Journal of Plant Growth Regulation
https://doi.org/10.1007/s00344-022-10725-y

Do Gibberellins Mediate Growth Responses of the Halophytic Woody *Prosopis Strombulifera* (Lam.) Benth Plants Exposed to Different Sodium Salts?

Analía Llanes[1,3] · Santiago Biava[1] · Claudia Travaglia[2,3] · Oscar Masciarelli[3] · Virginia Luna[1,3]

Received: 6 July 2021 / Accepted: 2 June 2022

Abstract
Phytohormones have essential roles in plant growth responses under salinity. A better understanding of gibberellin (GA) function in woody plant responses under different sodium salts could help to develop new strategies to improve plant tolerance to salinity. In this study, the role of GA in morpho-physiological responses of halophytic woody *Prosopis strombulifera* plants under salinity was analyzed. Plants were grown in hydroponic solutions and exposed to NaCl, Na_2SO_4, or their iso-osmotic mixture at − 1.0, − 1.9, and − 2.6 MPa. Control (without salt) and salt-treated plants were sprayed with gibberellin A_3 (GA_3), or chlormequat chloride (CCC), an inhibitor of its synthesis. Growth responses, anatomical alterations and ABA, active GA forms (GA_1, GA_3, and GA_4) and inactive GA forms (GA_8 and GA_{34}) endogenous levels were evaluated. The application of GA_3 increased growth in control plants more than in salt-treated plants. Roots and leaves of salt-treated plants showed high levels of ABA and active GA forms after exposure to GA_3, and lower endogenous levels of active GA when receiving the inhibitor. CCC triggered stress-alleviating responses in these plants, such as anatomical and hormonal changes that included an increase in spine length and the number of palisade cell layers, and a reduction in levels of ABA and GA_4. Na_2SO_4-treated plants showed reduced growth, high ABA levels and an active GA metabolism to control the levels of active GA. This study indicates that the suppression of GA signaling would contribute to sodium salts tolerance in the native halophytic woody *P. strombulifera* plants.

Keywords Abscisic acid · Gibberellin profile · Halophytic woody plants · Leaf anatomy · NaCl · Na_2SO_4

Introduction

Soil salinity is increasing around the world, rendering vast stretches of land unavailable for economic exploitation such as in the form of agriculture and forestation (Qadir et al. 2014). Although the phenomenon is observed mainly in arid and semi-arid regions, there are also large areas of saline soils in humid regions, particularly in coastal areas. In total, saline soils are estimated to cover one third of arable land worldwide (FAO 2017). Alterations identified at the anatomical, physiological, and molecular levels indicate that plants undergo a complex metabolism reprogramming in their cells under this stressful condition (Singh et al. 2017; Sancho-Knapik et al 2017; Tanveer and Shabala 2018; Liu et al. 2019). Thus, a better understanding of these metabolic changes could help to develop new strategies to improve plant tolerance to salinity.

Phytohormones are essential for plant growth regulation and developmental responses to different environmental conditions (Peleg and Blumwald 2011), including those aimed at adapting to salt. Abscisic acid (ABA) is known to be a key mediator in the plant's defense against abiotic stresses (Vishwakarma et al. 2017; Medina et al 2019), which explains why its levels rise rapidly under such conditions. ABA leads to plant responses such as water loss reduction via reduced

Handling Editor: Antonella Locascio.

✉ Analía Llanes
allanes@exa.unrc.edu.ar

[1] Laboratorio de Fisiología Vegetal-Interacción Planta-Ambiente, Universidad Nacional de Río Cuarto (UNRC), Río Cuarto, Córdoba, Argentina

[2] Laboratorio de Morfología Vegetal, Universidad Nacional de Río Cuarto (UNRC), Río Cuarto, Córdoba, Argentina

[3] Instituto de Investigaciones Agrobiotecnológicas (INIAB-UNRC)-Consejo Nacional de Investigaciones Científicas Y Técnicas (CONICET), Buenos Aires, Argentina

transpiration and, eventually, plant growth restriction (Sah et al. 2016). Gibberellins (GA), a large group of diterpenoid carboxylic acids, are compounds mediating plant growth and development, since they are involved in regulating root development, flowering, and fruit maturation, as well as in promoting shoot growth, among other functions (Hedden and Thomas 2016; Wang et al. 2017; Binenbaum et al. 2018).

Salinity induces biochemical responses that disrupt the regulation of biosynthetic ABA and GA pathways (Golldack et al. 2013; Shu et al. 2018). For instance, a reduction in levels and signaling of GA has been found to contribute to the restriction of plant growth upon exposure to different sources of abiotic stress, including salinity (Colebrook et al. 2014). Nevertheless, results on the role of GA under salinity has been quite controversial.

Most studies on this topic have been performed on non-woody plants, such as *Arabidopsis thaliana* (Zhang et al. 2018; Chen et al. 2019). Given that plants can share homologous genes and characteristics even if they have diverged from a common ancestor over millions of years (Shi et al. 2020), the role that GA play in non-woody plants might be conserved in woody plants. Further research is needed to clarify this topic, and one way to do it is by using plant growth retardants. These are products of chemical synthesis which delay cell division and elongation in stem tissues and one example of GA biosynthesis inhibition is chlormequat chloride (CCC or Cycocel; Han et al. 2011; Huang et al. 2015). Using CCC, endogenous levels of GA can thus be manipulated, and the anatomical and physiological effects brought about by the treatment can be reversed by applying exogenous GA (Huang et al. 2015; He et al. 2019). This method thus makes it possible to create suitable experimental conditions under which to explore the role of these hormones in woody plants under salinity, which will allow potential biochemical markers or genes for salt tolerance to be identified and used in breeding programs.

Halophytic woody plants have evolved to be naturally salt-tolerant and they can survive and complete their life cycles under salt concentrations over 200 mM NaCl (Flowers and Colmer 2015). A wide range of physiological, morphological, and molecular mechanisms are behind this adaptability, which at the same time varies significantly between species. *Prosopis strombulifera* (Lam.) Benth (Burkart 1976) is a native halophytic woody plant that grows as far up north as the Arizona Desert (USA) and as far down south as Patagonia (Argentina). In central Argentina, where salinized soils feature mostly NaCl and Na_2SO_4 in a similar proportion (Sosa et al. 2005), this species is abundant. Previous studies demonstrated that *P. strombulifera* was able to survive even when exposed to up to 1 M NaCl in hydroponic experiments. By contrast, when grown in the presence of Na_2SO_4, growth parameters were markedly reduced and were accompanied by senescence symptoms such as chlorosis, necrosis, and leaf abscission (Reinoso et al. 2005; Devinar et al. 2013). Plants growing in NaCl were able to filter the solution more efficiently than plants under Na_2SO_4 by preventing the passage of excess ions into the xylem through a sodium exclusion mechanism within the roots (this involves precocious development of a lignified endodermis; Reinoso et al. 2004). Also, NaCl-treated plants set in motion several adaptive responses such as ion compartmentation in vacuoles and increased synthesis of solutes such as proline, pinitol, and mannitol in the cytoplasm. By contrast, Na_2SO_4 caused a water imbalance and toxicity symptoms due to altered carbon metabolism (Llanes et al. 2013, 2016). These differential responses of *P. strombulifera* to the salts most commonly found in salinized soils thus provides an excellent model on which to continue building our knowledge about salt tolerance mechanisms. Therefore, our hypothesis was that manipulation of GA biosynthesis in halophytic woody *P. strombulifera* plants with differential responses to NaCl and Na_2SO_4 by the use of a GA biosynthesis inhibitor, would allow us to determine specific roles. Considering GA characteristics as plant growth promoters through the control of cell division, cell elongation and shoot growth induction, it can be expected that high levels of active GA would not be favorable for developing tolerance responses. This is because a considerable amount of resources need to be diverted to withstand stress (Leone et al. 2014; Bechtold et al. 2018). By contrast, inhibition of GA biosynthesis would then render a stress resistant plant, with morphological and physiological traits involved in salt tolerance mechanisms, at the expense of growth (Magome et al. 2008; Colebrook et al. 2014). The aim of this work was to gain insight into the role of GA in this plant's growth responses to salt stress, by observing the alterations in foliar anatomy, stem growth, and endogenous profiles of ABA and GA induced by the salt treatments and the hormonal applications. To achieve this the experiments initially exposed plants to increasing concentrations of NaCl, Na_2SO_4, or an iso-osmotic mixture of both salts. After reaching previously determined osmotic potentials, GA_3 and CCC (an inhibitor of GA biosynthesis) were exogenously applied. To our knowledge, this is the first study that focuses on the function of GA in a halophytic woody plant and broadens our knowledge on the hormonal regulation of sodium salt tolerance.

