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ORIGINAL ARTICLE

Effect of Boric Acid in *In Vitro* Conditions on the Salt Tolerance of Fox Grapes (*Vitis Labrusca* L.)

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Abstract
Shoots obtained from micro-cuttings of 'Balıkçı Siyahı' (*Vitis labrusca* L.) grape type were used as explants. The explants were cultivated in MS nutrient medium containing 1 mg/l benzyl adenine for shoot formation following surface sterilization. Shoots derived from the explants were transferred to MS medium containing 2 mg/l indole-3-butyric acid for rooting. The rooted plantlets were transferred to MS medium containing four doses (0, 0.5, 1, 2 mM) of H_3BO_3 and three doses (0, 100, 200 mM) of NaCl for determination of salt stress and the effectiveness of boric acid. In the study, some physiological parameters such as plant vitality (%), damage degree (0–3), some shoot growth parameters, chlorophyll content (SPAD), shoot tolerance ratio, leaf turgor weight (g), ion flux (%), cell membrane damage ratio (%) and explant relative water content (%) were evaluated. As salt doses increase, it has been observed that the damage, ion flux and cell membrane damage rate also increase. It has been determined that the most negative effect occurs in the plant with the application of 200 mM NaCl, significantly reducing growth and development. Overall, it has been determined that 0.5 mM and 1 mM H_3BO_3 doses reduce the negative effects caused by salt stress.

Keywords Abiotic stress · Tissue culture · Boron · Vine

Introduction

Plants continuously interact with their environment and thrive under optimal conditions. While gradual changes generally do not damage plant cells, intolerable stress can negatively impact vital plant functions (Culha and Cakırlar 2011). Abiotic stress is a major factor affecting plant growth, responsible for over half of global crop losses and yield reductions (Wang et al. 2003). In terms of the impact of different stress factors on agricultural land around the world, drought stress is in first place with a rate of 26%. The other stress factors consist of mineral stress at a rate of 20% (Kalefetoğlu and Ekmekçi 2005). Plant species and varieties differ greatly in their evolved strategies for coping with stress. Salt has more detrimental impacts on plant growth than toxic substances. Salinity adversely affects plant development by increasing intracellular ion concentrations and causing osmotic stress, which limits the uptake of essential nutrients like potassium, nitrate, phosphorus, and calcium. It also generates ion toxicity, impacting plant cells and organs (Batool et al. 2014; Muchate et al. 2016).

Salt stress generally reduces biomass, yield, leaf area, and cell development. Early leaf damage is crucial for identifying salt-tolerant genotypes. Chlorophyll levels decrease in stressed plants, leading to reduced photosynthesis (Manoj et al. 2011). Among the species is grapevine, a species with moderate tolerance to salt stress. (Baneh et al. 2013; Han and Li 2024). Soil salinity is a common problem in viticultural lands. Salinity issues are increasingly rising due to both human activities and climate change (Arora 2019). Various methods are being researched to increase plant resistance to salt stress or to produce plant types with high salt tolerance. Various plant growth regulators (such as salicylic acid and jasmonic acid) and plant nutrients (such as calcium, potassium, silicon, boric acid, etc.) are used to increase plant tolerance to abiotic stresses. Boric acid is vital for plant growth, as shown by Warington (1923), who found that boric acid deficiency in peas could be corrected with

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supplementation. It plays key physiological and morphological roles in crop plants (Marschner 2012; Ceylan et al. 2016). Four different forms of boric acid can be found in soils: as hydrated oxide surfaces of iron and aluminium, as adsorbed on organic matter, as rock and mineral particles, or as H_3BO_3 and $B(OH)_4$ ions in soil solution (Kaçar and Katkat 2015).

Boric acid supports key plant functions, including pollen tube formation, cell division in roots, shoot tips, and young leaves, and sugar transport. Both deficiency and toxicity can disrupt plant functions, as the optimal, deficient, and toxic concentrations are closely related (Uygan and Çetin 2004; Karataş and Ağaoğlu 2005). Boric acid deficiency is common in soils of 85 countries, including Turkey, often due to excessive rainfall or drought preventing its uptake (Huang et al. 2005; Marschner 2012).

