



# Positive impact of vermicompost leachate on salt stress resistance in tomato (*Solanum lycopersicum* L.) at the seedling stage: a phytohormonal approach

Salima Benazzouk · Petre I. Dobrev ·  
Zahr-Eddine Djazouli · Vaclav Motyka · Stanley Lutts

Received: 6 May 2019 / Accepted: 5 November 2019 / Published online: 14 November 2019  
© Springer Nature Switzerland AG 2019

## Abstract

**Background and aim** Vermicompost leachate (VCL) produced by earthworms is a valuable biostimulant but its hormonal impact on salt-treated plants remains elusive.

**Methods** Plants of *Solanum lycopersicum* L. were cultivated in nutrient solution and exposed during 7 days to 125 mM NaCl in the presence or absence of VCL (18 mL.L<sup>-1</sup>). Mineral nutrition, water and hormonal status were quantified in roots, young and old leaves and analyzed in relation to phytohormone content of VCL.

**Results** VCL improved plant growth and reduced Na<sup>+</sup> accumulation in salt-stressed plants. It delayed senescence in young leaves through a decrease in ethylene

synthesis and an increase in proline and anthocyanin contents. VCL contains high amounts of salicylic acid, benzoic acid and aminocyclopropane carboxylic acid (ACC) but low concentrations of jasmonates, cytokinins and proline. VCL did not increase abscisic acid content in salt-stressed plants and did not lead to ACC accumulation while it increased jasmonate accumulation and modified the pattern of cytokinin profile with an increase in dihydrozeatin-types in old leaves and N<sup>6</sup>-(Δ<sup>2</sup>-isopentenyl)adenine-types in young ones.

**Conclusion** VCL reduces the impact of salinity on leaf senescence, which is related to its impact on endogenous phytohormones rather than to a passive absorption of exogenous hormonal compounds.

**Keywords** Biostimulants · Phytohormones · Salinity · *Solanum lycopersicum* · Vermicompost

Responsible Editor: Wieland Fricke.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11104-019-04361-x>) contains supplementary material, which is available to authorized users.

S. Benazzouk · S. Lutts (✉)  
Groupe de Recherche en Physiologie Végétale, Earth and Life  
Institute - Agronomy (ELI-A), Université Catholique de Louvain,  
5 (Bte 7.07.13) Place Croix du Sud, 138 Louvain-la-Neuve,  
Belgium  
e-mail: stanley.lutts@uclouvain.be

S. Benazzouk · Z.-E. Djazouli  
Faculté des Sciences de la Nature et de la Vie, Université de Blida  
1, Ouled yaich, Blida, Algeria

P. I. Dobrev · V. Motyka  
Institute of Experimental Botany of the Czech Academy of  
Sciences, Prague, Czechia

## Introduction

Plants as sessile organisms are fixed to their substrate and use a wide range of biochemical strategies to permanently sense and adapt to their environment. Salinity is undoubtedly one of the major constraints limiting plant growth and development worldwide. Soil salinization may occur in coastal areas as a consequence of seawater intrusion and in inland areas as an impact of natural geological processes releasing soluble salt from the bedrock. It is aggravated by secondary salinization resulting from unadapted irrigation procedures and, on a long term basis, by global warming rising sea levels

(Bui 2013; Wassman et al. 2004). Salinity induces osmotic and ionic constraints on plants and drastically reduces yield in cultivated species. In most crops, however, an intraspecific variability exists in terms of salinity resistance and some cultivars appear to be more resistant to NaCl than others (Fita et al. 2015).

Beside their important functions in the regulation of all steps of plant growth and development, phytohormones also play crucial roles in environment sensing, signal transduction and elicitation of plant responses to environmental constraints (Ciura and Kruk 2018). Tomato (*Solanum lycopersicum* L.) is one of the most important crops in the world and its culture is expanding in relation to its high nutritive value and economical interest. It is considered as a glycophyte plant species but a comparison between cultivars exhibiting contrasting levels of resistance to salinity revealed that the phytohormonal status of the plant plays a critical role in its adaptation to high external NaCl doses (Manaa et al. 2014; Ghanem et al. 2008, 2011). This conclusion also results from the study of recombinant inbred lines issued from crosses between the cultivated glycophyte *S. lycopersicum* and closely-related wild halophyte species (Albacete et al. 2009). Gharbi et al. (2017) recently compared the hormonal profile of *Solanum lycopersicum* and the halophyte *Solanum chilense* which is able to cope with extremely high doses of NaCl and clearly showed contrasting status between the two species, especially regarding ethylene, polyamines, salicylic acid (SA) and abscisic acid (ABA) (Gharbi et al. 2016, 2017, 2018). The specific hormonal status of the halophyte species allowed it to respond very rapidly and in appropriate organs to detrimental doses of NaCl while the cultivated glycophyte reacted latter. Exogenous application of phytohormones such as SA (Mimouni et al. 2016), ABA (Gurmani et al. 2013), cytokinins (CKs; Wu et al. 2014) or gibberellins (GAs; Khan et al. 2010) were also shown to improve salinity resistance in various plant species.

The phytohormonal status may be an interesting target for improving salinity resistance in tomato. A breeding approach may help to achieve this goal on a long term basis but constitutes a complex and expensive task. An alternative would be to directly act on the phytohormonal status of the plant through exogenous application of plant growth regulators (PGRs; Rady and Mohamed 2015). Chemical synthesis of PGRs is rather expensive and application of synthetic compounds does not appear as an ecofriendly strategy. Biostimulants

constitute an attracting alternative and include diverse categories of products such as seaweed extracts, protein hydrolysate or humic acids (Alam et al. 2013; Calvo et al. 2014; du Jardin 2015). Vermicompost is obtained through digestion of organic wastes by red earthworms *Eisenia fetida*. This cheap process allowing biomass recycling produces a solid fraction (vermicompost sensu stricto) and a liquid fraction (vermicompost leachate; VCL). The increasing use of vermicompost products is due to their numerous beneficial effects on plant growth promotion and development (Singh et al. 2008; Hernandez et al. 2015; Rupani et al. 2018; Zarei et al. 2018), improvement of soil properties (Yang et al. 2015) and alleviation of nutrient deficiencies and abiotic stresses (Aremu et al. 2014; Chinsamy et al. 2013, 2014; Zhang et al. 2019).

