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Effects of 5-Aminolevulinic Acid (5-ALA) on Morphological and Physiological Characteristics of Grapevine against Salt Stress

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ABSTRACT

Salinity, one of the most significant abiotic stress factors restricting plant production, causes the destruction of agricultural lands and reduces productivity. In recent years, the utilization of 5-aminolevulinic acid (5-ALA) applications, which have important effects in terms of avoiding and providing tolerance to factors by impacting the physiology and metabolism of the plants, has been on the agenda. In this research, the impacts of foliar treatments of different levels of 5-ALA (0, 0.3, 0.6 and 0.9 mM) on morphological and physiological traits of 41 B American grapevine rootstocks under salinity stress (NaCl solution starting with 25 mM and reaching 150 mM concentration) were investigated. Salinity stress caused significant decreases in growth parameters, chlorophyll content, RWC and stomatal conductance, and significant increases in leaf temperature, proline and MDA content, physical damage and membrane damage degree. Under salinity stress, 0.9 mM 5-ALA treatments resulted in significant increases in shoot length (14.67 cm), root length (34.50 cm), leaf thickness (0.23 µm) leaf area (31.37 cm^2), leaf number (8.67 pieces), chlorophyll content (21.83 SPAD), RWC (80.20%), proline content (0.19 $\mu mol.g^{-1}$) and stomatal conductance (78.05 $mmol.m^{-2}.s^{-1}$); and significant decreases in physical damage degree (1.00 scale degree), membrane injury degree (15.46%) and MDA content (28.20 $nmol.g^{-1}$) compared to non-ALA treatments. According to the results of this study, 5-ALA can be recommended as an alternative application to provide salinity tolerance in plants in order to reduce the damage caused by salinity stress in agricultural lands.

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Introduction

Salinity, which is among the most significant abiotic stressors restricting plant production, causes the destruction of agricultural lands, reduces productivity and leads to significant economic losses (Qadir et al., 2014). Viticulture, with a global output of 77.4 million tons and an area of 7.3 million hectares, is among the major agricultural sectors that will be impacted by salinity damage.

On a global scale, it is estimated that more than 930 million hectares of land are confronted with the issue of soil salinity (Gregory et al., 2018). Due to climate change, increased evapotranspiration is predicted to accelerate soil salinization (Phogat et al., 2020). By 2050, there is a risk that 25-73% of existing vineyard areas under the Mediterranean climate will cease to be suitable for cultivation as a result of land desertification due to salinity and associated water scarcity (Hannah et al., 2013). Reports from leading grape-producing countries indicate that salinity is of particular concern for viticulture in some regions in Greece, India, Turkey, Italy, Australia, Iran, the US and Spain (Baneh et al., 2014; Phogat et al., 2020). In the Australian context, the prospective overall advantage of improving both soil salinity and sodicity was calculated to be $42 million annually for grape cultivation in 2005. This figure constitutes approximately 13% of the average production profit (Hajkowicz & Young, 2005).

The challenge of salinity, exacerbated particularly during hot and arid periods, typically arises due to inadequate rainfall, elevated evapotranspiration rates, or the utilization of irrigation water containing high concentrations of Na^+ and Cl^-. This leads to an escalation in salt concentration within the root zone (Tate, 2001; Hannah et al., 2013). Excess salt in the soil causes a number of metabolic disturbances in plants, particularly as a consequence of osmotic influences (dehydration), nutritional disorders and Na^+ toxicity (Munns, 2002). The early stage of the growth reaction in the presence of salt stress exhibits characteristics similar to those displayed by

plants experiencing water scarcity, attributed to disruptions in osmotic balance (Munns, 2002). Salt stress leads to growth inhibition, reduced photosynthetic activity and membrane stability, accumulation of specific osmolytes in tissues and induction of oxidative stress (Kozminska et al., 2018). As a result, Na^+ and Cl^- buildup on leaves and roots, as well as the induction of malondialdehyde (MDA), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) activities, and an increase in total phenolics and flavonoids are known to occur (Kumar et al., 2017). Salinity also significantly affects the yield and quality of plants through changes in cellular water content and osmotic potential (Yuanchun et al., 2015).

In the classification by Maas and Hoffman (1977), *Vitis vinifera* is categorized as a species moderately sensitive to salinity, with an upper limit of electrical conductivity (EC) around ~2.0 $dS.m^{-1}$, influencing factors such as fruit set and yield (Baby et al., 2016). In comparison to the majority of other plant species, this value is relatively low, as highlighted by Munns & Tester in 2008. On the flip side, the grapevine is recognized for its resilience to drought conditions (Charrier et al., 2018). Potential effects of high salinity on grapevine growth and productivity include reduced leaf expansion rates, leaf blight or mortality, reduced fruit set and significant yield losses (Munns & Tester, 2008; Baby et al., 2016).

Especially with the climatic changes that are predicted to continue in recent years, there is a need to develop new strategies that can eliminate or minimize the effects of increasing soil salinity on vine growth and development, yield and quality. In this regard, elicitors, which are naturally occurring and produced by organisms and play an important role in alleviating the effects of various stresses on plants, are seen as promising applications. Among the elicitors is 5-Aminolevulinic acid (5-ALA), which affects plant physiology and metabolism to prevent and tolerate stress factors. Acting as an important phytohormone that governs plant growth and development, 5-ALA functions as an environmentally friendly, biodegradable and non-toxic plant growth regulator (Xiong et al., 2020; Yang et al., 2021).

Studies have shown that exogenous 5-ALA applications are an effective strategy for increasing plant tolerance to different environmental stress factors, including salinity. The effectiveness of 5-ALA in enhancing salinity tolerance shows variation among different plant species, and the concentration range for optimal effectiveness also differs across these species. However, studies to evaluate the effects of 5-ALA on the defense mechanisms of grapevines under salt stress are quite limited. Especially in American grapevine rootstocks, which constitute the subsoil parts of the grapevine and will be primarily affected by salt stress conditions, no study has been found to examine the effects of 5-ALA applications. Therefore, there is a need to elucidate the mechanisms of 5-ALA applications on the salinity tolerance of grapevine and to determine the most effective 5-ALA concentration.

In this research, the impacts of various concentrations of exogenous 5-ALA foliar applications on morphological, physiological and biochemical parameters of grapevine rootstocks subjected to salt stress were investigated.