Studies have explored mitigating abiotic boric acid stress in various plant species (Neocleous and Vasilakakis 2008; Salim 2014; Torun et al. 2018; Yousefi et al. 2019; Rahman et al. 2021), but no *in vitro* research has focused on grapevines. Plant tissue culture techniques are increasingly valuable for their controlled conditions, which clearly show plant responses to stress and allow rapid growth and assessment of numerous plants. The aim of this study is to develop an alternative approach to address salinity stress in viticulture caused by increasing global warming and climate change worldwide. This study investigated the remedial effect of boric acid on grapevines under salinity stress by subjecting them to different doses of salinity and boric acid applications in vitro conditions. The aim was to determine the most appropriate dose(s) of boric acid.

Materials and Methods

Preparation of Plant Material

Post-pruning cuttings of 'Balıkçı Siyahı' (*Vitis labrusca* L.) genotype were maintained in water under laboratory conditions, and single-node microcuttings were used as explants. The shoots were immersed in a solution containing 20% commercial bleach and 1–2 drops of Tween 20 (P1379, Merck) for 20 min for surface sterilisation. They were then rinsed three times with sterile distilled water to remove the solution in a sterile cabinet.

Preparation of Plant Nutrient Medium

MS medium (Murashige and Skoog 1962; SIGMA, M5519 C) was used. As a growth regulator, 1 mg/l of BAP (6-benzylaminopurine, B3408, SIGMA) was used throughout the stage of shoot development, and 2 mg/l of IBA (indole-3-butyric acid, I5386, SIGMA) was used in the rooting and boric acid experiment stages. A volume of 30 g of sucrose and plant growth regulator were added to MS nutrient media. The pH of medium was adjusted to 5.8 using 0.1 N HCl (hydrochloric acid, K50244717821) and 0.1 N KOH (potassium hydroxide, B1485433829, Merck). Following this procedure, 8 g/L agar was added to the medium as a solidifying agent and boiled. After boiling, the medium was evenly distributed into borosilicate glass tubes (Z681784, Merck) of 25 mm × 150 mm, each containing approximately 10 ml, and the tube caps were sealed.

Cultivation of Explant

Explants were obtained from the shoots, leaving 1.5 cm below the node after surface sterilisation. The resulting single-node microcuttings were planted vertically in experimental tubes containing MS nutrient medium with 1 mg/l BAP. The tubes were then placed in a climate chamber. About 3 weeks after planting, when the shoots obtained from the nodule culture reached the 2–3 leaf stage, they were transferred to experimental tubes containing MS medium with 2 mg/l IBA in a sterile cabinet. After about 4 weeks, explants that had successfully rooted and developed were prepared for transfer to the salt and boric acid experiment.

Establishment of Boric Acid Trial

To examine the responses of explants to boric acid under different salt conditions, H_3BO_3 (1.00165 Merck) was added to MS nutrient medium at four different doses (0, 0.5, 1, 2 mM), along with three different doses (0, 100, 200 mM) of NaCl (Sod 106406), facilitating the investigation of interactions between boric acid and salinity. To accomplish this, rooted plantlets were introduced to the new medium by taking 2 cm stems from the top.

Sterilization of Used Equipment and Medium

The sterilisation of the forceps, lancets, test tubes, blotting paper, distilled water and experimental tubes used in the study was carried out in an autoclave (Core, NC 90M) at 1.05 atm and 121 °C for 15 min.

Culture Conditions

After planting, the explants, were kept in a growth chamber set to a photoperiod of 16 h of light and 8 h of darkness, illuminated by white fluorescent lamps with an intensity of 3000–4000 lx and a temperature of 25 ± 2 °C.