Benazzouk et al. (2018) recently demonstrated that VCL application on salt-treated tomato plants helps to maintain their net photosynthesis, to limit Na<sup>+</sup> translocation from the root to the shoot, and to improve osmotic adjustment mainly through proline synthesis. The involvement of phytohormones on the recorded improvement still remains an open question. VCL contains various nutrients (N, P, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>), humic acids and antioxidant compounds which may contribute to salt resistance. Canellas et al. (2002) and Trevisan et al. (2010) showed that humic substances such as those present in VCL may have an “auxin-like” effect and induce the expression of early auxin-responsive gene. According to Pizzeghello et al. (2013), CK-like activity could also be detected in humic substances produced by earthworms. Some recent studies also provided evidences that vermicompost and VCL contain substantial amounts of auxin, CKs, ABA and GAs (Aremu et al. 2015; Zhang et al. 2014, 2015).

Exogenously applied PGRs may be absorbed by the plants but may also have an impact on the endogenous phytohormone synthesis. Salinity may influence exogenous PGRs absorption and translocation, endogenous phytohormones synthesis, but also the impact of exogenous PGRs on internal hormonal synthesis. Since salinity is commonly considered to induce precocious senescence in plant tissues (Lutts et al. 1996; Ghanem et al. 2008), one may expect that VCL impact on phytohormonal status could be different in old and young leaves of salt-treated plants. A comprehensive approach aiming to understand the hormonal basis of VCL on salt-resistance improvement requires to quantify both the external and the internal concentrations of

phytohormones in different organs. Such an integrated approach remains poorly documented. Therefore, the aim of the present study is to establish a hormonal profile of plants exposed to salinity receiving or not an external supply of VCL. Phytohormones were quantified in VCL as well as in plant tissues and the obtained data are discussed in relation to the physiological status of the salt-stressed plants.

## Materials and methods

### Plant material and growth conditions

The VCL used in the present study was obtained from digestion of the food-wastes by red earthworm *Eisenia fetida* as indicated by Chaichi (2018) at a temperature comprised between 25 and 35 °C and a relative humidity of 90%. Seeds of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) were obtained from the Tomato Genetic Resource Centers (Davis, USA) and multiplied in the greenhouse of Université catholique de Louvain (Louvain-la-Neuve; Belgium). Seeds were germinated in a loam substrate in experimental greenhouse. Fifteen days after germination, the seedlings were transferred to a hydroponic culture system into a growth chamber under 16 h photoperiod at 150  $\mu\text{molm}^{-2} \text{s}^{-1}$  light intensity provided by Sylvania fluorescent tubes (F96 T12/CW/VHO). The temperature was set at 25 °C during the day and 20 °C during the night with corresponding relative humidity of 70% and 90%, respectively. Seedlings were fixed on polystyrene plates floating on half-strength aerated Hoagland nutrient solution containing (in mM): 5  $\text{KNO}_3$ , 5.5  $\text{Ca}(\text{NO}_3)_2$ , 1  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.5  $\text{MgSO}_4$ , and (in  $\mu\text{M}$ ) 25  $\text{KCl}$ , 10  $\text{H}_3\text{BO}_3$ , 1  $\text{MnSO}_4$ , 0.25  $\text{CuSO}_4$ , 1  $\text{ZnSO}_4$ , 10  $(\text{NH}_4)_6\text{Mo}_7\text{O}$  and 1.87  $\text{g.L}^{-1}$  Fe-EDTA. Solutions were renewed every week. Plants were distributed among twenty tanks (with five tanks per treatment and four seedlings per tank) containing 1.5 L of aerated nutrient solution in a complete randomized block design. Seedlings grew in control conditions for one week before NaCl and/or VCL treatments were applied. Four treatment groups were defined: (1) control: plants grown in half-strength Hoagland solution, (2) salt stress: plants grown in half-strength Hoagland solution containing 125 mM NaCl, (3) VCL: plants grown in half-strength Hoagland solution containing 18  $\text{mL.L}^{-1}$  VCL and (4) VCL + NaCl: plants grown in

half-strength Hoagland solution containing 18  $\text{mL.L}^{-1}$  VCL and 125 mM NaCl.

Plants were harvested and divided into roots and leaves for physiological and biochemical analyses after 7 days of treatment. Four specific leaves per plant were chosen: the old leaves identified as leaf number 2 and 3 (L2–3), and young leaves identified as leaf number 5 and 6 (L5–6) (acropetal numbering). Leaves 2 and 3 were fully expanded at the time of stress imposition while leaves 5 and 6 were still expanding during the time course of the experiment. All samples (except those used for analysis of mineral content, see below) were quickly frozen in liquid nitrogen and stored at  $-80$  °C until final analysis. Cations were also quantified in the xylem sap obtained from severing the shoot 2–3 cm above the roots and applying pressure (from 0.5 MPa for to 0.9 MPa) to the root system using a Scholander pressure chamber (Ghanem et al. 2011). The foremost efflux was discarded to avoid contamination that results from the radial pressure applied by collecting sleeve. Samples were then collected for 2 min.

### Plant growth, plant water content, osmotic potential and stomatal conductance

The roots and the shoots of six plants per treatment were weighed before and after incubation for 72 h in an oven at 70 °C. Water content was estimated as follow:  $\text{WC} (\%) = 100 * (\text{FW} - \text{DW}) / \text{FW}$ . For the osmotic potential determination ( $\Psi_s$ ), roots, old and young leaves samples were cut in small fragments, placed in perforated Eppendorf tubes, submitted to cycles of freeze/thawing, encased in a second Eppendorf tube, and centrifuged at 9000 x g. The collected sap was used to determine ( $\Psi_s$ ) using a Wescor 5500 vapour pressure osmometer. The osmotic potential ( $\Psi_s$ ; in MPa) was calculated according to the Vant't Hoff equation (Lutts et al. 1999). Leaf stomatal conductance ( $g_s$ ) was measured between 2 p.m. and 4 p.m. on leaves L2–3 and L5–6 on 6 plants per treatment using an AP4 diffusion porometer (DeltaTDevices Ltd., Cambridge, UK). The shoot water potential ( $\Psi_w$ ) was determined using a Scholander pressure chamber.

Senescence-related parameters involve estimation of malondialdehyde (MDA) quantified on the basis of thiobarbituric reaction according to Dhindsa and Matowe (1981) and membrane permeability assessed by relative leakage ratio (RLR) using the leakage of

UVA-absorbing substances and calculation of RLR according to Lutts et al. (1996).