Material and Method

Research Area and Plant Material

This experiment, conducted in the greenhouse and research laboratories of Yozgat Bozok University Faculty of Agriculture between 2022 and 2023, aimed to explore the effects of different concentrations of 5-ALA applications on grapevine rootstocks under salinity stress.

In the study, 1-year old cuttings of the *V. vinifera* × *V. berlandieri* hybrid 41 B (41 B Millardet et de Grasset, 41 B MGt) American grapevine rootstock, which is characterized by its sensitivity to salinity (Çelik, 1996), although it shows a very high resistance to lime in cultivation, and due to this feature, damage symptoms due to salinity stress can be observed significantly, were used as plant material.

Preparation of Growing Media, Planting of Cuttings and Cultivation of Plants

Prior to planting, 41 B American grapevine rootstocks were subjected to bud removal (a single bud was left) and bottom freshening. Rootstock cuttings were subjected to rapid dipping treatment with IBA (Indole Butyric Acid) at a concentration of 2000 ppm and then planted in 15×15×18 cm black PE pots made up of an equally large volume of sterilized peat and perlite. The cuttings were promptly watered following transplantation, and irrigation persisted until water began to drain out from the pot's drainage holes.

The research area where the plants were grown is a ~200 m^2 greenhouse with a spring roof, polycarbonate material, 70% shade screen, fan heater, fan & pad system and ventilation system with a concrete floor. In the greenhouse where the experiment will be established, there are rooting tables 5 m long, 1.20 m wide, 80 cm above the ground and 20 cm deep. The pots in which the cuttings were planted were placed on these tables.

5-ALA and Salinity Stress Applications

The study utilized 5-Aminolevulinic acid hydrochloride (CAS No: 5451-09-2) from the SIGMA company as the source of 5-ALA. Saplings at phenological stage 12-15 (shoot lengths of 10-15 cm) according to the modified Eichhorn-Lorenz (E-L) system introduced by Coombe (1995) were used in the experiment and 5-ALA solutions at concentrations of 0, 0.3, 0.6 and 0.9 mM were sprayed on the entire green surface of the plants ~6 weeks after planting.. Four weeks after 5-ALA applications, the growing media were irrigated with NaCl solution, which was started with 25 mM and increased by 25 mM weekly to 150 mM concentration. Purified water was used in control samples.

After a 120-day growing period in which adequate root and shoot development was achieved, the experiment was terminated and morphological, physiological and biochemical characteristics of the grapevine saplings were analysed.

The Effects of 5-ALA on Plant Growth Parameters

Shoot and root fresh weights were weighed using an analytical balance and the averages were expressed in g. The dry weights of shoots and roots were weighed using an analytical balance after drying in an air-circulating oven at 65°C for 72 hours and the averages were expressed in mg.

Shoot and root lengths were determined by measuring the distances from the tip to the base in cm using a ruler.

Leaf surface area was measured from mature leaves using an area meter (ADC BioScientific Area Meter AM 300) and the mean values were recorded in cm^2.

Leaf thickness was determined by mechanical micrometer (BTS-12051) and values were expressed in µm.

The degree of physical damage was determined using the scale (0-3 scale) developed by Sivritepe & Eriş (1999). Accordingly, those with no necrotic tissues on shoots and leaves caused by salinity stress were scored as "grade 0", those with necrosis on shoot tips and leaf margins were scored as "grade 1", those with necrosis on more than 50% of the leaf and/or part of the shoot were scored as "grade 2", and those with necrosis causing plant death were scored as "grade 3".

The Effects of 5-ALA on Physiological Characteristics

Chlorophyll content was assessed using a handheld chlorophyll meter (Konica Minolta SPAD 502) by measuring between the veins of the leaves. The values measured were represented in SPAD (Geravandi et al., 2011).

The relative water content of leaves was determined following the method outlined by Yamasaki & Dillenburg (1999). Accordingly, the fresh weight (FW) of the leaves was first determined. The leaves were immersed in distilled water for a duration of 6 hours, and their turgor weights (TW) were subsequently measured. Following this, the dry weights (DW) were determined by subjecting the leaves to 80°C for 24 hours. The relative water content (%) was calculated using the formula [(FW-DW)/(TW-DW)]×100.

Leaf temperature and stomatal conductance were measured between the veins of the leaves using a leaf porometer (Decagon/Pullman, WA, SC-1 Leaf Porometer) and recorded in $mmol.m^{-2}.s^{-1}$ and °C, respectively.

The membrane damage degree was computed by measuring the electrolyte removed from the cell. For this purpose, 3 discs of 6 mm in diameter were first removed from the leaves with the help of cork-borer. These discs were soaked in 20 ml distilled deionized water for four hours and EC1 was measured using an EC meter (Jenway-470 condimeter). After the same discs were kept at 100 °C for 10 min, EC_2 was measured and calculated as percentage (%) with the formula $(EC_1/EC_2)\times100$ (Nayyar, 2003).

The Effects of 5-ALA on Biochemical Characteristics

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) using the methodology outlined in the procedure by Lutts et al. (1996). MDA was measured by reading the color developing at 535 nm and 600 nm and the values were recorded as $nmol.g^{-1}$.

Proline was determined spectrophotometrically (Lambda 25, Perkin Elmer) at 520 nm using the ninhydrin assay according to the procedure of Bates et al. (1973) and the results were recorded as $\mu mol.g^{-1}$.

Experimental Design and Evaluation of Data

The study was designed based on the randomized plots trial design with three replicates and each replicate consisted of 20 plants. The numerical data obtained were processed using IBM SPSS 20.0 program. Analysis of variance (One-Way ANOVA) was applied to the data. The Duncan multiple comparison test (with a significance level of $p<0.05$) was employed to assess the distinctions among the means.

Results

The Effect of 5-ALA on Plant Growth Parametersand Chlorophyll Content

Salinity stress caused statistically significant decreases ($p<0.05$) in shoot length of grapevine saplings (16.33 cm) compared to non-stressed groups (7.83 cm). However, there was no significant difference in shoot fresh and dry weight between salinity stress and non-salinity stress groups. 5-ALA treatments resulted in statistically significant ($p<0.05$) increases in shoot length in both salinity stressed and non-salinity stressed groups compared to non-ALA treatments. In terms of shoot length under salinity stress, 0.6 and 0.9 mM 5-ALA treatments showed higher averages (12.50 cm and 14.67 cm, respectively) compared to the negative control (7.83 cm). In the non-salt-stressed groups, 0.9 mM 5-ALA treatment had a higher value in shoot length (28.17 cm) compared to the positive control (16.33 cm) (Figure 1) (Table 1).

