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Synergistic effect of seaweed-derived alginic acid and potassium sulfate fertilizer on the alleviation of salinity stress in Flame Seedless grapevine

Saeid Mansour Jafari
University of Tabriz
Seyed Yahya Salehi Laysar
y_salehi@tabrizu.ac.ir

University of Tabriz
Hassan Maleki Lajayer
University of Mohaghegh Ardabili
Hashem kazemzadeh-Beneh
Shahid Beheshti University
Ali Shahi Garelar
University of Mohaghegh Ardabili

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Abstract

This study was conducted to explore the effect of alginate acid (ALG) and potassium sulfate (PS) to alleviate salinity in Flame Seedless grapevine. Factorial completely randomized experimental design was directed to examine the 24 biostimulants including ALG at 0.1% and 0.2%, PS, and their co-applications with four replications under different levels of salinity (0, 50, 100, and 100 mM NaCl). Duncan's test ($p \leq 0.01$) exhibited that increasing salinity levels negatively decreased plant growth, photosynthetic pigments, carotenoid, stomatal conductance, and soluble sugar in salted grapevines; however, it induced electrolyte leakage and malondialdehyde product in cell membrane. Salted grapevines sprayed with ALG at 0.2% and 0.1%, and ALG 0.2% + PS enhanced plant growth parameters, chlorophylls (a, b, and total), carotenoid, stomatal conductance, and soluble sugar as well as decreased the lipid peroxidation by 7–21 mmol g^{-1} FW compared with their control counterparts, respectively. This proposes that ALG may improve these functionals by antioxidant systems regulation and Na^+/K^+ ions hemostasis. Under high salinity, not only ALG at 0.2% and 0.1% individually maintained a suitable content of phenolic and flavonoid compounds similar to controls, but also synergistically decreased the Na^+ toxicity by boosting K^+/Na^+ rate in leaf when they were co-applied with PS, respectively. Considering with enzymatic antioxidant in controls, ALGs + PS co-applications were more effective in synergistical promoting the superoxide dismutase, guaiacol peroxidase, polyphenol oxidase, and catalase activities (by mean of 60.47, 60.38, 55.97, and 49.70%, respectively) with arising salinity levels. Besides, secondary metabolites enhanced with increasing salinity levels; however, the highest concentration in proline (0.30 mg g^{-1} FW) by ALG 0.1%, viniferin (61.88 μg g^{-1} FW), resveratrol (132.17 μg g^{-1} FW), catechin (49.06 μg g^{-1} FW) was achieved by ALG 0.2% + PS in grapevine. Overall, our results highlighted ALGs and PS co-application as promising practice in mitigating salinity in grapevine cultivation.

Introduction

Grapevine (*Vitis vinifera* L.) is a widely cultivated tree, which offers several significant advantages globally such as economic impact, agriculture versatility, high value crop, product diversification, and health benefits [1]. Antioxidant compounds, aromatic compounds, minerals (magnesium, calcium, and iron) and vitamins (B1, B2, and C), and antimicrobial properties resulting from grapevine plays an important role in human health and the food industries [2]. Grapevine can growth in various climates and soil types, allowing cultivation along many regions worldwide in arid or semi-arid areas where soil salinization is a concern due to low rainfall and high evaporation, from traditional European vineyards to emerging areas in Asia and the Americas [1, 3, 4]. However, cold or high temperature, high light intensity, UV-B radiation, drought and salinity stress are the most important environmental factors that consistently challenge grapevine growth and yield in arid and semi-arid regions [5, 6]. Salinity stress is a major environmental restrictor with significant consequences for agricultural productivity in worldwide [6]. It has already been stated that fruit productivity, berry composition, and wine quality as well as biochemical, morphological and physiological responses in grapevine reduced or altered by salinity stress under climate change [7, 8]. Many studies have previously been highlighted that salinity stress

induces the accumulation of ion toxic in cell (Cl^- and Na^+), restricts photosynthetic pigment content and photosynthesis efficiency, impairing carbon source, modifies gas exchange and stomata conductance, reduce nutrient and water uptake, damages cellular membranes and change membrane integrity, intensify osmotic pressure, accelerates leaf senescence, promotes ROS (reactive oxygen species) generation and oxidative stress in grapevine plant cells [9–13]. Although grapevine cultivars indicate limited sensitivity to soil salinity up to a threshold, after which yield shows as considerable reduction with further increasing in salinity levels [4, 8, 9]. This progress in soil salinity will seriously threaten the potential of the main centers of viticulture to produce quality grapes and wines, especially in combination with climate change. Under this status, implementing the adaptation strategies and approaches such as viticultural practices (selecting grape cultivars with salt tolerance), agrochemicals and bio-stimulants treatments, and biotechnological techniques are vital to mitigate the undesirable effects of salinity stress on grapevine productivity [5].

Towards agriculture sustainability, the high potential application of various bio-stimulants such as plant-growth-promoting rhizobacteria, endophytes microorganisms, plant growth regulators, and natural bio-stimulants has extensively been proceeded to salinity resilience in plants [14–16]. Recently, the application of seaweed based-biostimulants to reduce the negative effects of plant stresses has been interested to as a new scientific [17]. This interest could be associated with a diverse range of bioactive compounds such as minerals, phytohormones, osmo-protectants, lipids, proteins, carbohydrates, amino acids, and antimicrobials in seaweed that directly promotes the plant growth [17, 18]. In this regard, studies have proven that seaweed extracted biomolecules induce a diverse response in plant when they either utilized to soil directly or applied to foliage indirectly [17–19]. Previous investigations have been confirmed that seaweed extractions act a signaling molecules to mitigate the salinity, drought, temperature, and plant disease pathogens stresses, when they absorb into plant cells [20–23]. Generally, alleviating the nutrient deficit, increasing uptake of soil nutrients, maintaining the phytohormone balance, accelerating the osmo-protectant accumulation, stimulating the enzymatic or non-enzymatic systems, reducing oxidative stress, providing the phytohormone balance, and acting as elicitors to induce the salicylic acid, jasmonic acid, and ethylene pathways are the most multiple functions of seaweed based-biostimulants that involved to stress mitigation [14, 17, 20, 24]. Many bioactive compounds can be found in seaweed biomass that have high bioactivities to apply as bio-stimulants treatment. Alginic acid or alginate acid (ALG), is a natural polysaccharide, originates primarily in the cell walls of brown seaweeds (such as *Ascophyllum nodosum*, *Laminaria* spp., and *Sargassum* spp.), where it constitutes up to 40% of the dry weight. ALG naturally exists in the cytoplasm of seaweed cells and plays a crucial role in structural integrity, ion exchange, and flexibility of cell wall against wave action [25]. Due to these critical roles, it has been proposed that ALG may modulate cell wall compositions and membrane fluidity against saline conditions and plant pathogenic agent [26–28]. It has been clarified that the plant cell wall constitutes a principal barrier to salt-induced stress and consequently, the alteration in cell compositions plays a vital role to determine the level of plant responses under salinity stress [29, 30]. Strengthening cell wall integrity by changing in cell wall compositions via the alginate treatment under salinity stress has been reported in rice [28]. Within the plant cell, alginic acid primarily exists as calcium alginate, but it

is also found in combined forms with magnesium, potassium, and sodium elements. The extraction obtained from seaweed typically contains sodium alginate [13]. The promising application of ALG to induce a resistance against salinity and pathogenic stresses has been confirmed in olive trees [27], pear [31], rice seedling [32], and *Brassica campestris* [33]. Therefore, not only ALG from seaweed stablish an important source of potential elicitors in plants but also it has high potential application in supplemented with other agricultural fertilizers. To date, there are not specified research on the application of ALG based-bio-stimulant to mitigate undesirable effects of salinity stress in grapevine cultivars. Therefore, viticultural practices of ALG as a new natural source of elicitors and discovering their effects on salinity stress is a significant approach to enhance plant growth.

Flame Seedless grapevine, as the earliest ripening red seedless cultivars, has been interested in worldwide due to high marketing value, suitable favorite grape for the consumer, good clusters, and high anthocyanin pigment [34]. In the current study, the potential application of ALG based-biostimulant from seaweed to mitigate the undesirable effect of salinity stress in Flame Seedless grapevine cultivar was explored at field conditions. Likewise, ALG treatment in supplemented with potassium sulfate was conducted to investigate their synergetic effect on salinity stress.

Material and Methods

Plant material, salinity, and biostimulant treatments

This work was performed on 3-years-old of rooted-cutting of grapevines (*Vitis vinifera*, L.) cv. Flame Seedless grown in big pots (80×80×65 cm) during one season, 2023–2024. The grapevines cutting were provided from a nursery in MeshkinShahr, Ardabil province, Iran. Grapevine trees of uniform growth and same vigor were planted 5 × 5 m apart under open-air conditions into plastic pots containing an equal amount of sandy soil enriched with animal manure, with a mean pH of 7.5–7.7. Irrigation practice was watered for regular program at every five days until the end of experiment. Irrigation process (every five days) was determined by saturating and weighting pots, drying them in an oven, and reweighting. For applying the salinity stress, pot-grown of Flame Seedless trees were exposed to the four levels of NaCl (0, 50, 100, and 200 mM) after seven months of the plant establishment. Salinity stress was continued for 15 days. After salinity treatment, the foliar applications of ALG (0.1% and 0.2%) and potassium sulfate (K_2SO_4: 0.2%; Merck KGaA, Darmstadt, Germany) were sprayed for 30 days at three steps (every 10 days). Ultimately, the morphophysiological, biochemical, and antioxidants traits were measured at 15 days after of the latest foliar treatments. All steps involved in experiment processes are shown in Fig. 1. The factorial completely randomized design (CRD) as experimental design was directed, where each experimental unit was linked to with a 24 × 4 (96 grapevine trees) factorial combination. Generally, 24 treatments including one control (K_2SO_4 0%/ ALG 0%/ NaCl 0%), three NaCl (50, 100, and 200 mM), two ALG (0.1% and 0.2%), three ALG 0.1% + NaCl, three ALG 0.2% + NaCl, one K_2SO_4 0.2%, three K_2SO_4 + NaCl, one ALG 0.1% + K_2SO_4 0.2%, one ALG 0.2% + K_2SO_4 0.2%, three ALG 0.1% + K_2SO_4 0.2% + NaCl, and

three ALG0.2% + K_2SO_4 0.2% + NaCl with four replication for each treatment was conducted. Tri-Kelp™ as supply of ALG (alginic acid at 0.1% and 0.2%) was provided from Sigma-Aldrich (St. Louis, MO, USA).