Parameters Examined

The number of surviving plantlets in *in vitro* salt and salt-free conditions and different boric acid doses was divided by the total number of plantlets and multiplied by 100 to determine the plant viability (%). At the end of experiment, the shoot lengths (cm) and leaf numbers (*n*) of the plantlets involved in the experiment were determined. Using a sensitive scale, the fresh weights of the shoots were measured with an accuracy of ±0.001 g. Following a 72-h drying period at 65 °C, the dry weights of the shoot samples were determined using a sensitive scale. The determination of chlorophyll in leaf samples taken from the middle part of the plantlets was carried out using a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan). The damage degree (0–3) was adapted from the scale applied to strawberry plants by Martinez Barroso and Alvarez (1997). In this scale, healthy plants are defined as '0 degree', slight drying and necrosis at leaf tips are categorized as '1st degree', more than half of the leaf and necrosis in the stem are labeled as '2nd degree', and necroses leading to plantlet death are classified as '3rd degree' damage. In the study, the shoot tolerance in salt and boric acid conditions was calculated separately for each salt and boric acid combination based on the formula provided by Turhan et al. (2005). TO: Tx / To, Tx: Shoot weights (g) of plantlets treated with specific concentrations of salt and boric acid; To: shoot weights (g) of plantlets not treated with salt and boric acid. Following application, the turgor weights of leaf samples obtained from the plantlets were determined by immersing them in clear water for a duration of 6 h. Leaf samples weighing 0.3 g were taken from the seedlings and placed in glass tubes measuring 25 mm × 150 mm. Then 15 ml of distilled water was added. The samples were kept in a shaker at 100 rpm for 24 h. Subsequently, the electrical conductivity (EC1) of the solution was determined using an EC meter. The same samples were autoclaved at 115 °C for 10 min and then left at room temperature for 24 h. The electrical conductivity (EC2) of the solution was measured again. Ion flux in the leaves was calculated using the formula (EC1 / EC2) × 100 (Özden et al. 2009). The cell membrane damage rate was calculated using the formula provided by Arora et al. (1998), utilizing ion flux. CMDR (%) = ([Ion Flux of Treatment (%) – Ion Flux of Control (%)]/ 100 – Ion Flux of Control) × 100. Following salt and boric acid applications, the fresh weights of the leaves carried by each plantlet were determined by weighing them with a sensitive scale with a precision of ±0.001 g during removal from nutrient medium. To calculate the relative water content (%) of the leaves, the fresh weights (FW), turgor weights measured after soaking in pure water for 6 h (TW), and dry weights measured after drying at 80 °C for 24 h (DW) were used (Yamasaki and Dillenburg 1999). LRWC: ([FW – DW] / [TW – DW] × 100).

Experimental Design and Statistical Analysis

The study was designed using a randomized complete block design with three replicates, each consisting of 10 explants. The LSD test was used at a significance level of 5% to examine differences between the treatments in the experiment, using JMP 13.2.0 statistical software. Prior to analysis, the percentage (%) values underwent angular transformation (ARCSIN).

Results

Shoot Growth Parameters

In examining shoot growth parameters, salt, boric acid, and their interaction significantly impacted shoot length. The highest shoot length (2.20 cm) was observed with 0 mM and 100 mM NaCl, while the lowest (1.90 cm) was at 200 mM NaCl. For boric acid, the highest shoot length (2.20 cm) was at 0.5 mM, and the lowest (2.00 cm) was at 2 mM. The highest shoot length from salt × boric acid interaction (2.44 cm) occurred with no salt or boric acid, and the lowest

Table 1 Effects of different doses of salt and boric acid (H_3BO_3) applications on shoot length (cm) and leaf number (*n*)

