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RESEARCH

Salt Stress Effects On Hybrid Bottle Gourd (*Lagenaria siceraria*) Rootstock Candidates Plant Growth, Hormones and Nutrient Content

Hakan Başak[1] · Alim Aydin[1] · Halit Yetişir[2] · Metin Turan[3]

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Abstract

Globally, salinity has a devastating effect on plant yield and quality. The breeding of salt-tolerant varieties/rootstocks is crucial to reducing these effects. This study involved 35 hybrid rootstock candidates, their female and male parent lines, 9 genotypes (*Lagenaria siceraria*). In hydroponic conditions, rootstock candidates were evaluated for the biomass, physicochemical parameters in leaf and root tissues under control [1.8 dS m^{-1}] and saline [10 dS m^{-1}] conditions. Salt stress reduced shoot dry weight by 63%, root dry weight by 43%, main stem diameter by 18%, number of leaves per plant by 41%, main stem length by 68%, and root length by 45%. Under salt stress, the highest amount of photosynthetic parameters were measured in genotypes 39-01 × 56-01 (16.17 µmol $CO_2/cm^2/s$) and 42-11 × 47-02 (16.47 µmol $CO_2/cm^2/s$), respectively. Salt stress decreased leaf tissue IAA (3%), ABA (63%), and root tissue IAA (28%), GA_3 (32%), and SA (63%) content, but increased leaf tissue GA_3 (196%), SA (27%) and root tissue ABA (47%) content. Salt stress decreased leaf K/Na and Ca/Na ratios by 100 and 97%, respectively, under salt stress. The increase in the amount of K/Na and Ca/Na in leaf tissues under salt stress conditions positively affected biomass and photosynthesis parameters. In this study, 15 *Lagenaria* hybrid rootstock candidates performed better under salt stress than watermelon and other hybrids salt-tolerant plants. It has been concluded that rootstock candidates selected as salt tolerant can be used as watermelon rootstocks in regions experiencing salt stress by determining their rootstock/scion interactions in terms of yield and quality in future studies.

Keywords Salinity · Rootstock · Bottle gourd · Watermelon · Biomass · Hormone and nutrient content

Introduction

Globally, salinity is one of the most important abiotic stress factors that limits crop production. A third of the world's food production is lost due to salt stress, which affects 20% of irrigated agricultural land (Manuel et al. 2017). Every year, approximately 10 million hectares of agricultural land are destroyed by salt deposition. Climate change, overuse of groundwater (especially near the sea), increased use of low-quality water for irrigation, and irregular irrigation are all factors contributing to this increase (Walczak 2021). Most vegetable species with low salinity tolerance are adversely affected by soil salinity. Despite very low levels of salt stress, most vegetables suffer significant yield losses as a result of salt stress (Munns 2011). Salt stress may also be caused by a variety of factors, such as species/variety, growing environment, environmental conditions, development period, and degree and duration of salinity (Munns and Tester 2008). As a result of salt stress, plant development is negatively affected by the following factors: (1) osmotic stress in rhizosphere because of low water potential in

Hakan Başak
hbasak@ahievran.edu.tr

✉ Alim Aydin
alim.aydin@ahievran.edu.tr

Halit Yetişir
yetisir1@erciyes.edu.tr

Metin Turan
metin.turan@yeditepe.edu.tr

[1] Faculty of Agriculture, Department of Horticulture, Kırsehir Ahi Evran University, Kırsehir, Turkey

[2] Faculty of Agriculture, Department of Horticulture, Erciyes University, Kayseri, Turkey

[3] Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey

the root zone, (2) ions toxicity such as Na^+, Cl^- and SO_4^{-2}, and (3) disruption of plant nutritional are regulation during different physiological events (Hasegawa et al. 2000; Manuel et al. 2017; Hernández 2019; Chen et al. 2020). Additionally, salt stress in plants causes oxidative stress, metabolic changes, membrane disruption, and decreased cell division, and growth (Hasegawa et al. 2000; Yamaguchi and Blumwald 2005; He et al. 2009). Physiological processes such as photosynthesis, protein synthesis, energy, and lipid metabolism are negatively affected by salt stress in plants (Tavakoli et al. 2019). Long-term salinity causes adult leaves to senescence prematurely, which causes ionic stress, which reduces the photosynthetic area available for growth (Gomes-Filho et al. 2008; Yang et al. 2013). High levels of chlorine and sodium can affect plant enzymes, resulting in cell swelling and decreased energy production in plants. High Na^+ causes ionic stress, competing with potassium, disrupts protein synthesis, and reduces enzyme activity, causing premature aging in old leaves and toxic symptoms (chlorosis, necrosis) in mature leaves (Hasegawa et al. 2000; Munns and Tester 2008).

In order to overcome the problem of soil salinity, there are different methods available. Among them are improving soil drainage (adding additives to the soil to ensure natural drainage, establishing a drainage system), using appropriate irrigation methods and systems, washing the soil with higher quality water, and using salt-tolerant varieties or rootstocks. Grafting salt-sensitive varieties on rootstocks that can grow under salt stress is a permanent, sustainable, and environmentally friendly solution to salinity, which causes significant yield loss in the *Cucurbitaceae* and *Solanneceae* vegetable species (Uygur and Yetisir 2009; Aydın and Yetişir 2022). A graft is the joining of a scion and rootstock with special methods to form a new plant. During grafting, the rootstock provides a suitable root system, while the scion ensures the production of harvestable yields (Lee et al. 2010; Keatinge et al. 2014). Vegetable grafting has the following benefits: (a) reducing the use of chemicals (fumigant, pesticides, fertilizers); (b) controlling soilborne diseases; (c) overcoming abiotic stresses like flooding, salinity and extreme soil temperatures; (d) extending the vegetative period and increasing yields and quality (Yamakawa 1983; Alexopoulos et al. 2007; Davis et al. 2008; Nawaz et al. 2016).

Grafted seedlings onto suitable rootstocks can handle with stress conditions more effectively by better uptake of water and nutrients, avoiding soil pathogens, and tolerating low soil temperatures, salinity, and high soil moisture (King et al. 2010). Grafted plants can develop different mechanisms to cope with salty conditions. These strategies; (1) excluding salt in rhizosphere (selective ion uptake), (2) retention of salt in the roots and inability to transport it to the shoots, (2) maintaining the potassium and calcium balance, (4) maintaining osmotic pressure, (5) activation of antioxidant defense systems and (6) counteracting stress conditions through hormonal regulation (Colla et al. 2010; Ulas et al. 2020; Aydın and Yetişir 2022). Several studies have examined the responses of grafted horticultural crops to salt stress and demonstrated that grafting onto salt tolerant rootstocks can improve salt tolerance in crops such as watermelon (Colla et al. 2006; Uygur and Yetisir 2009), cucumbers (Zhen et al. 2010; Aydın and Yetişir 2022), melon (Ulas et al. 2020), tomatoes (Martorana et al. 2007; Aydın 2024), peppers (Rastilantie et al. 2013), potatoes (Etehadnia et al. 2008), mandarine (Balal et al. 2017).

Salinity, which is a common abiotic stress, can occur when growing watermelon in arid or semi-arid regions. Watermelon seedlings can be grafted onto salt-tolerant rootstocks to reduce yield losses caused by salt stress. Watermelon can be grafted onto rootstocks belonging to the *C. moschata, Citrullus lanatus* var. *citroides, C. ficifolia* and *C. maxima* species, specifically *C. maxima* × *C. moschata* interspesific hybrids and *Lagenaria siceraria* species (hybrid and open pollinated rootstocks) (King et al. 2010). Generally known as the "white-flowered bottle gourd" the bottle gourd (*Lagenaria siceraria*) is a member of the *Cucurbitaceae* family. *L. siceraria* is one of the oldest (about 12,000 years) cultivated plants and is an annual monoecious plant (Robinson and Decker-Walters 1997). *L siceraria* is for Africa and widely distributed throughout the world, and ocean currents are thought to be responsible for its migration from Africa to the Americas (Kistler et al. 2014).

The bottle gourd's origin is being investigated by researchers from all over the world, including Turkey, to determine if it is African or Asian. According to Gürcan et al. (2015), Turkish bottle gourds are a mix of African and Asian varieties. Around the world, people are used the dried fruits of bottle gourds as utensils, musical instruments, or as fishing net holders (Robinson and Decker-Walters 1997). Bottle gourd leaves, shoots, seeds, and fresh sprouts are used in human nutrition or as a therapeutic agent (Loukou et al. 2011). Additionally, *L. siceraria* has the potential to be used as a rootstock for watermelon due to its tolerance to different biotic and abiotic stress factors such as Fusarium wilt (Yetişir et al. 2003), salt stress (Uygur and Yetisir 2009).

In watermelon species, however, salt resistance is dependent on uncertain and complex physiological interactions. It has been decades since the 'Quanneng Tiejia' (*C. moschata*; P1) rootstock was used in Northern China to grow watermelons. As a result, P1 is sensitive to many abiotic stresses, including salt stress, causing major yield losses in saline regions. In addition, the fruit quality of P1 was lower than that of *L. siceraria* or ungrafted watermelon when used as rootstock. 'Kaijia No. 1' (*C. moschata*; P2) and 'Hanzhen No. 3' (*L. siceraria*; G) rootstocks exhibit high growth ca-

Table 1 Plant material list

Hybrid Combinations	Hybrid Combinations	Female and Male Parent Lines	Controls
35-01 × 42-11	43-02 × 47-02	35-01	RS 841
35-01 × 43-02	43-02 × 56-01	39-01	Argentario
35-01 × 47-02	43-02 × 70-07	42-11	C. Tide
35-01 × 56-01	47-02 × 39-01	43-02	–
35-01 × 70-07	47-02 × 45-07	45-07	–
39-01 × 42-11	47-02 × 56-01	47-02	–
39-01 × 43-02	47-02 × 70-04	56-01	–
39-01 × 56-01	56-01 × 39-01	70-04	–
39-01 × 70-04	56-01 × 42-11	70-07	–
42-11 × 35-01	56-01 × 47-02	–	–
42-11 × 39-01	56-01 × 70-07	–	–
42-11 × 47-02	70-04 × 42-11	–	–
42-11 × 56-0	70-04 × 43-02	–	–
42-11 × 70-04	70-04 × 56-01	–	–
42-11 × 70-07	70-04 × 70-07	–	–
43-02 × 35-01	70-07 × 35-01	–	–
43-02 × 39-01	70-07 × 56-01	–	–
43-02 × 42-11	–	–	–

pacities, high yield capability, and tolerance to multiple stress conditions (Yan et al. 2018). Compared with ungrafted control plants, Crimson Tide watermelon variety grafted onto *C. maxima* and *L. siceraria* showed better vegetative growth under saline conditions (8 dS m^{-1}) (Uygur and Yetisir 2009). According to Colla et al. (2006), CO_2 assimilation decreased in both grafted and non-grafted plants with increasing salt concentration, and this decrease was greater in non-grafted plants. Grafted watermelons on pumpkins and bottle gourds lost less root dry weight in saline conditions than ungrafted ones (Colla et al. 2006; Uygur and Yetisir 2009). In Turkey and other countries, which are important watermelon producers, the rootstocks commonly used in watermelon are *C. maxima* and *C. moschata* hybrids. Some decreases in fruit quality have been reported due to rootstock interactions when *Cucurbita* hybrids were used as rootstocks. In terms of sustainability in watermelon production, it is important to develop plant materials that can be used as watermelon rootstocks. For this reason, studies on rootstock breeding in *Lagenaria* germplasm, which are not yet used sufficiently, are valuable.

In this study, the morphological, physiological, and biochemical tolerance levels of hybrid bottle gourds produced by crossing inbred lines developed from Turkish bottle gourd germplasm, which can be used as rootstocks for watermelons, were determined against salt stress under hydroponics conditions. Additionally, this study comprehensively evaluated the resistance of hybrid lines derived from Turkish bottle gourd germ plasm to salt stress for the first time.