Materials and Methods

Plant Materials

Proscopic strombulifera seeds were collected from a saline area in southwester San Luis province (Argentina), located at 33° 43´S, 66° 37´W, with an altitude of 400–500 m and

average annual temperatures of 15–20ºC. The soil has a sandy-loam texture, with abundant calcareous material and moderate salinity. The chemical composition of the soil in the sampling area indicates that NaCl and Na_2SO_4 are found at similar concentrations according to soil analysis (Sosa et al 2005; Llanes et al 2013). However, Na_2SO_4 was more abundant than NaCl in some soil samples. The soil profile from 0 to 35 cm depth shows an increase in EC (dS m^{-1}), from 8.4 to 11 (Sosa et al 2005). The voucher specimen was deposited in the herbarium of the Botanical Museum of Cordoba (CORD), National University of Cordoba (Argentina): voucher number CORD 00,085,768 (Cantero 7284; the specimen was identified by Cantero J.J.). *P. strombulifera* pods were collected at random from 100 plants belonging to the same population. Seeds were selected visually on the basis of uniform size and healthy appearance, and afterward scarified with sulphuric acid (100%) for 10 min and washed with distilled water. They were then placed on Petri dishes for 24 h at 35 °C with two layers of filter paper saturated in distilled water. Germinated seeds with 1 cm-long roots were cultured under hydroponic conditions, in three black trays per treatment per experiment (100 seedlings per tray), with 25% full-strength Hoagland solution (Hoagland and Arnon 1950). Seedlings were grown in a chamber with a cycle of 16 h light (200 µmol m-2 s-1) at 28 °C and 8 h dark at 20 °C, and with 70% relative humidity. The solutions, at pH 6, were changed every two days and continuously aerated by an aquarium tube system with a peristaltic pump. The experimental design consisted of nine black trays (three of each salt treatment). Trays were randomized periodically to minimize any variation within the culture chamber. At each sampling date, 24 h after reaching the final osmotic potentials desired for each salt treatment and spraying with GA_3, CCC or distilled water solutions, roots and leaves of at least 25 control plants and 25 treated plants were randomly collected. Samples were frozen with liquid nitrogen and stored at − 80 °C for hormonal analysis. The experiment was repeated four times.

Salt Treatments

When the plants had undergone 21 days of culture, the salt treatments were applied following a simple randomized design. Salt treatments consisted of pulses of NaCl (50 mmol L Hoagland solution-1), Na_2SO_4 (37.9 mmol L Hoagland solution-l), or their iso-osmotic mixture (obtained by mixing equal volumes of the monosaline solutions). Pulses of salts were performed every 48 h until reaching the final osmotic potentials evaluated (Ψo): − 1.0, − 1.9, or − 2.6 MPa for each salt treatment respectively, measured with a vapor pressure osmometer (Model 5500, Wescor Inc., Logan, UT, USA). The salt treatments are shown in Supplementary Information Table 1. Plants maintained in Hoagland solutions (Ψo of the medium = − 0.11 MPa) were kept as controls.

Treatments with GA_3 and its Biosynthesis Inhibitor

The plants were sprayed with solutions of GA_3 (100 ppm), chlormequat chloride (CCC), a GA synthesis inhibitor (2500 ppm), or distilled water (DW), to which 0.05% (v/v) Triton X-100 was added as a surfactant. A hand-held sprayer was used and the whole plant was sprayed. Spraying took place when the osmotic potentials (Ψo) mentioned above

Table 1 Effects of NaCl, Na_2SO_4, or the iso-osmotic mixture of both (NaCl + Na_2SO_4) on number of leaves, leaf length, and mesophyll thickness in *Prosopis strombulifera* plants sprayed with distilled water (DW), gibberellins (GA_3), or a gibberellin-synthesis inhibitor (CCC) at the lowest osmotic potential evaluated (− 2.6 MPa)

Treatments	Number of leaves	Leaf length (mm)	Mesophyll thickness (µm)			Adaxial/abaxial palisade mesophyll ratio	Palisade/spongy ratio
			Adaxial palisade	Abaxial palisade mesophyll	Spongy		
Control + DW	8.2 ± 0.92 a	2537.9 ± 11.2 e	77.8 ± 7.4 c	52.1 ± 1.1 c	38.6 ± 5.1 d	1.4	3.3
Control + GA_3	8.1 ± 0.83 a	1916.4 ± 18.3 a	30.5 ± 8.3 a	44.7 ± 6.8 c	27.1 ± 4.1 c	0.6	2.7
Control + CCC	8.4 ± 0.77 a	2118.2 ± 12.9 b	23.1 ± 6.7 a	17.4 ± 3.2 a	14.1 ± 2.7 a	1.3	2.9
NaCl + DW	6.5 ± 0.58 b	3099.3 ± 18.5 h	71.1 ± 11.7bc	90.2 ± 5.5 f	36.6 ± 4.8 d	1.2	4.3
NaCl + GA_3	6.1 ± 0.49 bc	2277.6 ± 18.8 c	85.3 ± 6.8 c	74.1 ± 6.2 e	36.8 ± 3.5 d	0.9	4.2
NaCl + CCC	6.4 ± 0.58 b	2531.5 ± 13.5 e	82.2 ± 4.5 c	51.8 ± 6.2 c	27.7 ± 5.1 bc	1.5	4.8
Na_2SO_4 + DW	5.3 ± 0.48 c	2310.2 ± 15.2 d	57.3 ± 8.2 b	33.5 ± 2.3 b	28.6 ± 3.1 c	1.7	3.3
Na_2SO_4 + GA_3	4.1 ± 0.32 d	2132.8 ± 17.0 b	23.6 ± 5.7 a	39.3 ± 4.2 b	28.6 ± 4.1 c	0.6	2.2
Na_2SO_4 + CCC	5.1 ± 0.51 c	2149.9 ± 13.2 b	62.2 ± 8.4 b	61.9 ± 2.9 d	41.2 ± 3.3 d	1.1	3.3
NaCl + Na_2SO_4 + DW	7.1 ± 0.81 ab	2817.5 ± 8.5 g	54.5 ± 6.3 b	50.1 ± 4.3 c	22.9 ± 3.2 b	1.2	4.5
NaCl + Na_2SO_4 + GA_3	6.7 ± 0.73 b	2607.1 ± 6.7 f	81.2 ± 2.1 c	106.9 ± 4.3 g	40.4 ± 2.3 d	0.7	4.6
NaCl + Na_2SO_4 + CCC	7.2 ± 0.53 ab	2618.7 ± 5,5 f	90.9 ± 8.4 c	101.2 ± 4.9 g	40.4 ± 1.7 d	0.9	4.7

Control indicates non-salt-treated plants. Data are from four replicated experiments ($n = 12$ true biological replications). Values are means ± SE. Different letters indicate significant differences ($p < 0.05$)

(− 1.0, − 1.9, or − 2.6 MPa) were reached. For each sampling, roots and leaves from controls and salt-treated plants were collected at random 24 h after the culture medium had reached the desired osmotic potentials and had been sprayed with the solutions of GA_3, CCC, or DW. Samples were frozen with liquid nitrogen and stored at -80 °C for hormonal analysis.

Determination of Growth Parameters

Root length, shoot height, first internode length, number of leaves, and spine length were monitored throughout the experiment. Measurements began at the same time as the application of the salt treatments (when the plants had undergone 21 days of culture). Growth parameters were measured in 20 plants (control and salt treated) collected at random 24 h after the culture medium had reached the desired osmotic potentials (− 1.0, − 1.9, or − 2.6 MPa) and plants were sprayed with the solutions of GA_3, CCC, or DW.

Leaf Anatomy

Leaf samples from control and salt-treated plants were collected 24 h after reaching osmotic potentials of − 1, − 1.9, and − 2.6 MPa and having been sprayed with solutions of GA_3, CCC, or DW. Samples were processed following Travaglia et al. (2012). They were fixed in FAA solution (ethyl alcohol, water, formalin, glacial acetic acid, 50:35:10:5 v/v) and then dehydrated in a series of ethyl alcohol-xylol mixtures. Next, they were placed in Histowax (highly purified paraffin wax with added polymers) (D'Ambrogio 1986), and 13 µm thick longitudinal and transversal cuts were made with a rotary microtome (Leitz®, Wetzlar, Germany). Afterward, they were stained with triple coloration of Hematoxylin-Safranin and Fast Green (Johansen 1940) and mounted using DPX (Distirene-Plasticizer-Xylene). The histological preparations were photographed with a camera AxioCam HRc, (Carl Zeiss, Göttingen, Germany) attached to a standard microscope (Model 16, Carl Zeiss). The boundary between xylem and phloem was identified by differential staining of the cell walls corresponding to sieve and vessel elements. The morpho-anatomical parameters evaluated were leaf length, mesophyll thickness, palisade and spongy parenchyma thickness, palisade/spongy mesophyll ratio, adaxial/abaxial palisade ratio, and characteristics of the central vascular bundles. The images were processed and analyzed using Image Pro-Plus software (Media Cybernetics Inc, Rockville, MD).