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	SL (cm)	LN (*n*)	SL (cm)	LN (*n*)	SL (cm)	LN (*n*)	SL (cm)	LN (*n*)
0 mM	2.44 a	3.13 abc	2.00 d	3.60 a	1.65 e	2.30 e	2.03 B	3.01
0.5 mM	2.27 abc	3.53 a	2.23 bc	3.37 a	2.10 bcd	2.43 de	2.20 A	3.11
1 mM	2.28 ab	3.00 a–d	2.13 bcd	2.73 b–e	2.09 cd	2.57 cde	2.17 A	2.77
2 mM	1.80 e	3.23 ab	2.42 a	3.00 a–d	1.77 e	2.55 cde	2.00 B	2.93
Average	2.20 A	3.22 A	2.20 A	3.17 A	1.90 B	2.46 B	–	–

$LSD_{P<0.05}$ for S (SL): 0.09; $LSD_{P<0.05}$ for B (SL): 0.11; $LSD_{P<0.05}$ for S × B (SL): 0.19
$LSD_{P<0.05}$ for S (LN): 0.31; $LSD_{P<0.05}$ for B (LN): N. S.; $LSD_{P<0.05}$ for S × B (LN): 0.63

Means not connected by same letter are significantly different ($P < 0.05$) level by LSD
S Salt, *B* H_3BO_3, *SL* Shoot length, *LN* Leaf number

Table 2 Effect of different doses of salt and boric acid applications on shoot sresh and dry weights (g)

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	SFW (g)	SDW (g)	SFW (g)	SDW (g)	SFW (g)	SDW (g)	SFW (g)	SDW (g)
0 mM	0.261 abc	0.030 a	0.238 c–f	0.022 c	0.213 g	0.017 f	0.24 B	0.023 A
0.5 mM	0.250 b–e	0.026 b	0.243 b–f	0.022 c	0.235 d–g	0.021 cd	0.24 B	0.023 A
1 mM	0.259 a–d	0.021 cd	0.268 ab	0.022 c	0.246 b–e	0.016 f	0.26 A	0.020 B
2 mM	0.219 fg	0.019 de	0.226 efg	0.016 f	0.276 a	0.017 ef	0.24 B	0.017 C
Average	0.250	0.024 A	0.240	0.020 B	0.240	0.018 C	–	–

$LSD_{P<0.05}$ for S (SFW): NS; $LSD_{P<0.05}$ for B (SFW): 0.01; $LSD_{P<0.05}$ for S × B (SFW): 0.02
$LSD_{P<0.05}$ for S (LN): 0.001; $LSD_{P<0.05}$ for B (LN): 0.001; $LSD_{P<0.05}$ for S × B (LN): 0.002

Means not connected by same letter are significantly different ($P<0.05$) level by LSD
S Salt, *B* H_3BO_3, *SFW* Shoot fresh weight, *SDW* Shoot dry weight

(1.65 cm) with 200 mM NaCl and no boric acid. At 200 mM NaCl, boric acid positively influenced shoot length, with the highest (2.10 cm) at 0.5 mM boric acid (Table 1).

According to general average data, the highest leaf number, determined as 3.22, was observed in the application of 0 mM NaCl. The lowest leaf number was observed to be 2.46 with the application of 200 mM NaCl. When salt and boric acid interactions were evaluated, the highest leaf number (3.6) was obtained from the application of 100 mM NaCl and 0 mM boric acid. According to the interaction findings, the lowest leaf number was observed to be 2.30 with the application of 200 mM NaCl and 0 mM boric acid. At the highest salt dose (200 mM), the maximum leaf number value of 2.57 was obtained from the application of 1 mM boric acid.