Plant growth parameters of grapevine

Seven weeks of after the start of salinity stress, new shoot length (NSL), new stem diameter (NSD), root fresh weight (RFW), root dry weight (RDW), leaf fresh weight (LFW), leaf dry weight (LDW), and leaf area (LA) were measured as growth parameters of grapevine seedlings. For measuring the growth parameters, the target tissues were incubated into oven at 50–60°C for 24 h and weighted with a digital scale (A&D Co. Ltd., Japan) with an accuracy of 0.01 g. Also, four fully developed leaves located at top of each grapevine was measured by a leaf area meter (Delta T Devices Ltd., Burwell and Cambridge, England), and the mean was computed.

Stomatal conductance and potassium (K)/sodium (Na) elements measurements

Stomatal conductance of leaf was evaluated by using a portable porometer (MK model, Delta-T, Cambridge, UK) on sunny day. The measurements were recorded between 10:00 a.m. and 14:00 pm on three leaves (three readings) of each pot comprising five grapevines per each treatment. Results of readings were expressed as μmol m^{-2} s^{-1}. The content level of K^+ and Na^+ were measured by flame photometric according to the method of Havre. [35].

Lipid peroxidation and electrolyte leakage

Malondialdehyde (MDA) content, is a breakdown product of lipid peroxidation, was used as a biochemical marker for assaying the lipid peroxidation rate of plants cell under salinity stress. In this regard, 200 μL of the harvested supernatant from the sample of leave extraction was mixed with 400 μL of 80% methanol following by 600 μL of 20% Trichloroacetic acid (TCA) containing 0.5% 2-thiobarbituric acid (TBA), respectively. Then the obtained solution was heated in a water bath at 99°C for 15 minutes. After that, solution was ice-cooled and then was centrifuged at 13000 rpm for 10 min at 4°C. Absorbance of the obtained solution was recorded at 600 and 540 nm based on colorimetric method [36]. MDA content was calculated using a standard curve prepared by different concentrations (0-100 nmol) of 3,1,1,3-tetraethoxy propane.

Additionally, the electrolyte leakage was distinguished according to the method previously described by Bajji et al. [37]. Fresh leaves were censored into 1 cm^2 pieces and placed in sterile falcons containing 20 mL of the deionized water. The electrolyte leakage assay was performed at two successive steps. For measuring the initial electrical conductivity of medium (EC1), falcons were incubated at 32°C for 2 h in a water bath and consequently, they were autoclaved at 121°C for 20 min to release electrolytes. For measuring the second electrical conductivity (EC2), the sample from prior step was cooled to 25°C.

Thus, relative electrolyte leakage (REL) was calculated as a percentage using the following formula: EL = (EC1/EC2) × 100.

Photosynthetic pigments content

Before harvesting the grapevine fruit, photosynthetic pigments content was examined by assaying the chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll (T Chl.) and carotenoids (Car.) contents were examined by methods described by Lichtenthaler. [38]. For this goal, 100 mg of fresh leaves samples was homogenized using 1 mL of 80% acetone and centrifuged at 8000 rpm for 15 min. Then, 100 µL of the harvested supernatant was transferred to tubes containing 900 µL of 80% acetone. Absorbance of the obtained solution was measured by using spectrophotometry method at 645 nm, 663 nm, and 470 nm (Aλ = absorbance at λ (nm)), respectively. Ultimately, the content of each target pigment resulted by using following equations: 1) Chl. a = 12.25 (A663) − 2.79 (A645); 2) Chl. b = 21.50 (A645) − 5.10 (A663); 3) Chl. a + Chl. b = 7.15 (A663) + 18.71 (A645); 4) Car. = 1000 (A4700 − 1.82 Chl. a − 85.02 Ch. b/198.

Total phenolic compound (TPC), total flavonoid compound (TFC) and, total soluble sugar (TSS)

To assay the TPC content, 50–100 mg of the crushed dry leaf tissue was homogenized with 1–3 mL of 80% methanol (v/v) and incubated at room temperature for overnight. Subsequently, each incubated sample was centrifuged at 13000 rpm for 15 min at 4°C to attain supernatants. 50 µL of the supernatant was mixed with 1.45 mL of distilled water. TFC was determined colorimetrically by applying sodium carbonate according to Folin-Ciocalteu method [39]. The color reaction was performed by adding 100 µL of Folin-Ciocalteu reagent and 350 µL of sodium carbonate to each sample. The absorbance of resulting mixture was determined spectrophotometrically at a wavelength of 765 nm after keeping them in dark condition for 90 min. TPC content expressed as mg gallic acid equivalents per gram of dry weight (mg gallic acid g^{-1} DW). Additionally, to assay TFC content, 50 µL of the extracted leaf tissue in 250 µL of 80% methanol was suspended in 440 µL of distilled water. The resulting mixture was subjected to 30 µL of 5% sodium nitrate and incubated on shaker at 90 rpm for 6 min. The reaction solution was provided to reading their absorbance when 200 µL of 1 M sodium hydroxide was added. Finally, the absorbance of reaction solution was measured at 510 nm against blank serving as reference [40]. Result for TFC content was expressed as mg catechin equivalents per g dry weight of grapevine leaf (mg catechin g^{-1} DW). Likewise, TSS of leaf was determined by colorimetric method based on protocol described previously by DuBois et al. [41].

Enzymatic antioxidant system

For assaying of the enzymatic activities, the crude enzyme extraction was attained by homogenizing the frozen-leaf tissue in 10 mL of 50 mM potassium phosphate buffer (pH 7.0) supplemented with 1 mM EDTA and 1% polyvinylpyrrolidone (PVP) (w/v). Then, homogenized sample was harvested by

centrifuging at 15000 rpm for 20 min at 4°C and thus, the supernatant was immediately considered as the crude enzyme extraction for all enzymatic assays.

For the assay of catalase (CAT) activity, firstly, 50 µL of crude enzyme extraction was added into 3 mL of reaction mixture supplemented with 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2 to start the target reaction for CAT activity. Secondly, the decrease in absorbance of reaction due to H_2O_2 decomposition was monitored spectrophotometry at 240 nm for 1 min at 25°C [42]. One unit (U) of CAT activity was defined as the amount of enzyme that decomposes 1 µmol H_2O_2 per min. result of CAT activity was expressed as U mg^{-1} protein (ε = 43.6 M^{-1} cm^{-1}).

For the assay of polyphenol oxidase (PPO) activity, firstly, 50 µL of crude enzyme extraction was added to 2 mL of reaction mixture supplemented with 125 µmol phosphate buffer (pH 6.8) and 50 µmol pyrogallol to start the target reaction for PPO activity. Secondly, after the incubation at 25°C for 5 min, the absorbance of purpurogallin formation was quantified at 420 nm through adding 0.5 mL of 5% (v/v) H_2SO_4 to stop the reaction [43]. One unit (U) of PPO activity was defined as the amount of enzyme causing an increase of 0.1 in absorbance per minute. PPO activity was expressed as U mg^{-1} protein.

Superoxide dismutase (SOD) activity was measured based on NBT (nitroblue tetrazolium) photoreduction method [44]. Firstly, 100 µL of crude enzyme extraction into 3 mL of reaction mixture supplemented with 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 12 mM methionine, 75 µM NBT (v/v), 0.025% Triton X-100, 100 µL enzyme extract, and 10 µL of 2 µM riboflavin. The beginning of reaction was started with riboflavin through illuminating under white light fluorescent at 350–400 µmol photons m^{-1} s^{-1} for 20 min at 25°C. Secondly, the absorbance of these fluorescent was recorded at 560 nm. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction. SOD activity was expressed as U mg^{-1} protein.

Guaiacol peroxidase (GPX) activity was assayed according to Hammerschmidt et al. [45] with some modifications. Firstly, 70 µL of crude enzyme extraction was introduced into 3 mL of reaction mixture supplemented with 50 mM potassium phosphate buffer (pH 5.2), 100 mM guaiacol, and 30 mM H_2O_2. Secondly, the assay was started by adding the enzyme crude extraction and subsequently, guaiacol oxidation was monitored by the increase in absorbance at 470 nm for 160 seconds (≈ 2.67 min) at 25°C, respectively. One unit (U) of GP activity was defined as the amount of enzyme that oxidizes 1 µmol guaiacol per minute. GA activity was expressed as U mg^{-1} protein (ε for tetra-guaiacol = 26.6 mM^{-1} cm^{-1}).

Free proline content

Free proline content was determined according to the method of Bates et al. [46] with minor modifications for scaling. Briefly, 0.5 g of grapevine leaf tissue was powdered by liquid nitrogen and homogenized with 5 mL of 3% (w/v) sulfosalicylic acid. The homogenized solution was centrifuged at

13000 rpm for 10 min at 25°C for subjecting to acidic ninhydrin reagent. Freshly acidic ninhydrin reagent was prepared by gentle-warm dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid to attain the fully dissolved (2.5% (w/v) ninhydrin final concentration). Afterward, 500 µL of the harvested supernatant was mixed with 500 µL of acid-ninhydrin reagent and 500 µL of glacial acetic acid. The mixture solution was incubated in a boiling water bath (100°C) for 1 h followed by immediate cooling in an ice bath for 10 min. After phase separation, 1 mL of toluene was added to the reaction mixture, vortexed vigorously for 2 min. Ultimately, proline content of grapevine leaf was measured by reading the absorbance of the upper (organic) phase at 520 nm. Proline concentration was quantified using a standard curve prepared with known concentrations of L-proline and expressed as mg proline g^{-1} FW).

HPLC chromatography analysis for stilbenes secondary metabolites characterization

Extraction of secondary metabolites components: grapevine leaf was homogenized in 10 mL of 90% ethanol. Then, the supernatant was three-times harvested by centrifuging at 12000 rpm for 15 min at 4°C and subsequently, the extraction was filtered using a 0.45 mm injection filter to expose on HPLC (high phase liquid chromatography). HPLC (Unicam-Cristal-200 model, USA) with UV-Diod-arrays detector was used to quantify the concentrations of delta-viniferin, trans-resveratrol, and catechin based on methods previously explained by Anna. [47] and Gabler et al. [48], respectively. For determining the concentrations of delta-viniferin and trans-resveratrol, 50 µL of extraction solution was injected into a ODS2 column (250 mm× 4.6 mm, 5 µm) connected with guard column. The acetonitrile/formic acid (50:50) as mobile phase, flow rate of 0.5 mL min^{-1}, and column temperature at 25°C were adjusted as the chromatographic conditions. For determining the concentrations of catechin, 25 µL of extraction solution was injected into a Phenomenex-P/NO 006-4097-Eo column (250 mm× 4.6 mm, 5 µm) connected with Phenomenex-P/NO 03A-4097-Eo guard column. The acetonitrile/glacial acetic acid (98:2) as mobile phase, flow rate of 1 mL min^{-1}, and column temperature at 25°C were adjusted as the chromatographic conditions.