#### Mineral elements and proline quantification

Dry roots, old and young leaves samples (100 mg each) were digested in 4 mL of 35% HNO<sub>3</sub> at 80 °C. The residue was re-dissolved with aqua regia (HCl 37%: HNO<sub>3</sub> 65% 3:1) and filtered on Whatmann N°1 filter paper (11 mm). The total concentration of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>+2</sup> and Mg<sup>+2</sup> were quantified by flame atomic absorption spectrophotometry (ICE 3300; Thermo Scientific; Waltham, MA).

Proline content was quantified according to the method of Bates et al. (1973): 200 mg FW of roots, old and young leaves samples were blended in 10 mL of 3% sulfosalicylic acid. After centrifugation at 1000 g for 5 min, the supernatant (2 mL) was held for 1 h in boiling water in the presence of 2 mL ninhydrin and 2 mL glacial acetic acid. The reaction mixture was extracted with 2 mL toluene. Proline concentration was measured spectrophotometrically (Beckman DU 640) at 520 nm and calculated against standards.

#### Total phenolics, flavonoids and anthocyanins content

Total phenolics and flavonoids were extracted from frozen old and young leaves with 80% methanol. The content of phenols from the methanolic extract was quantified using Folin–Ciocalteu method with gallic acid as standard and detection at 750 nm following the method of Singleton and Rossi (1965). The total flavonoid concentration from the methanolic extract was assayed with catechin as standard and detection at 510 nm according to the colorimetric method of Dewanto et al. (2002). The total anthocyanins were quantified from frozen old and young leaves according to the method of Mancinelli (1984), with detection at 530 nm and 657 nm. The anthocyanins content was calculated using 29,600 as extinction coefficient and 445.5 as the molecular weight.

#### Polyamines and ethylene quantification

Free polyamines were extracted and dansylated according to Quinet et al. (2014) with 3 mL VCL, 500 mg FW of leaves or 250 mg FW of roots. Extracts were re-suspended in methanol, filtered (Chromafil PES-45/15, 0.45 µm; Macherey-Nagel, Duren,

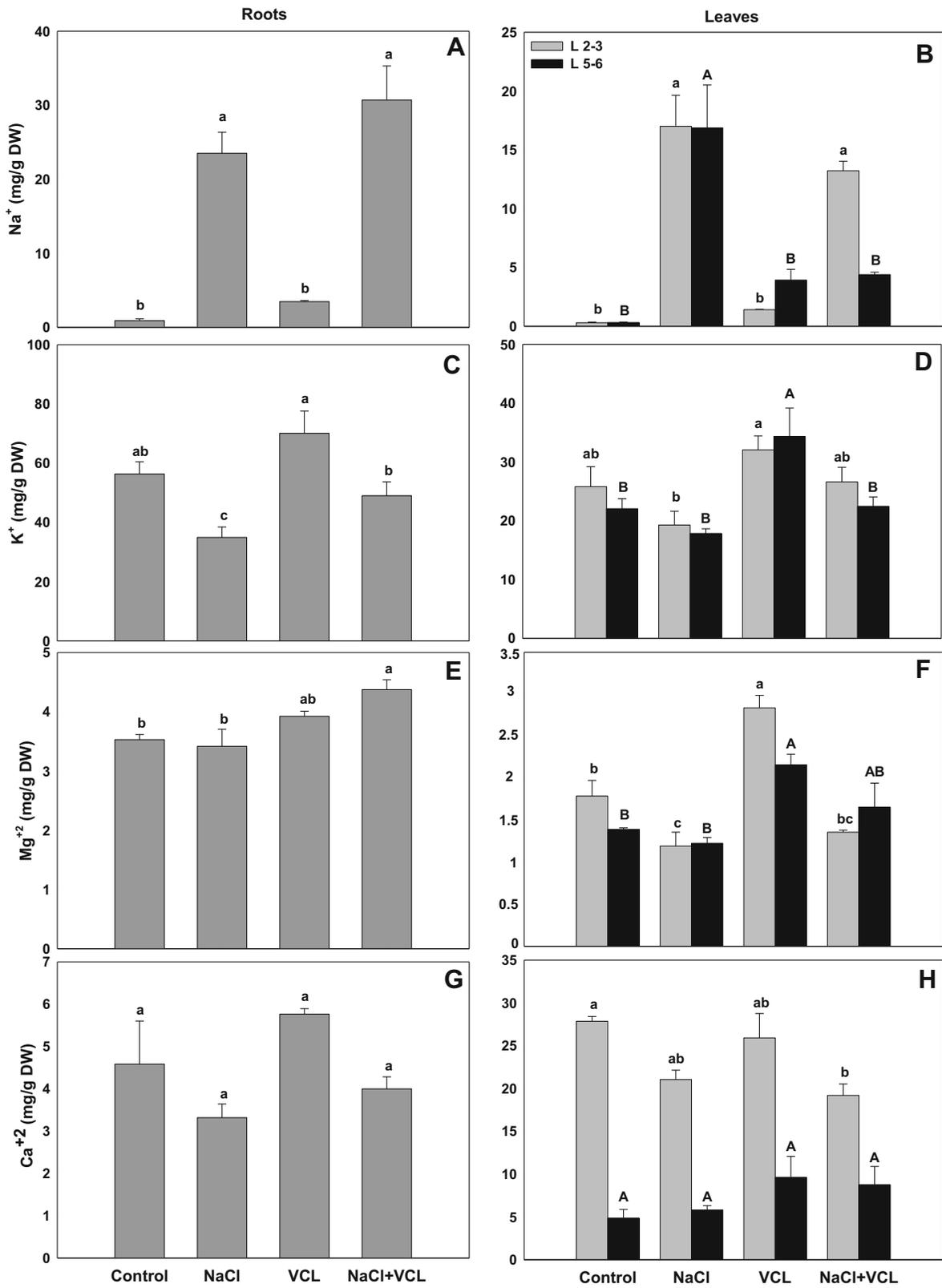
Germany) and injected onto a Nucleodur C18 Pyramid column (125 × 4.6 mm internal diameter, 5 µm particle size; Macherey-Nagel) maintained at 40 °C. Analyses were performed by a Shimadzu HPLC system coupled to a RF-20A fluorescence detector (Shimadzu, 's-Hertogenbosch, The Netherlands) with an excitation wavelength of 340 nm and an emission wavelength of 510 nm. The mobile phase consisted of a water/acetonitrile gradient from 40 to 100% acetonitrile and the flow was 1.0 mL.min<sup>-1</sup>. For each sample, three replicates were quantified.