Significant effects of salt, boric acid, and salt × boric acid interaction have been found on shoot dry weight. A volume of 1 mM boric acid produced the highest shoot fresh weight of 0.26 g, according to an analysis of the overall average outcomes of boric acid application. In terms of the salt × boric acid interaction, the highest shoot fresh weight of 0.276 g was determined with 2 mM H_3BO_3 dose and 200 mM salt application. In the application with the lowest salt dose (0 mM), the highest shoot fresh weight value of 0.261 g was determined in the treatment without boric acid. In the 100 mM NaCl application, the highest shoot fresh weight value was obtained from the application of 1 mM boric acid. According to the interaction findings, decreases in shoot fresh weight were observed with increasing salt doses. Compared to the application without boric acid at 200 mM salt, an increase in shoot fresh weight values was observed with all increasing boric acid doses. In this experiment, generally, an increase in shoot fresh weight was achieved with boric acid applications in salt conditions created with 100 mM and 200 mM NaCl. The maximum shoot dry weight value of 0.023 g was found in both the 0.5 mM H_3BO_3 and 0 mM H_3BO_3 after analyzing the overall average values for boric acid. The lowest shoot dry weight value, 0.017 g, was determined in the 2 mM H_3BO_3 application. The control application without salt produced the greatest shoot dry weight of 0.024 g when examining the overall average findings for salt. The lowest value was determined to be 0.018 g in the 200 mM NaCl application. The application without salt and H_3BO_3 produced the maximum shoot dry weight of 0.030 g when the salt × boric acid interaction values were examined. The lowest exudation dry weight was determined with a value of 0.016 g in applications of 100 mM salt and 2 mM H_3BO_3 together with 200 mM salt and 1 mM H_3BO_3. The highest shoot dry weight value at the highest salt dose (200 mM) was obtained from the application of 0.5 mM H_3BO_3, with a value of 0.021 g (Table 2).

Physiological Parameters

According to the overall average results, the highest plant vitality rate (90%) was achieved with 0 mM and 100 mM NaCl, while the lowest (73.89%) was with 200 mM NaCl. Salt × boric acid interaction analysis showed that all boric acid doses with 0 mM and 100 mM NaCl had the highest vitality rates (90%). Without boric acid, 200 mM NaCl resulted in the lowest vitality (67.86%). At the highest salt dose (200 mM NaCl), the maximum vitality rate (83.85%) was with 0.5 mM H3BO3. Boric acid positively affected plant vitality under salt stress. (Table 3).

The degree of damage was significantly influenced by salt, boric acid, and their interaction. The highest damage value of 0.99 was observed with 200 mM salt, while the lowest (0.14) was with 0 mM salt. For boric acid, the highest damage (0.85) was without boric acid, and the lowest (0.37) was with 1 mM H3BO3. In the salt × boric acid interaction, the highest damage (1.40) occurred with 200 mM NaCl and no boric acid. The lowest damage (0.03) was with 0.5 mM H3BO3 and no salt. Damage increased with higher salt doses in the absence of boric acid (Table 3).

The findings indicate that salt, boric acid, and their interaction significantly affect leaf chlorophyll content and shoot tolerance ratio. The highest chlorophyll content

Table 3 Effect of different doses of salt and boric acid applications on plant vitality (%) and damage degree (0–3)

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	PV	DD	PV	DD	PV	DD	PV	DD
0 mM	90.00 a	0.17 c	90.00 a	1.00 b	67.86 c	1.40 a	82.62	0.85 A
0.5 mM	90.00 a	0.03 c	90.00 a	0.30 c	83.85 ab	1.00 b	87.95	0.44 B
1 mM	90.00 a	0.27 c	90.00 a	0.13 c	75.00 bc	0.70 b	85.00	0.37 B
2 mM	90.00 a	0.10 c	90.00 a	0.27 c	68.85 c	0.85 b	82.95	0.40 B
Average	90.00 A	0.14 C	90.00 A	0.42 B	73.89 B	0.99 A	–	–

$LSD_{P<0.05}$ for S (PV): 6.38; $LSD_{P<0.05}$ for B (PV): NS; $LSD_{P<0.05}$ for S × B (PV): 12.76
$LSD_{P<0.05}$ for S (DD): 0.19; $LSD_{P<0.05}$ for B (DD): 0.22; $LSD_{P<0.05}$ for S × B (DD): 0.37

Means not connected by same letter are significantly different ($P<0.05$) level by LSD
PV Plant vitality, *DD* Damage degree, *S* Salt, *B* H_3BO_3, *SFW* Shoot fresh weight, *SDW* Shoot dry weight