Salinity stress caused statistically significant decreases ($p<0.05$) in root length of grapevine saplings (19.17 cm) compared to non-stressed groups (34.33 cm). However, there was no significant difference in root fresh and dry weight between salinity stress and non-salinity stress groups. In both salinity stressed and non-salinity stressed groups, 5-ALA treatments resulted in statistically significant increases in root length compared to non- 5-ALA treatments ($p<0.05$). All 5-ALA treatments under salinity stress resulted in significant increases in root length, especially 0.9 mM 5-ALA treatment had higher averages (34.50 cm) compared to other concentrations and negative control. In the groups without salinity stress, 0.9 mM 5-ALA application had higher values in terms of root length (59.00 cm) compared to the positive control (Figure 2) (Table 2).

Table 1. Effects of 5-ALA on shoot traits

Treatments	Shoot Fresh Weight (g)	Shoot Dry Weight (mg)	Shoot Length (cm)
Negative Control	2.81±2.03	0.87±0.60	7.83±2.84 f
Positive Control	3.36±0.65	1.08±0.28	16.33±1.26 bd
0.3 mM 5-ALA+NaCl	3.58±1.02	1.09±0.29	11.00±1.32 ef
0.3 mM 5-ALA	4.27±0.67	1.31±0.22	18.33±0.76 bc
0.6 mM 5-ALA+NaCl	3.39±1.04	1.03±0.32	12.50±0.50 de
0.6 mM 5-ALA	4.53±0.86	1.47±0.21	20.17±0.76 b
0.9 mM 5-ALA+NaCl	6.15±2.85	1.93±0.97	14.67±0.29 ce
0.9 mM 5-ALA	3.27±0.79	1.02±0.27	28.17±5.48 a
Mean	3.92±1.57	1.22±0.50	16.13±6.31

*Different letters indicate significant differences based on Duncan's post-hoc analysis at $p \leq 0.05$.

Table 2. Effects of 5-ALA on root traits

Treatments	Root Fresh Weight (g)	Root Dry Weight (mg)	Root length (cm)
Negative Control	6.54±0.78	3.34±1.09	19.17±3.33 f
Positive Control	5.50±2.05	2.27±1.39	34.33±0.58 cd
0.3 mM 5-ALA+NaCl	3.43±1.91	1.58±0.81	27.00±1.00 e
0.3 mM 5-ALA	4.96±1.94	1.85±0.39	36.83±1.89 c
0.6 mM 5-ALA+NaCl	6.08±2.82	2.43±1.27	30.67±2.31 de
0.6 mM 5-ALA	4.88±2.60	1.94±0.88	47.67±6.81 b
0.9 mM 5-ALA+NaCl	2.05±0.30	0.94±0.14	34.50±0.00 cd
0.9 mM 5-ALA	2.16±1.16	1.16±0.30	59.00±2.65 a
Mean	4.45±2.27	1.94±1.04	36.15±12.05

*Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

Table 3. Effects of 5-ALA on leaf characteristics and chlorophyll content

Treatments	Leaf Thickness (µm)	Leaf Area (cm^2)	Number of Leaves (piece)	Chlorophyll Content (SPAD)
Negative Control	0.17±0.01 c	19.70±2.52 e	6.33±1.15 d	17.90±2.26 c
Positive Control	0.15±0.01 c	31.64±1.54 c	8.67±0.58 bc	22.60±1.31 ab
0.3 mM 5-ALA+NaCl	0.19±0.02 b	25.25±1.97 d	7.67±0.58 cd	20.33±0.67 bc
0.3 mM 5-ALA	0.15±0.01 c	36.32±0.90 b	8.00±1.00 bc	22.93±2.01 ab
0.6 mM 5-ALA+NaCl	0.21±0.01 a	27.23±2.20 d	7.67±0.58 cd	20.73±1.55 bc
0.6 mM 5-ALA	0.15±0.01 c	39.48±2.20 ab	9.33±0.58 ab	22.87±0.64 ab
0.9 mM 5-ALA+NaCl	0.23±0.01 a	31.37±2.95 c	8.67±0.58 bc	21.83±1.55 ab
0.9 mM 5-ALA	0.15±0.01 c	41.74±1.61 a	10.33±0.58 a	23.93±1.99 a
Mean	0.18±0.03	31.59±7.32	8.33±1.31	21.64±2.27

*Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

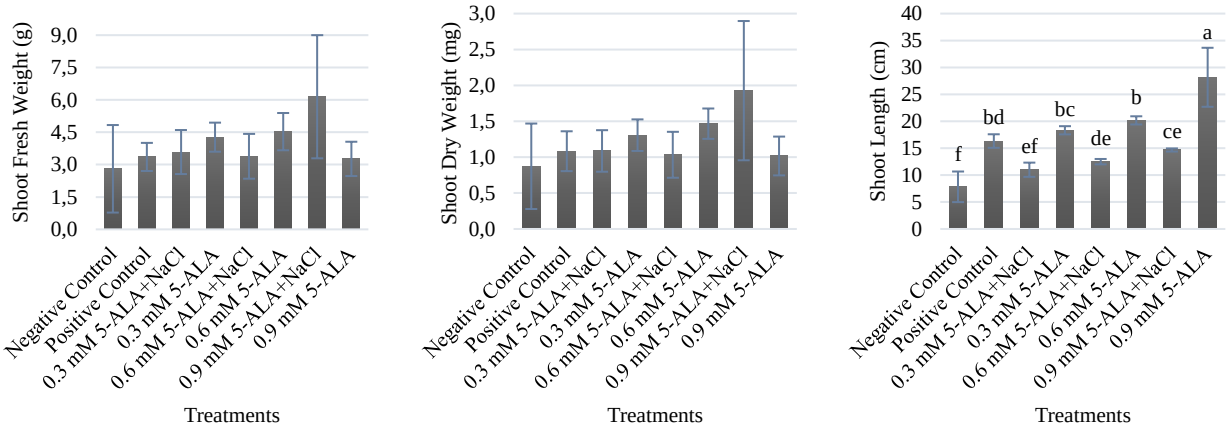

Figure 1. Effects of 5-ALA on shoot traits. Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