Material and Method

Plant Materials and Experimental Site

The experiment was conducted in the fully automated Venlo type glass R&D greenhouse of Kırsehir Ahi Evran University in 2022. Thirty five hybrid rootstock candidate, nine *L. siceraria* paternal lines (male and female), two commercial rootstocks [RS841 (*C. maxima* × *C. moschata*), Argentario (*L. siceraria)*] and Crimson Tide (*Citrullus lanatus*) watermelon variety were used as plant materials (Table 1). Commercial rootstocks and watermelon cultivars were used for comparison purposes.

Seedling Production and Establishment of The Experiment

Forty five seeds from each genotype were sown in multipot filled with peat: perlite (2v:1v) mixture in greenhouse conditions. The seedlings were planted in the hydroponic system consisting of 136 L plastic containers on June 14, 2022, when they had 2–3 true leaves. The nutrient solution used to grow plants was regularly aerated by a timed air pump. Modified nutrient solution was used according to Hoagland and Arnon (1950). The nutrient solution contained 1125 µM $Ca(N0_3)_2$, 375 µM $(NH_4)_2SO_4$ 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O_4$ and 80 µM Fe EDDHA. During the experiment, two different EC levels (1.8 dS m^{-1} and 10 dS m^{-1}) were used. The salt application was done gradually in an increasing manner (2 dS m^{-1} per day) 5 days after transplanting. The experiment was

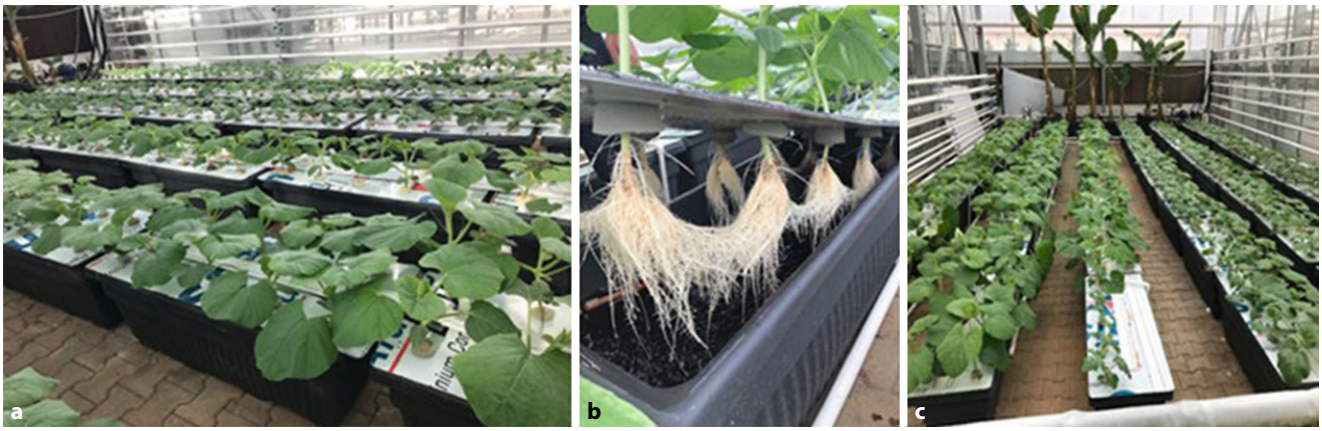

Fig. 1 Growing plants (bottle gourd rootstocks) in the hydroponic system under control and salt stress conditions

designed according to the randomized plot design with 3 replications and 3 plants in each replication (136 L pots of 1 m length are arranged in rows and the plants were planted 50 × 50 cm on tyrofoam plate) (Fig. 1). The study was continued for 30 days under controlled greenhouse conditions (22–24 °C day/16–18 °C night and 65% relative humidity).

Plant Growth Measurements

After four weeks of growing, plants were harvested and separated into shoots and roots. To determine shoot and root dry weight (g), plant materials were dried in a forced-air oven for 48 h at 65 °C. The main stem diameter (mm) was measured using a caliper. Main stem length (cm) was measured using a meter rule. The number of leaves per plant was counted in units. The root length (m), of the plants was determined by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada).

Chlorophyll a, b, Total Chlorophyll, Carotenoid Analysis and Photosynthesis Activity

Total chlorophyll, chlorophyll a, b were determined according to Arnon (1949) and total carotenoids were determined according to Witham et al. (1971) by reading on a spectrophotometer (Shimadzu 1208 Model, Tokyo, Japan) in three replicates from each application. Portable Photosynthesis System LI-COR was used to measure the photosynthesis activity of various plant samples every week commencing from the 2nd week to the 4th week.

Hormone Analysis in Plant Leaf and Root Tissues

Extraction and purification procedures were performed according to the protocol of Kuraishi et al. (1991). 80% methanol at –40 °C was added to one gram of fresh leaf sample. After homogenizing for 10 min using an Ultra-Turrax (IKA, T-25) homogenizer, the solution was incubated for 24 h in a dark environment and the samples were dried with evaporator pumps at 35 °C. Dried samples were solubilized using 0.1 M KHPO (pH 8.0) and a Sep-Pak C-18 (Waters) cartridge was used for further specific separation. The hormones adsorbed by the cartridge were transferred to the tubes using 80% methanol. Hormones were analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and the mobile phase was adjusted to pH 4.98 with 13% acetonitrile, the flow rate was 1.2 ml min^{-1} and the column temperature was 25 °C. A UV detector at 265 nm was used for the detection of gibberellic acid (GA_3), salicylic acid (SA), indole acetic acid (IAA), abscisic acid (ABA) (Turan et al. 2014).

Plant Nutrient Analysis in Leaf and Root Tissues

After harvest, fresh plant material was divided into two parts. One part was frozen in liquid nitrogen and stored at 80 °C for later use. The remaining fresh plant material was dried at 65 °C for 24 h. For determination of N, P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cu, Na, and Cl concentrations, 100 mg dried plant material was extracted by one hour boiling in 5 ml Milli-Q water. The solution was filtered through 0.2 mm filters (Whatman, England), and N, P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cu, Na, and Cl contents in the filtrate were analyzed using high-performance liquid chromatography (HPLC, Shimadzu Japan). The HPLC system was equipped with a ø 4.6 mm 6125 mm Shodex IC YS-50 column (Showa Denko). As an eluent, 4.0 mM methane sulfonic acid was used in HPLC-graded H_2O (J.T. Baker, The Netherlands) with a flow rate of 1 $mlmin^{-1}$. Final ion concentrations in the filtrate were calculated according to a calibration curve (Szalay et al. 2013; Bremner 2018; Gencer and Serçe 2022).

Table 2 Photosynthesis amount of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions

Genotype	Photosynthesis (μmol $CO_2/cm^2/s$)		
	Control	NaCl	% Change
35-01 × 42-11	23.17a-d	7.93f-j	66
35-01 × 43-02	21.77a-e	9.11c-j	58
35-01 × 47-02	20.57a-e	8.23e-j	60
35-01 × 56-01	24.00a-b	6.55 h-k	73
35-01 × 70-07	21.33a-e	11.17b-h	48
39-01 × 42-11	22.97a-d	13.03a-d	43
39-01 × 43-02	22.83a-d	12.20a-f	47
39-01 × 56-01	23.07a-d	16.17a	30
39-01 × 70-04	23.63a-d	7.97f-j	66
42-11 × 35-01	19.77a-e	9.00c-j	54
42-11 × 39-01	17.93c-e	12.87a-e	28
42-11 × 47-02	20.37a-e	16.47a	19
42-11 × 56-01	22.33a-e	8.99 d-j	60
42-11 × 70-04	20.90a-e	13.67a-c	35
42-11 × 70-07	22.17a-e	5.29jk	76
43-02 × 35-01	24.77ab	14.27ab	42
43-02 × 39-01	19.23a-e	2.39k	88
43-02 × 42-11	19.80a-e	5.95i-k	70
43-02 × 47-02	20.90a-e	6.99 h-k	67
43-02 × 56-01	25.47a	9.60b-j	62
43-02 × 70-07	17.23de	7.28 g-j	58
47-02 × 39-01	20.43a-e	8.18f-j	60
47-02 × 45-07	19.73a-e	10.33b-i	48
47-02 × 56-01	20.40a-e	10.33b-i	49
47-02 × 70-04	22.43a-e	11.83a-g	47
56-01 × 39-01	20.47a-e	9.00c-j	56
56-01 × 42-11	18.83a-e	9.00c-j	52
56-01 × 47-02	17.87c-e	11.83a-g	34
56-01 × 70-07	18.00c-e	11.97a-f	34
70-04 × 42-11	18.87a-e	12.87a-e	32
70-04 × 43-02	19.10a-e	11.00b-h	42
70-04 × 56-01	19.33a-e	10.13b-i	48
70-04 × 70-07	22.27a-e	10.05b-i	55
70-07 × 35-01	20.03a-e	9.17c-j	54
70-07 × 56-01	19.57a-e	10.00b-i	49
35-01	19.33a-e	9.48c-j	51
39-01	19.57a-e	9.83b-j	50
42-11	19.93a-e	9.55c-j	52
43-02	20.83a-e	8.92 d-j	57
45-07	18.12b-e	9.25c-j	49
47-02	21.50a-e	6.92 h-k	68
56-01	21.63a-e	6.01i-k	72
70-04	17.10de	7.13 h-j	58
70-07	18.07b-e	8.66 d-j	52
RS 841	17.44c-e	8.87 d-j	49
Argentario	20.43a-e	9.75b-j	52
C. Tide	15.70e	6.70 h-k	57
Genotype	***		
NaCl	***		
Genotype*NaCl	***		

Means that do not share a letter are significantly different

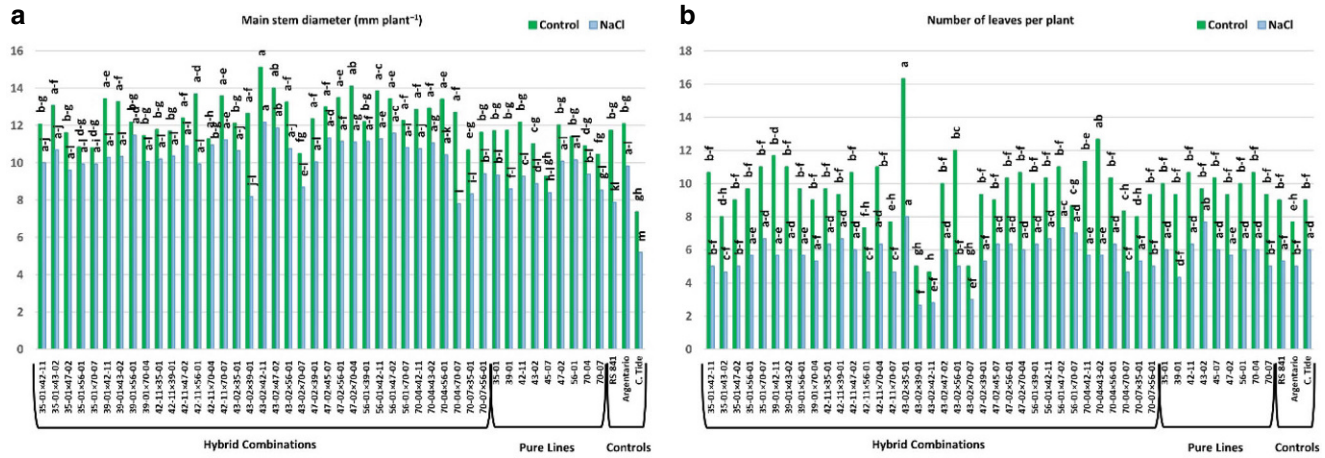

Fig. 2 Main stem diameter (**a**) and number of leaves per plant (**b**) of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

Statistical Analysis

Statistical analysis of the data was performed using the PROC GLM procedure of the SAS Statistical Software (SAS for Windows 9.1, SAS Institute Inc., Cary, NC, USA). A two-factor analysis of variance was performed to study the effects of genotype or grafting combination and salt and their interactions on the variables analyzed. The levels of significance are represented at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), or n. s. as not significant (F-test and Pearson correlation coefficients). Differences between the treatments were analyzed using Duncan's multiple range test ($p < 0.05$). Classification of genotypes was achieved by correlation and principal component analysis (PCA) using XLSTAT software (XLSTAT, New York, USA). Correlation analysis was done in SPSS 17.0 program.