ABA and GA (actives and inactive forms) Profile

To analyze phytohormones, 100 mg of root and leaf samples (dry weight) from control and salt-treated plants were collected 24 h after reaching osmotic potentials of − 1, − 1.9, and − 2.6 MPa and having been sprayed with solutions of GA_3, CCC, or DW. Samples were ground with liquid nitrogen, and ABA and GA were extracted with 3 ml of extraction buffer (water–methanol-acetic acid, 80:19:1 v/v, pH 2.8). 50 ng of deuterated ABA, GA_1, GA_3, GA_4, GA_8, and GA_{34} (OlChemIm Ltd, Olomouc, Czech Republic) were added as internal standards. The extracts were centrifuged at 8000 rpm for 15 min, and the supernatants were collected and mixed twice with ethyl acetate. The organic phase was separated and evaporated at 37ºC. The dried extracts were dissolved in methanol and 10 µl of each sample were injected into a Liquid Chromatograph (LC) (Waters Corp., New York, NY, USA). MS/MS experiments were performed on a Micromas Quattro UltimaTM PT double quadrupole mass spectrometer (Micromass, Manchester City, UK). LC analyses, MS/MS parameters and quantification of each hormone were performed as described by Llanes et al. (2014).

Experimental Design and Statistical Analysis

This study was carried out following a completely randomized experimental design in a 4×3 factorial scheme. Growth parameters and anatomical and hormonal data were analyzed as described by Di Rienzo et al. (2016). The intervening factors were as follows: (1) 4 levels of salinity: Control (without salt), NaCl, Na_2SO_4, and the mixture of both salts, and (2) 3 levels of exogenous spray applications: distilled water, GA_3, and CCC. A two-factor ANOVA (considering salinity and exogenous applications) was performed for each variable. Tukey's test ($\alpha = 0.05$) was used for post-hoc comparisons. InfoStat statistical software (version 2018, Centro de Transferencia InfoStat, Universidad Nacional de Córdoba, Argentina) was used to perform all data analyses.

Results

Growth responses in *P. strombulifera* were altered upon exposure to different types of salt (NaCl, Na_2SO_4 and $NaCl + Na_2SO_4$) and levels of salinity (− 1, − 1.9, and − 2.6 MPa) in the culture medium, as well as to the exogenous applications of GA_3, its biosynthesis inhibitor (CCC) or distilled water (DW). These two factors (salinity and hormonal applications) and the interactions between them had significant effects ($p < 0.05$) at the lowest osmotic potential evaluated (− 2.6 MPa). Therefore, only the results whose interaction was significant are shown (Fig. 1). In fact, plant growth behaved differently in response to the different salts in the medium after the plants were sprayed. GA_3 increased root length in all salt-treated plants but decreased it in the controls as compared to plants sprayed with distilled water. No significant changes in this parameter could be attributed

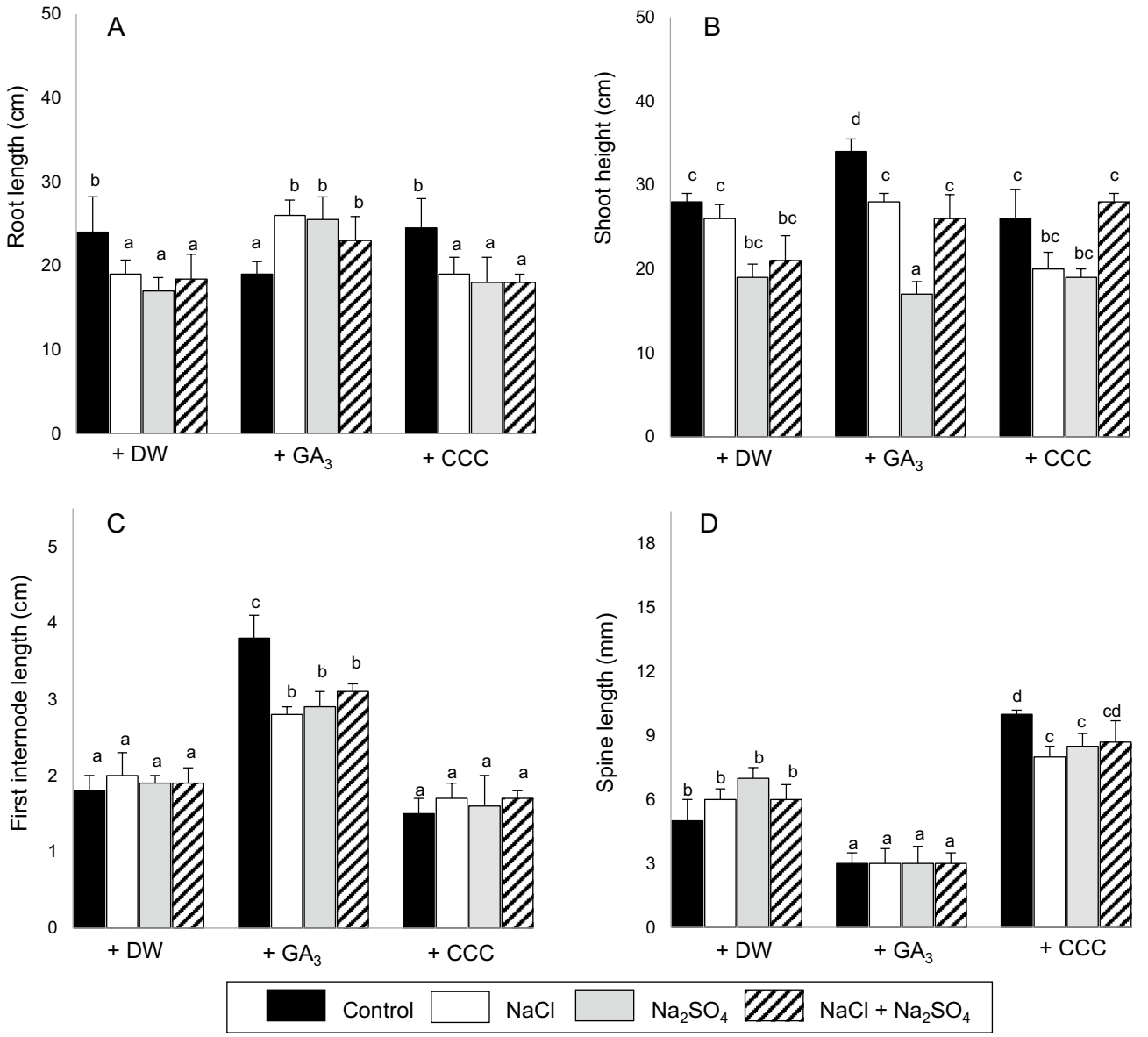


Fig. 1 Effects of NaCl, Na_2SO_4, or the iso-osmotic mixture of both (NaCl + Na_2SO_4) on root length (**A**), shoot height (**B**), first internode length (**C**), and spine length (**D**) in *Prosopis strombulifera* plants sprayed with distilled water (DW), gibberellins (GA_3), or a gibberellin-synthesis inhibitor (CCC) at the lowest osmotic potential evaluated (Ψo: -2.6 MPa). Control indicates non-salt-treated plants. Data are from four replicated experiments ($n = 12$ true biological replications). Values are means ± SE. Different letters indicate significant differences ($p < 0.05$)

to CCC in any of the plants (Fig. 1A). The lowest shoot height was found in Na_2SO_4− treated plants sprayed with GA_3, while similar values of shoot height were observed in NaCl and NaCl + Na_2SO_4− treated plants sprayed with GA_3. By contrast, this parameter significantly increased in the controls in response to the application of GA_3, reaching the highest shoot height (35 cm). Once again, CCC produced no changes in shoot height in controls and salt-treated plants as compared to plants sprayed with distilled water (Fig. 1B). The first internode length of controls and salt-treated plants sprayed with GA_3 was significantly longer (4 and 3 cm, respectively) as compared to plants sprayed with distilled water or CCC (2 cm). The first internodes of all CCC-sprayed plants were similar in length and no significant differences were observed between them (Fig. 1C). The application of CCC, however, brought about an increase in spine length regardless of the treatments. GA_3-treated plants had shorter spines than those plants receiving distilled water (3 and 6 mm, respectively; Fig. 1D).

The exposure to salinity and the applications of GA_3, CCC, or DW modified the number of leaves, leaf length, and mesophyll thickness, but the changes were only significant

($p < 0.05$) at the lowest osmotic potential (− 2.6 MPa) evaluated (Table 1 and Supplementary Information Fig. 1). The highest number of leaves was observed in non-salinized plants, regardless of the sprayed solution. No significant differences in number of leaves were observed in NaCl and NaCl + Na_2SO_4-treated plants sprayed with GA_3, CCC, or distilled water. Na_2SO_4-treated plants sprayed with GA_3 showed the lowest number of leaves (Table 1), which was accompanied by chlorosis and leaf abscission (data not shown). The leaf length in the controls and in all salt-treated plants sprayed with either GA_3 or CCC was less than in those plants sprayed with distilled water. Nevertheless, the lowest values for leaf length were observed in plants sprayed with GA_3. Plants treated with the iso-osmotic mixture of NaCl and Na_2SO_4 showed intermediate values in leaf length and mesophyll thickness to those observed in monosaline-treated plants, regardless of the sprayed solution. Examination of cross sections revealed an increased palisade/spongy ratio in salt-treated plants than in the controls sprayed with GA_3, CCC, or distilled water, except for Na_2SO_4-treated plants sprayed with GA_3, whose leaves had the lowest palisade/spongy ratio (Table 1 and Supplementary Information Fig. 1). The ratio between palisade mesophyll on the adaxial and the abaxial sides of the leaf was significantly higher in NaCl-, Na_2SO_4-, and iso-osmotic mixture-treated plants sprayed with CCC than in those receiving GA_3 (1.5, 1.1, 0.9 and 0.9, 0.6, 0.7, respectively). Leaf sections showed an isolateral organization and stomata were more abundant on the adaxial surface. Palisade cells tended to adopt an elongated shape, with similar size and arrangements toward both surfaces, mainly in salt-treated leaves, whereas plants sprayed with CCC had more epidermal cells with abundant tannins on the adaxial surface (data not shown).