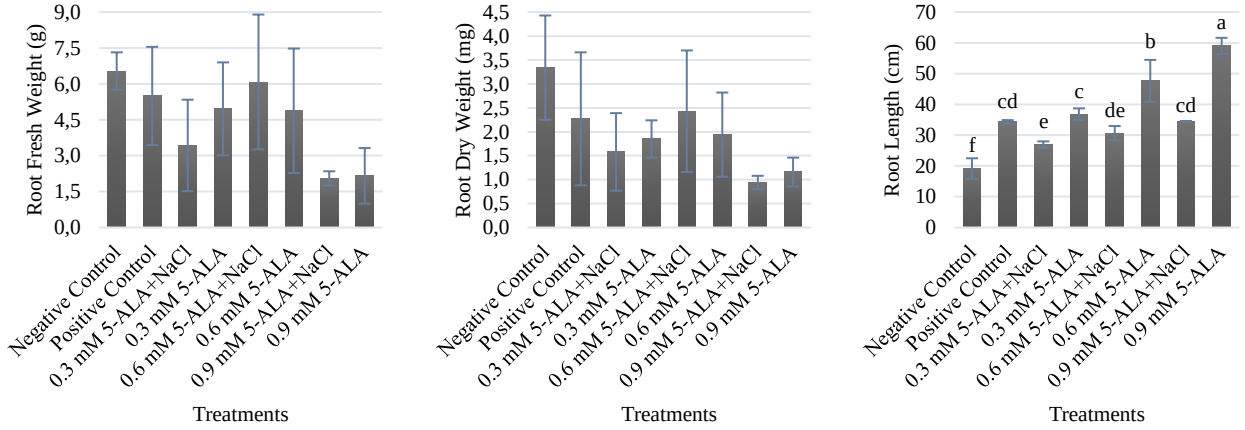

Figure 2. Effects of 5-ALA on root traits. Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

Salinity stress caused statistically significant decreases (19.70 cm^2, 6.33 pieces and 17.90 SPAD, respectively) in leaf area, leaf number and chlorophyll content of grapevine saplings (31.64 cm^2, 8.67 pieces and 22.60 SPAD, respectively) compared to non-stressed groups ($p<0.05$). Nevertheless, there was no notable alteration in leaf thickness observed between the positive and negative control groups. In both salinity stressed and non-salinity stressed groups, 5-ALA treatments resulted in statistically significant increases in leaf area and leaf number compared to non-ALA treatments ($p<0.05$). While all 5-ALA treatments under salinity stress provided significant increases in leaf thickness and leaf area, the most effective concentration was 0.9 mM 5-ALA treatment (0.23 µm and 31.37 cm^2, respectively) compared to the negative control (0.17 µm and 19.70 cm^2, respectively) ($p<0.05$). In terms of leaf number and chlorophyll content, only 0.9 mM 5-ALA treatment caused a statistically significant increase (8.67 pieces and 21.83 SPAD, respectively) compared to the negative control (6.33 pieces and 17.90 SPAD, respectively) ($p<0.05$). All 5-ALA treatments (0.3 mM 5-ALA: 36.32 cm^2; 0.6 mM 5-ALA: 39.48 cm^2; 0.9 mM 5-ALA: 41.74 cm^2) provided a statistically significant increase in leaf area compared to the positive control (31.64 cm^2) in the groups without salinity stress. In terms of leaf number, 0.9 mM 5-ALA treatment (10.33 pieces) had higher values compared to the positive control (8.67 pieces), while there was no statistically significant change in leaf thickness or chlorophyll content (Figure 3) (Table 3).

The Effect of 5-ALA on Stomatal Conductivity, Leaf Temperature, Proline Content and Leaf Relative Water Content

Salinity stress caused statistically significant decreases (59.04% and 72.38 $mmol.m^{-2}.s^{-1}$, respectively) in RWC and stomatal conductance of grapevine saplings (82.30% and 81.29 $mmol.m^{-2}.s^{-1}$, respectively) compared to non-stressed groups ($p<0.05$). However, leaf temperature and proline content increased significantly (22.33 °C and 0.13 $\mu mol.g^{-1}$, respectively) compared to non-stressed groups (21.40 °C and 0.08 $\mu mol.g^{-1}$, respectively) ($p<0.05$). In both salinity-stressed and non-salinity-stressed groups, 5-ALA treatments resulted in statistically significant increases in RWC ratio, stomatal conductance and proline content compared to non-5-ALA treatments ($p<0.05$). All 5-ALA treatments under salinity stress resulted in significant increases in RWC ratio (0.3 mM 5-ALA: 68.45%; 0.6 mM 5-ALA: 74.68%; 0.9 mM 5-ALA: 80.20%) compared to the negative control (59.04%). However, 0.6 and 0.9 mM 5-ALA treatments caused a statistically significant increase in proline content (0.16 and 0.19 $\mu mol.g^{-1}$, respectively) compared to the negative control (0.13 $\mu mol.g^{-1}$). In terms of stomatal conductance, 0.9 mM 5-ALA treatment caused a statistically significant increase (78.05 $mmol.m^{-2}.s^{-1}$) compared to the negative control (72.38 $mmol.m^{-2}.s^{-1}$). Nevertheless, there was no statistically significant alteration observed in leaf temperature. In the groups without salinity stress, 0.6 and 0.9 mM 5-ALA treatments had higher values for stomatal conductance (86.30 and 86.80 $mmol.m^{-2}.s^{-1}$, respectively) compared to the positive control (81.29 $mmol.m^{-2}.s^{-1}$). However, for RWC and proline content, 0.9 mM 5-ALA treatment showed higher values (93.48% and 0.11 $\mu mol.g^{-1}$, respectively) compared to the positive control (82.30% and 0.08 $\mu mol.g^{-1}$, respectively). In terms of leaf temperature parameter, 0.9 mM 5-ALA treatment had lower mean values (20.49°C) compared to the positive control (21.40°C) ($p<0.05$) (Figure 4) (Table 4).

