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INTERACTIVE EFFECT OF SALICYLIC ACID AND ASCORBIC ACID ON GASEOUS EXCHANGE AND MINERAL NUTRIENTS OF CHICORY (*CICHORIUM INTYBUS* L.) UNDER SALINE CONDITIONS

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Abstract

Chicory (*Cichorium intybus* L.) is used medicinally, nutritionally, and agriculturally. Salinity affects growth and quality. Salinity can be mitigated by shotgun methods such as plant growth regulators. To investigate the physiochemical role of salicylic acid (SA), ascorbic acid (AsA), and their combinations (SA + AsA) under salt stress in chicory genotypes, two chicory genotypes (Hamedan and Shiraz) were exposed to four salinity (0, 100, 150, 200 mM NaCl) levels after 45 days of sowing and on the very next day of salinity four levels of plant growth regulators i.e. water spray, SA (0.5 mM), AsA (0.5 mM) and SA (0.5 mM) + AsA (0.5 mM) were exogenously applied as a foliar spray. Salt stress considerably decreased the shoot dry weight, root length, chlorophyll content index, gas exchange attributes, shoot calcium, phosphorus, sodium and root potassium and root sodium. Exogenous applications of SA and AsA as foliar spray enhanced the growth by increasing the chlorophyll content, gas exchange parameters as well as ionic contents. Overall, the application of SA, AsA and their combination increased salt tolerance in chicory. Moreover, the performance of the Chicory genotype Shiraz was significantly better as compared to Hamedan.

Key words: Chicory (Kasni), Plant growth regulator, NaCl, Photosynthetic attributes.

Introduction

The main environmental factor that confines the production of agriculture is salinity (Etesami & Alikhani, 2019). Salinity reduces agricultural output. Under saline circumstances, plants must perform several metabolic processes (Parihar *et al*., 2015). Saline environments reduce shoot development and water intake and increase salt ions such as sodium and chloride (Parmoon *et al*., 2018). Salt limits crop productivity (Vaishnav *et al*., 2019). Salt stress is among the most pervasive limitations of food production, which eventually results in negative financial, environmental and social consequences (Dhillon *et al*., 2023).

Ascorbic acid (AsA) is considered as best growth regulator against salt stress (Wang & Huang, 2019). Ascorbic acid activates the complex biological defense mechanism and plays a role as an antioxidant (Taha *et al*., 2020). Many types of agricultural plants employ it to mitigate the damaging effects of salt stress (Khan *et al*., 2010).

The SA may also have additional physiological activities, such as influencing stomatal control, transpiration rate, chlorophyll contents, and respiratory routes (Zahra, 2010). Salicylic acid may be associated with some biochemical reactions in plants, moreover, it has vital performances in the development of plant regulatory mechanisms under salt stress (Grown, 2012). Unregulated transpiration may cause the accretion of ions in the shoot to maintain water status, the transformation of salt into shoot and root transpiration flux (Hasanuzzaman *et al*., 2013). Salicylic acid induces a change in the absorptivity of the plant membrane and plants show an immediate response to salinity by closing stomata and changing the ion flux into the shoot (Mimouni *et al*., 2016).

Chicory has valuable bio-monitor substantial metals like Cd, Zn and Pb (Duri *et al*., 2018). Chicory leaves are fleshy green, used as fodder for livestock and reduced the internal parasites in the animals (Nicoleta *et al*., 2015). It is known to be a vital medicinal plant that has great financial potential due to the presence of large concentrations of fructo-oligosaccharides just like inuline. Chicory roots contain 68% inulin and dried roots are used as sugar for diabetic patients. Moreover, it is also useful in maintaining the immunity of humans against serious diseases like diabetes, impotence, cancer, gallstones and insomnia (Das & Roychoudhury, 2014). It has been hypothesized that SA and AsA, either individually or in combination, can reduce chicory plants' sensitivity to salt. The objectives of this study were to investigate the effects of salicylic acid, and ascorbic acid, and their combination on the growth, gas exchange properties, and ionic levels of chicory when grown under saline conditions.

Materials and Methods

An experiment was performed in the Old Botanical Garden of the University of Agriculture Faisalabad, Pakistan to learn how salicylic acid and ascorbic acid interact in a saline environment to affect chicory (*Cichorium intybus* L.). The Hamedan and Shiraz chicory genotypes were obtained from the Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Eight kilograms of sterile sand were utilized in the experiment, contained in plastic containers. Ten seeds were planted in each plastic container, and after being thinned down to eight, the surviving plants were maintained. A full-strength Hoagland's nutrient solution was fed into the root development medium with regular interval of ten days. The experiment was arranged in CRD (Completely randomized design) manner. After 45 days of sowing, four salt stress (NaCl) treatments (0, 100, 150, 200 mM) were applied. After salt treatment, ascorbic acid (AsA 0.5 mM), salicylic acid (SA 05 mM), and its mixtures (SA 0.5 mM + AsA 0.5 mM) were applied as foliar spray.

Determination of plant growth parameters: Growth parameters i.e. shoot and root dry weight, shoot and root length, and total number of leaves were measured.

Determination of the chlorophyll content and carotenoids: Arnon (1949) protocol was employed to determine the chlorophyll content. Overnight at room temperature, a 0.1 g sample of fresh leaves was extracted in 5 milliliters of 80% acetone. Chlorophyll contents measured using a spectrophotometer (Thermo Fisher F1-01620 Vantaa, Finland) read the absorbance at the wavelength of 480, 663 and 645 nm.

Gas exchange attributes: IRGA (Infra-red gas analyzer, Model LCA-4) was used to record the readings. The third completely grown leaf from the plant's top was utilized for this.

Inorganic ions determination: Allen *et al*., (1986) protocol was employed for the determination of inorganic ions. After samples were oven-dried (0.1 g) and crushed, 5 ml sulphuric acid was let to sit overnight. At 250 degrees Celsius, the mixture became colorless after being treated with hydrogen peroxide drop by drop. Then, 50 ml of distilled water was added. Ions (shoot and root Na^+, K^+ and Ca^{2+}) were all measured by utilizing this substance with a flame photometer (Sherwood 410).

Phosphorous determination: Distilled water was used to bring the total amount up to 50 ml, then the digested solution and the Barton reagent 2 ml were added. After stirring, the liquid was left to sit for 30 minutes at room temperature. The spectrophotometer (IRMECO U2020) measurements were taken at 460 nm.

Statistical analysis

Statistix 8.1 and OriginPro-2021 were used to conduct a Fisher analysis of variance and a minimum significant different test LSD (Steel *et al*., 1997).

Results

Shoot dry weight: Salt stress notably ($p \leq 0.01$) decreased the shoot dry weight. Among all the four levels (0, 100, 150 and 200 mM), 200 mM showed a prominent decrease. Foliar application of 0.5 mM AsA and 0.5 mM SA + 0.5 mM AsA increased the shoot dry weight in Hamedan genotype (Table 1; Fig. 1). Foliar spray of SA + AsA behaved better as compared to SA and AsA under a controlled environment.