The ethylene production was measured by ethylene detector ETD-300 (Sensor Sense, Nijmegen, The Netherlands). Harvested leaves were placed in glass bottle dishes on two layers of filter paper moistened with 5 mL water. As a control from the obtained emission rates, the levels of ethylene were measured in a cuvette containing moistened filter papers with 5 mL water without leaves. Samples were treated with the method detailed by Cristescu et al. (2002) with slight modifications as recommended by Chmielowska-Bak et al. (2013). The measurements were conducted in a growth chamber (16 h photoperiod, 200 µmoles.m<sup>-2</sup> s<sup>-1</sup> irradiance, 22 °C) in a stop-and-flow mode with each cuvette being alternatively flushed with a flow of 3 L.h<sup>-1</sup> during 22 min.

#### Phytohormone analysis

Abscisic acid (ABA), auxins, salicylic acid (SA), jasmonates (JA), benzoic acid (BzA), cytokinins (CKs) and their metabolites were extracted and quantified from VCL, fresh tomato leaves (old and young) and roots according to Dobrev and Kaminek (2002), and Dobrev and Vankova (2012): 100 mg fresh sample were homogenized with 0.5 mL extraction mixture (methanol: formic acid: water, 15:1:4, by volume) and placed to cold (-20 °C). The following internal standards (10 pmol per sample) were added: <sup>13</sup>C<sub>6</sub>indole-3-acetic

**Fig. 1** Contents of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>) and calcium (Ca<sup>2+</sup>) in roots (**a**, **c**, **e** and **g**) and leaves (**b**, **d**, **f** and **h**) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. For each organ, values are means of six replicates ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at *P* < 0.05 (lower-case letter for old leaves (L2–3) and capital letter for young leaves (L5–6))



**Table 1** Shoot and root fresh weight and dry weight, osmotic potential ( $\psi_s$ ) in roots, old (L2–3) and young (L5–6) leaves, stomatal conductance ( $g_s$ ) in old and young leaves, shoot water potential ( $\psi_w$ ), malondialdehyde concentration (MDA) and relative leakage ratio of leaf segments of tomato (*Solanum lycopersicum* L.cv. Ailsa Craig) seedlings exposed to control

conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. For each organ, values are means of six replicates  $\pm$  SE. For a given parameter and a given organ, different letters indicate significant differences between treatments according to Student-Newman-Keuls test at  $P < 0.05$

	Control	NaCl	VCL	NaCl+VCL
Fresh weight (g)				
Root	3.06 $\pm$ 0.30 a	1.43 $\pm$ 0.07 c	3.46 $\pm$ 0.16 a	2.64 $\pm$ 0.11 b
Shoot	9.75 $\pm$ 0.76 a	6.34 $\pm$ 0.33 b	10.75 $\pm$ 0.77 a	11.37 $\pm$ 0.57 a
Dry weight (g)				
Root	0.156 $\pm$ 0.012 a	0.068 $\pm$ 0.003 b	0.152 $\pm$ 0.011 a	0.137 $\pm$ 0.013 a
Shoot	0.921 $\pm$ 0.112 a	0.620 $\pm$ 0.054 b	1.012 $\pm$ 0.077 a	1.018 $\pm$ 0.066 a
Osmotic potential ( $\psi_s$ ; MPa)				
Root	-0.434 $\pm$ 0.014 a	-0.804 $\pm$ 0.047 b	-0.445 $\pm$ 0.047 a	-1.005 $\pm$ 0.007 c
L2–3	-0.767 $\pm$ 0.004 a	-1.030 $\pm$ 0.041 b	-0.736 $\pm$ 0.027 a	-1.041 $\pm$ 0.039 b
L5–6	-0.747 $\pm$ 0.021 a	-1.077 $\pm$ 0.009 b	-0.748 $\pm$ 0.020 a	-1.022 $\pm$ 0.015 b
Stomatal conductance ( $g_s$ ; mmol.m <sup>-2</sup> s <sup>-1</sup> )				
L2–3	332 $\pm$ 27 a	82 $\pm$ 9 c	307 $\pm$ 18 a	178 $\pm$ 11 b
L5–6	458 $\pm$ 32 a	77 $\pm$ 7 c	411 $\pm$ 21 a	299 $\pm$ 22 b
Shoot water potential ( $\psi_w$ ; MPa)				
	-1.23 $\pm$ 0.07 a	-1.39 $\pm$ 0.04 b	-1.27 $\pm$ 0.08 a	-1.64 $\pm$ 0.11 c
Relative leakage ratio				
L2–3	15.3 $\pm$ 1.6 a	29.4 $\pm$ 2.1 c	14.8 $\pm$ 1.7 a	20.5 $\pm$ 0.8 b
L5–6	11.7 $\pm$ 2.4 a	28.3 $\pm$ 1.8 c	12.5 $\pm$ 1.4 a	16.2 $\pm$ 1.3 b
Malondialdehyde (nmol g <sup>-1</sup> FW)				
Root	7.8 $\pm$ 0.9 a	12.4 $\pm$ 2.1 b	6.4 $\pm$ 1.7 a	13.2 $\pm$ 1.8 b
L2–3	5.2 $\pm$ 0.3 a	14.9 $\pm$ 0.4 c	4.8 $\pm$ 0.3 a	10.1 $\pm$ 0.2 b
L5–6	6.0 $\pm$ 0.4 a	10.9 $\pm$ 0.7 c	5.3 $\pm$ 0.1 a	7.9 $\pm$ 0.4 b