Results

Plant Biomass

In plants, stem diameter and number of leaves per plant were significantly affected by genotype, salt, and genotype × salt interaction ($p < 0.001$) (Table 2). Salt application reduced stem diameter by 18% and the number of leaves per plant by 41% on average compared to control plants. In plants grown under control conditions, the highest stem diameter was measured in 43-02 × 42-11 and 47-02 × 70-04 hybrid combinations, while the lowest stem diameter was measured in the commercial watermelon variety (C. Tide) used as control. The highest stem diameter was measured under salt stress in the 43-02 × 42-11 hybrid combination, just as in control conditions, while the lowest stem diameter was determined in the C Tide watermelon variety. A significant portion of hybrid gourds (25) had thicker stems than commercial rootstocks and watermelon varieties under salt stress. Under control and saline conditions, the 43-02 × 35-01 hybrid combination produced the highest number of leaves. In control conditions, the 43-02 × 42-11 hybrid combination had the lowest number of leaves, and in saline conditions, the 43-02 × 39-01 hybrid combination had the lowest number of leaves. The number of hybrids with more leaves than commercial rootstocks and watermelon variety under salt stress was six. (Figure 2a, b).

Significant variation was detected among genotypes in terms of main stem length and root length both under control and salt stress conditions (Fig. 4). With salt application, reductions in plant length and root length were detected at different rates depending on the genotype. The average reduction recorded in plant and root length was 68 and 45%, respectively. (Table 2). In saline conditions, lines 39-01 and 47-02 had the shortest stem lengths among pure lines used as parents. The longest main stems in saline conditions were measured in 56-01 × 47-02, 70-04 × 43-02 and 35-01 × 70-07 hybrid combinations, respectively, while the shortest stems were measured in 43-02 × 42-11 and 43-02 × 70-07 hybrid combinations. Among hybrid combinations, the 43-02 × 35-01 and 42-11 × 70-04 hybrids had the longest roots, while the C. Tide variety produced the shortest roots. Under salt stress conditions, the longest root was measured in 70-04 × 43-02 hybrid combinations, while the shortest root was measured in C. Tide plants as in control conditions (Fig. 3a, b).

Genotypic variation was found to be significant in shoot and root dry weights both under control conditions and salt application ($p < 0.001$) (Table 2). With the application of salt stress, significant decreases in the dry weight of both shoots (63%) and roots (43%) were detected. This decrease was more evident in shoot dry weight by 63%

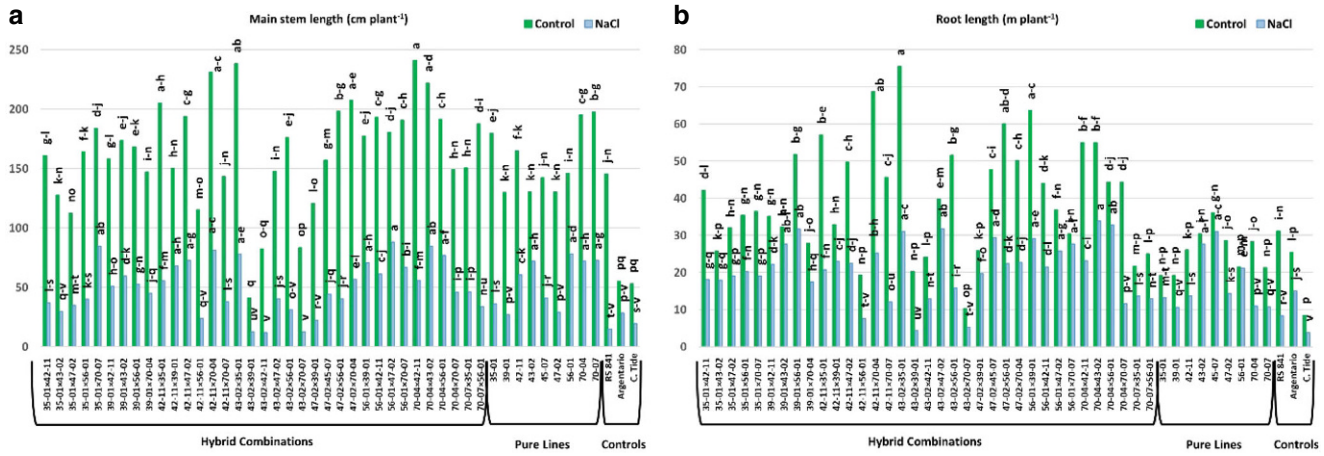

Fig. 3 Main stem length (**a**) and root length (**b**) of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

(Fig. 5a, b). Under control conditions, the highest shoot dry weight was recorded in 43-02 × 35-01, 70-04 × 43-02, and 70-04 × 42-11 hybrid combinations, while C. Tide plants produced the lowest shoot dry weight. Under salt stress, the 43-02 × 35-01 hybrid combination had the highest shoot dry weight, followed by the 56-01 × 47-02 hybrid and the 35-01 × 70-07 hybrid, respectively. Under salt stress, the lowest shoot fresh weight was recorded in C. Tide and 43-02 × 70-07 plants. Among pure lines under salt stress conditions, line 39-01 had the lowest shoot dry weight. Under saline conditions, Noticeably, a total of 22 gourds genotypes (hybrid and parent) produced higher shoot dry matter than commercial rootstock varieties and watermelon under salt stress (Fig. 4a, b).

As seen in Fig. 5b, root length was significantly affected by both genotype and salt application ($p < 0.001$) (Table 2). Under control conditions, the longest root was determined in the reciprocal hybrids of 56-01 × 39-01, while the shortest roots were measured in the C. Tide watermelon variety. Under control conditions, a significant portion (24) of the gourd genotypes (hybrids and parental lines) had longer roots than the commercial control varieties. Similarly to control conditions, the highest root dry weight was determined in the 56-01 × 39-01 hybrid combination, and the lowest root length was measured in the 42-11 × 56-01 hybrid combination. Under salt stress, 22 bottle gourd genotypes produced longer roots than commercial rootstocks and watermelon varieties, while the number of bottle gourd hybrids that produced longer roots than the parentel lines was 11.

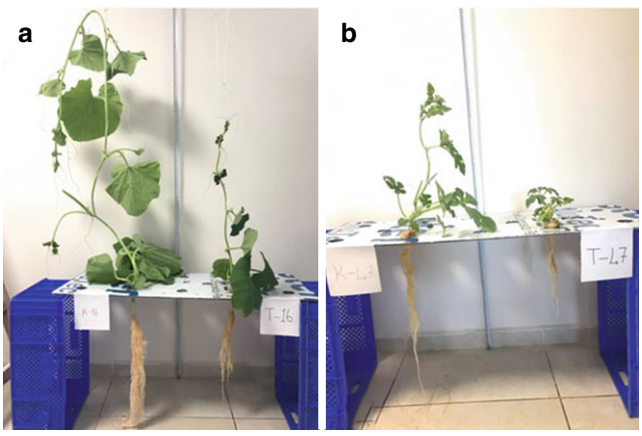

Fig. 4 **a** *Lagenaria* hybrid 43-02 × 35-01 grown under control (left) and saline conditions (right) 43-02 × 35-01 (*L. siceraria*), **b** Watermelon cultivar (C. Tide) grown control (left) and saline conditions (right)

Leaf Clorophyll and Carotenoid Content

Chlorophyll and carotenoid contents of plants grown under control and salt stress are given in Fig. 6. While there was no significant difference in chlorophyll content under control conditions, under salt stress the amount of chlorophyll increased significantly depending on the genotypes. Salt stress increased the chlorophyll content of leaves. Due to the effect of salt on the roots, this increase is thought to be caused by a decrease in the plant water intake. Chlorophyll-a contents under saline conditions was highest in the hybrid 47-02 × 45-07 combination and lowest in the commercial rootstock RS 841 variety. Under salt stress, chlorophyll-a content increased by an average of 25% in forty-seven genotypes; chlorophyll-b content increased by an average of 68%. Chlorophyll-a, chlorophyll-b and total chlorophyll contents were significantly affected by genotype, salt and genotype × salt interaction ($p < 0.001$). While carotenoid content was significantly affected by genotype and genotype × salt interaction ($p < 0.001$), it was not affected by salt application ($p > 0.05$) (Table 2). The RS-841 variety had the lowest chlorophyll-b content under salt stress conditions, while 39-01 × 56-01 hybrid combination

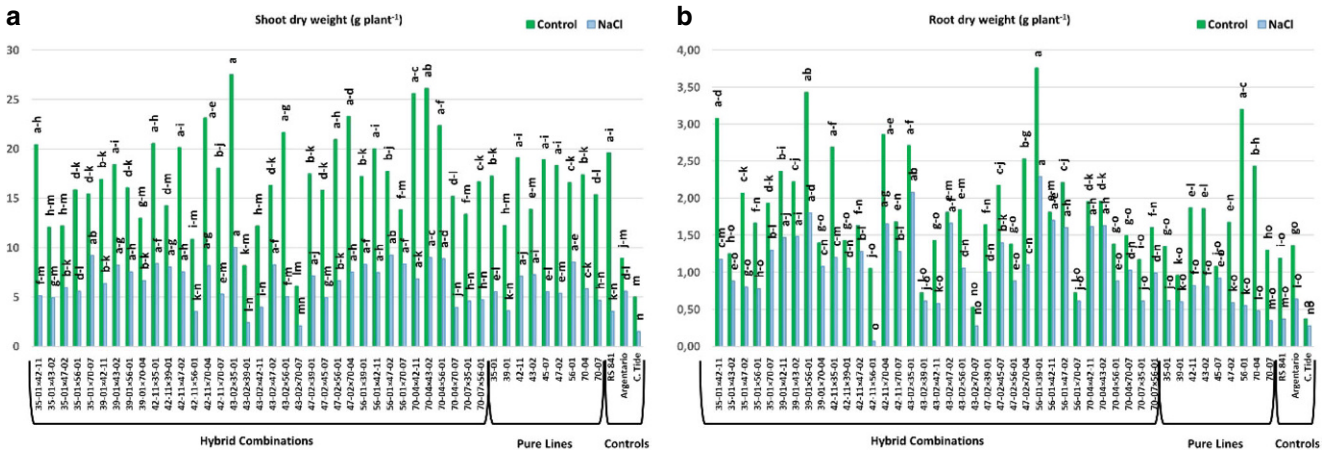

Fig. 5 Shoot (**a**) and root (**b**) dry weight of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

had the highest level. Salt-stressed plants with the highest total chlorophyll content were hybrid combinations 47-02 × 70-04 and 56-01 × 39-01 (Fig. 6a, b, c). The carotenoid content of the leaves showed significant genotypic differences in both control conditions and salt stress. The carotenoid content decreased in 22 genotypes under saline conditions, while twenty-five genotypes showed an increase. This suggests that saline conditions can modify the carotenoid content of plants. The commercial rootstock Argentario had the highest carotenoid content, while the 70-07 × 35-01 hybrid combination had the lowest carotenoid content. This suggests that the carotenoid content of rootstocks can be manipulated by selecting an appropriate rootstock (Fig. 6d).

Photosynthesis Activity

With the effect of salt stress, there was a decrease between 19 and 88% in the photosynthesis levels of plants compared to control plants. Under control conditions, the highest amount of photosynthesis was measured in genotype number 43-02 × 56-01 (25.47 µmol $CO_2/cm^2/s$). The lowest amount of photosynthesis was measured in watermelon plant C. Tide (15.70 µmol $CO_2/cm^2/s$). In plants under salt stress, the highest amount of photosynthesis was measured in genotypes 39-01 × 56-01 (16.17 µmol $CO_2/cm^2/s$) and 42-11 × 47-02 (16.47 µmol $CO_2/cm^2/s$), respectively. The lowest amount of photosynthesis in saline conditions was measured in genotypes 43-02 × 39-01 (2.39 µmol $CO_2/cm^2/s$) and 42-11 × 70-07 (5.29 µmol $CO_2/cm^2/s$) (Table 2).