Root dry weight: Root dry weight exposed a non-significant effect of salinity on both chicory genotypes. Genotype showed uniform behavior in root dry weight, dry weight remained unchanged by foliar application of either SA or AsA (Table 1; Fig. 1).

Table 1. Analysis of variance means for growth, chlorophyll content, and gas exchange characteristics of chicory (*Cichorium intybus* L.) plants subjected to salt stress and foliar applications of SA/AsA.

SOV	df	Shoot dry weight	Root dry weight	Shoot length	Root length	Chl. *a*
Genotype (G)	1	0.185**	0.024ns	0.13ns	55.51**	0.678**
Salinity (S)	3	0.236**	0.043**	129.64*	2.22**	0.032ns
(SA/AsA)	3	0.006ns	0.008ns	6.94ns	219.19ns	0.025ns
G× S	3	0.097**	0.028**	16.75*	14.23*	0.034ns
G× SA/AsA	3	0.008*	0.013ns	26.21**	17.33*	0.025ns
S× SA/AsA	9	0.018**	0.003ns	20.32**	6.83ns	0.066*
G×S× SA/AsA	9	0.036**	0.007ns	5.82ns	9.25*	0.0433ns
Error	96	0.003	0.007	5.97	4.67	0.026
SOV	**df**	**Chl. *b***	**Total chl.**	**Chl. *a*/*b* ratio**	**Carot.**	***E***
Genotype (G)	1	0.081**	0.869**	2.78**	0.0005**	18.89***
Salinity (S)	3	0.023ns	0.048ns	0.26**	8.08E-05	2.30***
(SA/AsA)	3	0.0111ns	0.139ns	0.0394ns	9.01E-05	7.20***
G×S	3	0.002ns	0.155ns	0.280**	8.47E-05	0.80**
G× SA/AsA	3	0.011ns	0.074ns	0.071	3.32E-05	0.77**
S× SA/AsA	9	0.022ns	0.203**	0.107*	0.00014**	0.08ns
G×S× SA/AsA	9	0.016ns	0.1111ns	0.077ns	7.30E-05	0.55***
Error	96	0.011	0.062	0.050	5.18E-05	0.15
SOV	**df**	g_s	C_i	**WUE**	***A***	
Genotype (G)	1	0.14**	24043.5**	29.89**	70.90**	
Salinity (S)	3	0.58**	4028.74**	11.93**	76.47**	
(SA/AsA)	3	0.38**	3586.35**	154.85**	144.88**	
G× S	3	0.04**	736.35ns	12.85**	0.49ns	
G× SA/AsA	3	0.016ns	567.211ns	0.85ns	2.43**	
S× SA/AsA	9	0.018**	1882.24*	2.13ns	1.14**	
G×S× SA/AsA	9	0.019**	1488.22*	7.05**	0.29ns	
Error	96	0.0089	735.61	1.78	0.24	

Where: ns (non-significant); *, ** = Significant at 0.05 and 0.01 respectively; df (degrees of freedom); chl. *a* (chlorophyll *a*); chl. *b* (chlorophyll *b*); total. Chl. (total chlorophyll); Car. (carotenoids); *A* (net CO_2 assimilation rate); *E* (transpiration rate); WUE (water use efficiency) *g*s (stomatal conductance); C*i* (substomatal CO_2 concentration)

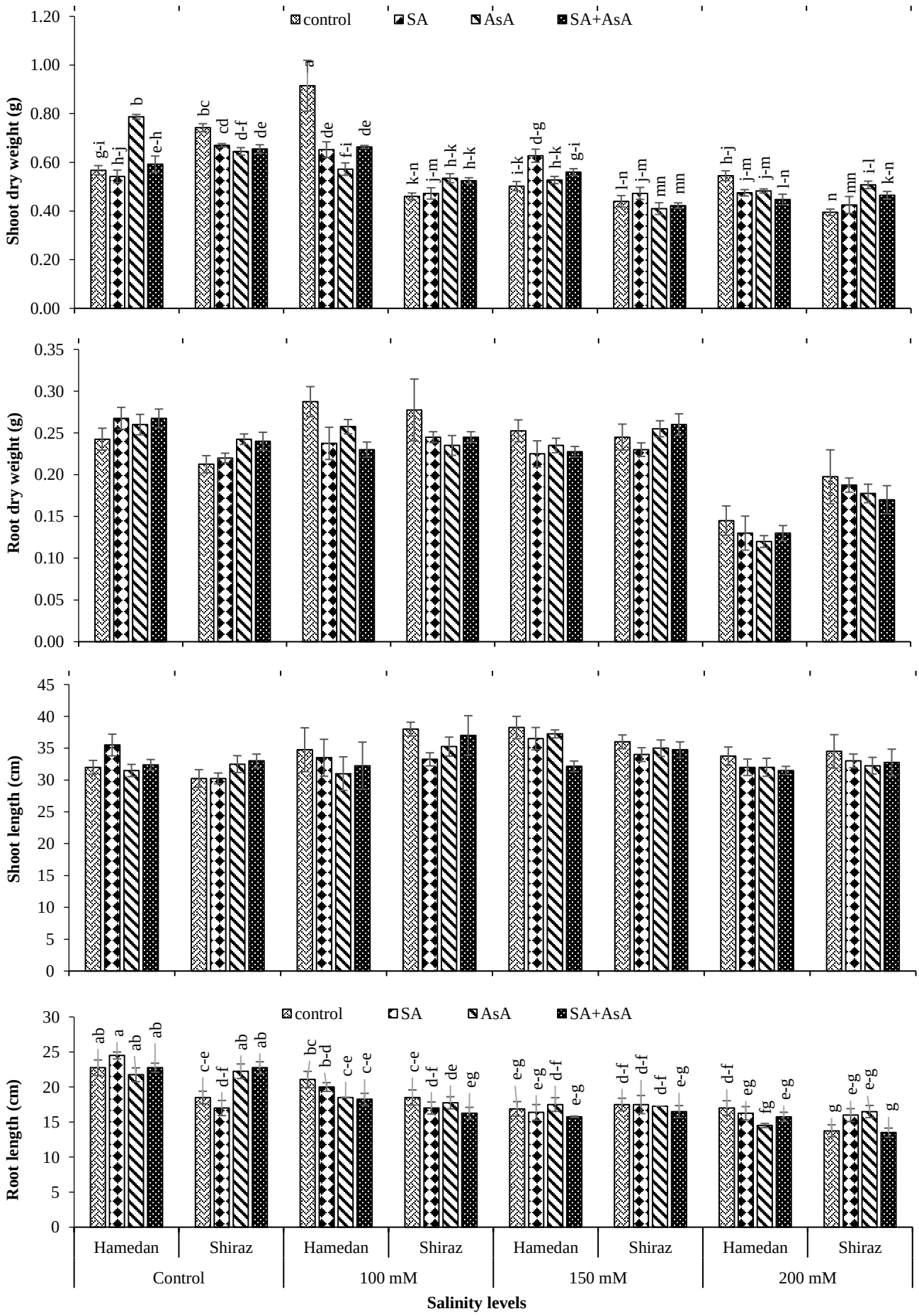

Fig. 1. Growth parameters of chicory (*Cichorium intybus* L.) plants when SA/AsA were foliarly applied under salt stress conditions.