Table 4. Effects of 5-ALA on RWC, stomatal conductance, leaf temperature and proline content

Treatments	RWC (%)	Stomatal Conductivity ($mmol.m^{-2}.sn^{-1}$)	Leaf Temperature (°C)	Proline Content ($\mu mol.g^{-1}$)
Negative Control	59.04±9.49 e	72.38±2.21 e	22.33±0.29 a	0.13±0.01 c
Positive Control	82.30±0.83 bc	81.29±1.87 bc	21.40±0.44 bc	0.08±0.01 e
0.3 mM 5-ALA+NaCl	68.45±2.36 d	75.19±1.42 de	22.33±0.29 a	0.14±0.01 c
0.3 mM 5-ALA	83.59±1.05 b	84.10±3.41 ab	20.70±0.57 cd	0.09±0.01 e
0.6 mM 5-ALA+NaCl	74.68±4.56 cd	76.20±2.62 de	21.90±0.44 ab	0.16±0.01 b
0.6 mM 5-ALA	85.48±2.86 b	86.30±3.63 a	20.69±0.53 cd	0.09±0.02 de
0.9 mM 5-ALA+NaCl	80.20±4.82 bc	78.05±1.24 cd	21.59±0.55 ab	0.19±0.02 a
0.9 mM 5-ALA	93.48±2.09 a	86.80±3.68 a	20.49±0.53 d	0.11±0.01 d
Mean	78.40±10.91	80.04±5.62	21.43±0.81	0.12±0.04

*Different letters indicate significant differences based on Duncan's post-hoc analysis at $p \leq 0.05$.

Table 5. Effects of 5-ALA on oxidative stress parameters

Treatments	Physical Damage Degree (0-3 scale)	Membrane Damage Degree (%)	MDA ($nmol.g^{-1}$)
Negative Control	3.00±0.00 a	17.94±0.29 a	37.25±0.33 a
Positive Control	0.00±0.00 d	14.83±0.74 d	16.85±0.18 e
0.3 mM 5-ALA+NaCl	2.00±0.00 b	17.00±0.82 ab	31.39±1.50 b
0.3 mM 5-ALA	0.00±0.00 d	14.80±0.70 d	22.39±1.33 d
0.6 mM 5-ALA+NaCl	1.00±0.00 c	16.24±0.31 bc	32.32±0.22 b
0.6 mM 5-ALA	0.00±0.00 d	14.38±0.32 d	21.49±0.64 d
0.9 mM 5-ALA+NaCl	1.00±0.00 c	15.46±0.88 cd	28.20±1.39 c
0.9 mM 5-ALA	0.00±0.00 d	12.85±0.25 e	21.29±0.52 d
Mean	0.88±1.08	15.44±1.61	26.40±6.69

*Different letters indicate significant differences based on Duncan's post-hoc analysis at $p \leq 0.05$.

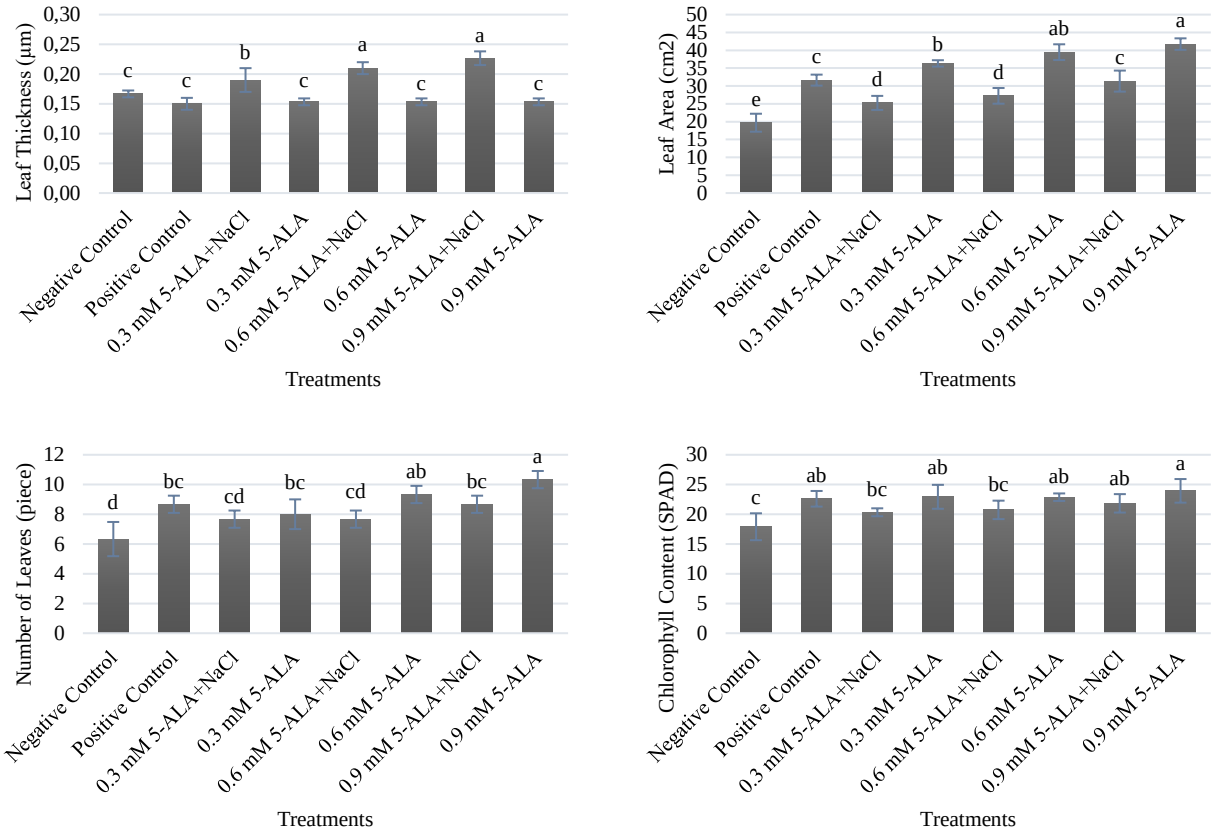

Figure 3. Effects of 5-ALA on leaf characteristics and chlorophyll content. Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