Statistical analysis

In this study, a factorial completely randomized design (CRD) with two main factors including salinity stress (with three levels), ALG (with two levels), and K_2SO_4 (with two levels) was conducted with four replications for each level. The normal distribution of variables was checked by Kolmogorov-Smirnov test. Two-way analysis of variance (ANOVA) was computed to detect the statistical differences in means between the effect of different salinity and biostimulants treatments and their interaction with the together on the measured traits. Data were analyzed using SAS and SPSS (version 22) software's. Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.01$ was performed to compare the means of measured traits. Principal component analysis (PCA) was conducted to investigate the pattern of relationship among traits by XLSTAT statistical software.

Results and discussion

Morphological growth parameters of grapevine under salt stress

As expected, the salinity stress induced a significant reduction in the growth parameters of Flame Seedless cultivar compared to control plants (Table 1). The negative effects of salinity stress on growth parameters of grapevine have previously been confirmed by many studies [8,9,12,15,17]. It was observed that the decrease in NSL, NSD, LFW, LDW, RFW, RDW, and LA under salinity stress mitigated by foliar single or co-application of ALG (at 0.2% and 0.1%) and PS treatments. Duncan's test (at $p \leq 0.01$) showed that foliar application of ALG and PS positively impacted grapevine growth parameters specially LA, leading to induction in pigment concentrations, chlorophyll content, photosynthesis rate, and growth. In this regard, the ALG 0.2% + PS and ALG 0.1% introduced as superior biostimulant treatments to mitigate salinity stress or improve the grapevine growth, respectively. Indeed, salinity stress induces undesirable challenges for grapevines such as ion hemostasis, ion toxicity, oxidative stress, nutrient reduction, water uptake reduction and Na^+/k^+ misbalancing [8,17,20,24]. Under this status, the positive effect of ALG and PS treatments on grapevine growth can be attributed to modulate the undesirable challenges by target treatments in help to improving the grapevine response against salinity stress.

Stomatal conductance, Na^+ and K^+ homeostasis, electrolyte leakage, and lipid peroxidation in grapevine leaves under salt stress

Primary result proved that Flame Seedless grapevine showed not only any reduction in stomatal conductance of leaf, but also the increase in stomatal conductance occurred when the level of salinity stress was intensified up to 100 mM NaCl (Fig. 2a). This indicates the moderate resistance of target cultivar against salinity stress according to Duncan's test at $p \leq 0.01$. Promising results associated with recent findings of studies on moderately sensitive to salt-resistance of grapevine cultivars confirm this aspect [8,12,15,49]. But the grapevine seedlings experienced a remarkable reduction in stomatal conductance under severe salinity stress with increasing the NaCl concentration up to 200 mM.

Table 1. The evaluation of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on morphological growth parameters in Flame Seedless grapevine under salinity stress.

Biostiumulants	NaCl (mM)	Plant growth parameters						
		NSL (cm)	NSD (mm)	LFW (mg)	LDW (mg)	RFW (mg)	RDW (mg)	LA (cm^2)
Non and salt controls	0	30 cde	0.6 bcd	1550.25 ef	581.24 de	1202.14 c	390.89 ef	101.23 de
	50	22.34 hi	0.62 abc	1423.14 g	561.35 ef	1201.35 c	400.24 e	98.56 ef
	100	17.27 jk	0.52 de	1378.91 g	487.26 f	110.97 d	395.19 ef	88.78 g
	200	13.78 l	0.44 e	1207.65 h	431.91 g	1059.45 d	351.84 g	75.68 h
PS	0	29.2 cde	0.59 bcd	1580.21 d-f	602.31 de	1242.36 c	402.63 e	103.41 de
	50	21.44 h-j	0.64 ab	1565.17 d-f	601.24 de	1235.69 c	405.95 e	100.25 ef
	100	19.56 i-k	0.54 d	1517.89 f	591.14 de	1237.14 c	400.57 e	99.87 ef
	200	14.89 kl	0.51 de	1489.08 f	521.42 ef	1217.53 c	381.99 f	94.36 fg
ALG 0.1%	0	34.21 ab	0.68 a	1674.52 bc	645.89 cd	1291.48 b	485.23 bc	109.35 bcd
	50	31.55 bcd	0.67 a	1643.78 bcd	638.24 cd	1301.86 ab	470.12 c	104.89 cd
	100	29.14 cde	0.58 bcd	1598.64 cde	612.75 de	1284.12 b	421.57 d	103.12 de
	200	24.66 gh	0.55 d	1560.53 def	603.49 de	1239.24 c	419.34 d	101.92 de
ALG 0.1% + PS	0	33.41 bc	0.63 ab	1568.09 def	655.47 cd	1275.61 b	467.55 c	109.21 bcd
	50	30.19 cde	0.55 d	1576.12 def	638.15 cd	1236.43 c	455.73 c	108.54 bcd
	100	28.02 d-f	0.52 de	1542.39 ef	633.81 cd	1241.99 c	419.28 d	102.3 de
	200	25.36 f-g	0.58 bcd	1562.49 def	627.56 cd	1240.02 c	426.93 d	99.47 ef
ALG 0.2%	0	36.88 a	0.72 a	1769.68 ab	741.67 a	1349.23 a	524.37 b	111.23 bc
	50	35.03 ab	0.68 a	1724.07 b	702.16 bc	1320.16 ab	519.25 b	111.02 bc
	100	32.69 bc	0.62 abc	1627.55 cd	685.43 bc	1301.47 b	481.09 bc	110.98 bc
	200	27.14 e-f	0.63 ab	1599.14 cde	643.21 cd	1288.27 b	472.33 c	105.32 cd
ALG 0.2% + PS	0	37.01 a	0.57 cd	1758.97 b	702.28 bc	1366.32 a	578.65 a	117.75 a
	50	34.78 ab	0.61 abc	1821.72 a	721.03 ab	1355.29 a	564.22 a	118.42 a
	100	32.48 bc	0.63 ab	1670.28 bc	690.33 bc	1344.45 a	515.62 b	112.36 b
	200	29.66 cde	0.57 cd	1688.77 bc	679.67 bc	1302.39 b	489.67 bc	104.23 cd

ALG: alginic acid; PS: potassium sulfate treatments. Columns with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

Thus, stomatal conductance decreased from 105 µmol m^{-2} s^{-1} to 50 µmol m^{-2} s^{-1}. Under these status, individual or integrated foliar applications of ALG and PS alleviated the negative effect of high salinity stress on the reduction of stomatal conductance, enhancing conductance by 10-15 µmol m^{-2} s^{-1} in high saline-treated grapevines (Fig. 2a). It inferred that the effeteness of foliar application on mitigating the negative effect of salinity stress on stomatal conductance was the level of salinity stress-, biostimulants integration-, and dose-dependents. Under non-saline conditions and increasing the level of salinity stress up to 100 mM NaCl, the individual and integrated application of ALG and PS treatments did not induce positive function on enhancing or mitigating the salinity stress on the reduction of stomatal conductance. Therefore, the positive effects of biostimulants application on maintaining or promoting the stomatal conductance was detected under high salinity stress (200 mM NaCl). Under high salinity, PS improved stomatal conductance, while any improvement was not induced by ALG application at 0.1% and 0.2%, even at integrated with PS. Besides, the synergistic effect of ALG 0.2% + PS showed lowest reduction in stomatal conductance under high salinity, maintaining levels at 60-70 µmol m^{-2} s^{-1} compared to saline controls. This supports the importance of biostimulants formulization to mitigate the

undesirable effects of high salinity stress. Notably, the reduction of stomatal conductance is attributed to leaf damage, leaf anatomical or morphological changing, closing stomatal, and the chlorosis emergence due to increasing Na^+ and lower K^+/Na^+ ratio in leaf of grapevines in response to high salinity stress [50,51]. The outcome results of K and Na measurements stated this aspect. The salt-treated grapevines indicated a drastic lowered K^+/Na^+ ratio and higher Na^+ as well as lower K^+ content with intensifying the salinity stress, compared to non-salt control (Fig. 3). The lowest K^+ (Fig. 3a) and K^+/Na^+ ratio (Fig. 3c) following by high Na^+ (Fig. 3b) was obtained by 200 mM NaCl treated grapevines. The undesirable status of K^+ and Na^+ cations due to salinity stress modulated by PS and ALG applications. In this regard, the performance of individual application of ALGs was higher than PS. In spite of the relative effect of PS, the performance of PS was increased synergistically when co-applied with ALGs. The higher K^+ and lower Na^+ following by higher K^+/Na^+ ratio was achieved to ALG 0.2% and ALG 0.2% + PS, respectively. However, the positive effects of ALG on Na^+ and K^+ homeostasis in grapevine are rarely stated. The Na^+ and K^+ ion homeostasis, water uptake, the degradation of cell wall, the fluidity or integrity modification of membrane, and membrane dysfunction are significant challenges for grapevine that poses by high salinity [49,52]. As it is predictable that electrolyte leakage indicated a stable membrane permeability relative to control, but electrolyte leakage promoted significantly with increasing the level of salinity stress up to 200 mM NaCl (Fig. 2b). Electrolyte leakage varied by 40.67%, 70.92%, and 82.13% at 50, 100, and 200 mM NaCl versus control, respectively. Result clearly exhibited that ALG and PS application was able to decrease the electrolyte leakage in saline control grapevines. The measured electrolyte leakage difference highlighted the effectiveness of foliar application of biostimulants, indicating enhanced membrane stability with significantly lower electrolyte leakage value ($p \leq 0.01$), especially at 100 and 200 mM NaCl concentrations, in saline control grapevines. Assessing the effectiveness of applied biostimulants, ALG 0.1%, PS, and ALG 0.2% + PS treatments showed a similar function at each specific level of salinity stress on decreasing the secretion of electrolyte leakage with lowest value compared to other foliar application. In contrast, ALG 0.2% and ALG 0.1% + PS indicated approximately a similar function at high salinity stress on preventing the secretion of electrolyte leakage in leaf grapevines. This signifies the non-synergistic effect of PS on maintaining the membrane stability when it was co-applied with two level of ALG biostimulant.