Leaf and Root Plant Hormone Content

IAA and ABA contents of leaves and roots are given in Fig. 7. IAA and ABA contents showed significant differences both under control conditions and salt stress ($p <$ 0.001) (Table 2). In thirty five genotypes, salt application increased the content of indole acetic acid (IAA) in the leaves, while it was decreased in twelve genotypes. There was an increase in IAA in the roots of eighteen genotypes and a decrease in the roots of twenty-nine genotypes. Under saline conditions, in IAA content was highest in both leaves and roots of hybrid combination 35-01 × 43-02 (Fig. 7a, b).

As shown in Fig. 7c, d, ABA content of leaves and roots showed significant genotypic variation in both control and salt stress conditions. An increase in leaf ABA content was observed in 18 of the genotypes used in the experiment. In the leaves of salt-stressed plants, the highest ABA content was recorded in 56-01 × 42-11 hybrid combination, while 47-02 × 56-01 and 39-01 × 56-01 hybrid combinations produced the lowest ABA content. As in leaf ABA content, root ABA content was significantly affected by both salt application, genotypes and genotype × salt interaction ($p < 0.001$) (Table 2). Salt stress caused an increase in the amount of root ABA in 25 genotypes. Root ABA levels were highest in the hybrid combination 43-02 × 47-02, while lowest in the hybrid combination 70-04 × 70-07 under saline conditions.

The contents of GA_3 and SA in leaves and roots of plants grown under salt stress and control conditions were significantly affected by genotype, salt, and genotype × salt interaction ($p < 0.001$) (Table 2). In salt stress application, 38 genotypes showed an increase in gibberellic acid (GA_3) in leaf tissues, while 39 genotypes showed a decrease in gibberellic acid in root tissues. 47-11 × 70-04, 43-02 × 56-01 and 42-11 × 56-01 hybrid combinations had the highest GA_3 content in the leaves of plants grown in saline conditions, while 56-01 × 42-11 hybrid produced lowest GA_3 in leaf. Under saline conditions, 70-04 × 70-07 and 70-04 × 56-01 hybrid combinations had the highest root GA_3 content, while a notable decrease was detected in 15 genotypes, including parental lines, commercial rootstocks and watermelon (Fig. 8a, b). While salt stress application caused an

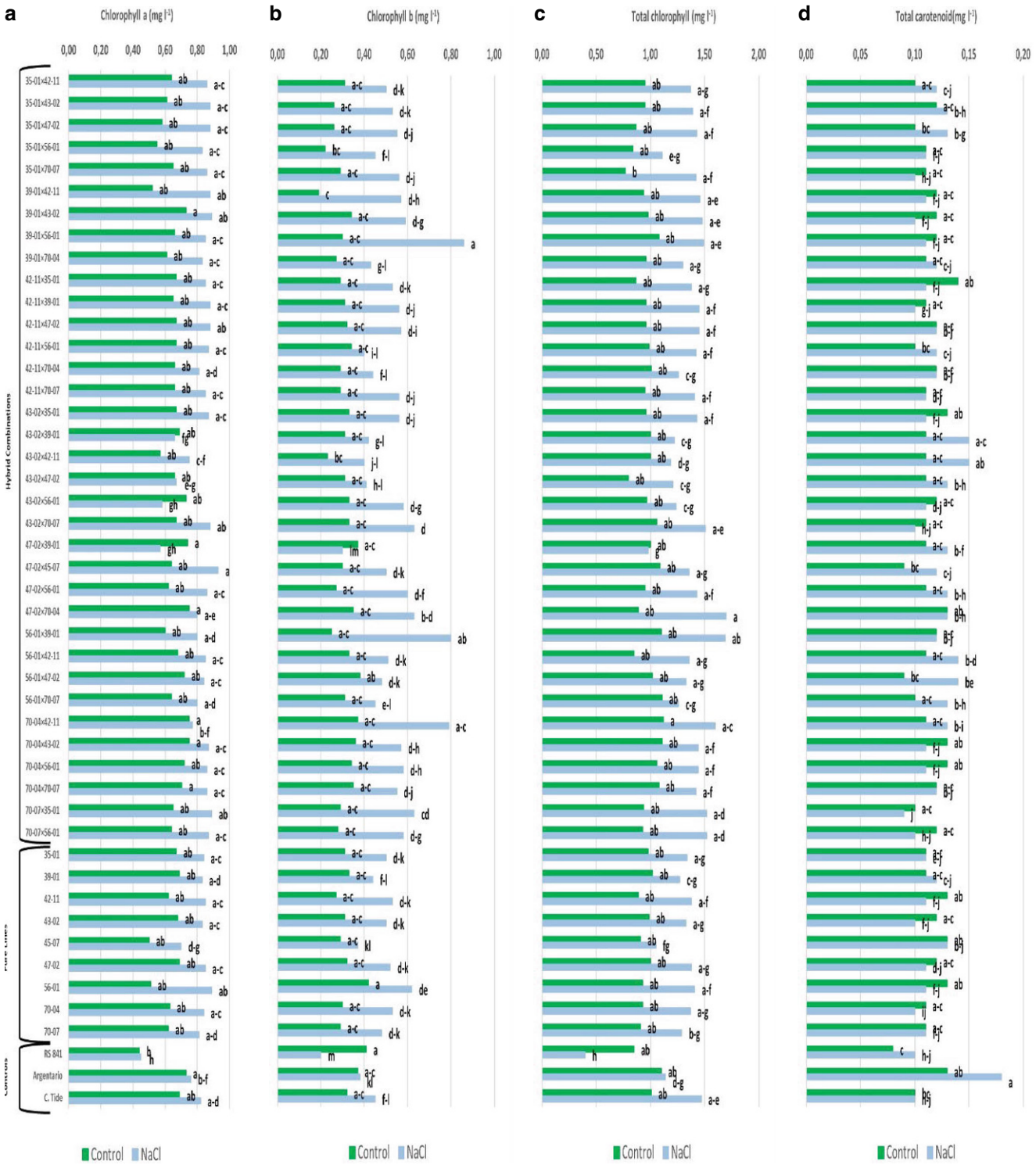

Fig. 6 Leaf chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**) and carotenoid (**d**) content of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

increase in leaf SA content in 27 genotypes, there was a decrease in the other genotypes. Salicylic acid levels in the leaf tissues of plants grown in saline conditions were highest in the 35-01 × 43-02 hybrid, and lowest in the 42-11 × 35-01 hybrid. The SA content of the roots was significantly affected by salt stress and except for 5 genotypes (70-04 × 43-02, 70-04 × 56-01, 70-04 × 70-07, 43-02 and 45-07), all genotypes under salt stress showed a decrease in root SA content. 70-04 × 56-01 and 70-04 × 70-07 hybrids had the

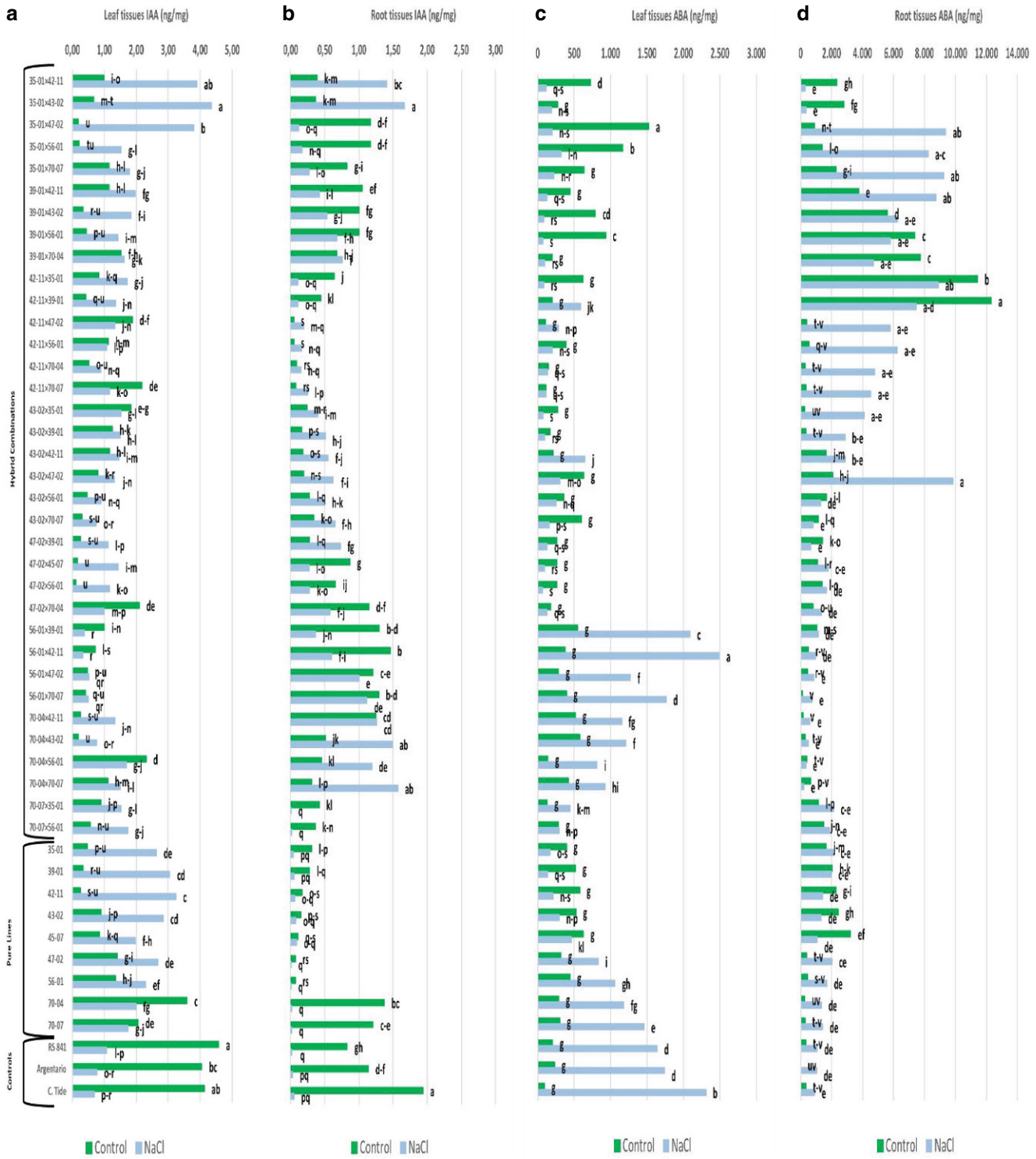

Fig. 7 Indole acetic acid (IAA) (**a**, **b**) and abscisic acid (ABA) (**c**, **d**) content in leaf and root tissues of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

highest root SA content under salt stress, while 47-02 pure line had the lowest SA in roots (Fig. 8c,d).