Shoot length: Means square values of shoot length showed that foliar-applied SA/AsA attenuated a non-significant effect in both chicory genotypes. Interactions for Genotype × salinity, genotype × SA/AsA and salinity × SA/AsA showed significant value (Table 1, Fig. 1).

Root length: Root length showed significant ($p \leq 0.01$) reduction under saline conditions. The minimum reduction was observed under 100 mM NaCl in Hamedan genotype while the maximum reduction was observed at 200 mM salinity level in both chicory genotypes. Salicylic acid SA (0.5 mM) alone caused maximum root length contrary the synergistic effect of SA 0.5 mM + AsA 0.5 mM showed similar behavior towards root length in both the genotypes (Table 1; Fig. 1).

Chlorophyll *a*: Data regarding chlorophyll *a* showed significant variation for genotypes as genotype Sheraz was better as compared to Hamedan. Salinity or foliar spray of SA 0.5 mM and AsA 0.5 mM did not cause any significant effect. However, the interaction of salinity and foliar application of SA 0.5 mM + AsA 0.5 mM showed a significant value ($P \leq 0.05$) (Table 1; Fig. 2).

Chlorophyll *b*: Chlorophyll *b* showed a significant response for chicory genotypes whereas, sodium chloride stress and exogenous application of SA 0.5 mM + AsA 0.5 mM showed a non-significant effect (Table 1). Among both genotypes, Shiraz was high in chlorophyll *b* than Hamedan (Fig. 2).

Total chlorophyll: Both the chicory genotypes fluctuated significantly ($P \leq 0.01$) in total chlorophyll as Shiraz was high than Hamedan (Table 1; Fig. 2). Imposition of sodium chloride or foliar application of SA and AsA showed a non-considerable effect.

Chlorophyll *a*/*b* ratio: The chlorophyll *a*/*b* ratio was shown to rise considerably ($p \leq 0.01$) in the presence of sodium chloride, although it was unaffected by the foliarly applied SA 0.5 mM + AsA 0.5 mM. Both the genotypes differed significantly as Shiraz was high as compared to Hamedan under both salinity and controlled environment (Table 1; Fig. 2).

Carotenoids: Data regarding carotenoids showed significant ($p \leq 0.01$) variation among genotypes whereas, foliar spray of SA and AsA and salt stress caused non-significant effect (Table 1; Fig. 2). However, interactions among salinity and foliar spray of SA (0.5 mM) + AsA (0.5 mM) showed significant ($p \leq 0.01$) value.

Net CO_2 assimilation rate (*A*): Imposition of four NaCl levels (0, 100, 150 and 200 mM) considerably ($p \leq 0.001$) decreased *A*. At 100 mM and 200 mM salinity Hamedan genotype unveiled more CO_2 assimilation rate than the Shiraz genotype. Overall, significant genotypic difference was observed. Hamedan genotype behaved better than the Shiraz genotype (Table 1; Fig. 3).

Transpiration rate (*E*): Application of salinity noticeably ($p \leq 0.01$) decreased the *E* (transpiration rate) in both chicory genotypes. Both genotypes showed variable behavior at different levels (0, 100, 150 and 200 mM) of salinity. Interaction between salinity and foliar revealed non-significant behavior while remaining interactions showed significant value. Application of alone SA (0.5 mM) in Hamedan genotype showed maximum increase at (100 mM) and (200 mM) salinity. While the interactive upshot of SA (0.5 Mm) + AsA (0.5 mM) application showed the minimum results at 200 mM salinity levels. In this parameter, Hamedan showed better behavior than Shiraz genotype (Table 1: Fig. 3).

Stomatal conductance (g_s): Imposition of salinity reduced the g_s (stomatal conductance) prominently ($p \leq 0.001$) in both chicory genotypes. A significant difference was unveiled among both genotypes. Foliar treatment of 0.5 mM SA + 0.5 mM AsA showed a significant ($p \leq 0.001$) decrease under different levels (0, 100, 150 and 200 mM) of salinity. Maximum reduction was observed in Shiraz genotype at 200 mM salinity level. Maximum stomatal conductance was recorded in Hamedan when 0.5 mM SA was applied under controlled conditions (Table 1; Fig. 3).

Sub-stomatal CO_2 concentration (C_i): C_i reduced ($p \leq 0.001$) when salinity was imposed in both chicory genotypes. A substantial ($p \leq 0.01$) decrease was shown in Shiraz genotype under 100 mM salinity levels by foliar application of AsA (0.5 mM) alone, while at 150 mM Shiraz genotype showed similar results under all foliar applications. Imposition of salinity at 200 mM Hamedan genotype showed similar behavior under all foliar applications. Regarding this parameter, no significant varietal difference was observed in both chicory genotypes (Table 1; Fig. 3).

Water use efficiency (WUE): Salinity application decreased the WUE significantly ($p \leq 0.001$) in both chicory genotypes. Both genotypes showed significant variation. Application of a combined spray of SA 0.5 mM + AsA 0.5 mM showed a prominent ($p \leq 0.001$) enhancement in WUE under salinity levels of Hamedan and Shiraz genotypes. Salinity 100 mM behaved maximum growth in Shiraz while minimum in Hamedan at 200 mM salinity. Overall, Shiraz genotype performed better than Hamedan genotype regarding this parameter (Table 1; Fig. 3).

Shoot sodium: Shoot Na^+ increased significantly ($p \leq 0.01$) in both chicory genotypes when sodium chloride stress with different levels was imposed. The genotypic difference was significant ($p \leq 0.01$) as Shiraz genotype showed high shoot Na^+ than Hamedan genotype. By the application of foliar applied SA and AsA Shiraz showed high sodium (Na^+). At 100 mM and 200 mM salinity Shiraz showed the highest shoot Na^+ concentrations (Table 2; Fig. 4).

Root sodium: Root sodium Na^+ increased considerably ($p \leq 0.01$) when salinity was applied. A slightly significant ($p \leq 0.05$) genotypic difference was observed as Hamedan genotype showed reduced Na^+ in the root. Exogenous application of SA 0.5 mM increased the root Na^+ in Shiraz genotype as compared to Hamedan. At 150 mM and 200 mM salinity Hamedan genotype showed the lesser root Na^+ than Shiraz under all foliar applications (Table 2; Fig. 4).