Overall, the highest membrane permeability in saline control grapevines can be associated with the occurrence of lipid peroxidation, leading to incrassation in the rates of MDA product [49-51]. Similar to electrolyte leakage, the content of MDA product indicated a stable lipid peroxidation relative to control, but MDA product encouraged significantly with increasing the level of salinity stress up to 200 mM NaCl, with the highest level by 41.59 μmol g^{-1} FW (Fig. 2c). Result revealed that not only ALG application prevented the MDA production in saline control grapevines but also it decreased the MDA production even at high salinity compared to control ($p \leq 0.01$). The MDA content decreased in saline control grapevines treated with target biostimulants with increasing the level of salinity stress, however; the lowest MDA content was obtained for ALG 0.1% (19.26 μmol g^{-1} FW) and ALG 0.2% (19.74 μmol g^{-1} FW) under high salinity stress, respectively. These reductions indicate that the ALG treatments mitigate lipid

peroxidation and oxidative membrane damage under high salinity stress in grapevine. In contrast, the PS application both in individual and integrated with ALG did not induce a significant positive effect on preventing the MDA production in salt-treated grapevines. This emphasized that the indiscriminate application of fertilizers such as PS can be intensifying the rate of salinity stress in grapevine, as has previously been mentioned [49,53]. Strangely, the many studies have been proven that the weakening of stomatal conductance is directly related to the imbalance of Na^+ and K^+ cations in plant cells under high salinity stress [50,54]. Therefore, result conformed that PS and ALG 0.2% + PS applications showed a superior performance on maintaining a workable stomatal conductance and K^+/Na^+ ratio under high salinity stress, suggesting the stress alleviation rely on biostimulants type- and dose-dependents. Possibly, the cation K^+ derived from PS and ALG-based seaweed supplies provide the high K^+ levels and consequently help to establish the threshold of K^+ /Na^+ ratio, prevent the ion toxicity, maintain the cell water status, keep suitable stomatal conductance, preserve the cell membrane permeability, and mitigate the lipid peroxidation under saline condition in grapevine [50,52,55-57]. Similarly, the preservation in stomatal conductance and CO_2 assimilation have been stated with ALG in *Artemisia annua* [58], *Brassica campestris* [33], and *Catharanthus roseus* [59]. Besides, potassium sulfate application in sunflower [60] and wheat [61] has been led to enhance the stomatal conductance by improving K^+/Na^+ ratios under salt stress. Furthermore, the ALG (0.2% and 0.1 %), PS, and their co-application mitigated remarkably the undesirable progressed of electrolyte leakage and lipid peroxidation owing to high MDA content in grapevines, which aligns with previous works in rice [28,32], maize [62], and cucumber [63]. These results The concentration-dependent responses highlighted the optimal application of lower ALG 0.1% for non-saline conditions to boost stomatal conductance, and higher ALG 0.2% with PS for severe salinity to minimize lipid peroxidation, supporting co-friendly biostimulant applications in viticulture. The maintained stomatal conductance and lipid peroxidation adaptations are vital for supporting photosynthetic efficiency and preserving cell membrane stability in grapevine under saline conditions.

Table 2. The evaluation of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on photosynthesis pigments, carotenoid and total soluble sugar in Flame Seedless grapevine under salinity stress.

Biostiumulants	NaCl (mM)	Chl. a	Chl. b	T Chl.	Carotenoids	TSS (mg g^{-1} FW)
Non and salt controls	0	6.637 b	7.818 b	11.79 c	2.08 c	0.75 ab
	50	8.616 a	9.505 a	13.65 b	2.57 b	0.78 ab
	100	5.874 c	6.04 c	10.03 d	2.41 bc	0.68 b
	200	4.637 d	4.038 d	8.23 e	2.04 c	0.49 c
PS	0	6.911 b	7.929 b	13.07 b	2.11 c	0.78 ab
	50	7.219 b	9.078 a	16.01 a	2.75 ab	0.69 b
	100	7.096 b	5.473 c	13.29 b	2.64 ab	0.69 b
	200	6.542 bc	4.376 d	12.16 c	2.54 b	0.58 c
ALG0.1%	0	5.494 c	4.640 d	12.02 c	2.60 b	0.79 ab
	50	10.525 a	6.178 c	18.25 a	3.03 a	0.70 b
	100	7.230 b	6.691 c	11.68 c	2.91 a	0.72 b
	200	7.252 b	7.829 b	14.38 ab	2.48 bc	0.68 b
ALG0.1% + PS	0	6.321 bc	8.407 ab	12.16 c	2.43 bc	0.80 ab
	50	8.929 a	9.274 a	16.01 a	2.62 b	0.76 ab
	100	8.287 ab	4.20 d	13.20 b	3.01 a	0.74 ab
	200	7.988 ab	6.425 c	12.43 c	2.50 bc	0.57 c
ALG0.2%	0	8.171 ab	9.158 a	14.59 ab	2.25 c	0.84 a
	50	6.781 b	6.311 c	12.07 c	2.03 c	0.70 b
	100	4.695 d	5.448 c	9.48 d	2.31 bc	0.74 ab
	200	5.091 cd	5.280 c	9.34 d	2.80 ab	0.57 c
ALG0.2% + PS	0	6.495 bc	3.684 d	12.55 c	2.59 b	0.82 ab
	50	5.874 c	6.274 c	10.20 d	2.74 ab	0.77 ab
	100	8.042 ab	4.111 d	9.50 d	2.64 ab	0.75 ab
	200	7.353 b	3.223 d	9.43 d	2.35 bc	0.68 b

TSS: total soluble sugar; ALG: alginic acid; PS: potassium sulfate. Columns with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

Salinity stress decreased the photosynthetic pigments and total soluble sugar accumulation in grapevines

Injury to chloroplast structure under salt stress is key factor to inhibit the grapevine photosynthesis due to decreasing in the content of photosynthetic pigments [50]. In non-saline controls, the chlorophyll pigments with adequate content were supported orderly the photosynthesis process (Table 2). While the chlorophyll pigments including Chl. a, Chl. b, and T Chl. in salted grapevine influenced adversely by boosting the salinity levels up to 200 mM NaCl, which is a sign of the photosynthesis inhibition. Results showed that the content of photosynthetic pigments in grapevine affected significantly by the salinity stress higher than 50 mM NaCl compared to non-saline control (at $p \leq 0.01$). The higher content of photosynthetic pigments at 50 mM NaCl concentration confirms this trend as well as being the moderate resistance of grapevine against salinity stress. Nevertheless, when ALG 0.1% + PS, ALG 0.1% and ALG 0.2% were foliar applied, respectively, the chlorophyll pigments of the salted grapevine revealed remarkable enhancement compared saline control plants. The results exhibited that after seven weeks of salinity stress, salted grapevines treated with PS, ALG 0.1%, ALG 0.1% + PS, and ALG 0.2% displayed

8.06%, 36.94%, 25.66%, and 18.77% increases in Chl. a, respectively, when compared to the non-saline control that did not apply by the target biostimulants (Table 2). Furthermore, ALG 0.1% and PS promoted T Chl. content by 17.97% and 11.21% at higher salinity compared to non-saline control, respectively. Although the synergistic effect of ALG and PS was no effective on Chl. b and other chlorophylls under high salinity, ALG 0.1% did prevent significantly the chlorophyll b degradation compared to non-saline control. At two levels of salinity (100 mM and 200 mM NaCl), the carotenoid content in grapevines sprayed with ALG 0.1%, ALG 0.2%, and PS enhanced by 30.94%, 28.59%, and 21.15%, respectively, compared to non-saline control.

Together, in this study, the salinity-induced reduction in stomatal conductance, promotion in Na^+ ion toxicity, enhancement in electrolyte leakage, and lipid peroxidation in Flame Seedless grapevine could be conceivably align with ROS (reactive oxygen species)-mediated membrane disruption and photosynthetic inhibition. As it has previously been proven in grapevine under stressful conditions [49-51,57]. The plant growth performance supplies by high photosynthesis efficiency under challenging conditions, which is dependent strongly on chlorophyll concentration in leaves [43,51]. In spite of the moderate resistance of target grapevine against salinity, the higher levels of salinity stress induced considerable reduction in chlorophylls contents compared to control; however, there was a stable or remarkable increase in chlorophyll concentrations when the salted grapevines treated with ALG 0.1, ALG 0.2, and PS, respectively. This exhibits the high efficiency of these biostimulants in preventing chlorophyll degradation and recovery of the plant vigor to resistant under salinity stress. These findings support previous studies on the potential bioactivity of ALG biostimulant to promote the plant chlorophyll content and photosynthesis efficiency under stressful conditions [19,20,63]. Our results proposed that ALG 0.1% is more effective than ALG 0.2% and PS biostimulants in preventing the chlorophylls degradation or enhancing their concentration in grapevines under higher salinity stress. Likelihood, the superior bioactivity of ALG 0.1% can be attribute to the higher its impact on the carotenoid accumulation, as shown in Table 2. It has been well-documented that carotenoid eliminates the generated ROS products resulted of the oxidative stress in PSII activity of the photosynthesis due to high antioxidant properties and consequently, it prevents the chlorophyll degradation via pigment photooxidation process [64]. However, to keep the appreciate photosynthesis capacity, the high maintenance of antioxidant activity in plant leaves is very vital for coping the oxidative stress and maintaining the suitable level of carbohydrate production under stressful conditions [20,65]. Result revealed that soluble sugar content decreased with increasing salinity levels. Contrary to our results, several studies found that soluble sugar increased in grapevine cultivars such as Thompson seedless and Sultana under salinity stress, which can be attributed to the integration among genetic background of Flame Seedless cultivar and environmental conditions [52,57,66]. Thus, at each level of salinity stress, ALG 0.2% + PS (ranged from 0.823 to 0.682 mg g^{-1} FW), ALG 0.1% (ranged from 0.789 to 0.678 mg g^{-1} FW), and PS (ranged from 0.782 to 0.581 mg g^{-1} FW) biostimulants induced higher soluble sugar accumulation in the leaves of grapevine, respectively, compared to their non and saline controls (ranged from 0.750 to 0.485 mg g^{-1} FW) (Table 2). Indeed, the intensity of photosynthesis affected directly by carbohydrate content in the plant body. The accumulation of soluble sugar, as a main form of carbohydrate metabolism and storage,

is monitored by leaf photosynthesis and linked enzymes [20]. However, maintaining higher total soluble sugar content is crucial for plant performance and photosynthesis efficiency under salinity stress, which reduces the undesirable effects of stressful condition through preserving the protein degradation, playing as a ROS scavenger signaling molecules, and maintaining the cell turgor [67]. In our result, enhancement in photosynthetic pigments also implied an increase in the accumulation and maintenance of appreciate soluble sugar in leaves of the salted grapevine under target biostimulants application. Grapevines treated by ALG 0.2% + PS acted as a superior biostimulant for maintaining the soluble sugar during salinity stress. Consequently, the promotion in soluble sugar content and photosynthesis capacity through ALG application under stressful condition has previously been stated in citrus [20], rice [32,68], and artemisia [58].

ALG and PS boosted the phenolic, flavonoid, and proline compounds in leaves of the salted grapevine

In response to plant salinity stress, the accumulation of osmo-protectant such as proline and trehalose, phenolic, flavonoid, and antioxidant enzymes activity stimulates in grapevine leaves to play a vigorous role as plant defense metabolites that can scavenge ROS, stabilizing membranes integrity, and monitoring cell redox potential [52,53,57,66]. Similar to total soluble sugar, accelerating in TPC, TFC, and proline contents in salted grapevines occurred at moderate level of salinity up to 50 mM NaCl, whereas their contents descended significantly at salinity levels higher than 50 mM NaCl (Fig. 4). This reduction could be associated with a high applied rate of the accumulated TPC, TFC, and proline contents in host cells to scavenge the highly generated ROS products and avoid dehydration severe salinity-induced indemnities. Primary result confirmed that the effeteness of the ALGs and PS application on TPC and TFC contents was levels of the salinity stress- and dose-dependents. Although ALGs and PS resulted in induce a more effect on accelerating the TPC and TFC accumulation at higher level of salinity, only ALG 0.1% was able to maintain the level of TPC (14.44 mg gallic acid g^{-1} DW) and TFC (2.71 mg catechin g^{-1} DW) similar to non-saline control (TPC=13.99 mg gallic acid g^{-1} DW; TFC=2.70 mg catechin g^{-1} DW at $p \leq 0.01$) under sever salinity (Fig. 4a and b). Likewise, it was observed that ALGs and PS application had no effect on TPC and TFC content in normal grapevines, whereas ALGs and PS application had a significant effect on increasing the proline accumulation in both normal and salted grapevines (Fig. 4c).

