/T_o)100$$

where,

x=0.00, 0.25, 0.50, 0.75. 1.00 g/l NaCl,

T_x=fresh weight or total chlorophyll content gain on x g/l NaCl,

T_o=fresh weight or total chlorophyll content gain on 0 g/l NaCl.

Moreover, percentage viability was also used as the third parameter to compare grapevine cultivars.

Statistical analyses of variance and LSD tests (mean separation of significant differences) were conducted at a 0.05 confidence level using the BARNES and MSTAT-C computer programs, respectively.

Results

The Effects of NaCl Treatments on Grapevine Cultivars

The effects of NaCl treatments and different treatment periods on the parameters determined in the Çavuş grapevine cultivar are shown in Table 1. It is clear from the data that the proliferation ratio, fresh weight, shoot length and number, 3-node shoots, leaf number of shoots, total chlorophyll content and viability of explants decreased due to the increase in NaCl concentrations and treatment periods. Moreover, explants had maintained their growth, total chlorophyll content and viability after 8 weeks of 0.25% NaCl treatment relative to the 4-week treatment. The response was similar during the 0.50% NaCl treatment except for a decrease in total chlorophyll content. Nevertheless, the 0.75 and 1.00% NaCl treatments inhibited the proliferation ratio and growth and decreased total chlorophyll content and the viability of explants. Furthermore, NaCl treatments caused necroses in explants and the severity of this injury varied depending on NaCl concentrations and treatment periods. Fig. 1 indicates that 4- and 8-week treatments of 0.25% NaCl induced 1st degree salt injuries 30% and 33%,

Table 1. The effects of NaCl treatments and different treatment periods on some physiological parameters in "cv. Çavuş".

NaCl (%)	Treatment period (weeks)	Proliferation ratio	Explant weight (mg)	Shoot length (mm)	Number of shoots/ explant	Number of 3-node shoots/expl.	Number of leaves/ shoots	Total chlorophyll (mg/100 g)	Viability (%)
0.00	4	7.16 b*	103.19 b	11.21 b	3.30 b	1.57 b	2.20**	13.16 a	100.00 a
	8	8.53 a	134.54 a	14.31a	3.93 a	2.30 a	2.65 A	13.34 a	100.00 a
0.25	4	5.13 d	80.94 c	8.82 d	1.80 cd	0.97 c	2.14	13.08 a	100.00 a
	8	6.25 c	104.65 b	9.68 c	3.60 ab	1.60 b	2.20 B	12.97 a	100.00 a
0.50	4	3.17 ef	57.63 e	8.18 e	1.57 de	0.77 d	2.07	10.75 b	100.00 a
	8	3.83 e	60.07 d	6.42 f	2.10 c	0.53 e	2.11 BC	8.34 c	100.00 a
0.75	4	3.04 f	55.33 f	6.58 f	1.53 de	0.53 e	1.75	8.79 c	100.00 a
	8	2.80 fg	58.84 de	5.32 g	1.63 cde	0.30 f	2.07 CD	4.26 e	80.00 b
1.00	4	1.93 h	27.51 g	6.25 f	1.17 e	0.20 f	1.62	5.56 d	76.67 c
	8	2.15 gh	22.06 h	5.43 g	1.17 e	0.17 f	1.94 D	2.78 f	30.00 d

* Interactions between NaCl x treatment periods (p<0.05)

** NaCl (P<0.05)

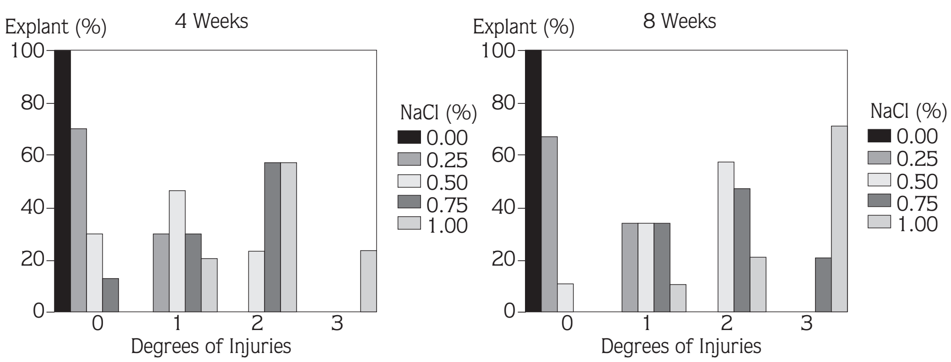

Figure 1. The effects of NaCl treatments on degrees of injuries in explants of "cv. Çavuş".

respectively. While 4-week treatment of 0.50% NaCl caused 1st degree injury (47%), 8-week treatment caused 2nd degree injury (57%) in explants. Injuries observed during 4 and 8 week treatments of 0.75% NaCl (57% and 47% respectively) were mostly rated as 2nd degree. The most injurious effect of NaCl on the Çavuş grapevine cultivar was observed during both treatment periods of 1.00% concentration.