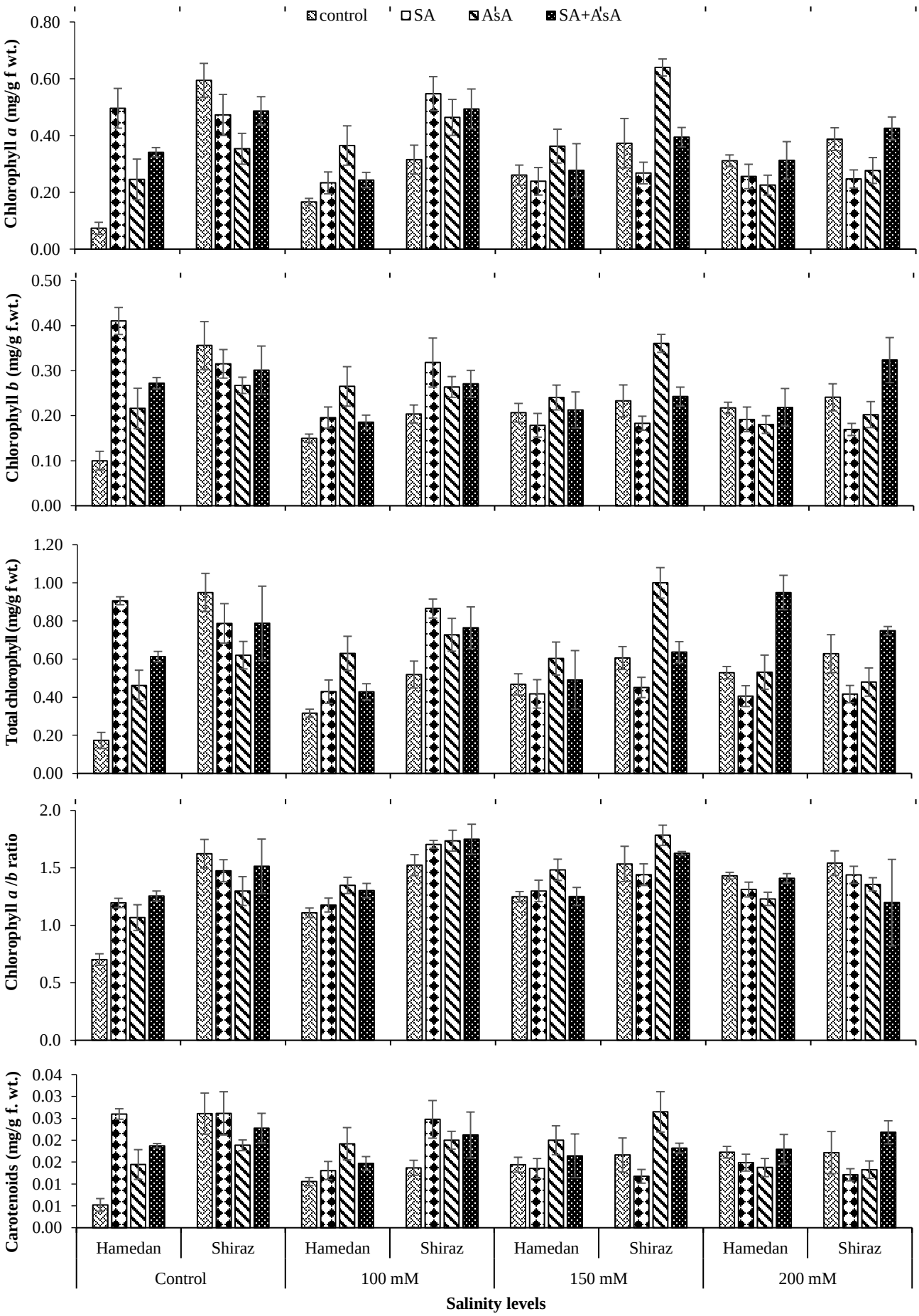

Fig. 2. Photosynthetic pigments of chicory (*Cichorium intybus* L.) plants when SA/AsA were foliarly applied under salt stress conditions.