acid (Cambridge Isotope Laboratories, Tewksbury, MA), <sup>2</sup>H<sub>4</sub>-SA (Sigma-Aldrich), <sup>2</sup>H<sub>3</sub>-phaseic acid (PA), <sup>2</sup>H<sub>6</sub>-ABA (both NRC-PBI, Saskatoon, Canada), <sup>2</sup>H<sub>5</sub>-jasmonic acid (JA; C-D-N Isotopes Inc., Pointe-Claire, Canada), <sup>2</sup>H<sub>5</sub>-*trans*Z, <sup>2</sup>H<sub>5</sub>-*trans*ZR, <sup>2</sup>H<sub>5</sub>-*trans*7G, <sup>2</sup>H<sub>5</sub>-*trans*Z9G, <sup>2</sup>H<sub>5</sub>-*trans*ZOG, <sup>2</sup>H<sub>5</sub>-*trans*ZROG, <sup>2</sup>H<sub>5</sub>-*trans*ZRMP, <sup>2</sup>H<sub>3</sub>-DHZ, <sup>2</sup>H<sub>3</sub>-DHZR, <sup>2</sup>H<sub>3</sub>-DHZ9G, <sup>2</sup>H<sub>6</sub>-iP, <sup>2</sup>H<sub>6</sub>-iPR, <sup>2</sup>H<sub>6</sub>-iP7G, <sup>2</sup>H<sub>6</sub>-iP9G, <sup>2</sup>H<sub>6</sub>-iPRM (all CK standards OlchemIm, Olomouc, Czechia; the system of CK abbreviations adopted and modified according to Kaminek et al. (2000), <sup>2</sup>H<sub>2</sub>-GA1, <sup>2</sup>H<sub>2</sub>-GA4, <sup>2</sup>H<sub>2</sub>-GA7, <sup>2</sup>H<sub>2</sub>-GA8, <sup>2</sup>H<sub>2</sub>-GA19 and <sup>2</sup>H<sub>2</sub>-GA20 (all GAs standards OlchemIm, Olomouc, Czechia). After 1 h extraction at -20 °C the pellet was separated by centrifugation (15,000 g, 15 min) and re-extracted with 0.5 mL of the same extraction buffer at -20 °C for 30 min. Both mixed supernatants were purified using the dual-mode solid-

phase method as described by Dobrev and Kaminek (2002). Two phytohormone fractions were obtained: fraction A, with acidic and neutral compounds (auxins, ABA, SA, JA), and fraction B, with basic compounds (CKs). Hormonal quantification was performed by HPLC (Ultimate 3000, Dionex) coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP; Applied Biosystems) as described previously by Djilianov et al. (2013) using isotope dilution method with multilevel calibration curves ( $r^2 > 0.99$ ). Data processing was carried out with Analyst 1.5 software (Applied Biosystems).

#### Data treatment

Normality distribution and homogeneity were verified using Shapiro-Wilk and Leven's test and data were

**Table 2** Cation ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) concentrations ( $\text{mg.L}^{-1}$ ) in the xylem sap of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) seedlings exposed to control conditions (Control), 125 mM NaCl (NaCl), 18  $\text{mL.L}^{-1}$  vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. Each value is the mean of six replicates  $\pm$  SE and different letters for a given element indicate significant differences between different treatments according to Student-Newman-Keuls test at  $P < 0.05$

Cation	Control	NaCl	VCL	NaCl+VCL
$\text{Na}^+$	70 $\pm$ 4 a	1011 $\pm$ 9 c	56 $\pm$ 5 a	707 $\pm$ 4 b
$\text{K}^+$	230 $\pm$ 4 a	721 $\pm$ 20 b	201 $\pm$ 16 a	645 $\pm$ 113 b
$\text{Mg}^{2+}$	39 $\pm$ 3 a	54 $\pm$ 4 a	35 $\pm$ 4 a	56 $\pm$ 10 a
$\text{Ca}^{2+}$	69 $\pm$ 4 a	59 $\pm$ 2 a	73 $\pm$ 4 a	70 $\pm$ 12 a

transformed when required. Data were subjected to one-way analysis of variance (ANOVA) using SYSTAT version 12. Differences between means were scored by the Student-Newman-Keuls test when the ANOVA was significant at  $P < 5\%$ . Data are presented as means  $\pm$  standards errors.

## Results

### Plant growth, water status and senescence-related parameters

Salt stress reduced the root and the shoot fresh and dry weights (Table 1). VCL applied in the absence of NaCl had no significant impact on plant growth while it significantly reduced the deleterious effect of NaCl. Salinity had no significant impact on water content, whatever the considered organ, and VCL did not modify this parameter (mean value of  $94 \pm 0.8\%$  for roots,  $89.8 \pm 1.0\%$  for L2–3 and  $88.7 \pm 0.9\%$  for L5–6; no significant difference was recorded between old (L2–3) and young (L5–6) leaves). Salt stress reduced  $\Psi_s$  values in all organs while VCL applied in the absence of NaCl had not significant impact comparatively to controls. VCL applied concomitantly with NaCl reduced the root  $\Psi_s$  compared with plants exposed to NaCl in absence of VCL. Stomatal conductance in control plants was higher in young leaves than in old ones. In both cases, salinity strongly reduced the  $g_s$  values. The additional presence of VCL reduced the deleterious impact of salt on  $g_s$ , especially in young leaves. Shoot water potential ( $\Psi_w$ ) decreased in response to NaCl and to lower values in presence than in the absence of VCL. The RLR and

MDA values also increased in response to NaCl, but they remained significantly lower in VCL + NaCl-treated leaves than in NaCl-treated ones. In contrast, VCL did not reduce MDA concentration in NaCl-treated roots.