(19.74) was observed with 0 mM NaCl, while the other NaCl doses showed lower chlorophyll levels. For boric acid, the highest chlorophyll content (18.84) was with 0 mM H_3BO_3, and the lowest (15.29) with 2 mM H_3BO_3. In the salt × boric acid interaction, the highest chlorophyll content (24.61) was seen with no salt or boric acid. At 200 mM NaCl, the highest chlorophyll content (18.43) was with 0.5 mM H3BO3. The highest shoot tolerance ratio (1.83) was with 0.5 mM H_3BO_3, while the highest ratio (1.37) for salt was with 100 mM NaCl, and the lowest (1.20) was with 200 mM NaCl. In the salt × boric acid interaction, the highest shoot tolerance ratio (1.93) was with 0.5 mM H_3BO_3 and 100 mM NaCl, and at 200 mM NaCl, it was 1.82 with 0.5 mM H_3BO_3 (Table 4).

According to the table, salt and boric acid applications did not significantly affect leaf turgor weight, but the salt × boric acid interaction was significant. Salt, boric acid, and their interaction significantly impacted ion flux. The highest leaf turgor weight (0.072 g) was observed with 100 mM NaCl and 0 mM H_3BO_3. At 200 mM NaCl, the highest turgor weight (0.045 g) was without boric acid. Based on the interaction findings, leaf turgor values closest to or greater

Table 4 Effect of different doses of salt and boric acid applications on chlorophyll content (SPAD) and shoot tolerance ratio

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	CC	STR	CC	STR	CC	STR	CC	STR
0 mM	24.61 a	1.00 fg	16.72 b–e	1.43 c	15.19 cde	1.10 ef	18.84 A	1.18 B
0.5 mM	17.66 bc	1.72 b	13.85 de	1.93 a	18.43 bc	1.82 ab	16.65 BC	1.83 A
1 mM	19.36 b	1.38 cd	15.66 cde	1.24 de	17.73 bc	0.89 g	17.58 AB	1.17 B
2 mM	17.33 bcd	1.27 d	15.26 cde	0.89 g	13.30 e	0.97 fg	15.29 C	1.04 C
Average	19.74 A	1.34 A	15.37 B	1.37 A	16.16 B	1.20 B	–	–

$LSD_{P<0.05}$ for S (CC): 1.78; $LSD_{P<0.05}$ for B (CC): 2.05; $LSD_{P<0.05}$ for S × B (CC): 3.56
$LSD_{P<0.05}$ for S (STR): 0.07; $LSD_{P<0.05}$ for B (STR): 0.09; $LSD_{P<0.05}$ for S × B (STR): 0.15

Means not connected by same letter are significantly different ($P<0.05$) level by LSD
S Salt, *B* H_3BO_3, *CC* Chlorophyll content, *STR* Shoot tolerance rate

Table 5 Effect of different doses of salt and boric acid applications on leaf turgor weight and ion flux

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	LTW	IF	LTW	IF	LTW	IF	LTW	IF
0 mM	0.027 ab	42.06 bc	0.072 a	42.75 bc	0.045 ab	63.05 a	0.048	49.29 A
0.5 mM	0.027 ab	24.64 d	0.019 b	33.73 cd	0.040 ab	64.49 a	0.029	40.95 AB
1 mM	0.022 b	24.36 d	0.037 ab	28.61 cd	0.033 ab	56.75 ab	0.030	36.57 B
2 mM	0.025 b	24.07 d	0.045 ab	42.69 bc	0.042 ab	66.50 a	0.037	44.42 AB
Average	0.025	28.78 C	0.044	36.94 B	0.040	62.70 A	–	–

$LSD_{P<0.05}$ for S (LTW): NS; $LSD_{P<0.05}$ for B (LTW): NS; $LSD_{P<0.05}$ for S × B (LTW): 0.047
$LSD_{P<0.05}$ for S (IF): 7.36; $LSD_{P<0.05}$ for B (IF): 8.50; $LSD_{P<0.05}$ for S × B (IF): 14.73

Means not connected by same letter are significantly different ($P<0.05$) level by LSD
S Salt, *B* H_3BO_3, *LTW* Leaf turgor weight, *IF* Ion flux

than the control were obtained from the applications of 1 mM (0.037 g) and 2 mM (0.045 g) H_3BO_3 with 100 mM NaCl dose, and from the applications of 0.5 mM (0.040 g) and 2 mM (0.042 g) H_3BO_3 with 200 mM NaCl dose.