Table 2 shows the effects of NaCl treatments and treatment periods on the Müşküle grapevine cultivar. During 4-week and 8-week treatments of 0.25-1.00% NaCl, significant decreases were determined in the proliferation ratio, growth and total chlorophyll content of the explants compared with 0.00% NaCl treatment. The explants had maintained their proliferation ratio, growth and viability at the end of 8 weeks of 0.25% NaCl treatment but, the total chlorophyll content of explants had decreased. There was a significant difference between the 4- and 8-week treatments of 0.50% NaCl. Although the explants continued to grow during the 8-week treatment, the effects of 0.50% NaCl treatments were more severe than the 0.25% NaCl treatments and they caused significant decreases in the total chlorophyll content and viability. The most inhibiting effect of salt was determined during the 0.75% and 1.00% NaCl treatments. Therefore, decreases in the proliferation ratio, growth, total chlorophyll content and viability were more pronounced in 8-week treatments of 0.75% and 1.00% NaCl. As shown in Fig. 2, salt treatment also had necrotic effects on "cv. Müşküle". The severity of salt injury observed in explants increased due to NaCl concentrations and treatment periods. While 0.25% treatments of NaCl induced 1st degree salt injury, 0.50% treatments caused 2nd degree injuries. The most injurious effect of NaCl (3rd degree) on the Müşküle grapevine cultivar was observed during 0.75% and 1.00% treatments.

Table 2. The effects of NaCl treatments and different treatment periods on some physiological parameters in "cv. müşküle".

NaCl (%)	Treatment period (weeks)	Proliferation ratio	Explant weight (mg)	Shoot lenght (mm)	Number of shoots/ explant	Number of 3-node shoots/expl.	Number of leaves/ shoots	Total chlorophyll (mg/100 g)	Viability (%)
0.00	4	13.13 b*	188.78 c	15.82 c	6.33 c	2.87 c	2.24 b	43.18 a	100.00 a
	8	25.53 a	480.54 a	21.11 a	16.20 a	7.87 a	2.73 a	37.44 b	100.00 a
0.25	4	6.48 d	98.60 e	13.67 d	3.37 d	1.20 e	1.83 d	36.88 b	100.00 a
	8	12.33 c	332.11 b	17.71 b	7.40 b	3.60 b	2.20 b	26.87 c	100.00 a
0.50	4	3.43 f	65.46 f	8.70 f	1.57 e	0.67 f	1.85 d	14.27 d	96.67 b
	8	4.53 e	114.08 d	11.66 e	3.33 d	1.73 d	2.05 c	8.71 e	70.00 c
0.75	4	2.07 g	44.42 h	6.13 h	1.23 fg	0.30 h	1.77 de	3.52 f	36.67 d
	8	2.17 g	60.99 g	7.64 g	1.30 ef	0.67 f	1.83 d	2.85 g	10.00 ef
1.00	4	1.33 h	28.34 i	5.57 h	1.00 g	0.17 h	1.50 f	3.41 fg	16.67 e
	8	1.47 h	29.04 i	6.10 h	1.13 fg	0.47 g	1.70 e	1.67 h	6.67 f

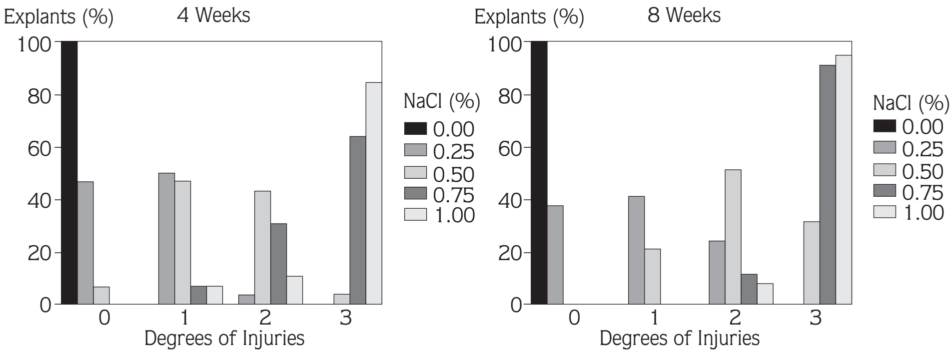

Figure 2. The effects of NaCl treatments on degrees of injuries in explants of "cv. Müşküle".