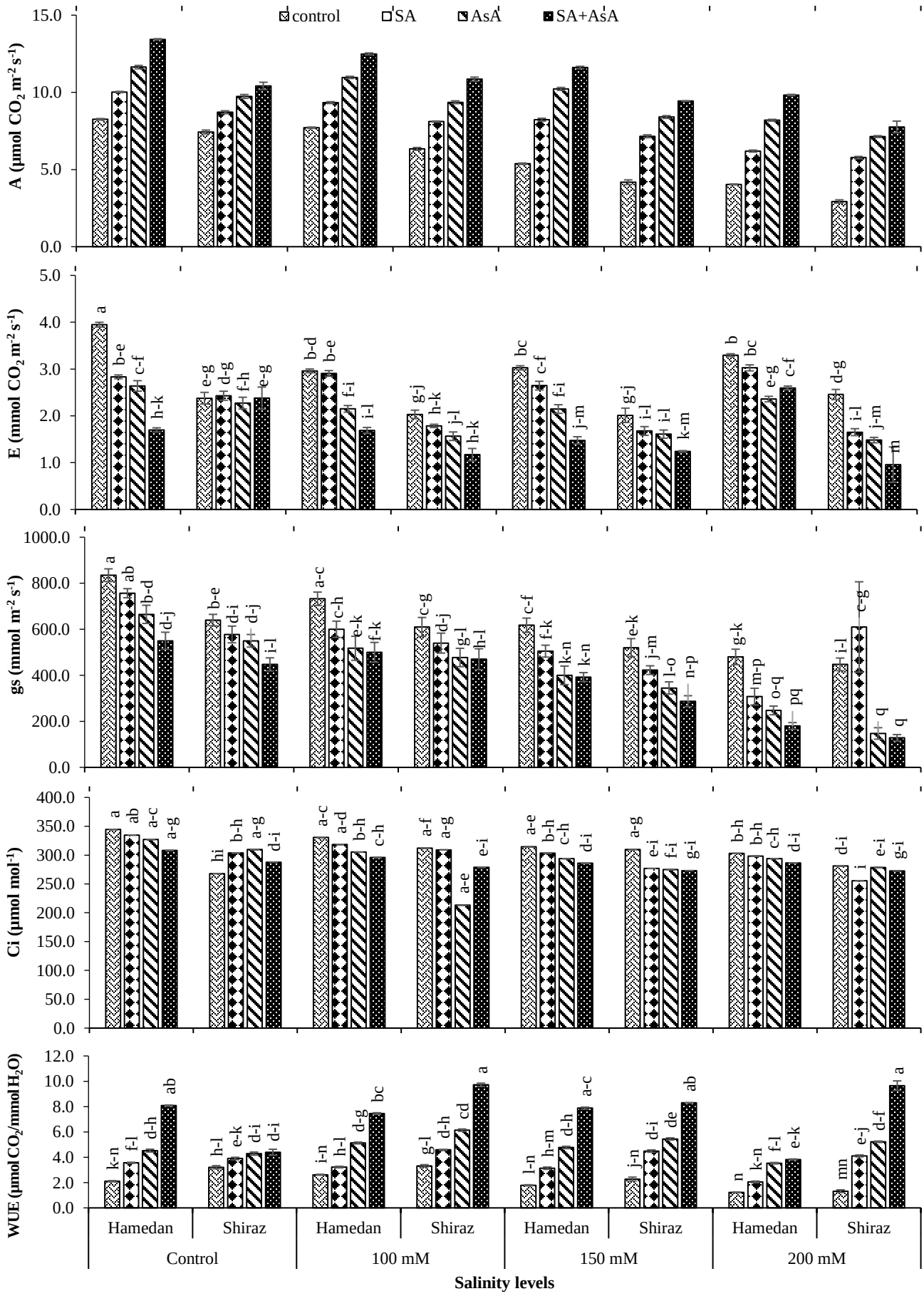

Fig. 3. Gas exchange attributes of chicory (*Cichorium intybus* L.) plants when SA/AsA were foliarly applied under salt stress conditions.

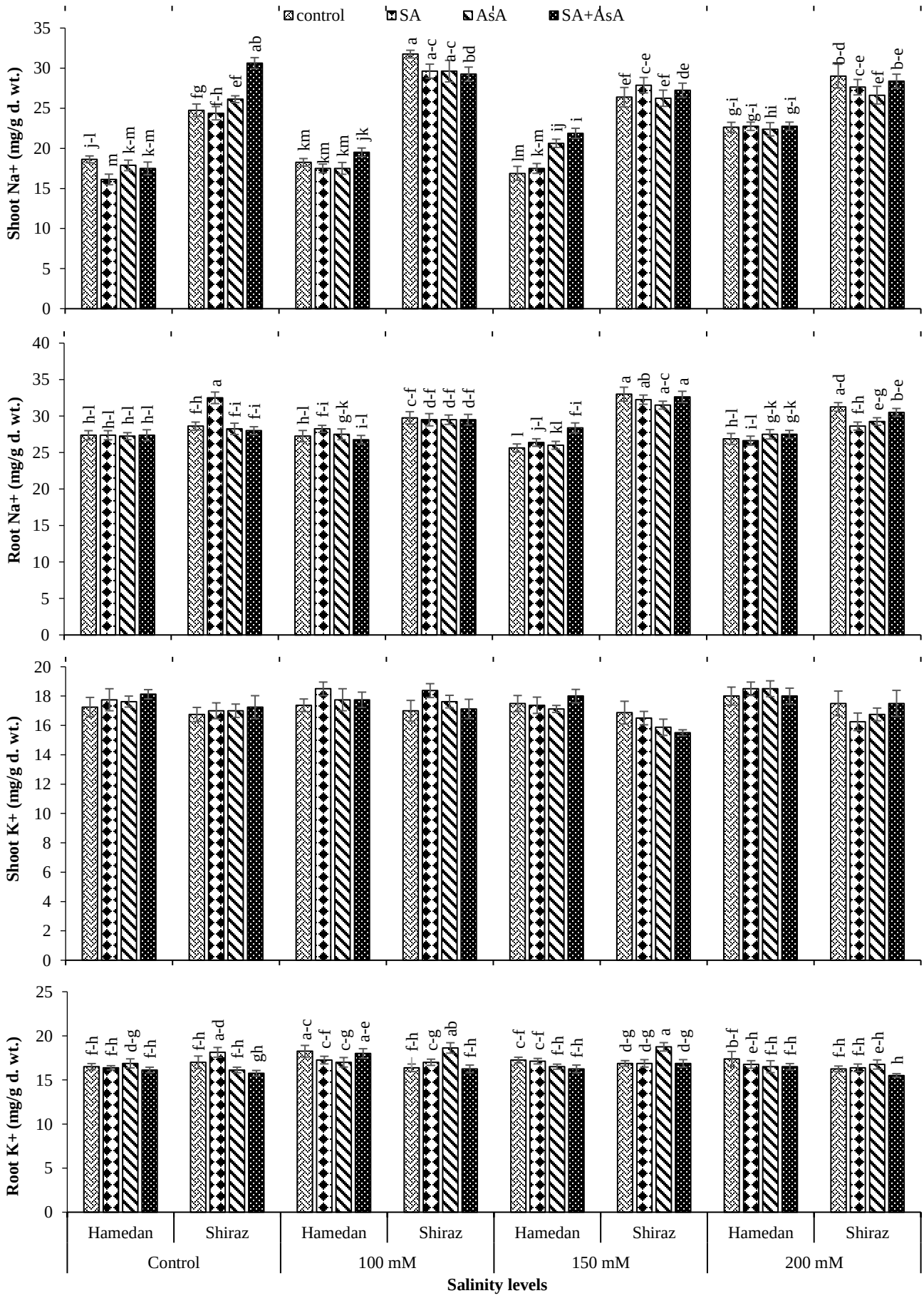

Fig. 4. Shoot and root sodium and potassium of chicory (*Cichorium intybus* L.) plants when SA/AsA were foliarly applied under salt stress conditions.

Table 2. Analysis of variance means for ionic contents of chicory (*Cichorium intybus* L.) when SA/AsA were foliarly applied under salt stress conditions.

SOV	df	Shoot Ca^{2+}	Shoot K^+	Shoot Na^+	Shoot P
Genotype (G)	1	59.13**	101.53**	2286.57**	0.16**
Salinity (S)	3	20.50**	18.91**	62.73**	0.01**
(SA/AsA)	3	3.87*	1.83ns	17.03**	0.004**
G× S	3	40.84**	7.28ns	60.12**	0.018**
G× SA/AsA	3	3.79*	2.36ns	3.131ns	0.004**
S× SA/AsA	9	2.44*	4.58ns	7.16**	0.002**
G×S× SA/AsA	9	3.98**	3.39ns	11.46**	0.002**
Error	96	1.21	5.18	2.66	9.40E-05
SOV	**df**	**Root Ca^{2+}**	**Root K^+**	**Root Na^+**	**Root P**
Genotype (G)	1	2.25ns	0.16ns	320.36**	0.017**
Salinity (S)	3	0.321ns	4.97**	8.44**	0.002**
(SA/AsA)	3	0.46ns	3.35*	2.13ns	0.0001ns
G× S	3	1.064ns	2.69*	24.71**	0.0012**
G× SA/AsA	3	1.86ns	4.39**	3.42ns	2.08E-05ns
S× SA/AsA	9	3.55**	0.794ns	5.24**	6.16E-05ns
G×S× SA/AsA	9	1.77*	2.38**	4.13*	4.62E-05ns
Error	96	0.74	0.86	1.74	0.00029

Where: ns (non-significant); *, ** = significant at 0.05 and 0.01 respectively; df (degree of freedom); shoot Ca^{2+} (shoot calcium); shoot K^+ (shoot potassium); shoot Na^+ (shoot sodium); shoot P (shoot phosphorous); Root Ca^{2+} (root calcium); Root K^+ (root potassium); Root Na^+ (root sodium) and Root P (root phosphorous)

Shoot potassium: Shoot potassium in chicory genotypes significantly ($p \leq 0.05$) decreased in both genotypes. However, Hamedan genotype showed the highest potassium content in the shoot as compared to Shiraz. On the other hand, significant leading potassium content was unveiled where 100 mM and 200 mM salinity levels were applied while the lowest was at 150 mM salinity level (Table 2; Fig. 4).