The 200 mM NaCl application had the highest ion flow (62.70%), while 0 mM NaCl had the lowest (28.78%), based on overall averages. Based on the total average results, the treatment without boric acid had the highest ion flow value (49.29%), whereas the application of 1 mM H_3BO_3 produced the lowest value (36.57%). Analyzing the salt × boric acid interaction data, 200 mM NaCl with 2 mM H_3BO_3 produced the highest ion flow (66.50%). At 200 mM NaCl, the lowest ion flux (56.75%) was with 1 mM H_3BO_3. The most efficient ion flux for both 100 mM and 200 mM NaCl was with 1 mM H_3BO_3. All boric acid doses reduced ion flux in the salt-free treatment (Table 5).

The highest membrane damage rate was 57.35% with 200 mM NaCl. For boric acid, the maximum cell membrane damage was 33.38% with 2 mM H_3BO_3, and the minimum was 20.39% with 1 mM H_3BO_3. The highest cell membrane damage from salt × boric acid interaction was 64.05% with 200 mM NaCl and 2 mM H_3BO_3. The lowest damage (0%) occurred with 0 mM, 0.5 mM, 1 mM, and 2 mM H_3BO_3 without NaCl. As salt doses increased, membrane damage also increased. The highest leaf explant water content (116.66%) was with 100 mM NaCl, while the lowest (97.13%) was with 200 mM NaCl. For boric acid, 2 mM H_3BO_3 resulted in the highest water content (113.77%), and 0.5 mM H_3BO_3 resulted in the lowest (93.92%) The highest relative water content of 132.32% was observed with 100 mM NaCl and 1 mM H_3BO_3. The lowest value (82.68%) occurred with 200 mM NaCl and the interaction of salt and boric acid. At 200 mM NaCl, 2 mM H3BO3 resulted in a relative water content of 109.33%. Increased salt doses significantly decreased relative water content (Table 6).

Discussion

The aim of the study was to determine both the tolerance by creating artificial salinity *in vitro* conditions and to determine the effectiveness of boric acid under salt conditions. In the study, necrosis in leaves and shoots was observed due to increasing salt doses. Plant viability, shoot fresh weight, shoot dry weight, shoot length, number of leaves on the shoot, chlorophyll content, and relative water content of explants were all found to decrease in the research in salinity conditions. Reduced plant growth and development in salt conditions has been related to increased osmotic pressure associated with the presence of sodium, chloride, magnesium and sulphate ions, which ultimately reduces the water available to the plant (Bybordi 2012). In this study, the relative water content of the leaves decreased as salt levels increased. This is due to the state of the stomata and the higher transpiration rate of the leaves. The osmotic regulation is used as a marker for the response to the osmotic stress. The osmotic potential decreases and the relative water content of the leaves decreases when salinity stress leads to water limitation. Evaluation of plant vitality values showed that all boric acid doses did not affect plant vitality values at either the 0 or 100 mM NaCl dose. The application without boric acid gave the lowest value at the 200 mM salt dose. The 0.5 mM H_3BO_3 dose showed the greatest positive effect. The lowest values in plants were obtained from the parameters of plant viability, shoot fresh weight, shoot dry weight, shoot length, number of leaves on the shoot, chlorophyll content, and explant relative water content in the application of 200 mM NaCl. Studies by Bulut (2019), Ekbic et al. (2021) and Ekbic et al. (2022) have also found the same decline in plant viability values in saline conditions. Demirtaş (2018) observed a decrease in shoot fresh weight in the some grape varieties with increasing salt doses. Similarly, Ekbic et al. (2021) stated a decrease in shoot fresh weight in 'Hamburg Misketi' and 'Isabella' grape varieties, while Ekbic et al. (2022) reported a decrease in shoot fresh weight with increasing salt doses in the 41B grapevine root-