Similar effects of salt treatments were also determined in the Sultani Çekirdeksiz grapevine cultivar (Table 3). Although explants had maintained their proliferation ratio and growth at the end of 8 weeks in 0.25% and 0.50% NaCl treatments relative to the 4-week treatments, decreases in total chlorophyll content and viability were significant. There was no significant difference between 8-weeks treatments of 0.25%, 0.50% and 0.75% NaCl in terms of viability. However, salt injury was more severe during 0.50% and 0.75% NaCl treatments than the 0.25% treatment. As shown in Fig. 3, while 1 st degree salt injury was observed during the 0.25% NaCl treatment, 0.50% and 0.75% NaCl treatments induced 2 nd degree injuries. Both 4- and 8-week treatments of 1.00% NaCl inhibited the proliferation ratio and growth in the Sultani Çekirdeksiz grapevine cultivar and caused significant decreases in the total chlorophyll content and viability of explants (Table 3). Moreover, injuries observed in these treatments were rated as 3 rd degree.

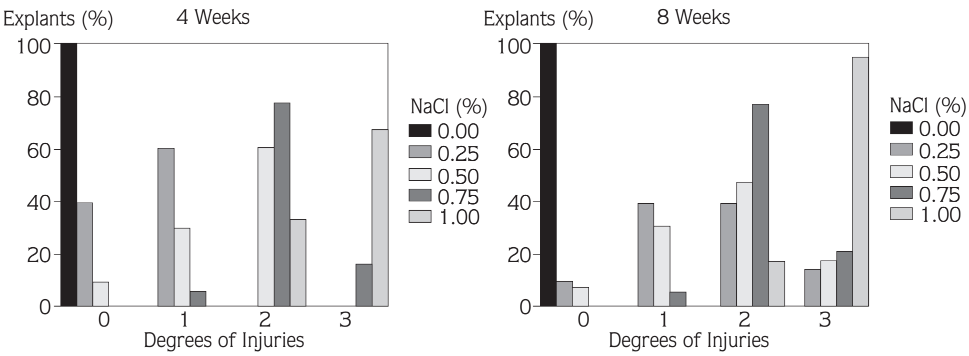

Figure 3. The effects of NaCl treatments on degrees of injuries in explants of "cv. Sultani Çekirdeksiz".

Table 3. The effects of NaCl treatments and different treatment periods on some physiological parameters in "cv. Sultani Çekirdeksiz".

NaCl (%)	Treatment period (weeks)	Proliferation ratio	Explant weight (mg)	Shoot lenght (mm)	Number of shoots/ explant	Number of 3-node shoots/expl.	Number of leaves/ shoots	Total chlorophyll (mg/100 g)	Viability (%)
0.00	4	17.33 b*	580.23 b	17.40 c	6.13 b	3.20 b	2.49 c	8.16 a	100.00 a
	8	33.89 a	783.23 a	28.03 a	14.50 a	5.00 a	3.20 a	7.86 a	100.00 a
0.25	4	7.40 e	442.56 c	14.39 d	4.13 d	1.60 e	2.45 c	7.97 a	100.00 a
	8	9.97 c	581.07 b	23.09 b	5.00 c	3.00 c	2.83 b	6.12 c	86.67 b
0.50	4	6.80 f	267.71 e	11.59 e	3.13 e	0.73 f	2.23 d	6.67 b	100.00 a
	8	8.43 d	289.37 d	16.63 c	4.13 d	1.87 d	2.45 c	3.34 e	83.33 b
0.75	4	3.80 g	182.19 f	7.21 g	1.93 f	0.53 g	1.84 f	4.66 d	83.33 b
	8	4.03 g	185.33 f	10.15 f	2.07 f	0.77 f	2.03 e	1.67 g	80.00 b
1.00	4	2.27 h	42.04 g	6.64 g	1.13 h	0.13 h	1.60 h	2.60 f	33.33 c
	8	2.27 h	43.84 g	6.87 g	1.37 g	0.17 h	1.76 g	1.11 h	16.67 d