Root potassium: Sodium chloride treatment with different levels resulted in a considerable ($p \leq 0.05$) reduction in root K^+ concentrations of chicory genotypes. Exogenously applied SA (0.5 mM) significant effect ($p \leq 0.05$) and increased root K^+. At (200 mM) salinity, the maximum root K^+ was exhibited in Shiraz when ascorbic acid was sprayed, whereas the least root potassium was driven from Shiraz genotype when treated with SA and AsAAt 150 mM salt regime Shiraz genotype showed maximum root K^+ by AsA (0.5 mM) application (Table 2; Fig. 4).

Shoot calcium: Analysed data of shoot Ca^{2+} projected a significant ($p \leq 0.05$) decrease under saline conditions. At 150 Hamedan genotype noticed a higher level of shoot Ca^{2+} by foliar application of AsA 0.5 mM and SA + AsA (0.5 mM) than Shiraz, while at 200 mM salt regime Shiraz showed a higher value by exogenously applied AsA 0.5 mM (Table 2; Fig. 5).

Root calcium: Root Ca^{2+} concentrations decreased considerably ($p \leq 0.05$) in both genotypes of chicory with an increase in salt concentration (Table 2). Describing under controlled conditions the maximum root Ca^{2+} concentration was determined in Hamedan genotype having SA (0.5 mM) + AsA (0.5 mM) except least value was recorded in Shiraz genotype when treated with a combination of SA and AsA at 200 mM salinity Hamedan showed maximum root Ca^{2+} by the application of SA + AsA while minimum in Shiraz genotype when 150 mM salinity was imposed (Table 2; Fig. 5).

Shoot phosphorus: Shoot phosphorus significantly ($p \leq 0.01$) enhanced under sodium chloride stress. Both the genotypes showed uniform behavior, However, Hamedan genotype showed the highest shoot phosphorus concentration when applied with SA and AsA 0.5 mM spray and the minimum content was studied with 0.5 mM SA under controlled conditions (Table 2; Fig. 5).

Root phosphorous: Data regarding root P exposed a significant ($p \leq 0.01$) increase under salt regimes. At 100 mM salinity Shiraz genotype accumulated high root phosphorous as compared to Hamedan. While at 200 mM salinity, the leading phosphorous content showed in Shiraz and the lowest amount was revealed in Hamedan genotype at 100 mM salinity stress. Under non-saline stress conditions when only foliar application was applied Shiraz genotype showed better phosphorous accumulation by the synergistic effect of (SA + AsA 0.5 mM) applications (Table 2; Fig. 5).

Pearson correlation analysis: (Fig. 6) shows the results of a Pearson correlation analysis of the associations between the chicory characteristics under study. Root length (RL) was considerably inversely connected with leaf number (NL), whereas root dry weight (RDW) was significantly positively correlated with RL, SL, and SDW. Additionally, the number of leaves, and amount of chlorophyll all showed positive correlations with root length and shoot dry weight, respectively. A similar positive association was seen between total chlorophyll (T. chl.) and carotenoid (carot.) levels and chlorophyll.