Table 6 Effect of different doses of salt and boric acid applications on cell membrane damage rate and leaf relative water content

H_3BO_3 doses	NaCl doses						Average	
	0 mM		100 mM		200 mM			
	CMDR	LRWC	CMDR	LRWC	CMDR	LRWC	CMDR	LRWC
0 mM	0.00 e	116.31 ab	42.41 bc	111.15 ab	57.27 a	97.02 bc	33.23 A	108.16 AB
0.5 mM	0.00 e	99.10 bc	15.93 d	100.00 bc	61.61 a	82.68 c	25.85 B	93.92 B
1 mM	0.00 e	100.00 bc	14.71 d	132.32 a	46.46 b	99.50 bc	20.39 B	110.60 A
2 mM	0.00 e	108.78 abc	36.08 c	123.20 ab	64.05 a	109.33 abc	33.38 A	113.77 A
Average	0.00 C	–	27.28 B	–	57.35 A	–	–	–

$LSD_{P<0.05}$ for S (CMDR): 5.10; $LSD_{P<0.05}$ for B (CMDR): 5.89; $LSD_{P<0.05}$ for S × B (CMDR): 10.20
$LSD_{P<0.05}$ for S (LRWC): 13.72; $LSD_{P<0.05}$ for B (LRWC): 15.84; $LSD_{P<0.05}$ for S × B (LRWC): 27.44

Means not connected by same letter are significantly different ($P<0.05$) level by LSD
S Salt, *B* H_3BO_3, *CMDR* Cell membrane damage rate, *LRWC* Leaf relative water content

stock. The decrease in shoot fresh weight values observed in this study due to the increase in salt dose was supported by the results of the mentioned study. In the application of 200 mM NaCl, the most effective boric acid dose was determined as 0.5 mM H_3BO_3 for plant viability, shoot dry weight, shoot length and chlorophyll content. Sotiropoulos et al. (2005) determined that shoot fresh weight increased in kiwifruit by applying different levels of salinity and boric acid. Neocleous and Vasilakakis (2008) have observed an increase in shoot fresh weight with boric acid applications *in vitro* saline conditions in raspberry.

It has been determined that the application of 1 mM H_3BO_3 is the most effective dose in mitigating salt damage for the damage degree, ion flux, and cell membrane damage rate in 200 mM salt application. The shoot tolerance rate has decreased with increasing NaCl doses. The highest shoot tolerance rate was determined with a value of 1.82 with 0.5 mM H_3BO_3 application in 200 mM NaCl application, while the highest shoot tolerance value of 1.72 was achieved with 0.5 mM H_3BO_3 application in 0 mM NaCl.

Conclusion

To evaluate the findings of the study in general, the salinity tolerance of 'Balıkçı Siyahı' and the effectiveness of boric acid in saline conditions were determined in *in vitro* conditions and whether boric acid has an improving effect on the salt tolerance of 'Balıkçı Siyahı' grape genotype was determined with the help of growth, development and physiological parameters. According to the results, it was noted that 200 mM salt application negatively affected the growth and development of the plant in all parameters and in some parameters, the plant was more resistant to 100 mM salt. When the effectiveness of boric acid under salt stress in plants was evaluated in general, 0.5 mM and 1 mM doses of H_3BO_3 were more efecctive. On the other hand, the application of 2 mM H_3BO_3 had a negative effect on the plant by causing a toxic effect in general. Boric acid application is recommended to mitigate the negative effects of high salt doses on vine growth, development and salt damage, and to increase resistance.

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Conflict of interest S. Akden, H.B. Ekbic and M. Ilhan declare that they have no competing interests.

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