Table 3 shows the investigated parameters [biomass, chlorophyll, carotenoid content of leaf tissues, indole acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA_3), salicylic acid (SA) content of root and leaf tissues] of plants grown under control and saline conditions, as well as their percentage changes under salt stress. Shoot dry weight was 16.74 g in control conditions, but 6.26 g in salty conditions. Shoot and root dry weights were reduced by

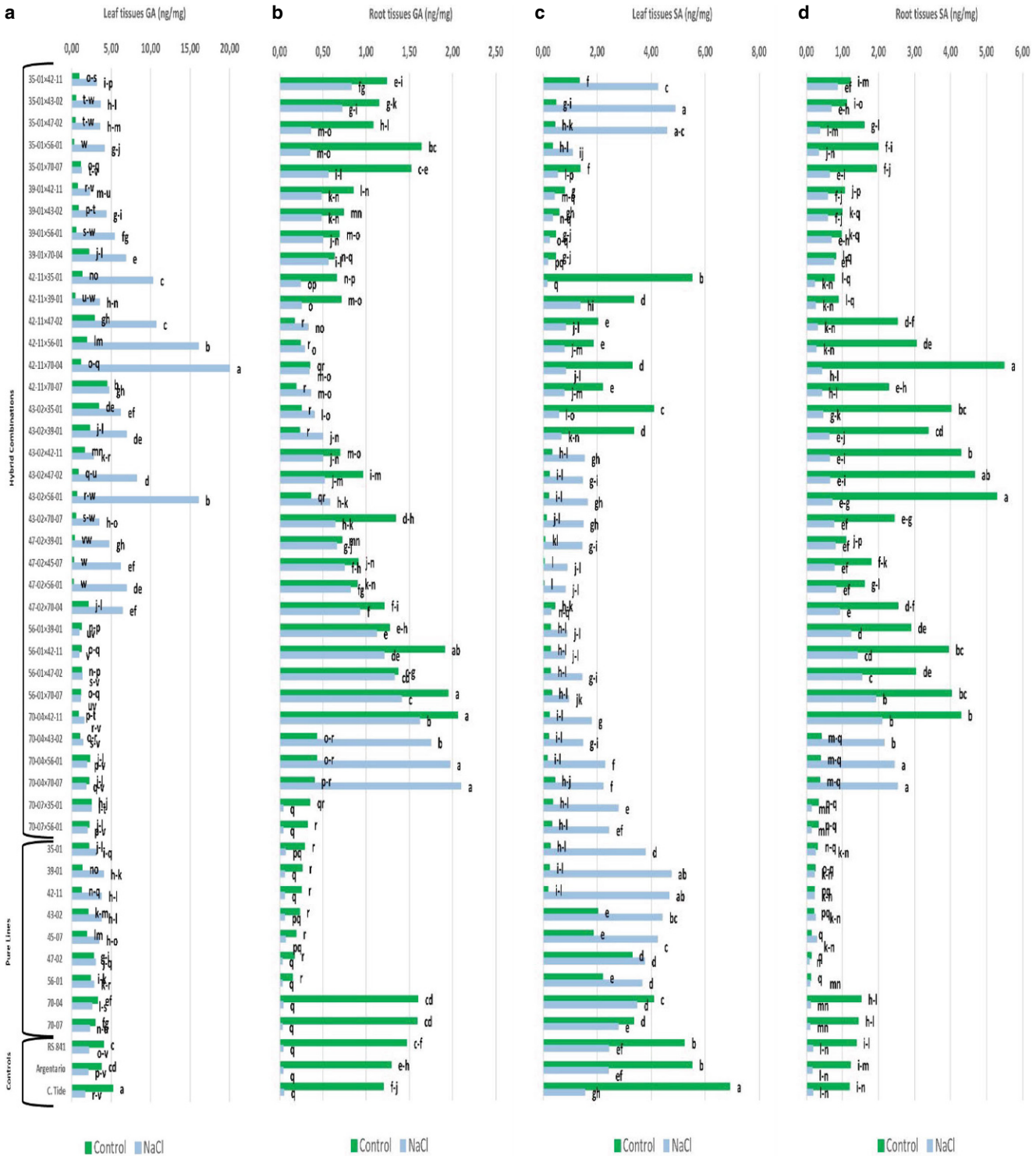

Fig. 8 Gibberellic acid (GA_3) (**a**, **b**) and salicylic acid (SA) (**c**, **d**) content in leaf and root tissues of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different

63 and 43%, respectively, by salt stress. As a result of salt stress, the main stem diameter decreased by 18% and the number of leaves per plant decreased by 41%. Plants under control conditions had a main stem length of 158.24 cm and a root length of 36.44 m, but under salty conditions they had a main stem length of 50.17 cm and a root length of 19.90 m. Generally, salt stress increases chlorophyll-a (25%), chlorophyll-b (68%), total chlorophyll (39%), and carotenoid content (3%). The salt stress caused a decrease in leaf tissue content of IAA (3%), ABA (63%), and root

Table 3 Average of biomass parameters, chlorophyll, carotenoid contents and hormone contents in leaf and root tissues of plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions, % change and *P* value

Parameters	Control	NaCl	% Change	Genotype	NaCl	Genotype*NaCl
Shoot dry weight	16.74	6.26	–63	***	***	***
Root dry weight	1.79	1.02	–43	***	***	***
Main stem diameter	12.16	10.00	–18	***	***	***
Number of leaves per plant	9.63	5.64	–41	***	***	***
Main stem length	158.24	50.17	–68	***	***	***
Root length	36.44	19.90	–45	***	***	***
Chlorophyll-a	0.65	0.82	25	***	***	***
Chlorophyll-b	0.31	0.52	68	***	***	***
Total chlorophyll	0.97	1.35	39	***	***	***
Total Carotenoid	0.11	0.12	3	***	n. s.	***
Leaf IAA	1.70	1.64	–3	***	***	***
Root IAA	0.63	0.45	–28	***	***	***
Leaf ABA	1667.17	611.53	–63	***	***	***
Root ABA	2096.85	3081.17	47	***	***	***
Leaf GA	1.77	5.26	196	***	***	***
Root GA	0.82	0.56	–32	***	***	***
Leaf SA	1.52	1.94	27	***	***	***
Root SA	1.84	0.69	–63	***	***	***

Values denoted by different letters are significantly different between genotypes within both columns at $P<0.05$. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$, n. s. not significant, indole acetic acid IAA, abscisic acid ABA, gibberellic acid GA_3, and salicylic acid SA

tissue content of IAA (28%), GA_3 (32%), and SA (63%), while it increased leaf tissue content of GA_3 (196%), SA (27%), and root tissue content of ABA (47%).

Leaf and Root Plant Nutrient Element Content

Figure 9 shows the K/Na (potassium/sodium) and Ca/Na (calcium/sodium) ratios in leaf and root tissues of plants grown in control and saline conditions. K/Na and Ca/Na ratios in the leaf and root tissues of plants grown under both control and saline conditions were significantly affected by genotypes, salt treatment and genotype × salt interaction ($P<0.01$) (Table 4). Salt application caused a decrease in the K/Na ratio of leaves in 39 genotypes. 35-01 genotype and 70-04 × 70-07 hybrid had the highest K/Na ratio in leaf tissues, while 56-01 and RS 841 rootstocks had the highest K/Na ratio in root tissues (Fig. 9a, b). Among the hybrid combinations tested under salt stress, the 42-11 × 39-01, 42-11 × 56-01 and 35-01 × 42-11 hybrid combinations had the highest leaf K/Na ratios, while the 42-11 × 35-01 hybrid combinations had the lowest ratio. Under saline conditions, hybrid combinations 56-01 × 47-02 and 35-01 × 47-02 had the highest root K/Na ratios. Under control conditions, the 70-04 × 70-07 hybrid combination had the highest leaf Ca/Na ratio, while the 43-02 × 70-07 hybrid combination had the highest root Ca/Na ratio. Under salt stress, the highest leaf Ca/Na ratio was determined in 35-01 × 42-11 and 35-01 × 56-01 hybrid combinations, while the highest Ca/Na ratio in root tissues was determined in 56-01 × 47-02, 39-01, and 42-11 plants (Fig. 9c, d).

Table 4 shows the nitrogen (N), phosphorus (P), potassium, calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), iron (Fe), zinc (Zn), boron (B) in root and leaf tissues, copper (Cu), sodium (Na), chlorine (Cl) and copper (Cu) contents, K/Na and Ca/Na ratios of root and leaf tissues of plants grown under control and saline conditions, as well as their percentage changes under salt stress. Salt stress reduced the N content of the genotypes' leaf and root tissues by 32 and 40%, respectively. Under control conditions, mean leaf P content was 0.17 mg kg^{-1}, K content is 0.91 mg kg^{-1}, while root P and K content are 0.18 and 0.60 mg kg^{-1}. Salt stress caused a 19% decrease in Ca content in plant leaf tissues and a 2% decrease in root tissue. There was a reduction in mean leaf Mg (38%), S (58%), Mn (45%), Fe (36%) and root Mg (15%), S (7%), Mn (27%), Fe (39%), Zn (44%) and B (25%) contents in salt-stressed plants compared to control plants, but an increase in leaf mean Na (13248%), Cl (2202%), and root; Na (2069%) and Cl (5898%). As a result of salt stress, leaf K/Na and Ca/Na ratios decreased by 100%, while root K/Na and Ca/Na ratios decreased by 97%.

Principal Component Analysis of Nutrient Element Content

Based on plant nutritional elements in leaf tissues under salt stress, 35 hybrid combinations, 9 parental lines, 2 commer-

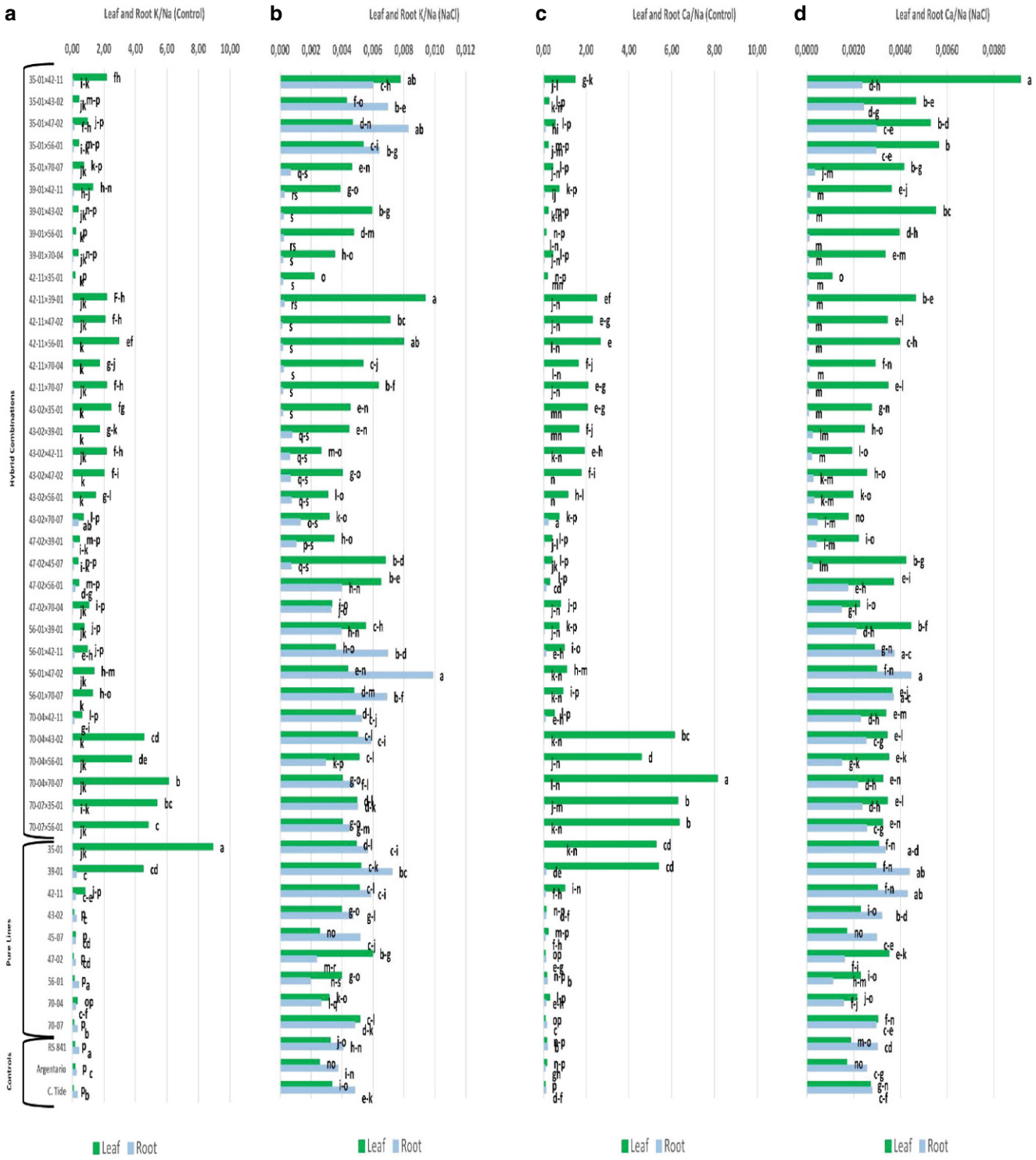

Fig. 9 Leaf and root tissues K/Na (**a**, **b**) and leaf and root tissues Ca/Na (**c**, **d**) rate of bottle gourd and control plants grown in control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions. Means that do not share a letter are significantly different, *K/Na* potassium/sodium, *Ca/Na* calcium/sodium

cial rootstocks used as controls, and C. Tide watermelon cultivars were categorized using principal component analysis (PCA) (Fig. 10a). Two principal components (68.22% for PC1 and 26.59% for PC 2) explained 94.81% of the variation. Plants were positioned on the coordinate plane in different regions based on the nutrients they contained in their leaves. Plants that stand out in terms of leaf K/Na, Ca/Na, Ca, and P contents are located in the I. region of the graph. As shown in the III. region of the graph, genotypes with the highest levels of Zn, Fe, B, Cu, and Cl nutritional