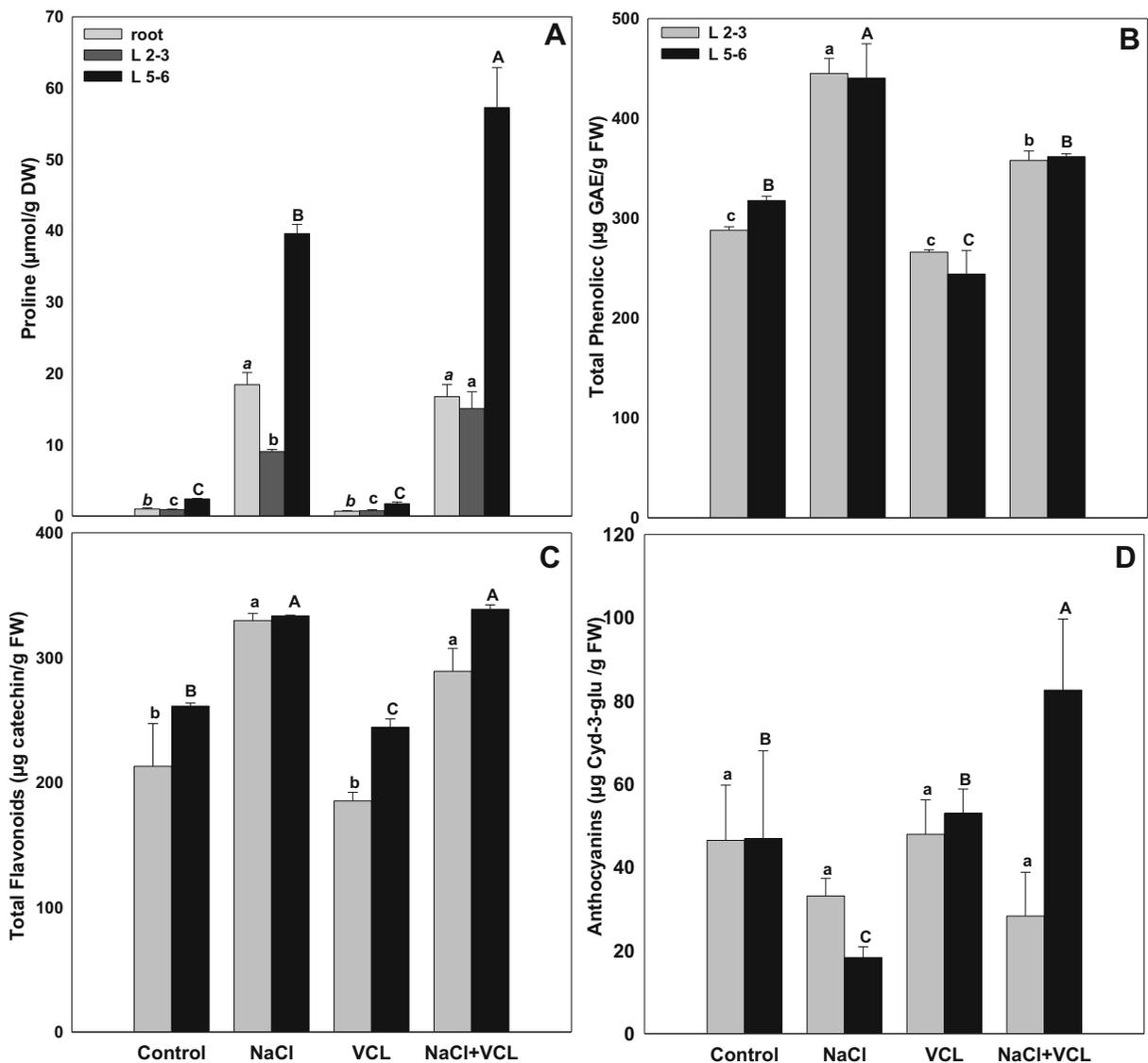
### Ion content

VCL contains 618  $\text{mg.L}^{-1}$   $\text{Na}^+$ , 2200  $\text{mg.L}^{-1}$   $\text{K}^+$ , 114  $\text{mg.L}^{-1}$   $\text{Mg}^{2+}$  and 101  $\text{mg.L}^{-1}$   $\text{Ca}^{2+}$ . The total VCL electrical conductivity before dilution was 15.90  $\text{dS.cm}^{-1}$ . Exposure to NaCl increased the  $\text{Na}^+$  concentration in all organs (Fig. 1). Plants cultivated in the presence of VCL and in the absence of salt tended to contain higher  $\text{Na}^+$  concentration than controls, however, the difference was not significant. VCL applied under salt conditions increased  $\text{Na}^+$  concentration in the roots (Fig. 1a) and decreased it in the leaves (Fig. 1b), the effect being significant for young leaves only where a strong decrease in  $\text{Na}^+$  was recorded in L5–6.

Salinity significantly reduced  $\text{K}^+$  concentration in roots only (Fig. 1c). Exposure to VCL in the absence of salt led to the highest  $\text{K}^+$  concentration in leaves (Fig. 1d) but NaCl reduced the impact of VCL on  $\text{K}^+$  content in roots and young leaves. Salinity reduced  $\text{Mg}^{2+}$  content in old leaves while treatment of plants with VCL in the absence of salt increased the leaf  $\text{Mg}^{2+}$  concentration (Fig. 1f). Calcium concentration was not significantly modified in roots and young leaves while it slightly decreased in old leaves in NaCl+VCL-treated plants (Fig. 1g & h). Data on ion concentrations in the xylem sap are provided in Table 2: salinity increased  $\text{Na}^+$  concentration in the xylem sap to a higher extent in NaCl than in NaCl+VCL-treated plants. Surprisingly, salinity also increased the  $\text{K}^+$  concentration in the xylem sap but VCL had no significant effect on  $\text{K}^+$  content on xylem sap. Magnesium and  $\text{Ca}^{2+}$  concentration in the xylem sap remained similar in all treatments.

### Proline and phenolic compounds concentration

No proline was detected in the vermicompost. Proline concentration increased in the roots of plants exposed to NaCl (Fig. 2a) and VCL had no impact on the root proline content in salt-treated plants. Proline also increased in the shoots of plants exposed to NaCl and to a higher extent in young leaves than in old ones. In both cases, proline accumulation was increased by VCL treatment. Total phenolic compounds were found to be 27.11  $\mu\text{g.mL}^{-1}$  in VCL. Their concentrations were



**Fig. 2** Proline (a), total phenolics (b), flavonoids (c) and anthocyanins (d) content in tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. For each organ, values