The interaction between salinity and exogenous hormonal applications had a significant effect ($p < 0.05$) on central vascular bundles features in leaves of *P. strombulifera* plants at the lowest osmotic potential reached (Table 2 and Supplementary Information Fig. 2). The fiber wall was thickest around them in NaCl-treated plants, regardless of the sprayed solution. GA3 decreased the thickness of fibers in controls and NaCl-treated plants as compared to those sprayed with distilled water (54.3, 74.1 and 67.2, 85.5 µm, respectively). By contrast, GA_3 increased it in Na_2SO_4-treated plants as compared those sprayed with distilled water (60.1 and 49.4 µm, respectively). No significant effects were observed in central vascular bundles features in leaves of NaCl + Na_2SO_4-treated plants sprayed with the different solutions. CCC significantly increased the thickness of wall fibers in leaves of Na_2SO_4-treated plants in comparison with those sprayed with distilled water (68.5 and 49.5 µm, respectively). NaCl-treated plants also had the fewest parenchyma layers surrounding the bundles, regardless of the sprayed solution. The number of layers in all salt-treated plants was not affected by distilled water, GA_3, or CCC, but the hormone inhibitor did increase them in the controls. Parenchyma cells were larger on the abaxial side and most contained tannins. One to three minor vascular bundles could be observed at the sides of the central vascular bundles (data not shown).

Table 2 Effects of NaCl, Na_2SO_4, or the iso-osmotic mixture of both (NaCl + Na_2SO_4) on central vascular bundle features in the leaves of *Prosopis strombulifera* plants sprayed with distilled water (DW), gibberellins (GA3), or a gibberellin-synthesis inhibitor (CCC) after being cultured hydroponically at the lowest osmotic potential evaluated (− 2.6 MPa)

Treatments	Thickness of fibers (µm)	Number of parenchyma layers
Control + DW	67.2 ± 1.1 d	5 ± 0.1 b
Control + GA_3	54.3 ± 2.8 b	5 ± 0.2 b
Control + CCC	69.2 ± 1.1 d	6 ± 0.2 c
NaCl + DW	85.5 ± 0.5 f	4 ± 0.3 a
NaCl + GA_3	74.1 ± 3.3 e	4 ± 0.2 a
NaCl + CCC	87.7 ± 2.5 f	4 ± 0.4 a
Na_2SO_4 + DW	49.4 ± 2.1 a	5 ± 0.1 b
Na_2SO_4 + GA_3	60.1 ± 0.9 c	5 ± 0.2 b
Na_2SO_4 + CCC	68.5 ± 0.9 d	5 ± 0.2 b
NaCl + Na_2SO_4 + DW	66.4 ± 2.5 d	5 ± 0.3 b
NaCl + Na_2SO_4 + GA_3	67.1 ± 2.1 d	5 ± 0.2 b
NaCl + Na_2SO_4 + CCC	69.7 ± 2.2 d	5 ± 0.3 b

Control indicates non-salt-treated plants. Data are from four replicated experiments ($n = 12$ true biological replications). Values are means ± SE. Different letters indicate significant differences ($p < 0.05$)

Changes in endogenous levels of ABA and GA (active and inactive forms) also took place in roots and leaves under the different experimental conditions and, like the anatomical changes, they were significant ($p < 0.05$) at the lowest osmotic potential (− 2.6 MPa) reached (Fig. 2 and 3). The endogenous levels of hormones in sprayed plants varied under exposure to different types of salts in the culture medium. In general, the levels of ABA were higher in salt-treated plants than in the controls (Fig. 2A, B). Roots of salt-treated plants had the highest ABA level when sprayed with GA_3, but the application of CCC did not modify this parameter in comparison with salt-treated plants sprayed with distilled water (Fig. 2A). Similarly, the leaves of salt-treated plants showed an increase in ABA when sprayed with GA_3, with the highest level being observed in Na_2SO_4-treated plants (11.6 ng.gDW-1). The leaves of NaCl + Na_2SO_4-treated plants sprayed with GA_3 showed intermediate values of ABA to those observed in the NaCl- and Na_2SO_4- treated plants (9.3 and 7.6, 11.6 ng.gDW-1, respectively). On the other hand, ABA content was lower in leaves exposed to NaCl and Na_2SO_4 solutions and sprayed with CCC than in those sprayed with

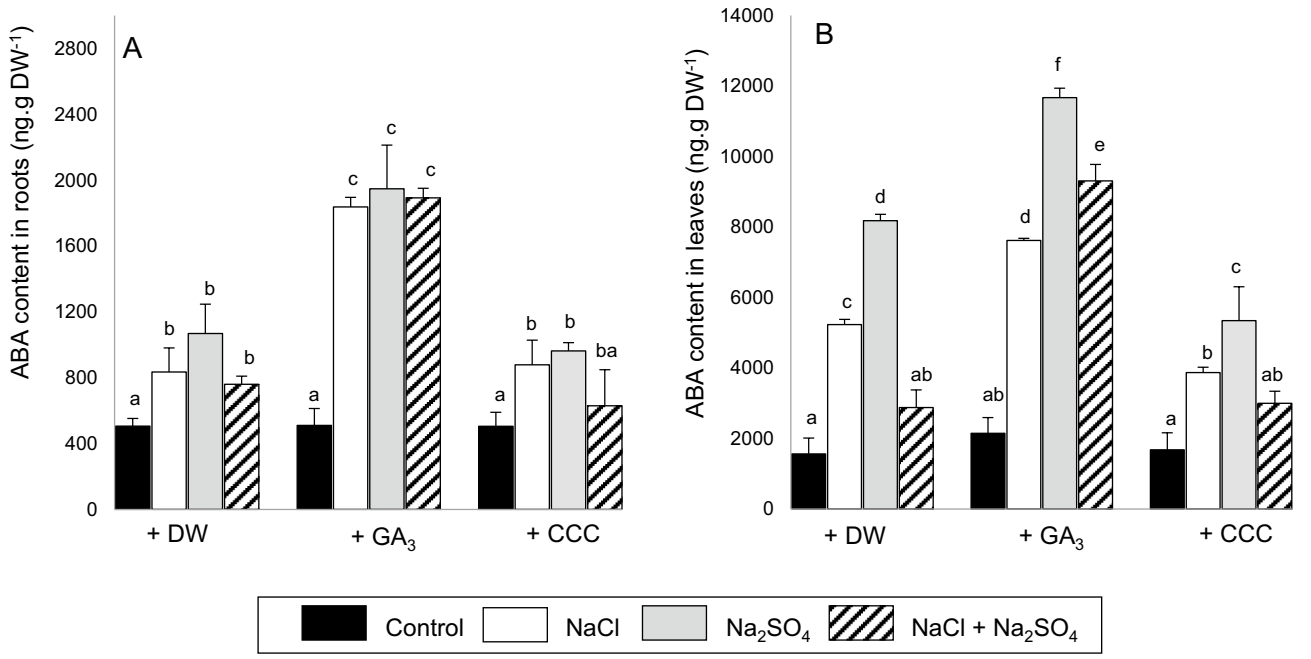
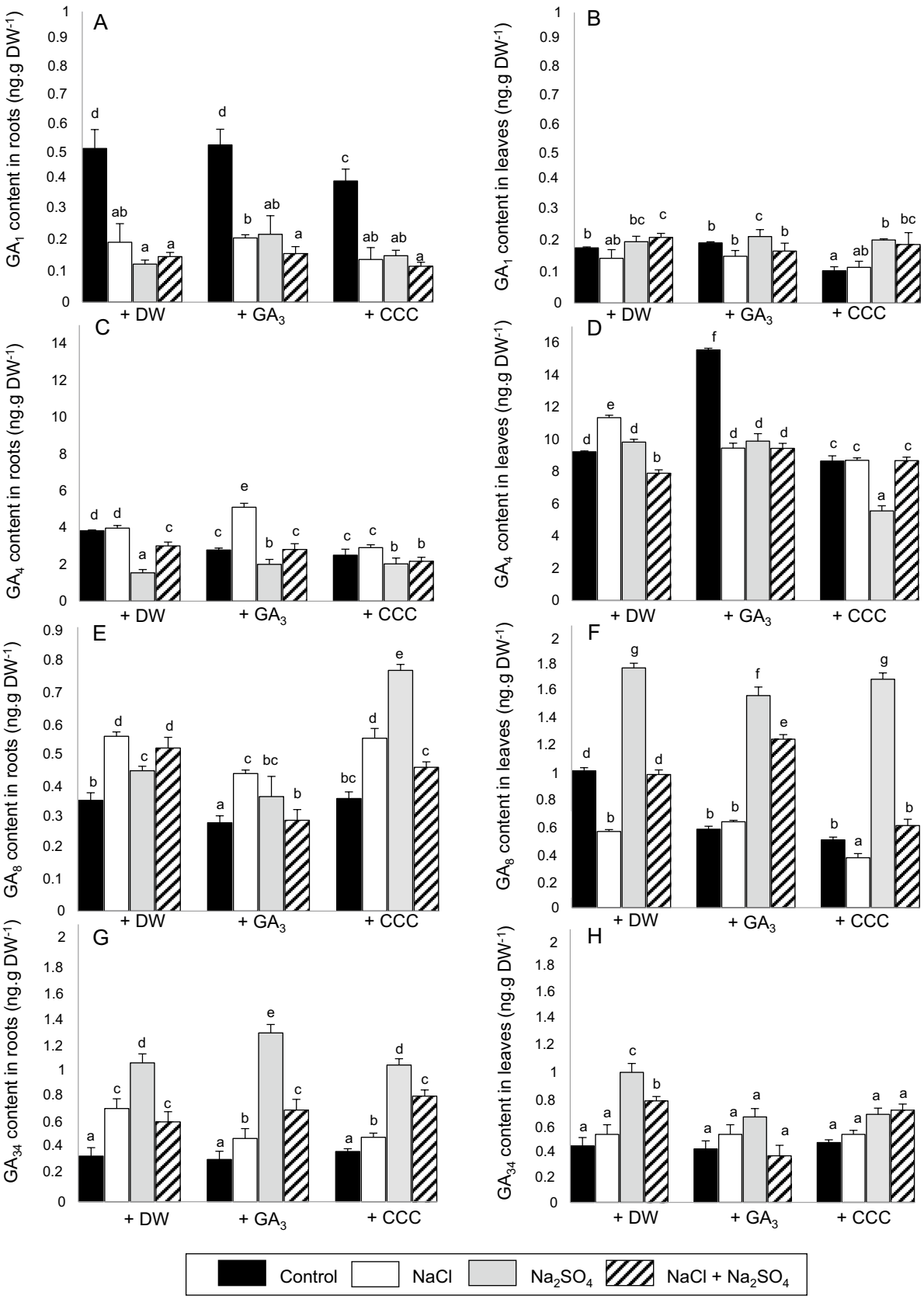