Table 4 Mean values of nutrient contents in leaf and root tissues of plants grown under control (1.8 dS m^{-1}) and NaCl (10 dS m^{-1}) conditions, % change and *P* value

Parameters	Control	NaCl	% Change	Genotype	NaCl	Genotype*NaCl
Leaf N	1.76	1.20	–32	***	***	***
Root N	0.83	0.50	–40	***	***	***
Leaf P	0.17	0.18	4	***	***	***
Root P	0.09	0.09	8	***	***	***
Leaf K	0.91	1.52	66	***	***	***
Root K	0.60	0.64	7	***	***	***
Leaf Ca	0.82	0.67	–19	***	***	***
Root Ca	0.35	0.34	–2	***	***	***
Leaf Mg	0.21	0.13	–38	***	n. s.	***
Root Mg	0.08	0.07	–15	***	***	***
Leaf S	0.23	0.10	–58	***	***	***
Root S	0.06	0.06	–7	***	***	***
Leaf Mn	25.37	13.89	–45	***	***	***
Root Mn	9.64	7.06	–27	***	***	***
Leaf Fe	30.39	19.39	–36	***	***	***
Root Fe	33.84	20.62	–39	***	***	***
Leaf Zn	3.53	4.02	14	***	***	***
Root Zn	10.77	6.08	–44	***	***	***
Leaf B	2.42	2.93	21	***	***	***
Root B	2.81	2.11	–25	***	***	***
Leaf Na	1.60	214.20	13248	***	***	***
Root Na	9.22	200.03	2069	***	***	***
Leaf Cl	3.61	83.13	2202	***	***	***
Root Cl	3.74	224.15	5898	***	***	***
Leaf Cu	3.70	0.56	–85	***	***	***
Root Cu	0.40	0.12	–69	***	***	***
Leaf K/Na	1.63	0.0047	–100	***	***	***
Root K/Na	0.12	0.0033	–97	***	***	***
Leaf Ca/Na	1.61	0.0033	–100	***	***	***
Root Ca/Na	0.06	0.0016	–97	***	***	***

Values denoted by different letters are significantly different between genotypes within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, *n. s.* not significant, *B* boron, *Cl* chlorine, *Cu* copper, *Fe* iron, *Zn* zinc, *Na* Sodium, *Mg* magnesium, *Mn* Manganese, *N* nitrogen, *K* potassium, *S* sulfur, *P* phosphorus, *Ca* calcium, *K/Na* potassium/sodium, *Ca/Na* calcium/sodium

elements in leaf tissues were found, whereas genotypes with the highest levels of S, K, N, Mn, Mg, and Na nutritional elements in leaf tissues were found in the IV. region. The plants (especially 35-01 × 42-11, 35-01 × 70-07, 35-01 × 56-11, 39-01 × 42-11, 39-01 × 43-02 and 39-01 × 70-04) in the first region of the graph are the plants that contain the highest amount of leaf plant nutrients in this region. The plants in the third region of the graph are the plants that contain the lowest levels of plant nutrients Ca, P, K, and S in their leaf tissues. The highest leaf Na content was found in plants (especially 42-11, 47-02, 70-07, and C. Tide), located in regions III. and IV. of the graph. Plants in regions I. and II. of the graph are the plants with the lowest Na and Cl contents in their leaf tissues (Fig. 10a, b).

Based on plant nutritional elements in root tissues under salt stress, 35 hybrid combinations, 9 parental lines, 2 commercial rootstocks used as controls, and C. Tide watermelon cultivars were categorized using principal component analysis (PCA) (Fig. 11a). Two principal components (69.21% for PC1 and 28.74% for PC 2) explained 97.85% of the variation. While there are plants with high root Na and Cl content in the first region of the graph, especially pure lines and plants used as control are located in this region. In the III. region of the graph, plants with the lowest Na and Cl content in their root tissues are located. In the IV. region of the graph, the plants containing the highest plant nutritional elements (B, Ca, Fe S, K, N, Mn, Mg, Zn, and P) in their root tissues are located. Additionally, plants in this region had the highest root K/Na and Ca/Na ratios (Fig. 11a, b).

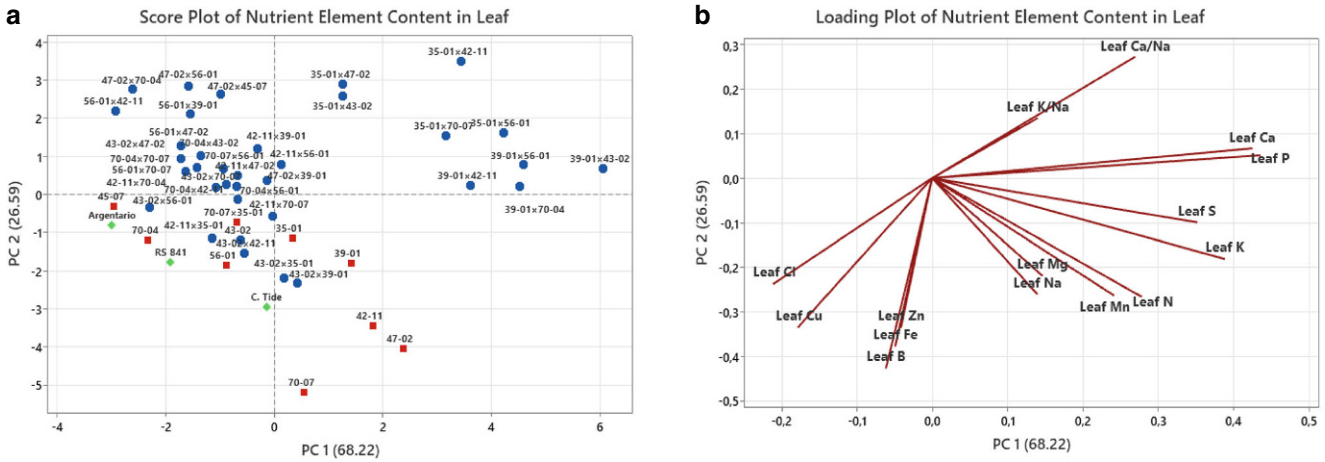

Fig. 10 PCA of bottle gourd and control plants grown under salt stress (10 dS m^{-1}) conditions based on nutrient element content in leaf tissues. *PCA* , principal component analysis; *B* boron, *Cl* chlorine, *Cu* copper, *Fe* iron, *Zn* zinc, *Na* Sodium, *Mg* magnesium, *Mn* Manganese, *N* nitrogen, *K* potassium, *S* sulfur, *P* phosphorus, *Ca* calcium, *K/Na* potassium/sodium, *Ca/Na* calcium/sodium

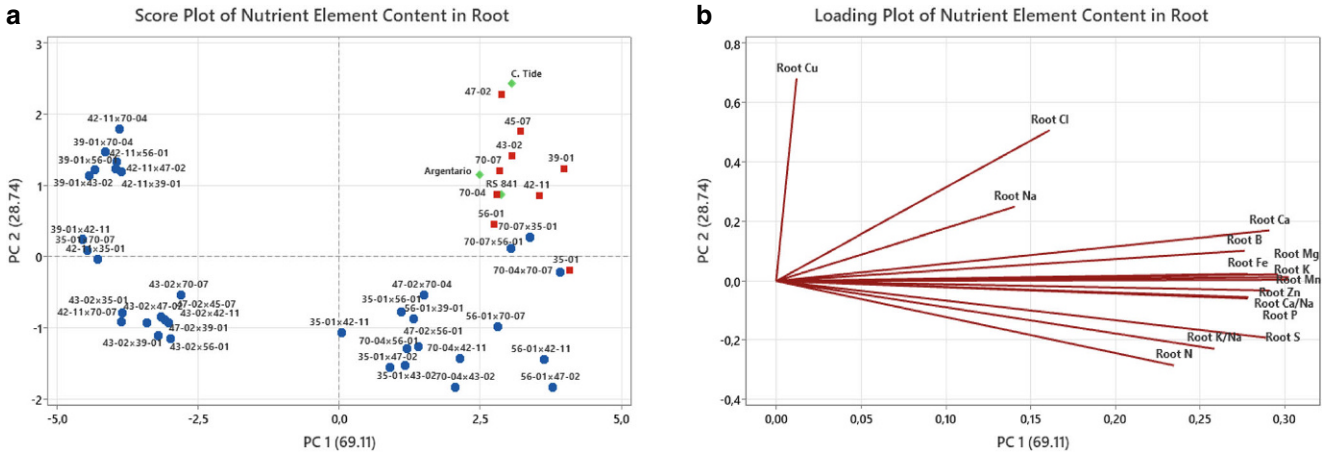

Fig. 11 PCA of bottle gourd and control plants grown under salt stress (10 dS m^{-1}) conditions based on nutrient element content in root tissues. *PCA* principal component analysis, *B* boron, *Cl* chlorine, *Cu* copper, *Fe* iron, *Zn* zinc, *Na* Sodium, *Mg* magnesium, *Mn* Manganese, *N* nitrogen, *K* potassium, *S* sulfur, *P* phosphorus, *Ca* calcium, *K/Na* potassium/sodium, *Ca/Na* calcium/sodium

Correlation Analysis

A high positive correlation was detected between plant biomass parameters. A high positive correlation was detected between chlorophyll-b and other biomass parameters except the number of leaves per plant. A highly positive correlation was detected between the amount of photosynthesis and shoot and root dry weight, stem length, number of leaves, and root length ($p < 0.01$). A positive correlation was detected between root IAA hormone and root dry weight, stem diameter and root length ($p < 0.05$). A positive correlation was detected between root GA hormone and shoot and root dry weight, stem diameter, root length and chlorophyll b content. While a negative correlation was detected between leaf SA and root dry weight, stem diameter and photosynthesis amount, a positive correlation was detected between root SA and shoot and root dry weight and root length. While IAA and GA in root tissues helped to protect plants from salt stress, IAA and GA in leaf tissues had no effect on reducing the effect of salt stress. If the amount of SA acid is high in root tissues, it reduces the effect of salt stress, but when it is high in leaf tissues, it does not reduce the effect of salt stress. Since no correlation was detected between the IAA and ABA hormones in the root tissues and the ABA and GA hormones in the leaf tissues and the biomass and photosynthesis content, it was determined that they had no effect on salt stress (Table 5).

While a negative correlation was detected between leaf N content and total carotenoid content, a positive correlation was detected with leaf K content. A negative correlation was detected between leaf Mn content and shoot dry weight, stem length, number of leaves and root length.