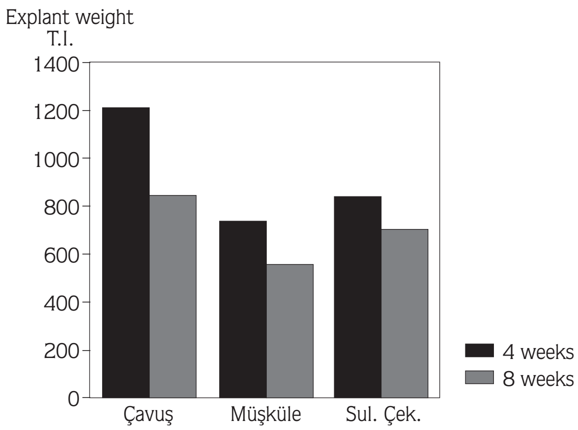

Figure 4. Tolerance indexes of grapevine cultivars on the basis of explant weight (LSO=61.04)

Salt Tolerance In Cultivars and Differences Between the Cultivars

Concerning the tolerance indexes calculated on the basis of fersh weight of explants, differences both in treatment periods and between the grapevine cultivars were significant (Fig. 4). However, the order of the cultivars in terms of salt tolerance did not change due to treatment periods. At the end of 4- and 8-week treatments of NaCl, the highest TIs, were obtained from "cv. Çavuş" i.e. 1214 and 843, respectively. This was followed by "cv. Sultani Çekirdeksiz". The minimum TIs were determined in "cv. Müşküle" i.e. 731 (4 weeks) and 547 (8 weeks).

Regarding the tolerance indexes calculated on the basis of total chlorophyll content of explants, similar differences were seen between the cultivars (Fig. 5). At the end of 4- and 8-week treatments of NaCl, “cv. Çavuş” had the highest TI values i.e. 1681 (4 weeks) and 1105 (8 weeks). This was followed by “cv. Sultani Çekirdeksiz” (1516 and 784) and “cv. Müşküle” (619 and 497), respectively.

The findings related to tolerance ratios, calculated on the basis of fresh weight and total chlorophyll content of explants are shown in Figs. 6 and 7. It is clear from the data that tolerance ratios decreased in all the cultivars due to increases in NaCl concentrations and treatment periods. In all the NaCl concentrations, differences which occurred both in treatment periods and between the grapevine cultivars were significant. Nevertheless, the order of the cultivars in terms of salt tolerance did not change due to NaCl concentrations and treatment periods. According to tolerance ratios, the highest salt tolerance was observed in “cv. Çavuş”. This was followed by “cv. Sultani Çekirdeksiz”. The most sensitive cultivar to salt was Müşküle. The differences which occurred between the cultivars in trems of percentage viability were consistant with the results of TI and TR.

Discussion

It is evident from the results that NaCl treatments caused growth inhibition in explants due to decreases in proliferation ratio, fresh weight, shoot length and number, 3-node shoots, and leaf number of shoots. The effects of *in vitro* NaCl treatments on grapevine cultivars supported the previous findings of *in vitro* and *in vivo* NaCl treatments on grapevine cultivars Sultani Çekirdeksiz (9, 10, 12, 17, 22, 29), Yuvarlak Çekirdeksiz (30), Deisss Anz, Black Corint, Emperor (9, 29), Perlette, Beaty Seedles and Delight (22).

Salt-induced osmotic stress leads to a rapid decline in water and the osmotic potential of cells and a decrease both in cell volume and cell expansion (5, 31, 32). From the present results, it

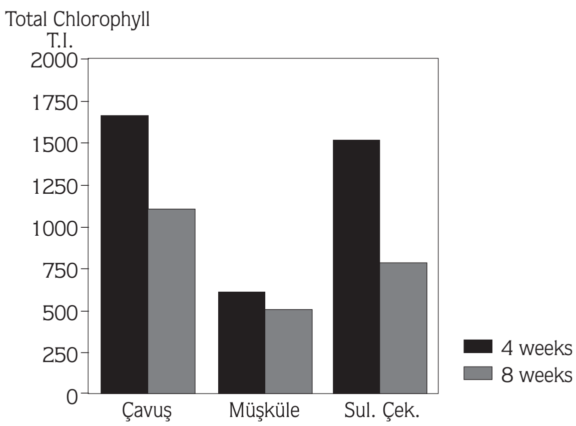

Figure 5. Tolerance indexes of grapevine cultivars on the basis of total chlorophyll (LSO=61.04)