Fig. 2 Effects of NaCl, Na_2SO_4, or the iso-osmotic mixture of both (NaCl + Na_2SO_4) on levels of ABA in roots (**A**) and leaves (**B**) in *Prosopis strombulifera* plants sprayed with distilled water (DW), gibberellins (GA_3), and a gibberellin-synthesis inhibitor (CCC) at the lowest osmotic potential evaluated (Ψo: -2.6 MPa). Control indicates non-salt-treated plants. Data are from four replicated experiments ($n = 12$ true biological replications). Values are means ± SE. Different letters indicate significant differences ($p < 0.05$)

distilled water. CCC had no significant effects on controls or on plants treated with the mixture of both monosaline solutions (Fig. 2B). For its part, endogenous levels of GA_3 in roots and leaves was either very low or undetectable by LC–MS-MS, no matter the salt treatment or the application (data not shown). The values for GA_1 were the highest in roots of controls, and decreased significantly when they were exposed to CCC. Although its content tended to increase in the roots of salt-treated plants sprayed with GA_3, the differences were not significant with respect to plants sprayed with distilled water (Fig. 3A). In leaves, GA_1 decreased only in controls sprayed with CCC in comparison with those sprayed with distilled water. Overall, this hormone was not altered in leaves of salt-treated plants in response to CCC or GA_3 (Fig. 3B). An increase in GA_4, the most abundant biologically active GA in *P. strombulifera*, was observed in the roots of NaCl- and Na_2SO_4-treated plants sprayed with GA_3, as compared to those sprayed with distilled water (5.1, 2 and 3.9, 1.5 ng. gDW-1, respectively). However, no effects on levels of GA_4 were observed in roots of NaCl + Na_2SO_4-treated plants sprayed with GA_3. The application of CCC, by contrast, decreased GA_4 levels in control and all salt-treated plants (Fig. 3C). In leaves, the highest level was detected in GA_3-sprayed controls, while a reduction from distilled water values took place in both control and salt-treated plants upon application of CCC. Thus, the lowest values of GA_4 were registered in Na_2SO_4-treated plants sprayed with CCC (Fig. 3D).

Given that the 2beta-hydroxylation of GA_1 results in an inactive GA_8 form, while that of GA_4 produces inactive GA_{34}, both GA_8 and GA_{34} were analyzed. GA8 was generally higher in the roots of salt-treated plants than in the controls, regardless of the exogenous applications, but it was significantly reduced in roots when GA_3 was used. In the roots of Na_2SO_4-treated plants sprayed with CCC, its content increased with respect to the distilled water treatment (0.76 and 0.44 ng.gDW-1, respectively, Fig. 3E). The leaves of Na_2SO_4-treated plants also showed the highest level of GA_8 when sprayed with distilled water or CCC. On the other hand, GA_3 and CCC exogenous applications reduced GA_8 in leaves of controls plants as compared to those sprayed with distilled water. As far as plants treated with the iso-osmotic mixture of monosaline solutions are concerned, there was an increase in GA_8 in their leaves upon application of GA_3 and a decrease upon application of CCC in comparison with distilled water application. A reduction of GA_8 content was found in leaves of NaCl-treated plants sprayed with the inhibitor in comparison with GA_3 or distilled water applications (Fig. 3F). As regards GA_{34}, its highest level in roots was observed in Na_2SO_4-treated plants sprayed with GA_3. The applications of GA_3 and CCC reduced it in the roots of NaCl-treated plants, but did not affect either the controls or NaCl + Na_2SO_4-treated plants (Fig. 3G). In leaves, its highest


A
B
C
D
E
F
G
H
GA1 content in roots (ng.g DW-1)
GA1 content in leaves (ng.g DW-1)
GA4 content in roots (ng.g DW-1)
GA4 content in leaves (ng.g DW-1)
GA8 content in roots (ng.g DW-1)
GA8 content in leaves (ng.g DW-1)
GA34 content in roots (ng.g DW-1)
GA34 content in leaves (ng.g DW-1)
+ DW
+ GA3
+ CCC
Control
NaCl
Na2SO4
NaCl + Na2SO4

◂**Fig. 3** Effects of NaCl, Na_2SO_4, or the iso-osmotic mixture of both ($NaCl + Na_2SO_4$) on the gibberellin profile in roots (**A**, **C**, **E**, and **G**) and leaves (**B**, **D**, **F**, and **H**) in *Prosopis strombulifera* plants sprayed with distilled water (DW), gibberellins (GA_3), or a gibberellin-synthesis inhibitor (CCC) at the lowest osmotic potential evaluated (Ψo: -2.6 MPa). A and B correspond to GA_1 content, C and D to GA_4 content, E and F to GA_8, G and H to GA_{34}. Control indicates non-salt-treated plants. Data are from four replicated experiments ($n = 12$ true biological replications). Values are means ± SE. Different letters indicate significant differences ($p < 0.05$)

values were found in Na_2SO_4 and $NaCl + Na_2SO_4$-treated plants sprayed with distilled water, but GA_3 and CCC did not modify it, regardless of the salt treatments (Fig. 3H).

Discussion

The present work seeks to make a contribution to the knowledge gap on how the exogenous application of an active gibberellin (GA_3) or an inhibitor of GA synthesis (CCC) affects the woody halophytic plant *P.strombulifera* and modifies growth in response to different salts (NaCl, Na_2SO_4 or the iso-osmotic mixture of both) present in a hydroponic culture medium. NaCl and Na_2SO_4 are the most commonly found salts in soils of many countries (Moreno-Izaguirre et al. 2016; Reich et al. 2017; Taleisnik et al 2021). Thus, it is important to compare both NaCl and Na_2SO_4 effects on physiological and biochemical responses that woody plants develop to cope with soil salinity in natural environments. It has been reported that plant growth responses depend on the type of salt and the osmotic potential generated, as well as on the physiological and phenological state of the plant, among other factors (Flowers and Colmer 2015; Hniličková et al. 2019; Tanveer et al. 2020). In the present study, results demonstrated that *P. strombulifera* is a halophytic species that shows changes in growth responses, morpho-anatomical parameters, and hormonal profile only when plants were exposed at the lowest osmotic potential evaluated (− 2.6 MPa), without significant modifications at − 1 and − 1.9 MPa in the hydroponic culture. This is relevant considering that plants able to survive in salinized areas with more than 500 mM NaCl are scarce (Flowers and Colmer 2015). Therefore, *P. strombulifera* plants are within the maximum tolerance levels reported for halophytic plants (700 mM NaCl). However, previous results in this species also demonstrated that *P. strombulifera* is less tolerant to Na_2SO_4 than to NaCl, and that sulfate toxic effects were partially reversed with the iso-osmotic mixture ($NaCl + Na_2SO_4$). The presence of large amounts of sulfate anion in the cytosol and cell compartments affected metabolism, ion compartmentation, and hormonal levels (Llanes et al. 2013, 2016; Reginato et al. 2014). Furthermore, Reich et al. (2017) reported a higher toxicity of Na_2SO_4 over NaCl in *Brassica napa* plants by altering the sulfate transporters in cell membranes and leading to a detrimental efflux of sulfate into the cytosol and the chloroplasts.