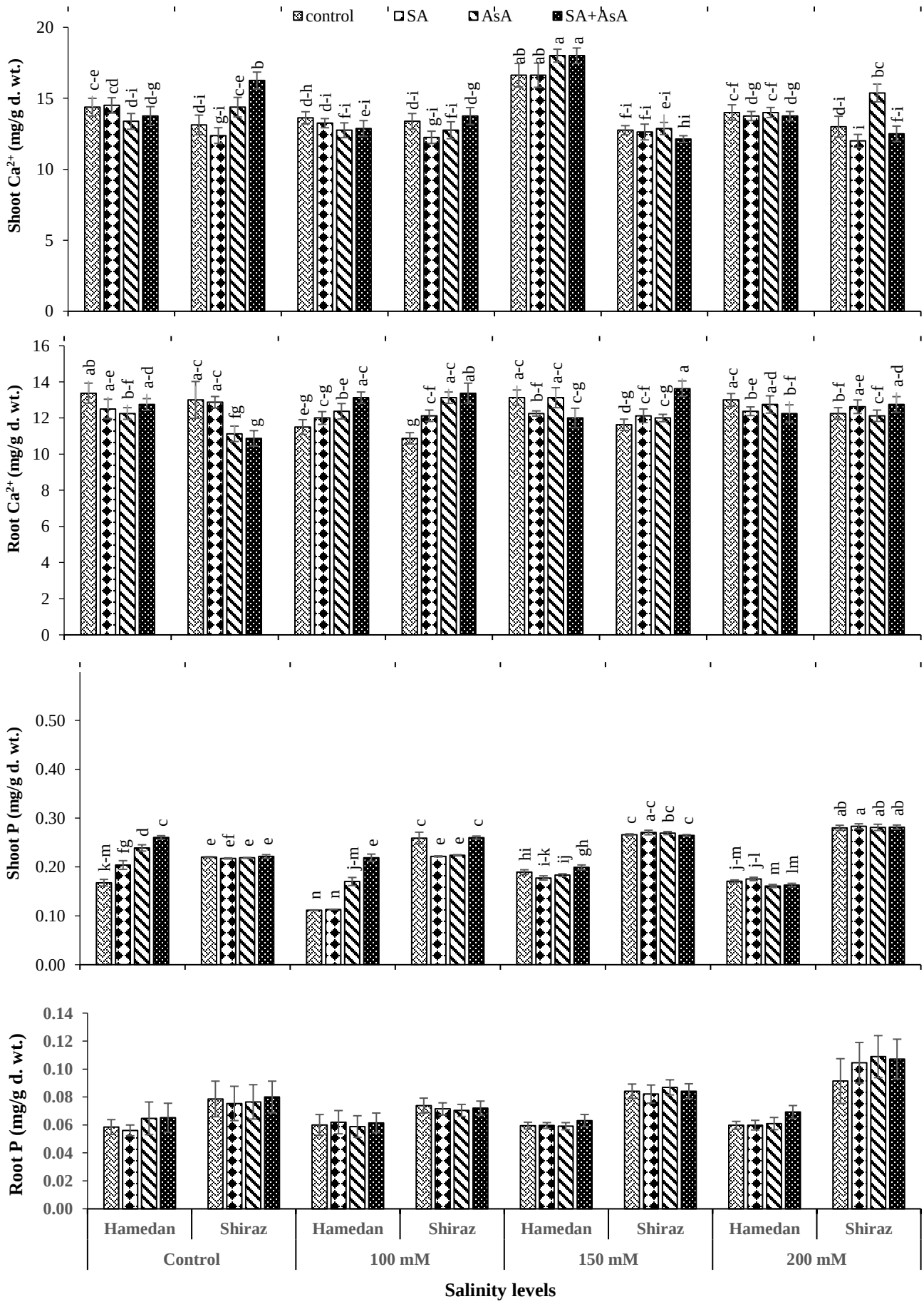

Fig. 5. Shoot and root calcium and phosphorus of chicory (*Cichorium intybus* L.) plants when SA/AsA were foliarly applied under salt stress conditions.

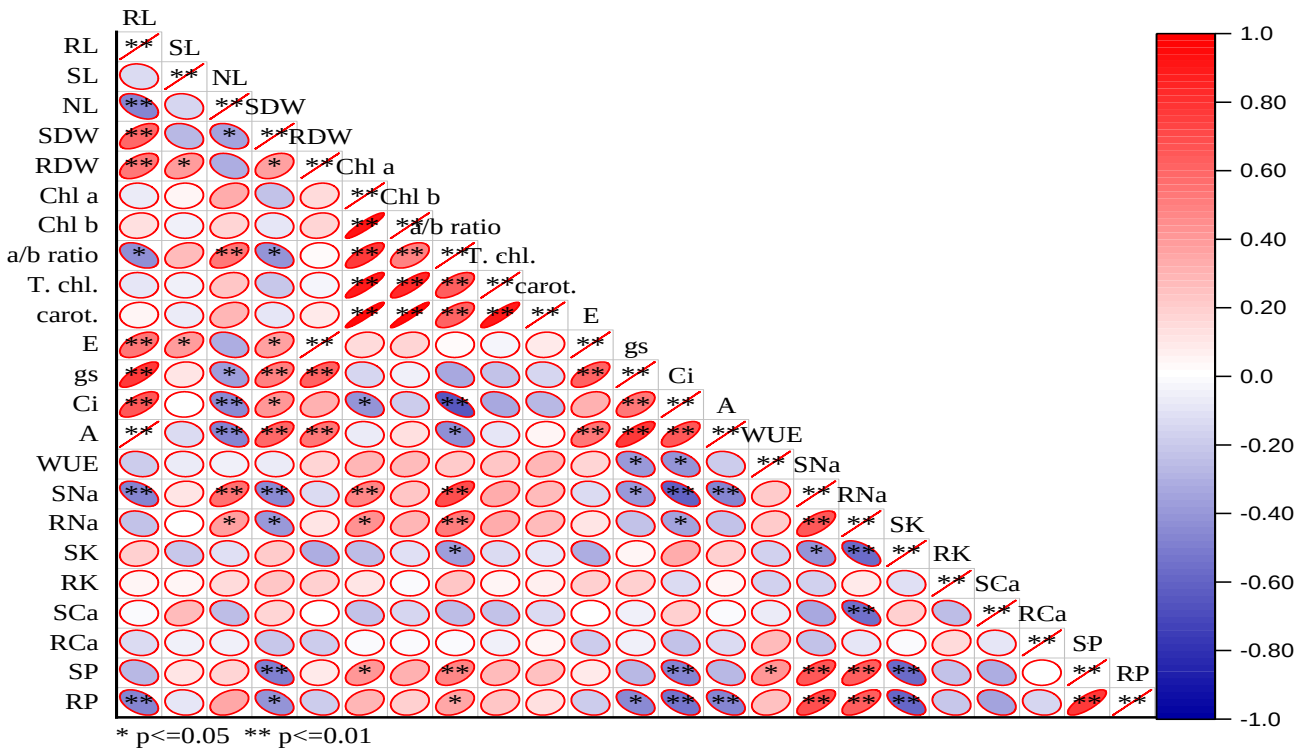

Fig. 6. Pearson correlation matrix for a morphological, chlorophyll, gas exchange attributes and ionic contents i.e. *A* = CO_2 assimilation rate, g_s - stomatal conductance, *E*- transpiration rate, C_i - sub stomatal conductance, Chl. *a*- chlorophyll *a*, Chl. *b*- chlorophyll *b*, T. Chl- total chlorophyll, Caro- carotenoids, SDW- shoot dry weight, RDW- root dry weight, SL- shoot length, RL-root length, SNa- shoot sodium, RNa, root sodium, S Ca^{2+}-shoot calcium, R Ca^{2+}-root calcium, SK-shoot potassium, RK-root potassium, SP-shoot phosphorous and RP-root phosphorous.

Transpiration (*E*) and stomatal conductance (g_s) revealed a positive relationship with RL (root length), SL (shoot length), SDW (shoot dry weight), and RDW (root dry weight). Moreover, WUE (water use efficiency) exhibited a negative correlation stomatal conductance and sub-stomatal conductance (C_i). In addition, shoot sodium content (SNa^+) unveiled a strongly negative correlation with root length, shot dry weight, stomatal and sub-stomatal conductance and CO_2 assimilation rate (*A*) and positively correlated with a number of leaves, chlorophyll *a*, and *a*/*b* ratio. Moreover, shoot phosphorus (SP) concentration showed that negative correlation between shoot dry weight, sub-stomatal conductance and shoot potassium content whereas, it was positively influenced by the chlorophyll *a*, a/b ratio water use efficiency, shoot sodium (SNa^+) and root sodium (RNa^+) and the similar repose was also studied in root phosphorus (RP) content.

Discussion

Salinity stress inhibits the many physiological roles that are the main cause of dysfunction of biological and morphological characteristics in plants like reduction in photosynthesis due to reduced enzymes activity and protein content as well as it also disrupts the osmotic gradient and ionic concentrations (Muhammad *et al.*, 2021). Impedance effects of salinity on growth traits have been observed on different crops. like okra (Ashraf.*et al*., 2019), wheat (Khan *et al*., 2013) and *Chenopodium quinoa* (Riaz *et al*., 2020). Growth. reduction. is possibly due to enhanced osmotic stress (Alsahli *et al*., 2019). PGR (Plant Growth regulators) are compounding that control development of plant by increasing branching, suppressing growth shoots, increasing flowering, eliminating fruit surplus, or altering fruit maturity. PGRs are organic compounds that showed a crucial role in plant growth and development (Raddadi *et al.*, 2018).