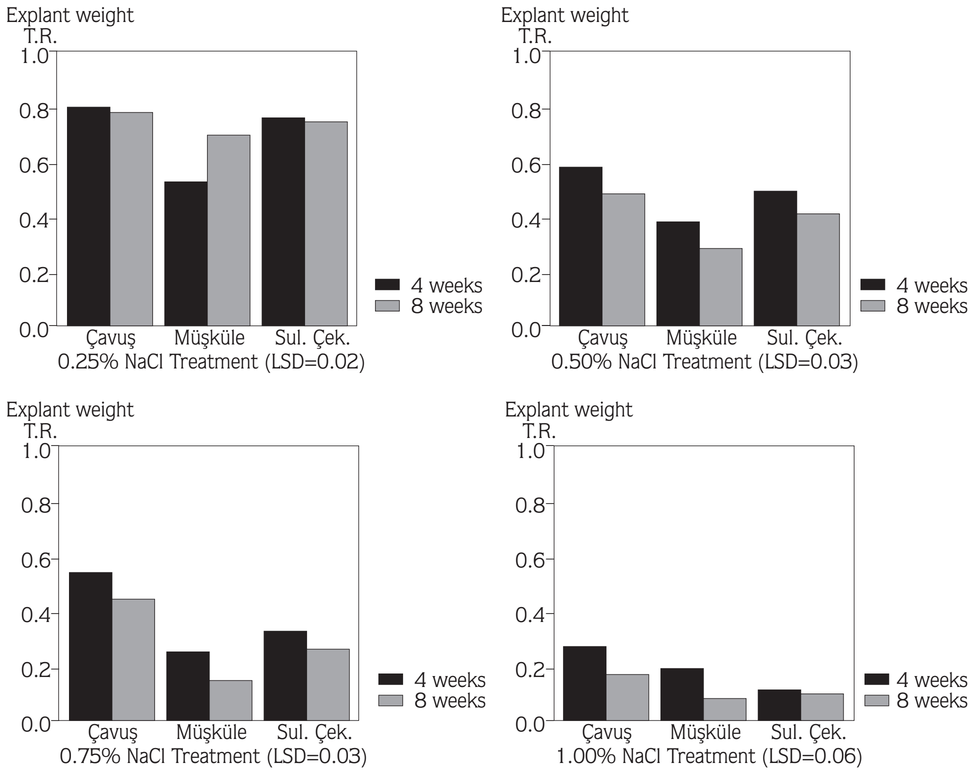

Figure 6. Tolerance ratios of grapevine cultivars on the basis of explant weight.

can be deduced that the lower explant weights due to higher NaCl concentration and treatment periods can be attributed to the osmotic effects of salt.

The growth inhibition due to salinity has also been explained by a suppression of nutrient absorbtion due to uptake of NaCl in competition with nutrient ions (8, 9, 33, 34). Sivritepe (35), working with the same grapevine cultivars, has concluded that increases in Na uptake. Sivritepe has also concluded that growth inhibition related to nutrient deficiency could be a result of an osmotically induced inhibition of root growth.

Specific toxic effects of salt also reduce plant growth. Walker *et al.* (4) have reported that decreases in shoot growth of grapevines under saline conditions are related to Cl accumulation in the plant.

Munns and Termaat (36) have suggested that the limited growth due to short term exposure is probably due to the water status of the root. However, growth inhibition in the long term is related to lower photosynthetic area. If the rate of leaf death approaches the rate of new leaf