The application of GA_3 fostered growth in non-salinized plants (controls) but negatively affected growth in salt-treated plants; specifically, despite an increase in root length, stressed plants treated with GA_3 had shorter shoots (although their first internode length increased in all plants). Interestingly, additional growth inhibition upon GA_3 application in Na_2SO_4-treated plants was evidenced by a reduction in the number of leaves, with increased leaf chlorosis and abscission, and a decrease in palisade/spongy ratio, which indicate that sulfate toxicity symptoms were accentuated by GA_3. Overall, these results indicate that exogenous applications of GA_3 exacerbated the effects of salinity in *P. strombulifera* plants. The applications of CCC produced no changes in shoot height, root length, and first internode length in controls and salt-treated plants as compared to plants sprayed with distilled water or GA_3, although CCC triggered modifications in spine length, leaf thickness, number of palisade cell layers, number of leaves, and endogenous hormone levels.

Roots and leaves of salt-treated plants sprayed with GA_3 showed an accumulation of ABA, which suggests that more ABA may be needed for appropriate responses to salinity. ABA has been demonstrated to play important roles in the regulation of plant responses to salinity, such as limiting water loss through the adjustment of stomatal aperture (Umezawa et al. 2010; Finkelstein 2013; Sah et al. 2016; Cai et al. 2017; Medina et al. 2019). However, regardless of the hormonal applications, the fact that the highest ABA levels were observed in leaves of Na_2SO_4-treated plants confirms previous results which proposed that the presence of sulfate in mesophyll cells interferes with ABA signaling, since it causes stomata to remain open and transpiration to be high (Llanes et al. 2014). *P. strombulifera* plants sprayed with CCC in our study showed lower ABA levels than those sprayed with GA_3, which could be associated with a reduced impact of ions under salinity in CCC-treated plants. In effect, previous reports have shown that ABA is necessary for the maintenance of intracellular ion homeostasis and the accumulation of compatible solutes, and that it can improve the activity of tonoplast antiporters and the expression of salt tolerance responsive genes (Fukuda et al. 2011; Anschütz et al. 2014; Osakabe et al. 2014; Vishwakarma et al. 2017; Ullah et al. 2018). The lower endogenous levels of GA also resulted in improved growth responses to salt stress. Much in the same way, *Arabidopsis* plants subjected to salt conditions have been observed to undergo a reduction in levels of GA active. This physiological adjustment in GA contents under stressful conditions is presumed to have a protective role, as evidenced by the enhanced tolerance to salt stress of GA-deficient mutants (Magome et al. 2008).

An interesting result is that CCC application caused an increase in spine length. Spines are modified leaves or leaf parts that can work as an adaptive strategy under adverse conditions (Mauseth 1988). For instance, plants can develop spines as a consequence of water shortages to reduce transpiration, which is why spiny plants are more frequently found in arid than in humid regions (Nobel 2003; Bagella et al. 2019). The presence of greater spines in salt-treated plants sprayed with CCC in our experiments could therefore represent an adaptation trait in response to salinity, while in control plants, it may be a symptom brought on by the growth retardant. Importantly, our results revealed significantly thicker palisade tissues in salt-treated plants sprayed with CCC, which could be related to higher photosynthetic activity (Nandy et al. 2005) as a means of coping with the salt conditions. Thicker leaves are another helpful trait for some plants growing under salt conditions, because they make it possible for more water to be retained by mesophyll tissues, thus counteracting salt toxicity (Naz et al. 2016). Moreover, thicker palisade tissue contributes to a higher light energy capture and mesophyll conductance and hence to CO_2 diffusion that can increase the photosynthesis rate (Nandy et al. 2005). Since the process of photosynthesis takes place mainly within these cells, increased parenchyma thickness enables higher photosynthetic activity and carbohydrate production. It is worthy to mention here that all these effects observed upon CCC application are known by plant physiologists as rustication, a typical response to this group of plant growth regulators, the growth retardants, due to their capacity to inhibit GA biosynthesis. Rustication is a process that involves biochemical modifications in the metabolism to produce complex carbohydrates such as lignin, suberin, tannins at the expense of growth, providing a greater plant resistance to stress (Taiz et al. 2015). By contrast, the application of GA_3 on Na_2SO_4-treated plants caused the lowest palisade/spongy ratio registered. This obvious impact on the photosynthesizing tissue could be related to the toxic effects of the sulfate anion, which has also been found to remarkably decrease net photosynthetic rate, maximum quantum efficiency of PSII, and quantum yield in *P. strombulifera* plants at − 2.6 MPa Na_2SO_4 (unpublished data). Thus, the application of GA_3 intensified the negative effects of Na_2SO_4. Lastly, although the formation of fibers in the xylem and phloem of maturing leaves is known to be controlled by hormones such as auxins and citokinins (Aloni 2013), GA have also been pointed out as likely participants in the process (Dayan et al. 2012). However, in the present study, results did not show a larger number of fibers in the vascular bundles after the application of GA_3, which suggests that salinity might trigger other signals that inhibit this specific role of GA. In addition, the fibers in the leaf vascular bundles became thicker after exposure to the GA synthesis inhibitor. Further clarity on this particular point could be gained by comparing levels of active GA in the histological leaf sections of plants under all treatments and applications, but finer image resolution than the one used in this study would be needed.

Among the more than 130 GA identified, only a few are known to be active forms (GA_1, GA_3, GA_4, and GA_7) which can induce physiological functions, while inactive forms act as precursors for the active ones or are deactivated metabolites in plants (Yamaguchi et al. 2008; Kim et al. 2014; Magome and Kamiya 2016). In this study, GA_4 was found to be the most abundant biologically active GA in *P. strombulifera* plants. Its content was significantly higher than that of other active forms like GA_1 and GA_3 independently of the treatment and most of the morpho-physiological responses observed could be related to GA_4 action. The exogenous application of GA_3 produced no changes in endogenous levels of GA_3, which were very low or not detectable, probably due to its immediate metabolism once entering the cell. However, application of GA_3 induced a high GA_4 accumulation in the non-stressed tissues of control plant leaves, but in Na_2SO_4-treated plants, the synthesis and metabolism of GA_1 were stimulated producing high levels of its catabolite GA_8. CCC, on the other hand, decreased the levels of the active GA_4 in Na_2SO_4-treated plants and also increased GA_1 synthesis and metabolism, producing high levels of its catabolite GA_8. GA are inactivated through at least three different pathways, as evidenced by some studies in rice and Arabidopsis plants (Zhu et al. 2006; Rieu et al. 2008; Zhang et al. 2011). More precisely, precursors and active forms are substrates of catabolic enzymes gibberellin-2-oxidase (GA2ox), gibberellin methyltransferase (GAMT), and CYP714A. Some GA2ox enzymes can hydroxylate C-2 in active C_{19}-GA (GA_1 and GA_4) to produce inactive GA forms, such as GA_8 and GA_{34}, among others (Hedden and Phillips 2000; Sakamoto et al. 2004). Recently, Shufang et al. (2018) demonstrated that GA2ox expression may be involved in NaCl-controlled root growth in Arabidopsis plants, because root elongation was positively correlated with enzyme expression levels. In the present study, salt-treated plants had higher levels of GA_8 and GA_{34} than the controls, which suggests that GA2ox pathways are functioning in these plants. In addition, result of endogenous levels of active and inactive form of GA in control and salt-treated plants sprayed with CCC indicates that inhibitor application was not effective reducing the biosynthesis of GA. However, CCC modified the spine length, leaf thickness, number of palisade cell layers, number of leaves, and endogenous ABA levels in sprayed plants. The low efficacy of CCC as inhibitor of GA biosynthesis in *P. strombulifera* plants suggests that the presence of this inhibitor is not enough to block the earlier steps of the GA biosynthesis pathways (Rademacher, 2016; He et al. 2019). This effect could be related to responses depending on the dose of the inhibitor or activation of GA independent

processes. Results of study not exclude the possibility that woody plants evolved multistep pathways of GA regulation and the use of other GA inhibitors to deepen the role of GA in plants under salinity.