At 0 mM NaCl, ALGs and PS treatments induced same increase in proline accumulation in normal controls but their induced different effects on proline accumulation in salted grapevines rely on salinity levels. Signifying the critical role of these ALGs and PS treatments in maintaining the highly non-enzymatic antioxidant system against salinity stress. At 100 mM NaCl, content of proline in salted grapevines sprayed with ALG 0.2% promoted by 35.36% and at 200 mM NaCl, the promotion was 28.42% and 20.93% for ALG 0.1% and PS, respectively (Fig. c). The enhancement in proline content under higher NaCl concentrations confirms the preventive role of ALGs and PS biostimulants in alleviating the effects of salinity stress in grapevine. Therefore, the highest increase in proline concentration was attained by

ALG 0.2% (0.526 mg g^{-1} FW) at 100 mM NaCl, ALG 0.1% (0.475 mg g^{-1} FW), and PS (0.430 mg g^{-1} FW) at 200 mM NaCl, respectively. The accelerating the proline concentration by ALG biostimulant to mitigating unfavorable effects of plant stress is in accordance with several studies [58,69,70]. Consideration with the promotive effect of ALG 0.1% on proline accumulation under high salinity stress, the target biostimulant maintained the TPC and TFC concentration as well as non-saline grapevine to mitigate the high salinity stress. Phenols and flavonoids are key components of the non-enzymatic antioxidant system that have high potential to neutralize free radicals and scavenge the ROS products of oxidative stress [71-73]. Our results showed that ALG at 0.1% and 0.2% played a vital role in mitigating high salinity stress through preventing the TPC and TFC concentration following by accelerating in the proline biosynthesis in grapevine to inhibit the lipid peroxidation and ROS-oxidative degradation, as shown in Fig. 2. In align with these results, Bouissil et al. [26] reported that the alginate application, as an elicitor, induces the natural defence system in data palm roots through enhancing the phenolic compound with antiviral activity. According to our results, studies have previously been announced that the promotion of proline, phenolic, and flavonoid concentrations through biostimulant applications in grapevine cultivars inhibits practically the unfavorable effects of abiotic challenges such as salinity and water stresses [66,74-77].

ALG and PS boosted the activates of antioxidant enzymes in leaves of the salted grapevine

As shown in Fig. 5, enzymatic antioxidant activities including SOD, CAT, GPX, and PPO in salted grapevine leaf increased powerfully by escalating salinity levels. Besides, when ALG and PS biostimulants were applied, the power of antioxidant activities in target enzymes boosted during salinity stress, specially at higher salinity levels. In both the absence and presence of the salinity stress, ALG 0.1% + PS and ALG 0.2% + PS treatments promoted synergistically the activities of antioxidant enzymes in grapevine leaves; however, co-applications of ALGs and PS increased superiorly the SOD, CAT, GPX, and PPO activities with arising the salinity levels up to 200 mM NaCl compared to non and saline controls, respectively (at $p \leq 0.01$). There were corresponding percent intensifications of 60.71% and 60.24% in SOD activity for ALG 0.2% + PS and ALG 0.1% + PS, respectively, compared to non-saline control (Fig. 5a). In this regard, ALG 0.2% + PS and ALG 0.1% + PS shared a same response to increase the CAT activity (49.79%) in grapevine leave under high salinity level (Fig. 5b). Compared to non-saline controls, ALG 0.2% + PS and ALG 0.1% + PS applications promoted significantly the GPX activity by 60.93% and 59.83% and PPO activity by 55.30% and 56.65%, respectively, in the grapevine leaves. Overall, considering with the superior effect of ALGs and PS co-applications, the activities of target four enzymes were partially or not significantly affected by the individual application of ALG (at 0.1% and 0.2%) and PS. This aspect demonstrates that ALG and PS co-amplify the salinity-induced upregulation of antioxidant enzymes.

Generally, the obtained results emphasized that plant growth and performance relied on the maintenance of non-enzymatic and enzymatic antioxidant systems and preserve metabolic balance in salted grapevine leaves to alleviate the ROS-mediated oxidative and lipid peroxidation. The moderate

salt-resistance of Flame Seedless grapevine was also confirmed by the highly preservation of SOD, CAT, GPX, and PPO activities in leaf during salinity stress. ALGs and PS co-applications induced the power of salinity resistance in grapevine by stimulating the activities of target antioxidant enzymes, which in turn promote grapevine resistance to lipid peroxidation and oxidative stress. This is a strengthened enzymatic defense system by target biostimulants application in grapevine. Salinity stress triggers excessive ROS production, causing oxidative damage that plants counteract through upregulation of antioxidant enzymes such as SOD, CAT, PPO, and GPX [78]. SOD serves as the first line of defense by converting superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2), which is subsequently detoxified by CAT and GPX into water and oxygen, thereby preventing cellular damage [79]. PPO contributes to ROS scavenging and lignin formation for cell wall reinforcement, while GPX plays a dual role in H_2O_2 removal and cell wall cross-linking [80]. It has been stated that alginate functions as a signaling molecule that upregulates gene expression of antioxidant enzymes, boosts overall antioxidant capacity, and reduces oxidative injury in various crops [17,20,81]. Likewise, potassium sulfate supports these effects by acting as a cofactor in enzymatic reactions, improving K^+/Na^+ homeostasis, improving osmotic adjustment, and stress signaling pathways [82,83]. The synergistic interaction between ALGs and PS results in a superior enzymatic antioxidant activation compared to individual treatments, providing integrated protection against ROS accumulation and oxidative damage due to lipid peroxidation. According to our results, the powerfully role of ALG and PS application in the induction of resistance or alleviation of unfavorable effects of stressful conditions due to activating the antioxidant enzymes has been stated in several studies [14,50,68].

Biosynthesis of the stilbene's secondary metabolites under salinity stress in grapevine

Stilbenes phytoalexins, including resveratrol, catechin, delta-viniferin, alpha-viniferin, trans-pterostilbene, trans- and cis-piceid, trans- and cis-astringin, and trans- and cis-resveratrol-oside, are the most secondary metabolites that induced by biotic or abiotic stresses as a part of grapevine defense mechanism [84]. HPLC chromatography analysis exhibited that the concentration of trans-resveratrol and catechin increased with arising salinity levels, while the concentration of delta-viniferin decreased significantly (at $p \leq 0.01$) in salted grapevines compared to controls (Fig. 6). According to our findings in Syrah cultivar and American wild grapevines, it has reported that salinity stress led to increase the resveratrol and its derivatives [85,86]. In the absence of salinity stress, ALGs (at 0.1% and 0.2%), PS, ant their co-applications induced a positive impact on promoting the biosynthesis of trans-resveratrol, delta-viniferin, and catechin in grapevine leaves compared to control. However, the power of treatments on promoting the biosynthesis of target stilbene metabolites was significantly intensified in the presence of salinity stress as well as with arising NaCl concentrations. In this regard, the co-applications of ALG 0.2% + PS and ALG 0.1% + PS induced a superior power to promote the biosynthesis of stilbenes metabolites including trans-resveratrol, delta-viniferin, and catechin during salinity stress, respectively (Fig. 6 a, b, and c). A study on grapevine by Hanzouli et al. [86] has been suggested that not only resveratrol and its

derivatives increase total antioxidant capacity in salted grapevines but also, they protect the cell membrane against ROS product and oxidative stress by redox homeostasis. Equally, the application of plant growth regulators such as ABA (abscisic acid), methyl jasmonate as elicitor for improving the biosynthesis of resveratrol and its derivatives in grapevine to mitigate abiotic stresses has been stated [84,87]. Considering with these points, the elicitor role of seaweed based ALG to stimulate different defence such as salicylic acid, jasmonic acid, and plant hormones pathways under biotic and abiotic stresses has been mentioned apparently [17-19]. Therefore, in this study, the intensified power of accumulation of TPC, TFC, and antioxidant enzymatic activities induced by ALGs and ALGs + PS biostimulants in grapevine leaves can be associated with accelerating the biosynthesis of stilbenes metabolite such trans-resveratrol, delta-viniferin, and catechin. The increase in the concentration of stilbenes metabolite can be probably attributed to the positive role of ALG on plant growth regulators-signaling pathway.

Principal component analysis of ALG and PS on grapevine leaf-related indicators

The PCA biplot designed to discover the pattern relationship and simplify complexity of the achieved data attributed to non-enzymatic and enzymatic antioxidant systems, photosynthetic pigments, nutrient uptake, secondary metabolites, membrane stability, and lipid peroxidation in grapevine under salinity stress (Fig. 7). PCA biplot exhibited remarkable alterations in the content of leaf-related traits including enzymatic or non-enzymatic antioxidant systems, carotenoid, trans-resveratrol, catechin, delta-viniferin, and K^+ uptake depending on biostimulant type and salinity levels in salted grapevines. These alterations accounted for 64.13% of the total variance sources based on the PC1 and PC2 (components with eigenvalues ≥ 1.00). considering with alterations, PC1 was described by a comparatively big proportion of the variance (44.06%), whereas a small proportion of the variance (20.07%) was explained by PC2.