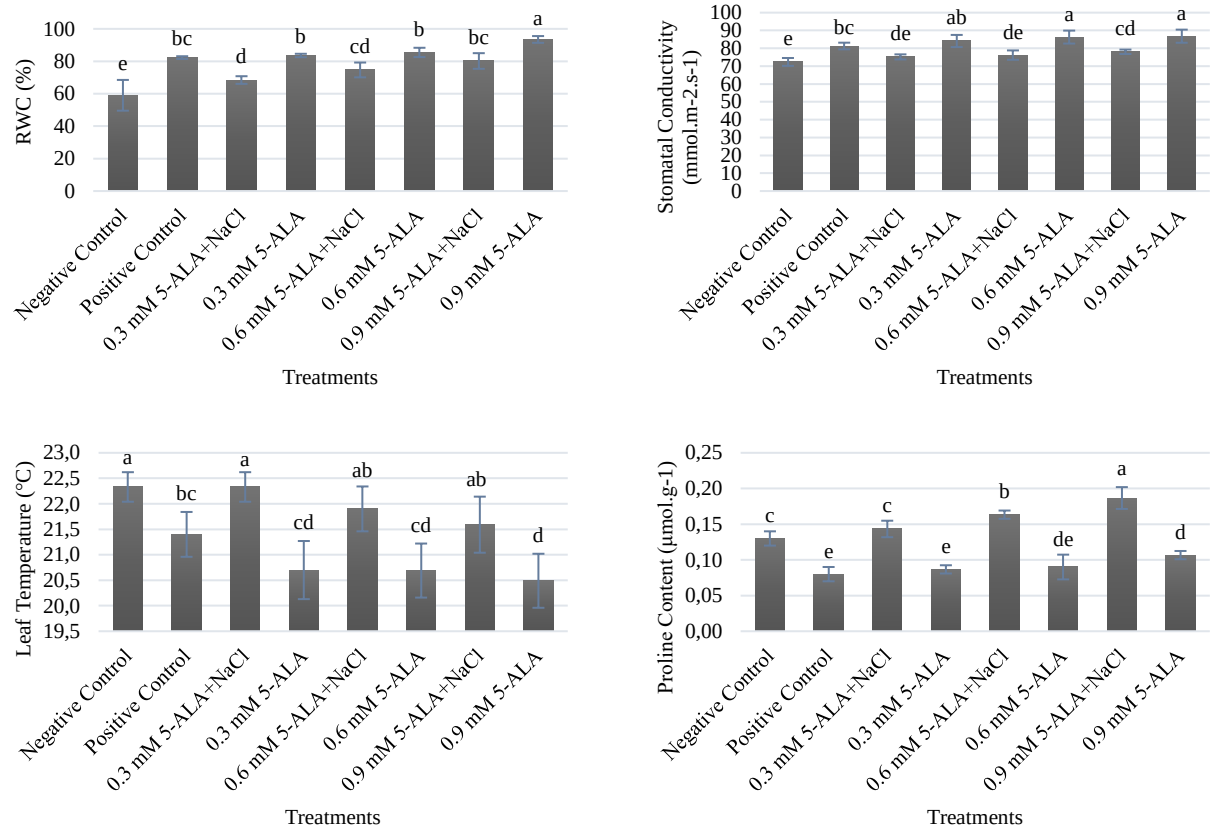

Figure 4. Effects of 5-ALA on RWC, stomatal conductance, leaf temperature and proline content. Different letters indicate significant differences based on Duncan's post-hoc analysis at p ≤0.05.

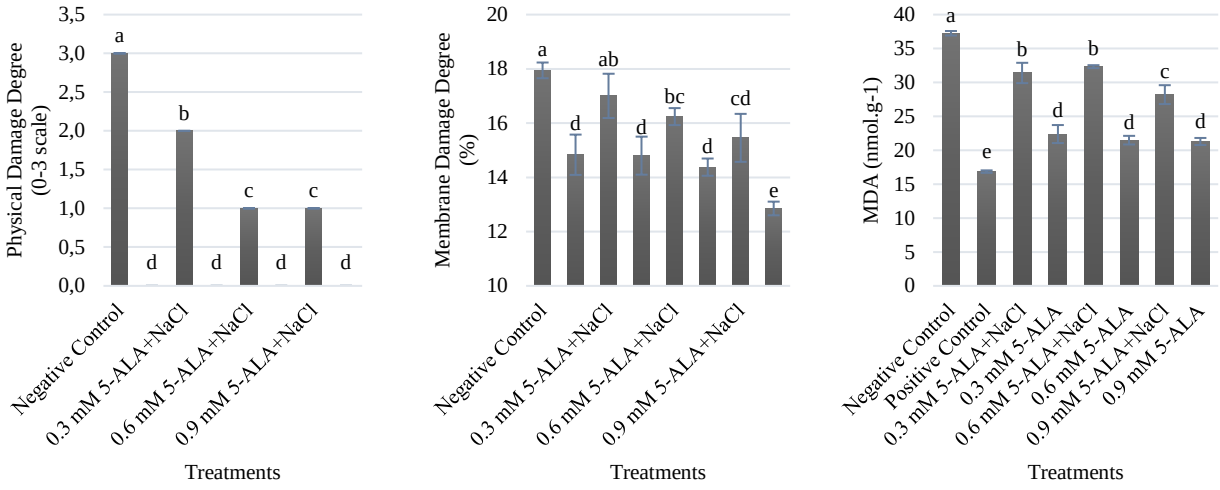

Figure 5. Effects of 5-ALA on oxidative stress parameters. Different letters indicate significant differences based on Duncan's post-hoc analysis at $p \leq 0.05$.

The Effect of 5-ALA on Oxidative Stress Parameters

Salinity stress caused statistically significant increases (3.00 scale degree, 17.94% and 37.25 nmol.g^{-1}, respectively) in the degree of physical damage, membrane damage and MDA content of grapevine saplings (0.00 scale degree, 14.83% and 16.85 nmol.g^{-1}, respectively) compared to non-stressed groups ($p<0.05$). All 5-ALA treatments under salinity stress showed significant decreases (0.3 mM 5-ALA: 2.00 scale degree and 31.39 nmol.g^{-1}; 0.6 mM 5-ALA: 1.00 scale degree and 32.32 nmol.g^{-1}; 0.9 mM 5-ALA: 1.00 scale degree and 28.20 nmol.g^{-1}) in the degree of physical damage and MDA content compared to the negative control (3.00 scale degree and 37.25 nmol.g^{-1}, respectively) ($p<0.05$). In terms of the degree of membrane damage, 0.6 and 0.9 mM 5-ALA treatments caused a statistically significant decrease (16.24% and 15.46%, respectively) compared to the negative control (17.94%) ($p<0.05$). While 0.9 mM 5-ALA application provided a significant decrease (12.85%) in the degree of membrane damage in the groups without salinity stress compared to the positive control (14.83%), all 5-ALA applications showed a significant increase in MDA content (0.3 mM 5-ALA: 22.39 nmol.g^{-1}; 0.6 mM 5-ALA: 21.49 nmol.g^{-1}; 0.9 mM 5-ALA: 21.29 nmol.g^{-1}) compared to the positive control (16.85 nmol.g^{-1}) ($p<0.05$). No statistically significant difference was found in the degree of physical damage (Figure 5) (Table 5).