Conclusion

The present study demonstrates, for the first time, how the exogenous application of an active GA form (GA_3) or an inhibitor of GA synthesis (CCC) affects the woody halophytic plant *P. strombulifera* and modifies growth in response to different sodium salts present in a hydroponic culture medium. These growth responses were affected not only by the low osmotic potential generated in the culture medium (− 2.6 MPa), but also by the chemical composition of the salts, being highly tolerant to NaCl (i.e., with no compromise to growth up to concentrations higher than 700 mM NaCl). By contrast, Na_2SO_4-treated plants showed growth inhibition and high ABA levels related to accumulation of sulfate anion in the cytosol causing metabolic toxicity, which can be partially reversed in the presence of NaCl when both salts are combined in an iso-osmotic mixture, probably due to ion antagonism and mutual competence at the membrane level (Reginato et al. 2014).

The high endogenous levels of GA_4 in leaves of all salt-treated plants, together with the low levels of its catabolite GA_{34} and high levels of ABA, suggest that both hormones act in concert in the regulation of plant growth under salinity conditions. It has been clear for some time that a major role of GA signaling in the response to abiotic stress is to integrate information from a number of other hormone signaling pathways, and ABA is known to be closely integrated with GA signaling in a number of systems (Colebrook et al. 2014).

GA_1 was also present in leaves and roots of all salt-treated plants but in much lower concentrations that GA_4. Its catabolite GA_8 was nevertheless found in higher concentrations than GA_{34}, especially in Na_2SO_4-treated plants, suggesting that the levels of the active form are tightly controlled.

The application of application of CCC, an inhibitor of GA synthesis, triggered morpho-anatomical and hormonal changes such as an increase in spine length, leaf thickness, and number of palisade cell layers, and a reduction in endogenous ABA levels in all salt-treated plants. All these traits, characteristic of a 'rustic' or stress resistant plant, coincided with a significant decrease in GA_4 in the most stressed plants, which were treated with Na_2SO_4. In agreement with our results, evidence is accumulating that suppression of GA signaling is a general response to abiotic stress, with transcriptional upregulation of *GA2ox* genes, encoding GA-inactivating enzymes. Furthermore, the action of GA cannot be considered in isolation of the other hormone signals, because of the rapidly emerging evidence for interactions between hormone pathways, in many cases mediated by DELLA proteins (Liu et al. 2016; Shu et al. 2018).

These findings contribute to our knowledge about the role of GA in the physiology of woody plants growing in saline soils, such as the *Prosopis* genus studied here. Future research could be carried out at the transcriptomic level to continue elucidating the relevance of these hormones in these plants' response to salinity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-022-10725-y.

Acknowledgements We thank UNRC for allowing us to use its facilities.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Analía Llanes and Santiago Biava; Claudia Travaglia contributed to the anatomical assays and data analysis. Oscar Masciarelli contributed to the hormonal data collection and analysis. The first draft of the manuscript was written by Analía Llanes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was partially supported by Secretaría de Ciencia y Técnica UNRC (SCyT-UNRC), Fondo para la Investigación Científica y Tecnológica (FONCyT) (PICT 2018–3148) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Data Availability Data and material analyzed are not publicly available but are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

References

Aloni R (2013) Role of hormones in controlling vascular differentiation and the mechanism of lateral root initiation. Planta 238(5):819–830. https://doi.org/10.1007/s00425-013-1927-8

Anschütz U, Becker D, Shabala S (2014) Going beyond nutrition: regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. J Plant Physiol 171(9):670–687. https://doi.org/10.1016/j.jplph.2014.01.009

Bagella S, Filigheddu R, Benesperi R, Giordani P, Minuto L, Viciani D, Casazza G (2019) Thorn, spine and prickle patterns in the Italian flora. Plant Biosyst 153(1):118–133. https://doi.org/10.1080/11263504.2018.1474961

Bechtold U, Ferguson JN, Mullineaux PM (2018) To defend or to grow: lessons from Arabidopsis C24. J Exp Bot 69(11):2809–2821. https://doi.org/10.1093/jxb/ery106

Binenbaum J, Weinstain R, Shani E (2018) Gibberellin localization and transport in plants. Trends Plant Sci 23(5):410–421. https://doi.org/10.1016/j.tplants.2018.02.005

Burkart A (1976) A monograph of the genus Prosopis (Leguminosae subfam. Mimosoideae). Catalogue of the recognized species of Prosopis. J Arnold Arbor 57:450–525

Cai R, Dai W, Zhang C, Wang Y, Wu M, Zhao Y, Cheng B (2017) The maize WRKY transcription factor ZmWRKY17 negatively regulates salt stress tolerance in transgenic Arabidopsis plants. Planta 246(6):1215–1231. https://doi.org/10.1007/s00425-017-2766-9

Chen HI, Li PF, Yang CH (2019) NAC-Like Gene GIBBERELLIN SUPPRESSING FACTOR regulates the gibberellin metabolic pathway in response to cold and drought stresses in arabidopsis. Sci Rep. https://doi.org/10.1038/s41598-019-55429-8

Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. J Exp Bot 217(1):67–75. https://doi.org/10.1242/jeb.089938

D'Ambrogio de Argüeso A (1986) Manual de técnicas en histología vegetal. Hemisferio Sur, Buenos Aires, p 83

Dayan J, Voronin N, Gong F, Sun TP, Hedden P, Fromm H, Aloni R (2012) Leaf-induced gibberellin signaling is essential for internode elongation, cambial activity, and fiber differentiation in tobacco stems. Plant Cell 24(1):66–79. https://doi.org/10.1105/tpc.111.093096

Devinar G, Llanes A, Masciarelli O, Luna V (2013) Different relative humidity conditions combined with chloride and sulfate salinity treatments modify abscisic acid and salicylic acid levels in the halophyte *Prosopis strombulifera*. Plant Growth Regul 70(3):247–256. https://doi.org/10.1007/s10725-013-9796-5

FAO (2017) FAO soils portal, salt-affected soils. http://www.fao.org/soils-portal/soil-management/management-of-some-problem-soils/salt-affected-soils/more-information-on-salt-affected-soils/en/ (Accessed February, 2020).

Finkelstein R (2013) Abscisic acid synthesis and response. The Arabidopsis book/American society of plant biologists

Flowers TJ, Colmer TD (2015) Plant salt tolerance: adaptations in halophytes. Ann Bot 115(3):327–331. https://doi.org/10.1093/aob/mcu267

Fukuda A, Nakamura A, Hara N, Toki S, Tanaka Y (2011) Molecular and functional analyses of rice NHX-type Na+/H+ antiporter genes. Planta 233(1):175–188. https://doi.org/10.1007/s00425-010-1289-4

Golldack D, Li C, Mohan H, Probst N (2013) Gibberellins and abscisic acid signal crosstalk: living and developing under unfavorable conditions. Plant Cell Rep 32(7):1007–1016. https://doi.org/10.1007/s00299-013-1409-2

Han LP, Wang X, Guo X, Rao MS, Steinberger Y, Cheng X, Xie GH (2011) Effects of plant growth regulators on growth, yield and lodging of sweet sorghum. Res Crops 12(2):372–382

He H, Liang G, Lu S, Wang P, Liu T, Ma Z, Mao J (2019) Genome-wide identification and expression analysis of GA2ox, GA3ox, and GA20ox are related to gibberellin oxidase genes in grape (Vitis vinifera L). Genes 10(9):680. https://doi.org/10.3390/genes10090680

Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci 5:523–530

Hedden P, Thomas SG (2016) Annual plant reviews, the gibberellins. Wiley. https://doi.org/10.1016/S1360-1385(00)01790-8

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circular, 2nd edn. California Agricultural Experiment Station, p 347

Huang M, Fang Y, Liu Y, Jin Y, Sun J, Tao X, Zhao H (2015) Using proteomic analysis to investigate uniconazole-induced phytohormone variation and starch accumulation in duckweed (*Landoltia punctata*). BMC 15(1):81. https://doi.org/10.1186/s12896-015-0198-9

Johansen DA (1940) Plant microtechnique. McGraw-Hill Book Company Inc, London

Kim SK, Kim HY (2014) Effects of gibberellin biosynthetic inhibitors on oil, secoisolaresonolodiglucoside, seed yield and endogenous gibberellin content in flax. Korean J Plant Res 27(3):229–235

Leone M, Keller MM, Cerrudo I, Ballaré CL (2014) To grow or defend? Low red:far-red ratios reduce jasmonate sensitivity in arabidopsis seedlings by promoting DELLA degradation and increasing JAZ10 stability. New Phytol 204:355–367. https://doi.org/10.1111/nph.12971

Liu X, Hu P, Huang M, Tang Y, Li Y, Li L (2016) The NF-YC–RGL2 module integrates GA and ABA signaling to regulate seed germination in Arabidopsis. Nat Commun 7:12768. https://doi.org/10.1038/ncomms12768

Liu J, Shabala S, Shabala L, Zhou M, Meinke H, Venkataraman G, Zhao Q (2019) Tissue-specific regulation of Na+ and K+ transporters explains genotypic differences in salinity stress tolerance in rice. Front Plant Sci 10:1361. https://doi.org/10.3389/fpls.2019.01361