Considering with the distribution of NaCl concentrations, PCA analysis confirmed obviously that Flame Seedless grapevine showed a moderate salt resistance up to 50 mM NaCl and it was able to preserve the suitable levels of photosynthesis pigments, TPC, TFC, K^+/Na^+ ratio, and stomatal conductance under this condition (Fig. 7a and b; quarter 2). However, the undesirable effects of salinity stress in grapevine were observed obviously at higher concentrations of NaCl (100 mM and 200 mM) due to the highly uptake of Na^+, electrolyte leakage, and lipid peroxidation (Fig. 7a and b; quarters 3 and 4). Considering with the distribution of target biostimulants, ALG (at 0.1% and 0.2%) and their co-applications with PS (ALG 0.1% + PS and ALG 0.2% + PS) were separated from non and saline controls, exhibiting a noticeable districted positive effect of target biostimulants on grapevines indicator traits such as SOD, CAT, PPO, GPX enzymes activities, carotenoid, trans-resveratrol, catechin, delta-viniferin, and K^+ uptake under the high levels of salinity stress as well as the negative effects on Na^+ uptake, MDA, and electrolyte leakage occurrence in cell membrane (Fig. 7a: quarters 1 and 4; and Fig. 7b: quarters 1, 3, and 4). As displayed in Fig. 7b, the conducted traits could be destinated into four main groups, namely, the relationship among

secondary metabolites and enzymatic activities (group A), the relationship among photosynthetic pigments, sugar metabolism, and non-enzymatic activities (group B), lipid peroxidation (group C), and the relationship among Na^+ uptake and electrolyte leakage of cell membrane (group D). The relationship among secondary metabolites and enzymatic activities was at an acute angle to the photosynthetic pigments, sugar metabolism, and non-enzymatic activities due to the positive correlation. While they exhibited a significant negative correction with lipid peroxidation (group C) and membrane instability due to the presence of high Na^+ uptake (group D) in grapevine leaves. PCA analysis confirmed that ALG (at 0.1% and 0.2%) and their co-applications with PS (ALG 0.1% + PS and ALG 0.2% + PS) increased positively the components of groups A and B as well as decreased negatively the components of groups C and D, and thus, they mitigated the salinity stress at higher levels of NaCl concentrations (100 mM and 200 mM) in Flame Seedless grapevine. Therefore, since ALG based seaweed can acts as elicitors to stimulate the biosynthesis of metabolic and induced the signaling in jasmonic acid, salicylic acid and ethylene pathways in plants, they can induce multiple functionals in plant to mitigate biotic and abiotic stresses, which it is uncleared yet [17-19]. To dissect mechanism involved in mitigating salinity stress by ALG biostimulants in future research, designing and developing the Genosensor or biosensor based on monoclonal and polyclonal antibodies for the related-genes expression in response to the ALGs application can be provide the accurate information about their muti-functionals in mitigating abiotic or biotic stresses in plants [88-92].

Conclusion

The current study exhibited that the sequential spraying of Flame Seedless grapevine seedlings with the ALGs (at 0.1% and 0.2%) and PS, individually or co-applications, enhanced plant growth, proline and stilbenes secondary metabolites, TPC, TFC, enzymatic activity, K^+/Na^+ hemostasis, stomatal conductance, and photosynthetic pigments under different levels of salinity stress. In this regard, findings underscored the co-application of ALG 0.1% +PS and ALG 0.2% + PS was indicated a high potential in mitigating salinity stress, followed by individual application with ALG 0.1% and then ALG 0.2%. The efficiency of target biostimulants promoted by increasing the levels of salinity stress; thus, they were able to prevent the highly uptake of Na^+ ion, electrolyte leakage, and lipid peroxidation under high salinity in grapevines. These warrants the field-scale validation of ALG biostimulant for sustainable grape production under stressful conditions. In conclusion, the results highlighted the biostimulant application of ALGs in alleviating the salinity stress on horticultural crops. Due to multiple functionals of ALG based seaweed in plants, future research should be conducted to investigate for discovering their possible involved mechanisms in mitigating salinity stress using omics technologies.

Declarations

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Authors' contributions

S.M.J., S.Y.S.L., H.M.L and H.K.B designed research, S.M.J and H.M.L. performed research, S.Y.S.L., H.K.B., and H.M.L. performed writing-review & editing, A.S.G., S.Y.S.L., H.M.L. and H.K.B. analyzed data, and methodology handled by S.M.J., H.M.L. and S.Y.S.L. All authors read and approved the manuscript.

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Data availability

The datasets generated and / or analyzed during the current manuscript will be available from corresponding authors upon reasonable request.

Ethics approval and consent to participate

No specific permissions were required for the collection of plant material in this study. All methods and experimental research were carried out in compliance with local and national guidelines of Iran.

Clinical number

Not applicable.

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Competing interests

The authors declare no competing interests.

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Figures

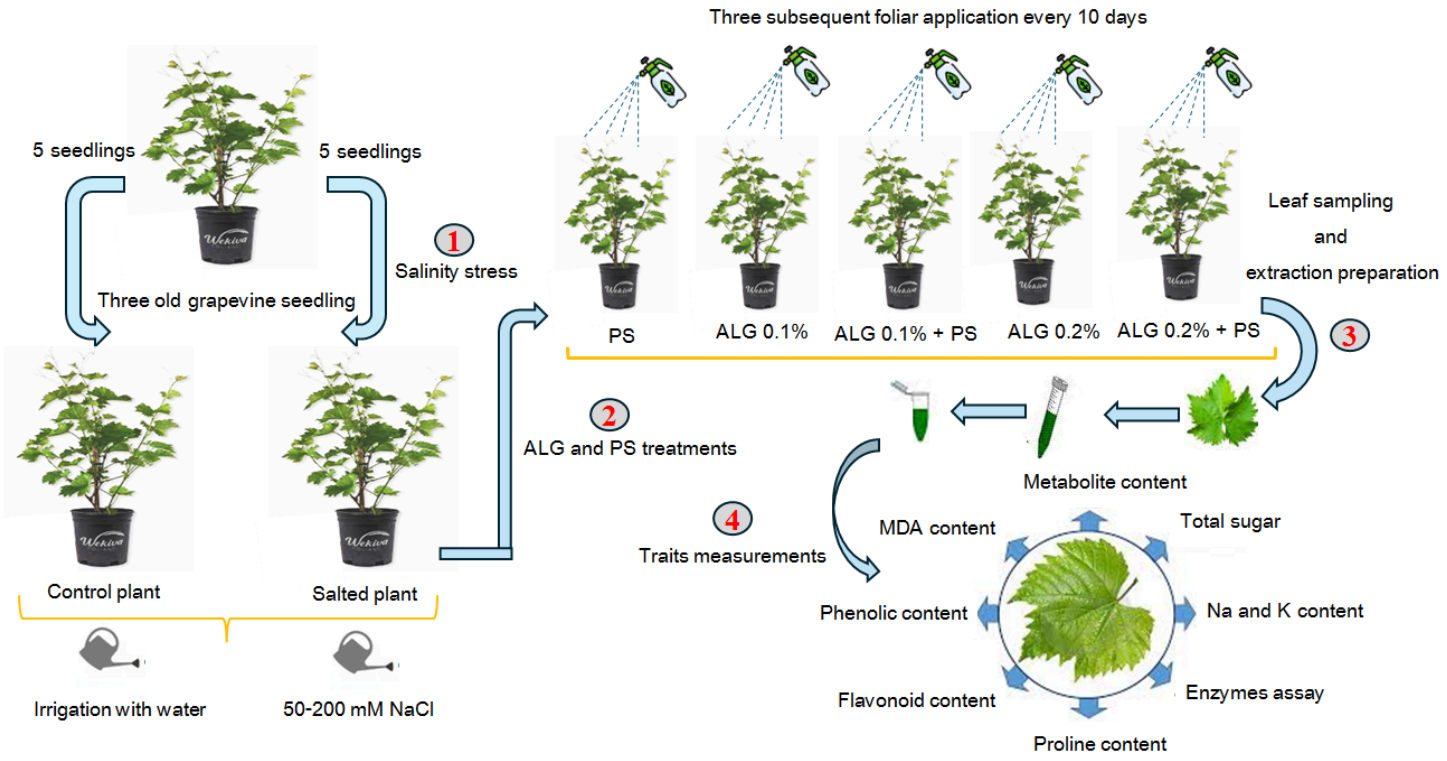


Figure 1

Schematic graphic of all steps involved in the experiment processes of current study from salinity stress and target biostimulants treatments to traits measurements in the Flame Seedless grapevines.

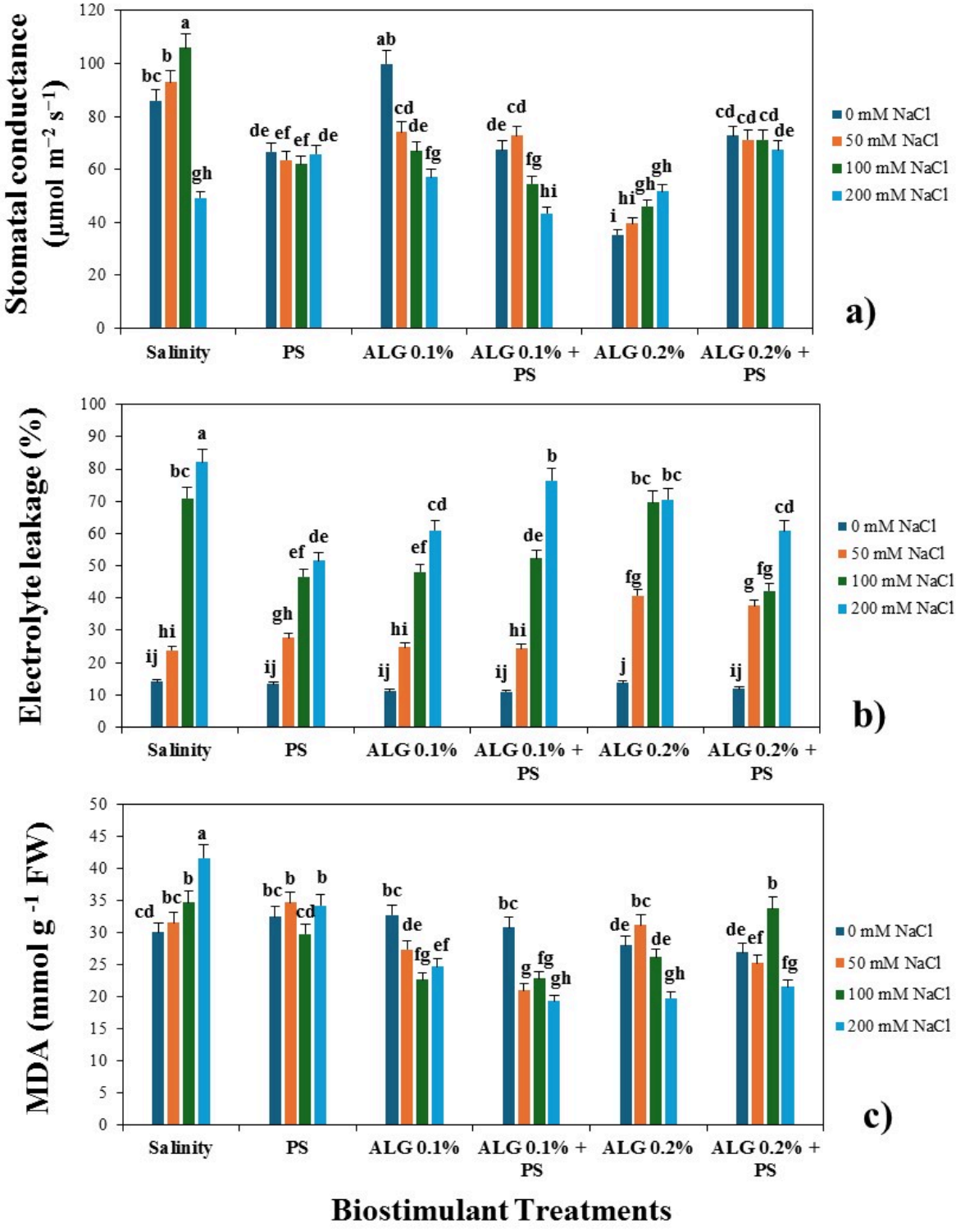


Figure 2

Effects of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on **(a)** stomatal conductance, **(b)** electrolyte leakage (%), and **(c)** malondialdehyde (MDA) content (µmol g^{-1} DW) in Flame Seedless grapevine under salinity stress. Values are means ± SE (n=4). Bars with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