Discussion

In the present study, salinity stress caused significant reductions in the growth characteristics (shoot length, leaf area, leaf number and root length) of grapevine saplings compared to non-stressed groups. This growth inhibition in salinity-affected grapevine saplings was thought to be due to osmotic and ionic responses such as oxidative stress, water loss and photoinhibition (Zhou-Tsang et al., 2021). Studies have reported that the osmotic potential of saline soils causes loss of cellular turgor, leading to dehydration of grapevine tissues and consequent growth inhibition, biomass loss and cell death (Munns & Tester 2008; Stevens et al. 2011; Baby et al. 2016).

5-ALA treatments significantly increased growth traits (shoot length, root length, leaf area and number of leaves) in both salinity stressed and non-salinity stressed groups compared to non-ALA treatments. In addition, 5-ALA treatments under salinity stress were effective in increasing leaf thickness. While all 5-ALA concentrations were found to be effective in increasing root length, leaf thickness and leaf area under salinity stress, 0.6 and 0.9 mM 5-ALA treatments were found to be the most effective concentrations in terms of shoot length and 0.9 mM 5-ALA treatment in terms of leaf number. In the groups without salinity stress, all 5-ALA concentrations were found to be effective in terms of leaf area, while 0.9 mM 5-ALA was found to be the most effective concentration in terms of shoot length, root length and number of leaves. Studies have shown that 5-ALA are regulatory substance that promotes plant growth and development under both normal and stressful conditions (Wang et al., 2004; Korkmaz, 2012). Tavallali et al. (2019) reported that foliar application of different concentrations (0, 25 and 50 mg.l^{-1}) of 5-ALA had positive effects on shoot biomass, shoot length, total phenolic content and antioxidative activity in purslane (*Portulaca oleracea* L.); however, the most effective concentration was obtained from 50 mg.l^{-1} concentration. Watanabe et al. (2000) reported that among 12 different plant growth regulators examined against salinity stress in cotton, 5-ALA was the most effective application in terms of increasing plant tolerance. Nishihara et al. (2003) reported that 5-ALA treatments improved growth and increased antioxidative enzyme activity in spinach (*Spinacia oleracea*) under NaCl stress. On the other hand, Yang et al. (2021) reported that leaf size and leaf thickness increased in *Buxus megistopphylla* Levl exposed to various stress factors as a result of foliar spraying of 5-ALA at a concentration of 20 mg.l^{-1}. Manafi et al. (2015) determined that exogenous 5-ALA applications at different concentrations (0, 0.3, 0.6 and 0.9 mM) applied from seed and leaves against cold stress in soybean (*Glycine max* L. Merr) increased plant height, shoot fresh and dry weight and chlorophyll content at 0.3 mM dose. The same researchers found that foliar spray application of 5-ALA was more effective than seed

application. In the present study, it was thought that the positive effect of 5-ALA applications on the growth characteristics of grapevine saplings may be related to the increase in chlorophyll content, photosynthesis and the proportion of enzymatic or non-enzymatic antioxidant systems (Naeem et al., 2010, 2011). Indeed, Hotta et al. (1997a) reported that 5-ALA (10-300 $mg.l^{-1}$) applied at the early growth stage increased growth rate and photosynthesis in different plant species such as paddy, faba bean, barley, potato, radish, garlic and kidney bean. In another study, 5-ALA was found to promote plant growth by increasing the photosynthetic capacity of melon seedlings under low temperatures and low light intensity (Wang et al., 2004). Similar findings were recorded by Xu et al. (2010) in Kudzu (*Pueraria phaseoloides*) and persimmon (*Phoenix dactylifera* L.) and reported that the growth enhancement was related to chlorophyll content and photosynthetic rate.

In the present study, salinity stress caused significant decreases in the chlorophyll content of grapevine saplings compared to the non-stressed groups. Previous studies have shown that chlorophyll fluorescence is attenuated in grapevines under salinity stress due to inhibition of electron transport in photosystem II (Downton, 1983) and this effect has been attributed to ROS-induced peroxidation of membrane lipids (Fozouni et al. 2012).

5-ALA treatments provided significant increases in terms of increasing chlorophyll content under salinity stress compared to 5-ALA untreated groups. The most effective concentration for increasing chlorophyll content under salinity stress was 0.9 mM 5-ALA treatment. In this study, the positive effect of 5-ALA applications on the chlorophyll content of grapevine saplings was evaluated as a result of the fact that 5-ALA constitutes the initial step in the chlorophyll synthesis chain in plants (Scheer, 2004). In a similar study conducted on grapevine, Watanabe et al. (2006) found that the application of 100 $mg.l^{-1}$ 5-ALA increased plant growth and CO_2 assimilation. On the other hand, Hotta et al. (1997b) found that low concentrations (0.06-0.6 $\mu mol.l^{-1}$) of 5-ALA increased chlorophyll content in horseradish (*Armoracia rusticana*) and golden pothos (*Epipremnum aureum*). It has also been reported by various researchers that exogenous 5-ALA applied at low concentrations increases the photosynthetic capacity and yield by increasing the chlorophyll content in leaves (Watanabe et al., 2000; Youssef & Awad, 2008).