Llanes A, Bertazza G, Palacio G, Luna V (2013) Different sodium salts cause different solute accumulation in the halophyte *Prosopis strombulifera*. Plant Biol 15:118–125. https://doi.org/10.1111/j.1438-8677.2012.00626.x

Llanes A, Masciarelli O, Ordoñez R, Isla MI, Luna V (2014) Differential growth responses to sodium salts involve different abscisic acid metabolism and transport in *Prosopis strombulifera*. Biol Plant 58(1):80–88. https://doi.org/10.1007/s10535-013-0365-6

Llanes A, Arbona V, Gómez-Cadenas A, Luna V (2016) Metabolomic profiling of the halophyte *Prosopis strombulifera* shows sodium salt-specific response. Plant Physiol Biochem 108:145–157. https://doi.org/10.1016/j.plaphy.2016.07.010

Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2008) The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis. Plant J 56(4):613–626. https://doi.org/10.1111/j.1365-313X.2008.03627.x

Magome H, Kamiya Y (2016) Inactivation processes. Ann Plant Rev 49:73–93

Mauseth JD (1988) Plant Anatomy. Benjamin Cummnings Pub. Co., Inc., California, pp 1–560

Medina EF, Mayrink GC, Dias CR, Vital CE, Ribeiro DM, Silva IR, Merchant A (2019) Physiological and biochemical responses of Eucalyptus seedlings to hypoxia. Ann for Sci 76(1):4

Moreno-Izaguirre E, Ojeda-Barrios D, Avila-Quezada G, Guerrero-Prieto V, Parra-Quezada R, Ruíz-Anchondo T (2016) Sodium sulfate exposure slows growth of native pecan seedlings. Phyton 84(1):80–85

Nandy P, Das S, Ghose M (2005) Relation of leaf micro-morphology with photosynthesis and water efflux in some Indian mangroves. Acta Bot Croat 64(2):331–340

Naz N, Fatima S, Hameed M, Naseer M, Batool R, Ashraf M, Ahmad KS (2016) Adaptations for salinity tolerance in *Sporobolus ioclados* (Nees ex Trin.) Nees from saline desert. Flora 223:46–55. https://doi.org/10.1016/j.flora.2016.04.013

Nobel PS (2003) Environmental biology of agaves and cacti. Cambridge University Press, Cambridge

Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2014) ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. New Phytol 202(1):35–49. https://doi.org/10.1111/nph.12613

Qadir M, Quillérou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Noble AD (2014) Economics of salt-induced land degradation and restoration. Nat Resour Forum 38(4):282–295. https://doi.org/10.1111/1477-8947.12054

Rademacher W (2016) Chemical regulators of gibberellin status and their application in plant production. Ann Plant Rev 49:359–403. https://doi.org/10.1002/9781119312994.apr0541

Reginato M, Sosa L, Llanes A, Hampp E, Vettorazzi N, Reinoso H, Luna V (2014) Na2SO4 and NaCl determine different growth responses and ion accumulation in the halophytic legume *Prosopis strombulifera*. Plant Biol 16:97–106

Reich M, Aghajanzadeh T, Helm J, Parmar S, Hawkesford MJ, De Kok LJ (2017) Chloride and sulfate salinity differently affect biomass, mineral nutrient composition and expression of sulfate transport and assimilation genes in *Brassica rapa*. Plant Soil 411(1–2):319–332. https://doi.org/10.1007/s11104-016-3026-7

Reinoso H, Sosa L, Ramirez L, Luna V (2004) Salt-induced changes in the vegetative anatomy of *Prosopis strombulifera* (Leguminosae). Can J Bot 82:618–628. https://doi.org/10.1139/b04-040

Reinoso H, Sosa L, Reginato M, Luna V (2005) Histological alterations induced by sodium sulfate in the vegetative anatomy of *Prosopis strombulifera* (Lam.) Benth. World J Agr Sci 2:109–119

Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., Robledo, C., 2016. InfoStat. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar/. (Accessed February, 2020).

Rieu I, Eriksson S, Powers SJ, Gong F, Griffiths J, Woolley L, Phillips AL (2008) Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. Plant Cell 20(9):2420–2436. https://doi.org/10.1105/tpc.108.058818

Sah SK, Reddy KR, Li J (2016) Abscisic acid and abiotic stress tolerance in crop plants. Front Plant Sci 7:571. https://doi.org/10.1105/tpc.108.058818

Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Agrawal GK, Takeda S, Abe K, Miyao A, Hirochika H, Kitano H, Ashikari M, Matsuoka M (2004) An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol 134:1642–1653. https://doi.org/10.1104/pp.103.033696

Sancho-Knapik D, Sanz MÁ, Peguero-Pina JJ, Niinemets Ü, Gil-Pelegrín E (2017) Changes of secondary metabolites in Pinus sylvestris L. needles under increasing soil water deficit. Ann for Sci 74(1):24

Shi T, Rahmani RS, Gugger PF, Wang M, Li H, Zhang Y, Li Z, Wang Q, Van de Peer Y, Marchal K, Chen J (2020) Distinct expression and methylation patterns for genes with different fates following a single whole-genome duplication in flowering plants. Mol Biol Evol 37(8):2394–2413. https://doi.org/10.1093/molbev/msaa105

Shu K, Zhou W, Chen F, Luo X, Yang W (2018) Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. Front Plant Sci 9:416. https://doi.org/10.3389/fpls.2018.00416

Shufang LS, Yu D, Sun Q, Jiang J (2018) Activation of gibberellin 20-oxidase 2 undermines auxin-dependent root and root hair growth in NaCl-stressed Arabidopsis seedlings. Plant Growth Regul 84(2):225–236. https://doi.org/10.1007/s10725-017-0333-9

Singh D, Singh CK, Kumari S, Tomar RSS, Karwa S, Singh R, Pal M (2017) Discerning morpho-anatomical, physiological and molecular multiformity in cultivated and wild genotypes of lentil with reconciliation to salinity stress. PLoS ONE 12(12):e0190462. https://doi.org/10.1371/journal.pone.0177465

Sosa L, Llanes A, Reinoso H, Reginato M, Luna V (2005) Osmotic and specific ion effects on the germination of *Prosopis strombulifera*. Ann Bot 96:261–267. https://doi.org/10.1093/aob/mci173

Taiz L, Zeiger E, Møller IM, Murphy A (2015) Plant physiology and development, 6th edn. Sinauer Associates Incorporated, pp 6–761

Taleisnik E, Rodríguez A, Bustos D, Luna D (2021) Plant Tolerance Mechanisms to Soil Salinity Contribute to the Expansion of Agriculture and Livestock Production in Argentina. In: Taleisnik E, Lavado R (eds) Saline and alkaline soils in latin America. Springer, Cham, pp 381–397

Tanveer M, Shabala S (2018) Targeting redox regulatory mechanisms for salinity stress tolerance in crops. In: Kumar V, Wani SH, Suprasanna P, Tran L-SP (eds) Salinity Responses and Tolerance in Plants, vol 1. Springer, Cham

Travaglia C, Balboa G, Espósito G, Reinoso H (2012) ABA action on the production and redistribution of field-grown maize carbohydrates in semiarid regions. Plant Growth Regul 67(1):27–34. https://doi.org/10.1007/s10725-012-9657-7

Ullah A, Sun H, Hakim Y, Zhang X (2018) A novel cotton WRKY gene, GhWRKY6-like, improves salt tolerance by activating the ABA signaling pathway and scavenging of reactive oxygen species. Physiol Plant 162(4):439–454. https://doi.org/10.1111/ppl.12651

Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K (2010) Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol 51(11):1821–1839. https://doi.org/10.1093/pcp/pcq156

Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, Sharma S (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. Front Plant Sci 8:161. https://doi.org/10.3389/fpls.2017.00161

Wang Y, Zhao J, Lu W, Deng D (2017) Gibberellin in plant height control: old player, new story. Plant Cell Rep 36(3):391–398. https://doi.org/10.1007/s00299-017-2104-5

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Ann Rev Plant Biol 59:225–251

Zhang ZL, Ogawa M, Fleet CM, Zentella R, Hu J, Heo JO, Sun TP (2011) Scarecrow-like 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis. PNAS 108(5):2160–2165. https://doi.org/10.1073/pnas.1012232108

Zhang S, Yang R, Huo Y, Liu S, Yang G, Huang J, Wu C (2018) Expression of cotton PLATZ1 in transgenic Arabidopsis reduces sensitivity to osmotic and salt stress for germination and seedling establishment associated with modification of the abscisic acid, gibberellin, and ethylene signalling pathways. BMC Plant Biol 18(1):1–11. https://doi.org/10.1186/s12870-018-1416-0

Zhu Y, Nomura T, Xu Y, Zhang Y, Peng Y, Mao B, Zhu X (2006) ELONGATED UPPERMOST INTERNODE encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. Plant Cell 18(2):442–456. https://doi.org/10.1105/tpc.105.038455