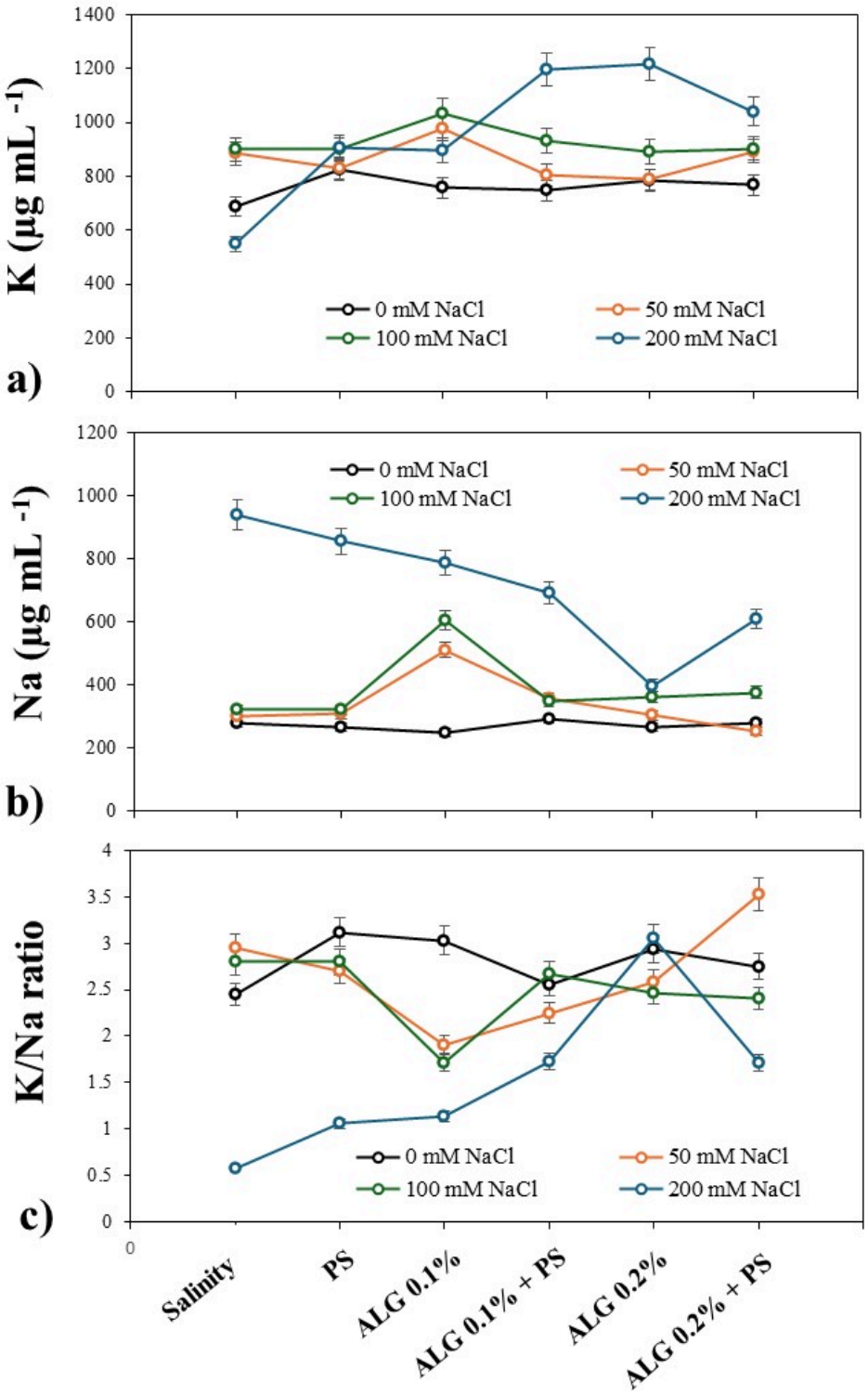


Figure 3

Effects of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on **(a)** K, **(b)**Na, and **(c)** K/Na ratio in Flame Seedless grapevine under salinity stress.

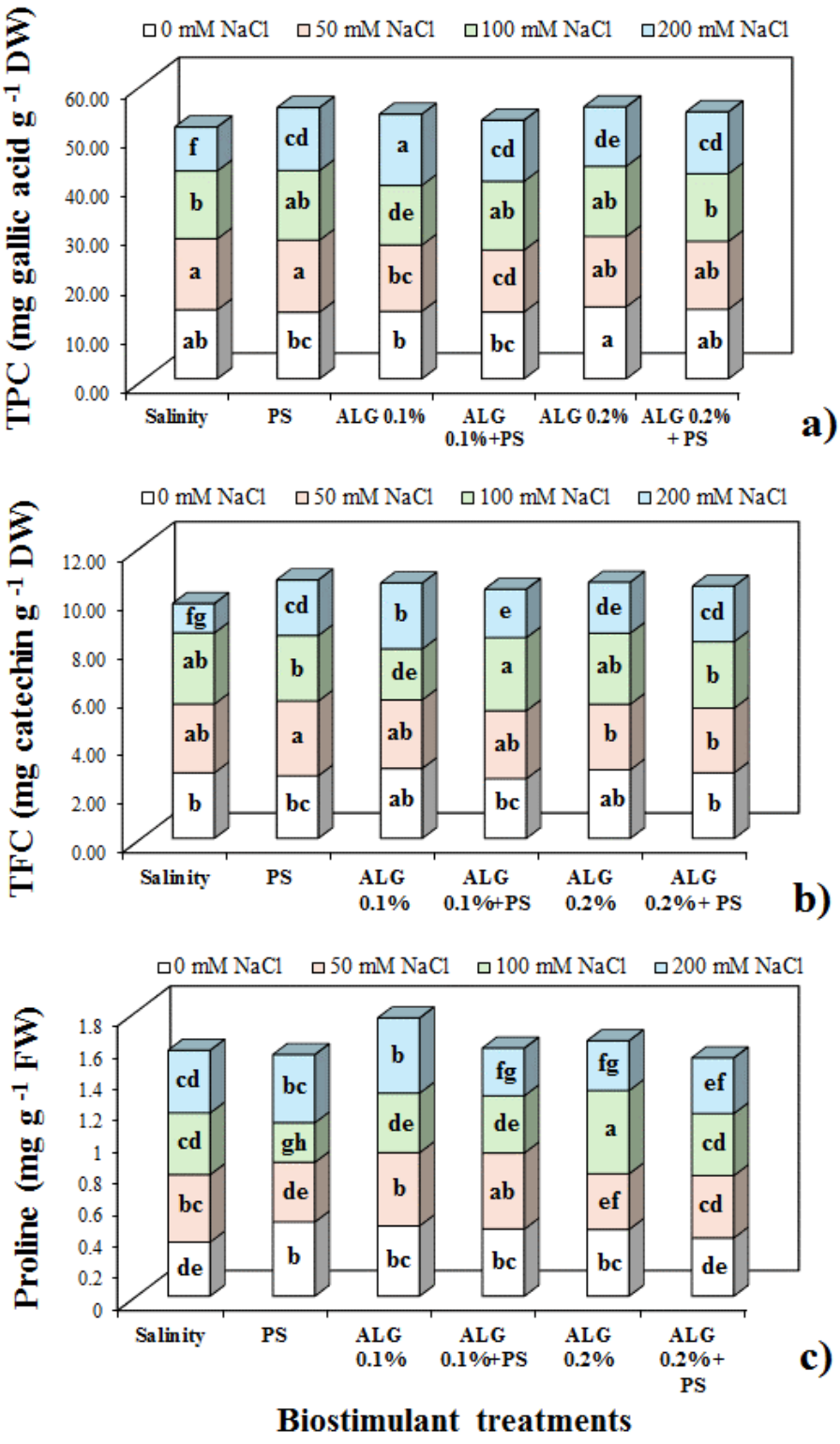


Figure 4

The evaluation of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on **(a)** total phenolic content (TPC), **(b)** total flavonoid content (TFC), and **(c)**proline content in Flame Seedless grapevine under salinity stress. Values are means ± SE (n=4). Bars with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