Table 5 Correlation analysis (biomass/chlorophyll/photosynthesis/hormone) of bottle gourd and control plants grown under salt stress

Parameters	Shoot dry weight	Root dry weight	Main stem diameter	Main stem length	Number of leaves per plant	Root length	Chloro-phyll-a	Chloro-phyll-b	Total chlorophyll	Total Carotenoid	Photosyn-thesis
Shoot dry weight	1	*0.670***	*0.622***	*0.817***	*0.745***	*0.814***	0.197	*0.303**	0.225	–0.014	*0.466***
Root dry weight	*0.670***	1	*0.601***	*0.512***	*0.457***	*0.716***	0.129	*0.458***	*0.317**	0.069	*0.489***
Main stem diam-eter	*0.622***	*0.601***	1	*0.339**	0.213	*0.612***	0.137	*0.294**	0.208	*0.317**	0.262
Main stem length	*0.817***	*0.512***	*0.339**	1	*0.730***	*0.623***	*0.402***	*0.383***	*0.359**	–0.223	*0.483***
Number of leaves per plant	*0.745***	*0.457***	0.213	*0.730***	1	*0.649***	0.217	0.153	0.137	–0.238	*0.509***
Root length	*0.814***	*0.716***	*0.612***	*0.623***	*0.649***	1	0.159	*0.310**	0.180	0.055	*0.515***
Chlorophyll-a	0.197	0.129	0.137	*0.402***	0.217	0.159	1	*0.513***	*0.748***	–0.241	0.245
Chlorophyll-b	*0.303**	*0.458***	*0.294**	*0.383***	0.153	*0.310**	*0.513***	1	*0.812***	–0.268	*0.370**
Total chlorophyll	0.225	0.317*	0.208	*0.359**	0.137	0.180	*0.748***	*0.812***	1	–0.172	0.273
Total Carotenoid	–0.014	*0.069*	*0.317**	–0.223	–0.238	0.055	–0.241	–0.268	–0.172	1	–0.125
Photosynthesis	*0.466***	*0.489***	0.262	*0.483***	*0.509***	*0.515***	0.245	*0.370**	0.273	–0.125	1
Leaf IAA	–0.119	–0.222	–0.163	–0.124	–0.109	–0.110	0.253	–0.015	0.034	–0.139	–0.218
Root IAA	0.167	*0.315**	*0.289**	0.096	–0.099	*0.291**	0.038	0.185	0.162	0.239	0.143
Leaf ABA	–0.010	–0.045	–0.184	0.163	0.178	–0.055	–0.153	–0.061	–0.105	0.177	–0.029
Root ABA	0.256	0.214	0.223	0.085	0.092	0.154	0.169	0.030	0.063	–0.153	0.145
Leaf GA	0.063	0.120	0.206	0.039	0.002	0.048	–0.105	–0.127	–0.061	0.025	0.193
Root GA	*0.302**	*0.412***	*0.340**	0.286	0.086	*0.399***	0.087	*0.294**	0.262	0.233	0.220
Leaf SA	–0.262	*–0.431***	*–0.373***	–0.147	–0.099	–0.215	0.016	–0.161	–0.156	–0.056	*–0.329**
Root SA	*0.290**	*0.373***	0.281	0.286	0.091	*0.391***	0.046	0.269	0.218	0.219	0.215

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 6 Correlation analysis (biomass/chlorophyll/photosynthesis/leaf and root nutrient element) of bottle gourd and control plants grown under salt stress

Parameters	Shoot dry weight	Root dry weight	Main stem diameter	Main stem length	Number of leaves per plant	Root length	Chloro-phyll-a	Chloro-phyll-b	Total chloro-phyll	Total Carotenoid	Photosyn-thesis
Leaf N	0.035	–0.080	–0.190	0.064	–0.021	–0.132	0.172	–0.043	–0.014	*–0.330**	–0.008
Leaf P	0.045	0.112	0.001	–0.043	–0.072	0.044	0.235	0.120	0.062	–0.223	0.103
Leaf K	–0.017	–0.121	–0.138	0.017	–0.058	–0.123	0.243	0.065	0.026	*0.394***	0.024
Leaf Ca	0.007	0.056	–0.057	–0.028	–0.066	0.023	0.276	0.137	0.083	–0.245	0.039
Leaf Mg	–0.178	–0.119	–0.175	–0.133	–0.100	–0.228	–0.099	–0.059	–0.029	–0.210	–0.048
Leaf S	0.035	0.075	–0.055	0.015	–0.031	–0.042	0.175	0.127	0.082	–0.240	0.128
Leaf Mn	*–0.319**	–0.195	–0.150	*–0.345**	*–0.451***	*–0.352**	–0.039	–0.118	–0.086	–0.082	–0.032
Leaf Fe	–0.092	–0.285	*–0.503***	0.158	0.233	–0.186	0.029	–0.046	–0.048	–0.228	–0.108
Leaf Zn	–0.142	*–0.386***	*–0.417***	0.105	0.046	–0.233	–0.032	–0.097	–0.175	–0.155	–0.189
Leaf B	–0.260	*–0.397***	*–0.344**	–0.110	–0.077	*–0.401***	–0.093	–0.181	–0.076	–0.162	–0.265
Leaf Cu	*–0.300**	*–0.335**	–0.234	–0.158	–0.161	*–0.390***	*–0.439***	*–0.352**	*–0.364**	0.053	–0.240
Leaf Na	–0.128	–0.246	*–0.297**	–0.219	–0.197	–0.239	–0.212	–0.201	–0.287	–0.095	*–0.365**
Leaf Cl	–0.054	–0.124	–0.214	0.158	–0.039	–0.059	–0.174	–0.122	–0.141	0.047	0.006
Leaf K/Na	*0.439***	0.109	0.208	0.185	0.138	0.122	*0.418***	0.197	0.282	–0.207	*0.414***
Leaf Ca/Na	*0.386***	0.209	0.155	0.087	0.055	0.170	*0.411***	0.233	0.265	–0.133	*0.405***
Root N	0.000	–0.144	0.017	–0.011	0.106	0.037	–0.067	0.010	–0.104	0.311*	–0.187
Root P	–0.259	*–0.365**	*–0.356**	–0.073	0.029	–0.217	–0.134	–0.156	–0.154	0.083	–0.279
Root K	–0.187	*–0.326**	*–0.424***	0.027	0.135	–0.155	–0.002	–0.083	–0.070	0.015	–0.126
Root Ca	–0.205	*–0.383***	*–0.485***	0.004	0.160	–0.207	–0.073	–0.167	–0.173	–0.036	–0.158
Root Mg	–0.158	*–0.339**	*–0.404***	0.072	0.104	–0.135	0.032	–0.060	–0.059	0.008	–0.166
Root S	–0.109	–0.198	–0.247	0.058	0.096	–0.059	0.058	0.090	0.073	0.107	–0.159
Root Mn	–0.144	*–0.318**	*–0.390***	0.072	0.139	–0.121	0.016	–0.022	–0.023	0.041	–0.155
Root Fe	–0.021	–0.171	*–0.298**	0.156	0.213	–0.047	0.011	0.085	0.041	0.011	–0.154
Root Zn	–0.040	–0.245	*–0.318**	0.161	0.170	–0.034	0.078	0.029	0.010	0.005	–0.160
Root B	–0.250	*–0.368**	*–0.482***	0.025	0.013	–0.286	0.023	–0.038	–0.066	–0.073	–0.159
Root Cu	–0.087	–0.231	*–0.298**	–0.006	0.128	–0.064	0.043	–0.176	–0.182	–0.152	*0.300**
Root Na	–0.265	*–0.452***	*–0.291**	–0.149	–0.068	–0.287	–0.140	–0.105	–0.090	–0.113	*–0.326**
Root Cl	–0.222	*–0.322**	*–0.554***	0.035	0.150	–0.268	0.129	–0.113	–0.006	–0.134	–0.079
Root K/Na	–0.083	–0.145	*–0.223*	0.050	0.117	–0.029	0.074	–0.058	–0.044	0.169	–0.091
Root Ca/Na	–0.108	–0.225	*–0.330**	0.052	0.172	–0.093	–0.009	–0.144	–0.148	0.109	–0.114

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

A negative correlation was detected between leaf Fe content and stem diameter, and between leaf Zn and B content and root dry weight and stem diameter. A negative correlation was detected with the amount of Cu in leaf tissue and shoot and root dry weight, root length, and chlorophyll (a, b, total) content. A negative correlation was detected between leaf Na content and photosynthesis amount. A positive correlation was detected between leaf K/Na and Ca/Na and shoot dry weight, chlorophyll and photosynthesis amount. A negative correlation was detected between root Na and photosynthesis. Negative correlation was detected between Na and Cl in root tissues and root dry weight and stem diameter. In our study, as leaf nitrogen content increased under salt stress conditions, carotenoid content decreased and as potassium content increased, total carotenoid content increased. In our study, salt stress caused an increase in the amount of Cu. The genotypes that showed the highest increase in some genotypes were plants that were naturally sensitive to salt stress. Cu may have become toxic due to salt stress, and as a result, chlorophyll and root length parameters were negatively affected. While the amount of Na in leaf and root tissues negatively affected the dry weight of plant shoots and roots, at the same time, the increase in Na content under salt stress conditions caused a decrease in the amount of photosynthesis. In our study, plants selected for salt tolerance had lower Na content in their tissues and higher photosynthesis amount. While the increase in the amount of K/Na and Ca/Na in leaf tissues positively affects biomass parameters under salt stress conditions, it also reduces the extent to which the amount of photosynthesis is affected by salt stress (Table 6).

Cluster Analysis

In this study, a hierarchical clustering chart was generated based on the shoot and root dry weight, stem diameter, stem length, number of leaves, root length, chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid, leaf and root hormone, and plant nutrient content of forty seven genotypes (Fig. 12). Of the thirty-five gourd hybrids, eight were classified as susceptible, twelve as semi tolerant, and fifteen as tolerant. according to their performance under salt stress. While the watermelon variety C. Tide was in the sensitive group, commercial rootstocks RS841 and Argentaria were in the semi tolerant tolerant group. While three of the parental lines were in the semi tolerant group, six were in the sensitive group. In the analysis of forty-seven plant materials, including commercial rootstocks, it was determined that fifteen of these plant materials, consisting of gourd hybrids, were rootstock candidates with higher salt tolerance.

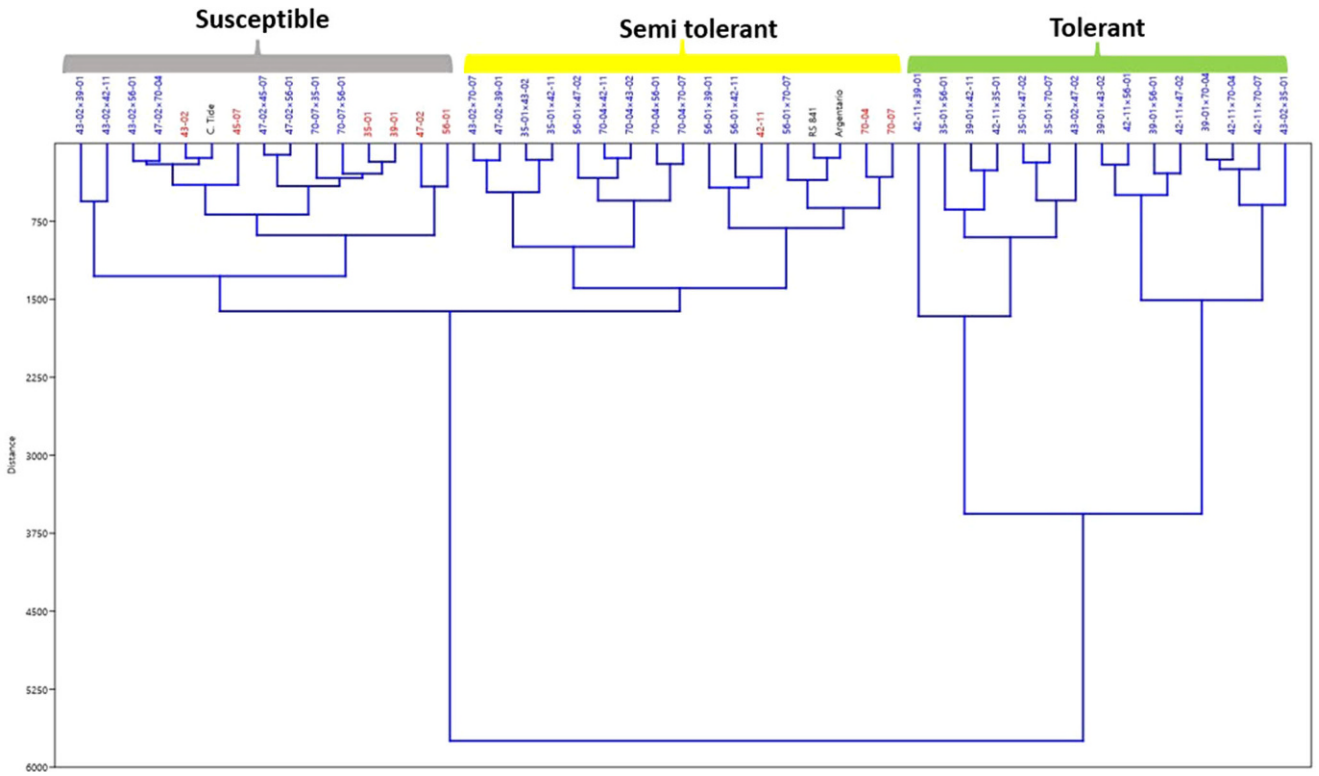

Fig. 12 UPGMA of bottle gourd and control plants grown under salt stress (10 dSm^{-1}) conditions based on biomass parameters and nutrient element and hormone content in leaf and root tissues. *UPGMA* unweighted pair group mean arithmetic