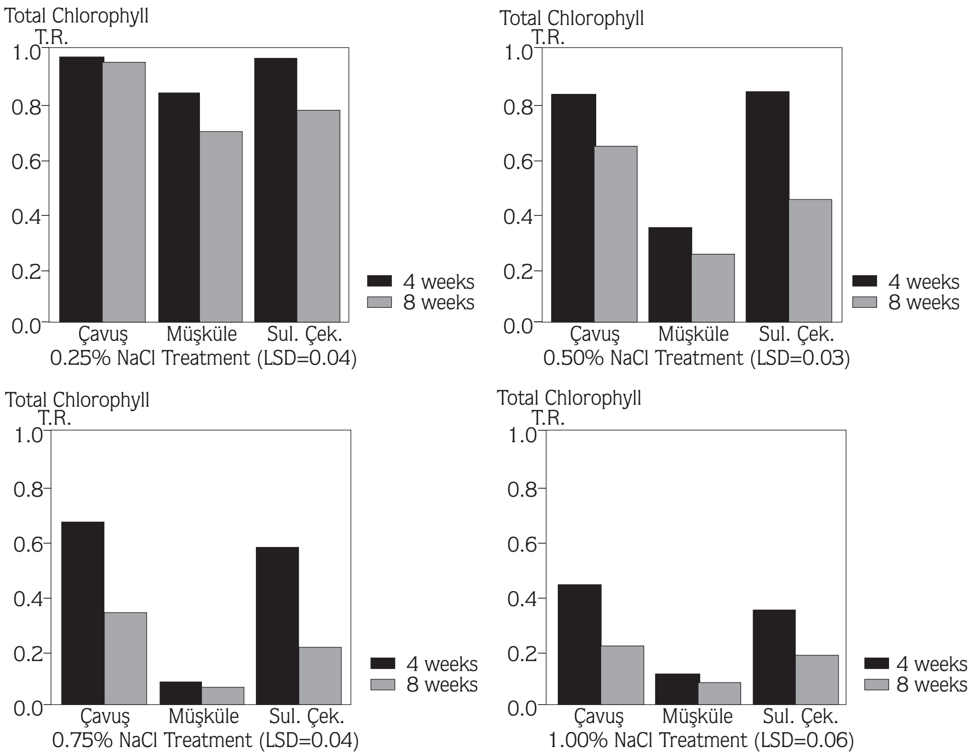

Figure 7. Tolerance ratios of grapevine cultivars on the basis of explant weight.

expansion, the photosynthetic area will eventually become too low to support continuing growth. The present study showed that NaCl treatment caused significant decreases in leaf number of shoots. These findings are compatible with those of Göktürk (19), working with grapevine rootstocks under *in vitro* conditions. However, decreases in the number of leaves were not only related to the growth inhibiting effects of salt, but also to the injurious effects of salt due to defoliation of the damaged leaves. In fact the first visual symptoms of injurious effect of salt on grapevines were withered shoot-tips and leaf edges. The severity of this injury increased as salinity levels and the length of treatment periods increased. These results supported the previous findings for grapevines (12, 19, 30). Salt-induced water deficiency, osmotic dehydration, high transpiration and ion toxicity have been suggested as the main causes of salt damage in plants (37, 38).

Salt-induced growth inhibition is accompanied by one or more metabolic disturbances. The most dramatic disturbance is on photosynthesis (33). Most research on the Sultani Çekirdeksiz grapevine cultivar has shown that NaCl treatment decreases photosynthesis (4, 6, 11, 17). Behboudian *et al.* (39) have also concluded that lower photosynthesis due to salinity is correlated with chlorophyll content. The present results in grapevine cultivars show that NaCl treatment causes significant decreases in the chlorophyll content of explants due to salt levels and treatment periods. These findings are compatible with those of Downton and Millhouse (31), working with grapes.

However, the severity of salt damage varied depending on NaCl concentration, treatment period and the grapevine cultivar. These results were consistent with those of Joolke *et al.* (20), Alsaidi *et al.* (7, 23), Charbaji *et al.* (24), and Ercan and Gülcan (30). Comparison of the salt tolerance between the cultivars showed that the most tolerant grapevine cultivar to salt was Çavuş, and this was followed by Sultani Çekirdeksiz and Müşküle. However, previous findings on 'cv. Sultani Çekirdeksiz" have suggested that this cultivar was more tolerant than the Black Corinth, Emperor, Perlette, Beauty Seedless and Delight grapevine cultivars (21, 22, 29).

The results of the present study show the advantages and usefulness of salt tests conducted under *in vitro* conditions using shoots propagated with axillary bud culture. The axillary bud culture has some advantages in grape growing. However, the most important of these is maintaining genetic stability (40). Therefore, *in vitro* salt tests could be used as an early diagnostic method to screen grapevine cultivars for response to salinity or to identify salt tolerant grapevine cultivars for genetic improvement.

The utilization of tolerance ratios and tolerance indexes was found to be a good evaluation method for classifying different cultivars. These parameters can be uset to give a coefficient in evaluated cultivars. Thus, an inventory or grapevine clutivars to screen their salt tolerance could be compiled in Turkey.

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