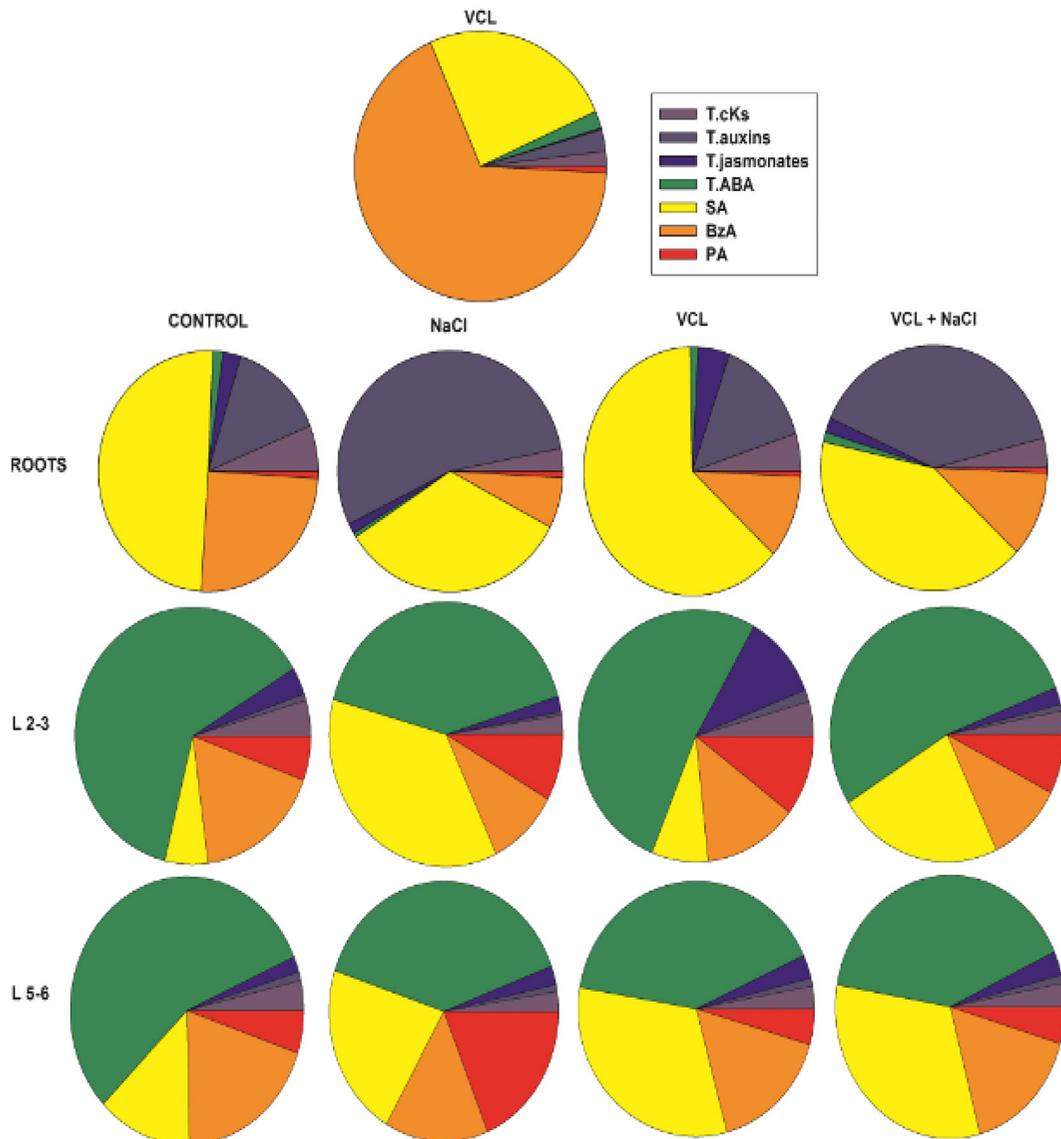
are means of three replicates  $\pm$  SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at  $P < 0.05$  (italic letters for roots, lower-case letter for old leaves (L2–3) and capital letter for young leaves (L5–6))

always similar in young and old leaves (Fig. 2b). Salinity increased levels of total phenolics but VCL slightly reduced their contents in salt-treated plants. Total flavonoids (Fig. 2c) content was slightly higher in young than in old leaves, except in plants exposed to NaCl in the absence of VCL. Salinity increased the total flavonoids and VCL had no impact on this parameter. Vermicompost contained 8.41  $\mu\text{g.mL}^{-1}$  anthocyanins and the two types of leaves behaved differently regarding anthocyanins accumulation (Fig. 2d). Anthocyanins

indeed remained unaffected in old leaves while it significantly decreased in response to NaCl in young leaves in the absence of VCL but increased by more than 100% comparatively to controls in NaCl+VCL-treated plants.

#### Phytohormonal profile

The relative proportions of soluble phytohormones (except for the precursor of the gaseous hormone aminocyclopropane carboxylic acid [ACC]) expressed as a

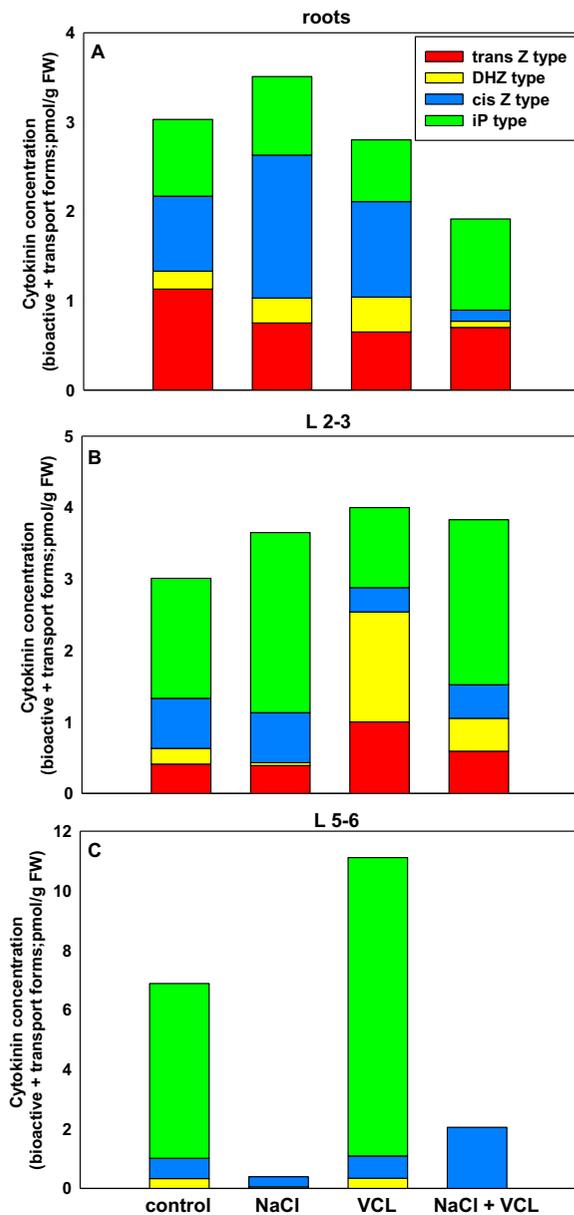


**Fig. 3** Relative proportion (in % of total content expressed on a molar basis) for soluble phytohormones quantified in vermicompost leachate (VCL) and in roots, old leaves (L2–3) and young leaves (L5–6) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL)

and combined treatments (NaCl+VCL) for 7 days. Ethylene and its immediate precursor aminocyclopropane carboxylic acid (ACC) were omitted from the calculation due to the gaseous nature of ethylene (SA: salicylic acid; ABA: abscisic acid; BzA: benzoic acid; PA: phaseic acid; CKs: cytokinins)

percentage of the total content calculated on a molar basis are shown in Fig. 3 for VCL as well as for roots, old and young leaves of plants exposed to the various treatments. In VCL, BzA represented almost 70% of the total phytohormone pool followed by SA whereas auxins, ABA and CKs exhibited only a low and equivalent proportion. SA represented 50% of the detected hormones in the roots of control plants and even more in

the roots of the VCL-treated ones. Exposure to NaCl reduced the relative proportion of SA but obviously increased the proportion of auxins at the root level. In old leaves (L2–3), ABA was the most represented hormone from a relative point of view, although its proportion slightly decreased in response to NaCl at the expense of enhanced proportion of SA. Phaseic acid (PA) was also detected in the leaves in significant amounts in