Discussion

Breeding programs aimed at developing salt-tolerant varieties or rootstocks require effective selection of salt-tolerant genetic material. Genetic material is selected based on its ability to survive in saline conditions. This can be done by testing plants in saline soils and comparing their performance to non-saline plants. Plants that survive in saline conditions are more likely to be salt-tolerant and suitable for inclusion in breeding programs. It is important to breed salt-tolerant rootstocks for cucurbit crops because salinity adversely affects plant growth, yield, and fruit quality. Genetically modified rootstocks are an effective way to combat salinity, as they can produce more resilient plants. Additionally, soil management practices such as drainage and irrigation can help reduce the effects of salinity. This study aimed to determine the ones with the highest tolerance to salt stress among the rootstock candidate hybrid combinations obtained by crossing pure lines selected as salt tolerant from the Turkish bottle gourd germplasm. Salt stress caused an average decrease of 63% in shoot dry weight, 43% in root dry weight, 68% in main stem length, 45% in root length, 18% in main stem diameter and 41% in the number of leaves per plant in 47 genotypes (Table 2). The salt stress resulted in reduced root and shoot lengths, possibly resulting from limited nutrient and water uptake due to osmotic stress and toxicity (Majid et al. 2013; Ouji et al. 2015). As roots absorb water and shoots provide water to aboveground tissues, these data support previous evidence that root and shoot lengths are key determinants of salt tolerance (Abbas et al. 2010; Janghel et al. 2020). Plant growth is inhibited by high NaCl concentrations due to osmotic stress and sodium toxicity in the cytoplasm, resulting in a reduction in photosynthetic function. This can also cause a decrease in root growth and inhibition of stomatal movement, leading to a decrease in transpiration and CO_2 supply for photosynthesis. This ultimately leads to a decrease in water uptake, resulting in dehydration (Taffouo et al. 2010). In salt-stressed plants, water uptake potential is significantly reduced, root development and stem elongation are inhibited, and stem diameters, plant heights, and leaf area are lower than in control plants (Aydın and Yetişir 2022). According to Alzahrani et al. (2018), abiotic stress factors like salinity can negatively affect root development and root anatomy. As a result of salt stress, we observed decreases in biomass parameters, however, stem length was negatively affected at higher rates than stem diameter proportionally compared to the control. Furthermore, shoot dry weight was more affected than root dry weight. Previous studies have also reported that shoot development is more affected than root development under salt stress (Colla et al. 2006; Uygur and Yetisir 2009; Munns 2011).

Similarly to our findings, Munns and Tester, (2008) found that the green parts of plants were more susceptible to saline conditions than the roots. Although all hybrid combinations were greatly affected by salt stress, their stress responses varied significantly. This indicates the existence of significant genetic diversity related to salt tolerance in the studied germplasm. According to Naseer et al. (2022), Under salt stress, "Nuefield" and "Crystal long" bottle gourd genotypes had more leaves, shoots, roots, plant fresh weight, and plant dry weight than other genotypes. The genotypic variation among species may explain the differences between plants' responses to salt stress, as reported by Saadallah et al. (2001). Photosynthetic pigments Chla, chlb, total chl, and carotenoid content were significantly affected by NaCl. Salt stress decreased chlorophyll-a content in three genotypes (43-02 × 56-01, 47-02 × 39-01 and 43-02 × 39-01), chlorophyll-b content in two genotypes (47-02 × 39-01 and RS 841), and total chlorophyll content in RS 841 commercial rootstocks (Fig. 5). Salt stress increased the chlorophyll content of other genotypes. *Thellungiella salsuginea* increased Chla and Chlb significantly at 300 mM NaCl concentration, which is similar to our results (Goussi et al. 2018). The chlorophyll content is generally affected by NaCl concentration, exposure time, and their interaction (Shin et al. 2020).

In our study, while salt stress caused a decrease in the amount of photosynthesis in all genotypes, the amount of photosynthesis was found to be lower especially in genotypes with low biomass parameters. Ulas et al. (2020) reported that high salt levels negatively affect photosynthetic activity, and this may be due to stoma-related limitations due to the closure of stomata, non-stomal-related limitations, or both limitations. One of the most important reasons for the limitation in growth and development in plants under stress is the closure of stomata, reducing carbon dioxide uptake and limiting photosynthesis (Ulas et al. 2020).

In our study, different genotypes showed different hormone levels under salty conditions. Salt stress decreased leaf tissue IAA (3%), ABA (63%), and root tissue IAA (28%), GA_3 (32%), and SA (63%) content, but increased leaf tissue GA_3 (196%), SA (27%) and root tissue ABA (47%) content (Table 2). This suggests that genetic variation may influence the plant's response to salinity. Further studies are necessary to better understand the relationship between genotype and hormone levels under different salinity conditions. IAA maintains cell division in the apical root meristem, but ABA increases the synthesis of antioxidant enzymes and antistress proteins. By effectively functioning, the defense system reduces the production of reactive oxygen species (ROS), protects membrane integrity, and regulates stomatal conductance. This provides greater CO_2 availability in leaf mesophyll cells, thus increasing photosynthetic activity, transpiration rate, and water use efficiency

(Sarwar et al. 2021). In case of stress, the ABA hormone stimulates genes that play a role in mitigating the negative osmotic potential caused by salt (Botella et al. 2007). Salt stress has also been reported to increase ABA levels in cucumber leaves, similar to our findings (Talanova and Titov 1994). Another study showed that increased ABA synthesis in bean plants under salt stress increased salt tolerance by reducing Na and Cl content (Montero et al. 1997). The salt stress resulted in an increases and decreases in GA_3 content depending on the genotype. In plants, GA_3 regulates developmental processes. These include germination, stem elongation, leaf expansion, and flowering. GA_3 also helps to regulate auxin transport, which is involved in plant growth and development (Binenbaum et al. 2018). Additionally, GA_3, helps the plant grow better against salinity by improving membrane permeability and nutrient levels in the leaves. As a result of exogenous GA_3 application, the negative effects of salt on summer squash plants were alleviated by reducing the accumulation of reactive oxygen species, which increases with salinity stress, regulating nutrient uptake, and increasing the activity of antioxidant enzymes (Al-Harthi et al. 2021).

Plants respond differently to Na toxicity in different ways; Sodium accumulates in the leaves of some plants, while it is excreted at the roots of others (Tejera et al. 2006). In addition to increasing potassium intake, sodium influx can also be prevented by increasing potassium intake (Serrano et al. 1999). Combined with cytotoxicity and nutrient imbalances, high salt stress limits water use. As a result, plants cannot uptake enough water and nutrients, leading to reduced growth and productivity. Additionally, sodium ions inhibit nutrient uptake in plants, resulting in nutrient deficiency. As Na accumulates in the cytoplasm and vacuoles, K/Na homeostasis is disrupted; In the presence of excessive Na and Cl, ion imbalance leads to a K deficiency due to damage to the cell membrane. As a result, the oxidation process in the cells is impeded, resulting in deterioration of the photosynthetic mechanism, growth rate, and biomass (Hafsi et al. 2017; Khoshbakht et al. 2018; Guo et al. 2020). In our study, while the leaf K/Na ratio was 1.63 and the root Na/K ratio was 0.12 under control conditions, the leaf K/Na ratio was determined as 0.0047 and the root K/Na ratio was 0.0033 under salty conditions, and salt stress caused a significant decrease in the K/Na ratio in roots and leaves. Likewise, under control conditions, the leaf Ca/Na ratio was 1.61 and the root Ca/Na ratio was 0.06, while in saline conditions, the leaf and root Ca/Na ratios were determined as 0.033 and 0.0013, respectively (Table 3). A positive correlation was determined between Ca-P, S-K, N-Mn, and Zn-Fe-B in the leaf tissues of 47 genotypes under salt stress conditions. In root tissues, a positive correlation was determined between plant nutritional elements B-Ca-Fe-S-K-N-Mn-Mg-Zn-P. A generally negative correlation was found between these plant nutrients and Na and Cl in leaf and root tissues (Fig. 9 and 10). Our study also found lower levels of Na and Cl ions in the tissues of salt-tolerant plants. According to researchers, Na and Cl compete with nutrients such as K, Ca and NO_3 under salt stress, resulting in ionic toxicity and impaired plant growth (Ahmad et al. 2018; Guo et al. 2020). Compared to control plants, Mg, K, and Ca contents decreased by 10.5%, 53.8%, and 13.9%, respectively, while Na content increased by 85.9% and the Na/K ratio increased significantly in cucumber plants under salt stress (He et al. 2022).

In our study, while IAA, GA and SA in root tissues helped protect plants from salt stress, IAA, GA and SA in leaf tissues had no effect on reducing the effect of salt stress. Leaf nitrogen content increased under salt stress conditions, carotenoid content decreased and as potassium content increased, total carotenoid content increased. Salt stress has caused the amount of Cu to increase. In some genotypes, the genotypes with the highest increase are plants that are naturally sensitive to salt stress. While the amount of Na in leaf and root tissues negatively affected the dry weight of plant shoots and roots, at the same time, the increase in Na content under salt stress conditions caused a decrease in the amount of photosynthesis. In our study, plants selected to tolerate salt stress had lower Na content in their tissues and had higher photosynthesis amount. The increase in the amount of K/Na and Ca/Na in leaf tissues under salt stress conditions positively affected biomass and photosynthesis parameters.

Conclusion

The development of resistant or tolerant rootstocks for vegetable cultivation is a rational and sustainable strategy because it increases yield and quality by combating biotic and abiotic stress factors. Therefore, the use of resistant rootstocks is an important tool for sustainable vegetable production, especially in fruits-bearing vegetables. In this study, hybrid *Lagenaria siceraria* rootstock candidates showed superior performance in terms of salt tolerance compared to commercial rootstocks (RS841 and Argentario) used in Turkey in terms of plant growth parameters, leaf chlorophyll content, plant nutrients, and hormone metabolism. When all the investigated parameters in this study were evaluated together, it was revealed that grafting on hybrid *L. siceraria* rootstock candidates could be used as an alternative to *C. maxima* × *C. moschata* interspecific hybrid rootstocks, which are commonly used commercially, in watermelon to provide salt tolerance. However, it is necessary to investigate the effects of hybrid rootstock/scion combinations on watermelon yield and fruit quality parameters, and to determine the most suitable rootstock/scion combinations. Our

studies about the effect of selected *Lagenaria* hybrids as salt tolerant in this study on the plant growth, yield and quality of watermelon under salt stress are continuing in hydroponic and open field conditions.

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Conflict of interest The authors of the article declare that they have no conflict of interest.

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