Salinity stress caused significant decreases in RWC ratio and stomatal conductance of grapevine saplings compared to non-stressed groups. On the contrary, leaf temperature and proline content showed an opposite trend and increased significantly compared to the non-stressed groups. Responses to salinity consist of changes in plant physiology or biochemistry as a result of damage or plant responses that attempt to prevent or mitigate damage (Munns et al. 2020). Stomatal regulation (usually closure) is a well-known early response to osmotic and/or drought stress (Zhou-Tsang et al., 2021). In this response, Walker et al. (1981) reported that 5-ALA reduced water loss through transpiration, but caused a decrease in photosynthetic activity by limiting CO_2 diffusion through the stomatal pores to the leaf and increasing photorespiration. Downton et al. (1990) reported a decrease in stomatal conductance and photosynthetic activity of salt-affected Sultana vines. On the other hand, Meggio et al. (2014) reported that Na^+ and Cl^- accumulation in grapevine tissues under salt stress caused a decrease in stomatal conductance and water potential. Some osmoprotectants, such as proline, observed in grapevines under salt stress are known to provide antioxidative properties, emphasizing the close relationship between water and oxidative stresses, especially under salinity, and the importance of a combined response accordingly (Ozden et al. 2009; Haider et al. 2019). Indeed, Fozouni et al. (2012) observed an increase in the concentration of compatible solutes such as soluble sugars and proline in grapevine leaves after saline irrigation.

5-ALA treatments provided significant increases in RWC ratio, stomatal conductance and proline content in both salinity stressed and non-salinity stressed groups compared to 5-ALA untreated groups. While all 5-ALA concentrations under salinity stress were found to be effective in increasing RWC ratio, 0.6 and 0.9 mM 5-ALA applications were found to be the most effective concentrations in terms of proline content and 0.9 mM 5-ALA application was found to be the most effective concentrations in terms of stomatal conductance. In the groups without salinity stress, 0.6 and 0.9 mM 5-ALA treatments were the most effective concentrations in terms of stomatal conductance; 0.9 mM 5-ALA treatment was the most effective in terms of RWC, proline content and leaf temperature. The reason why 5-ALA increased proline content, stomatal conductance and leaf relative water content in grapevine saplings was thought to be due to its ability to regulate osmotic balance in plant cells, water regulation and increase their ability to cope with stress (Tan et al., 2022). In parallel with our findings, Yang et al. (2014) reported that 5-ALA (0.5 $mg.l^{-1}$) sprayed on leaves against 200 mM NaCl stress in creeping bentgrass (*Agrostis stolonifera*), a salinity-sensitive perennial grass species, increased chlorophyll content, net photosynthetic rate, leaf relative water content and stomatal conductance, and was effective in alleviating membrane electrolyte leakage and lipid peroxidation damage caused by salinity stress. In a similar study, Youssef & Awad (2008) reported that 5-ALA increased the rate of photosynthesis by increasing leaf relative water content and stomatal conductance in date palm seedlings (*Phoenix dactylifera*) exposed to salinity stress. Tang et al. (2016) reported that foliar application of 5-ALA at concentrations of 0, 12.5, 16.7, 25.0 and 50.0 $mg.l^{-1}$ increased the fresh weight, chlorophyll content (SPAD), stomatal conductance and antioxidant enzyme activities of leaves and roots against salinity stress in *I. indigotica*. Manafi et al. (2015) determined that exogenous 5-ALA applications at 0.3 mM concentrations applied from seed and leaves increased stomatal conductance and relative water content in soybean (*Glycine max* L. Merr) against cold stress. On the other hand, Yang et al. (2021) reported that proline content and antioxidant enzyme activity increased in *Buxus megistopphylla* Levl exposed to various stress factors as a result of spraying leaves with 5-ALA at a concentration of 20 $mg.l^{-1}$.

Salinity stress caused significant increases in the physical damage, membrane damage and MDA content of grapevine saplings compared to non-stressed groups. This effect was thought to be due to increased oxidative stress associated with reactive oxygen species (ROS) (Fozouni et al. 2012).

5-ALA treatments provided a significant reduction in physical damage, membrane damage and MDA content under salinity stress compared to 5-ALA untreated groups. While all 5-ALA concentrations were found to be effective in reducing physical damage and MDA content under salinity stress, 0.6 and 0.9 mM 5-ALA treatments were found to be the most effective concentrations in reducing membrane damage. In the groups without salinity stress, 0.9 mM 5-ALA application was found to be the most effective concentration in terms of reducing membrane damage. In the present study, it was thought that the effect of 5-ALA applications on the reduction of salinity stress damage may be related to the increase in the amount of cell antioxidants and protection of plasma membranes against free radicals (Nishihara et al., 2003). Indeed, Wongkantrakorn et al. (2009) noted that a decrease in lipid peroxidation (MDA) was observed in NaCl-treated paddy (*Oryza sativa* L.) due to 5-ALA-induced activation of antioxidative enzymes.

In a similar study, Genişel & Erdal (2016) found that 5-ALA applications (10 and 20 $mg.l^{-1}$) significantly increased protein content and SOD, CAT and APX enzyme activities in wheat seedlings against 150 mM NaCl stress and significantly alleviated lipid peroxidation and stress-induced oxidative damage. On the other hand, Manafi et al. (2015) determined that 5-ALA applications at 0.6 mM concentrations applied from seed and leaf against cold stress in soybean (*Glycine max* L. Merr) increased SOD and CAT enzyme activities and proline amounts and reduced membrane damage. Hotta et al. (1998) and Zhang et al. (2006) reported that low 5-ALA concentrations increased cold tolerance in paddy and potato. In the present study, interestingly, all 5-ALA concentrations caused an increase in MDA content in the non-salinity stressed groups. In this increase in MDA content, it was thought that the excessive accumulation or unbalanced distribution of compatible solutes in the cell may have led to increased oxidative stress in cell membranes and triggered lipid peroxidation (Shen et al. 1999, Singh et al. 2015).

Conclusion

This study investigated the effects of exposure of 41 B American grapevine rootstocks to salinity stress and foliar application of different 5-ALA concentrations on morphological, physiological and biochemical traits. Salinity stress caused significant decreases in growth parameters, chlorophyll content and water balance, whereas 0.9 mM 5-ALA treatments resulted in significant increases in plant growth characteristics, chlorophyll content and water holding capacity. Moreover, the improvement in oxidative stress parameters emphasizes the potential of 5-ALA to increase salinity tolerance in plants. These results suggest that 5-ALA may be a promising alternative application for enhancing tolerance to salinity stress in agricultural fields. Among the different concentrations used in this study, 0.9 mM 5-ALA (high concentration) was found to give the best results. Therefore, it is recommended that higher concentrations be tested in future studies on the use of 5-ALA in grapevine.

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