Usually, photosynthetic pigments decreased under higher salinity (Ruiz *et al.*, 2016). Photochemical processes are protected and stabilized by the antioxidant ability of carotenoids (Abbasi *et al.*, 2014). Imposition of salinity through rooting medium decreased chlorophyll content in the current investigation. Our results are matched with similar findings on okra (Ashraf *et al*., 2019), thyme (Bistgani *et al*., 2019), and Quinoa (Amjad *et al.*, 2015); Riaz *et al.*, 2020). The index of chlorophyll reduction was owed to a rise in chlorophyllase activity (Kumar *et al.*, 2016). ROS construction and ion accumulation in cytoplasm disturbed the structure of chloroplast, which eventually tends to decrease chlorophyll contents (Fayez & Bazaid, 2014). Reduced uptake of Mg in NaCl-affected soil or increased level of proline might be the other reason for photosynthetic pigments reduction (Hassanein *et al.*, 2009). Foliar treatment of AsA and SA significantly boosted total chlorophyll and carotenoids in mung beans in a prior study. Sweet pepper, on the other hand, has been reported to have AsA-enhancing effects on chlorophyll content (Barzegar *et al*., 2018). Antioxidant activities of AsA and SA to scavenge ROS may be accountable for the increase in leaf chlorophyll concentration Ma *et al*., (2017). Foliar application of SA stimulates the biosynthesis of enzyme

protein kinase involved in organogenesis, differentiation of cell, and division of cells to manage salinity in plants (Kang *et al*., 2014).

To prevent water loss, under osmotic stress, plants rapidly decrease their stomatal conductance (Isayenkov & Maathuis, 2019). In the current study, salinity stress considerably declined the gas exchange of both chicory genotypes by decreasing carbon assimilation, conductance of stomata, and increase the rate of transpiration. Lessening in stomatal conductance following previous studies on gas exchange attributes (Khan *et al.,* 2010; Kausar & Shahbaz, 2017) like carbon assimilation and transpiration rate (Shaheen *et al.,* 2013; Hussein and Khursheed, 2014). Citrus (Khoshbakht *et al*., 2017), cucumber (Wu *et al*., 2018), tomato (Gharbi *et al*., (2018), *Nicotiana benthamiana* (Qin *et al*., 2020), onion (Da-Silva *et al*., 2019), and *Vigna radiata* (Ahanger *et al*., (2019) all showed this decrease in gas exchange characteristics under salt. Reduction in water potential owing to an increase in the ionic strength of soil solutions, the plant water uptake is impeded by an increase in soil salt content (Noreen *et al*., 2010). Many physiological problems in plants may be traced back to the osmotic strength, which limits water absorption (Ashfaq *et al*., 2014). Long-term salinity resulted in the accumulation of salts in vacuoles, cell membrane, and outer membrane (cell wall) of the leaves resulting in cell death of the mature leaves, ultimately a smaller number of leaves for gaseous exchange and photosynthetic activity (Acosta-Motos *et al.,* 2017). In on-going research, WUE and g_s showed more prominent in Shiraz genotype and *E*, sub-stomatal conductance and net CO_2 showed better in Hamedan genotype. Both the WUE and the net CO_2 assimilation rate improved when supplemented with exogenous ascorbic acid. The *A*, stomatal conductance, and CO_2 content were all boosted internally by ascorbic acid at a 0.5 mM concentration in both genotypes. Improved gas exchange in wheat (Al-Hakimi, 2001; Ishaq *et al*., 2021) corresponded with higher CO_2 assimilation rates in rice (Kobayakawa & Imai, 2017) and stomatal conductance (Chen & Gallie, 2004).

The growth medium absorbs inorganic ions like Cl^- and Na^+ during salt stress, disrupting ionic concentration (Misra and Gupta, 2006; Taffouo *et al*., 2009). Salinity stress enhanced root and shoot sodium, calcium, and potassium accumulation in this study. Many investigations reported the same results (Shalata & Neumann 2001; Azooz *et al*., 2009; Ramezani, 2011; Munns, 2012; Wang, 2014; Roy, 2014; Patishtan, 2018; Suhaib, 2018).

When the inorganic ions sodium and chloride accumulate in leaves in large quantities, these ions become toxic to the plants and disturb the cytoplasmic processes of plants like protein synthesis and photosynthetic reactions (Bakht *et al.,* 2006; Marschner, 2012; Ahanger & Agarwal, 2017). Na^+ and Cl^- in large quantities in soil disturb the captivation of important ions like K^+ and Ca^{2+} and NO_3 (Ben Ahmed *et al.,* 2010; Nemati *et al.,* 2011). High salt concentrations raise soil osmotic pressure, which prevents plant cells from absorbing water and nutrients potassium and calcium (Munns *et al*., 2006; Silva *et al*., 2010; Maimaiti *et al*., 2014). Plant growth and metabolism require K^+ (Annunziata, 2017). The potassium level of plants controls the physiological process in plants Ashraf *et al*., (2012). Chicory treated with ascorbic acid lowered Na^+ uptake in plants.

As previously documented, decreased Na^+ absorption increased shoot K^+ and root K^+ and Ca^{2+} levels. SA modulates the uptake of nutrients and Na^+ concentrations in higher plants under salinity (Tohma & Esitken, 2011; Per *et al*., 2017). Roshdy *et al*., 2021) originate that increasing SA to 90 ppm under 40 mM NaCl availability reduced the Na^+ level of strawberry leaves and protected them from salt damage. In seedlings of cotton grown in (150 mM) NaCl, foliar salicylic acid supplementation at (0 mM, 1.0 mM, 1.5 mM and 2.0 mM) decreased plant Na^+ absorption and increased nutritional uptake (Gaber *et al*., 2020). Nutrient accumulation was greatly enhanced and Na^+ and Cl^- accumulation were reduced in the SA 1.0 mmol/L plus treatment of sodium chloride (NaCl) treatment (Jinni & Joseph, 2017).

Conclusion

In conclusion, different levels of sodium chloride 0 mM, 100 mM, 150 mM and 200 mM NaCl reduced the growth parameters of chicory. Moreover, exogenously applied 0.5 mM SA, 0.5 mM AsA and their combinations (SA + AsA) increased the growth in the chicory plant. However, levels of foliar applications including salicylic acid ascorbic acid) and their combinations (SA+AsA) improved gas exchange attributes and ionic contents. The Shiraz chicory genotype was shown to be more resistant to salt stress than the Hamedan variety.

Future Perspectives

Chicory plants have great medicinal properties and are used as a coffee substitute. Due to its great medicinal properties, there is a need to make inorganic products that have no side effects on human health and make herbal industries where chicory is used as herbal medicine. There is a need to further study the role of various genes in the defensive mechanisms of the Chicory plant.

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