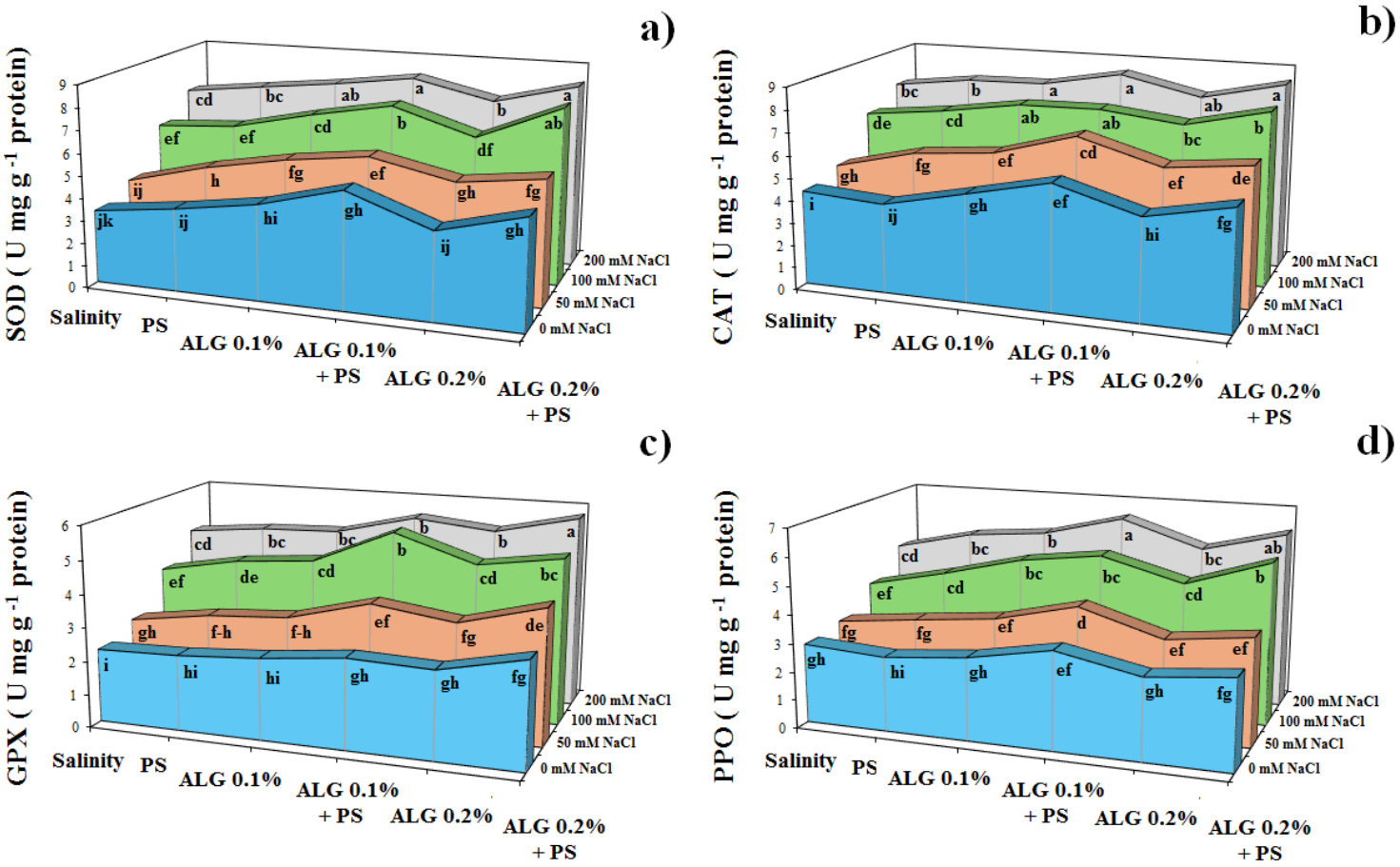


Figure 5

The evaluation of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on **(a)** superoxide dismutase (SOD), **(b)** catalase (CAT), **(c)** guaiacol peroxidase, and (**d**) polyphenol oxidase (PPO) enzymatic activities in Flame Seedless grapevine under salinity stress. Values are means ± SE (n=4). Bars with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

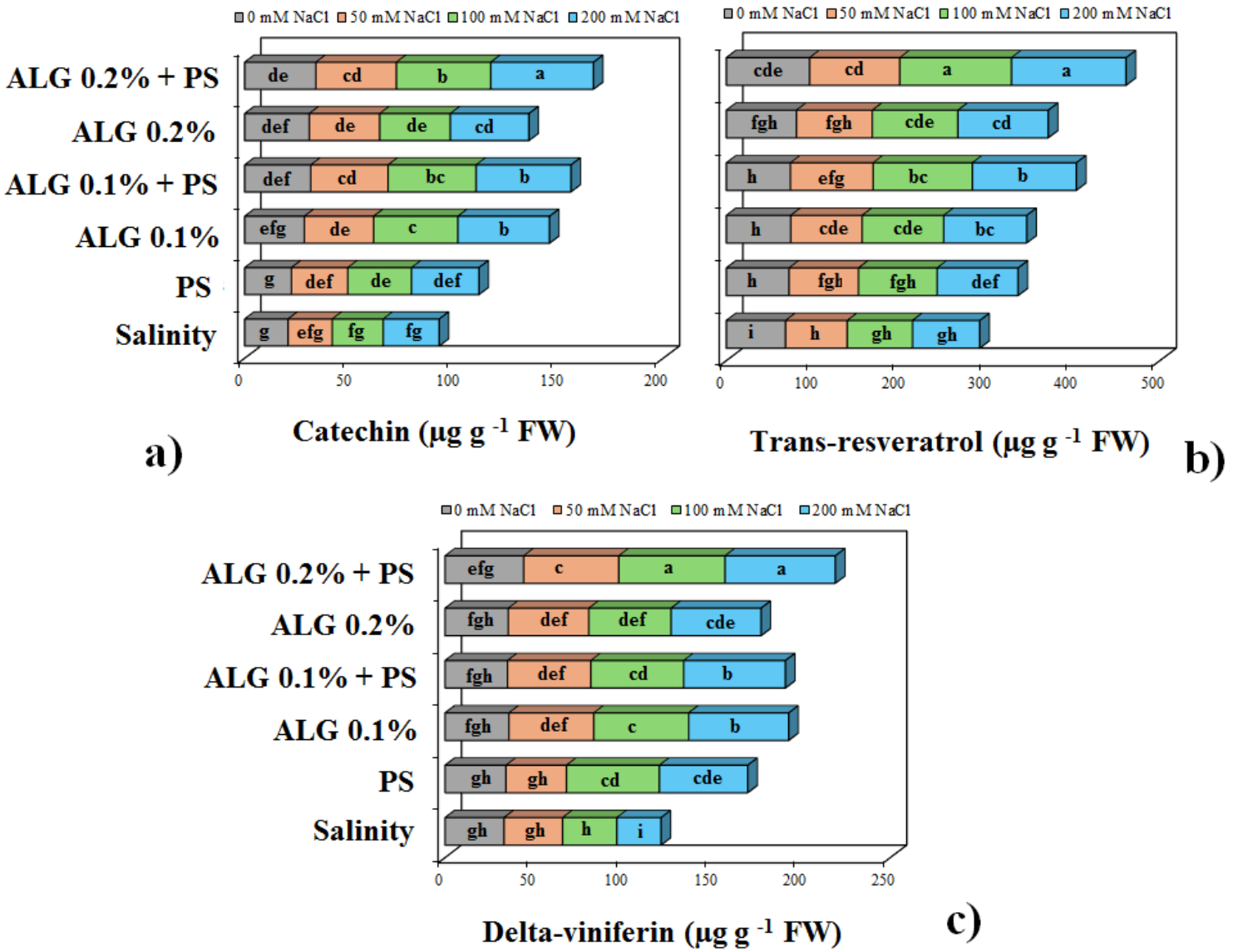


Figure 6

The evaluation of individual and synergetic effects of alginic acid (ALG: 0.1% and 0.2%) and potassium sulfate (PS (K_2SO_4: 0.2%)) on secondary metabolites biosynthesis including **(a)**, trans-resveratrol **(b)** catechin, and **(c)** delta-viniferin in Flame Seedless grapevine under salinity stress. Values are means ± SE (n=4). Bars with the same letter are not significantly different at $p \leq 0.01$ according to Duncan's test.

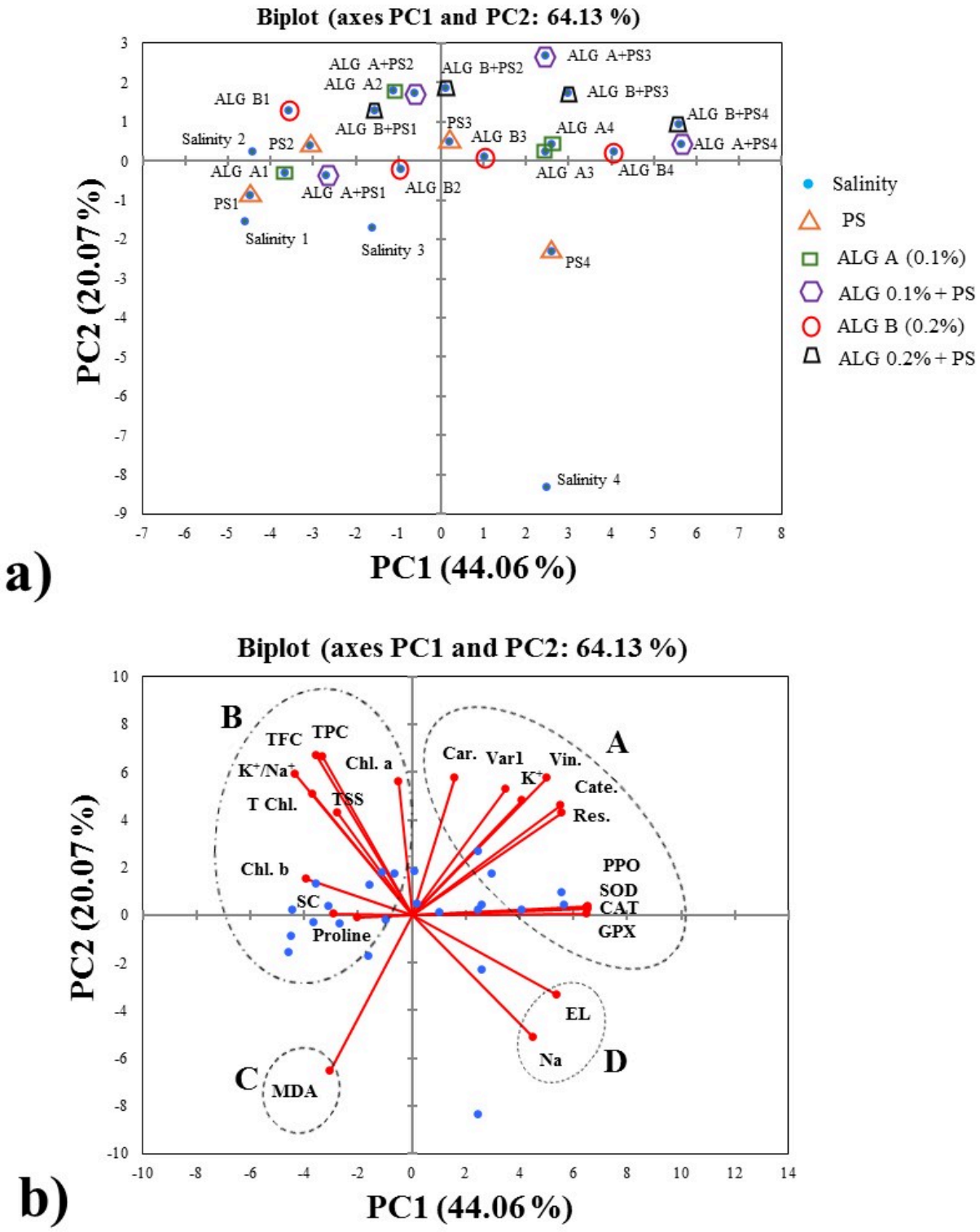


Figure 7

Principal component analysis of relevant traits in Flame Seedless grapevine under salinity stress. (**a**) The relationship among the scores of principal component analysis of related traits and the rate of ALGs and PS applied and **(b)** principal component loading plot. PG: plant growth, Car.: carotenoid, Vin.: delta-viniferin, Res.: trans-resveratrol, Cat.: catechin, Chl. a, b, and T: chlorophyll pigments, TPC: total phenolic compound, TFC: total flavonoid compound, TSS: total soluble sugar, SC: stomatal conductance, PPO:

polyphenol oxidase, CAT: catalase, SOD: superoxidase, GPX: guaiacol peroxidase, MDA: malondialdehyde, EL: electrolyte leakage.