**Fig. 4** Concentration of cytokinins (bioactive + transport forms) in roots (a), old leaves (L2–3; b) and young leaves (L5–6; c) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. Data are provided for *trans*-zeatin (*transZ*), dihydrozeatin (DHZ), *cis*-zeatin (*cisZ*) and *N*<sup>6</sup>-( $\Delta^2$ -isopentenyl)adenine (iP) types of cytokinins

contrast to the roots. The proportion of jasmonates increased in VCL-treated plants, however, this increase was abolished by simultaneous exposure to NaCl. Young leaves (L5–6) presented a higher proportion of

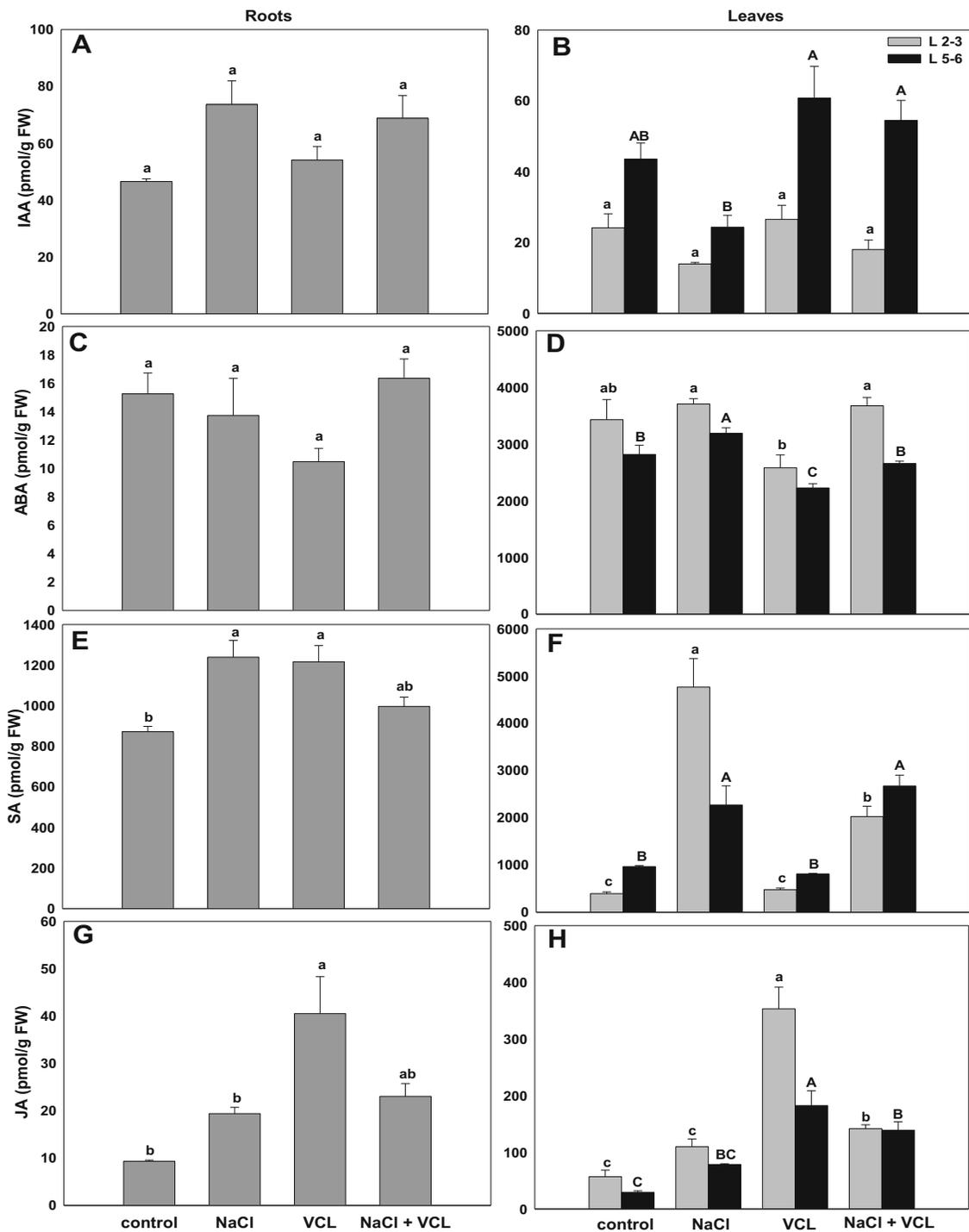
SA than old ones in plants maintained in the absence of NaCl. Salinity reduced the proportion of ABA and increased the proportion of SA and PA in young leaves.

CKs *trans*-zeatin (*transZ*) and *trans*-zeatin riboside (*transZR*) were not detected in the VCL. Dihydrozeatin (DHZ), *cis*-zeatin (*cisZ*) and *N*<sup>6</sup>-( $\Delta^2$ -isopentenyl)adenine (iP) exhibited a concentration of 0.4, 0.65 and 0.33 pmol.mL<sup>-1</sup> while their riboside forms (*cisZR* and iPR) represented 0.23 and 0.31 pmol.mL<sup>-1</sup>, respectively. Bioactive and transport cytokinins are represented by free and riboside cytokinin forms and their concentrations are shown in Fig. 4 for each organ, considering separately *transZ*, DHZ, *cisZ* and iP-types. The pattern of endogenous CKs was different in various considered organs. VCL reduced *cisZ*-type CK levels in NaCl-treated roots (Fig. 4a). Salinity increased contents of iP-types in old leaves while VCL strongly increased the DHZ-types in the absence of salt (Fig. 4b). In young leaves (Fig. 4c), iP-types represented the main CK fraction recorded in the absence of salt which was significantly increased by VCL. Salinity, however, strongly reduced concentrations of bioactive CKs in young leaves and only *cisZ*-types were recorded in these organs.

The complete profiles of CKs including their individual metabolites are given in Tables S1 and S2 for roots and leaves, respectively, and indicate a NaCl-

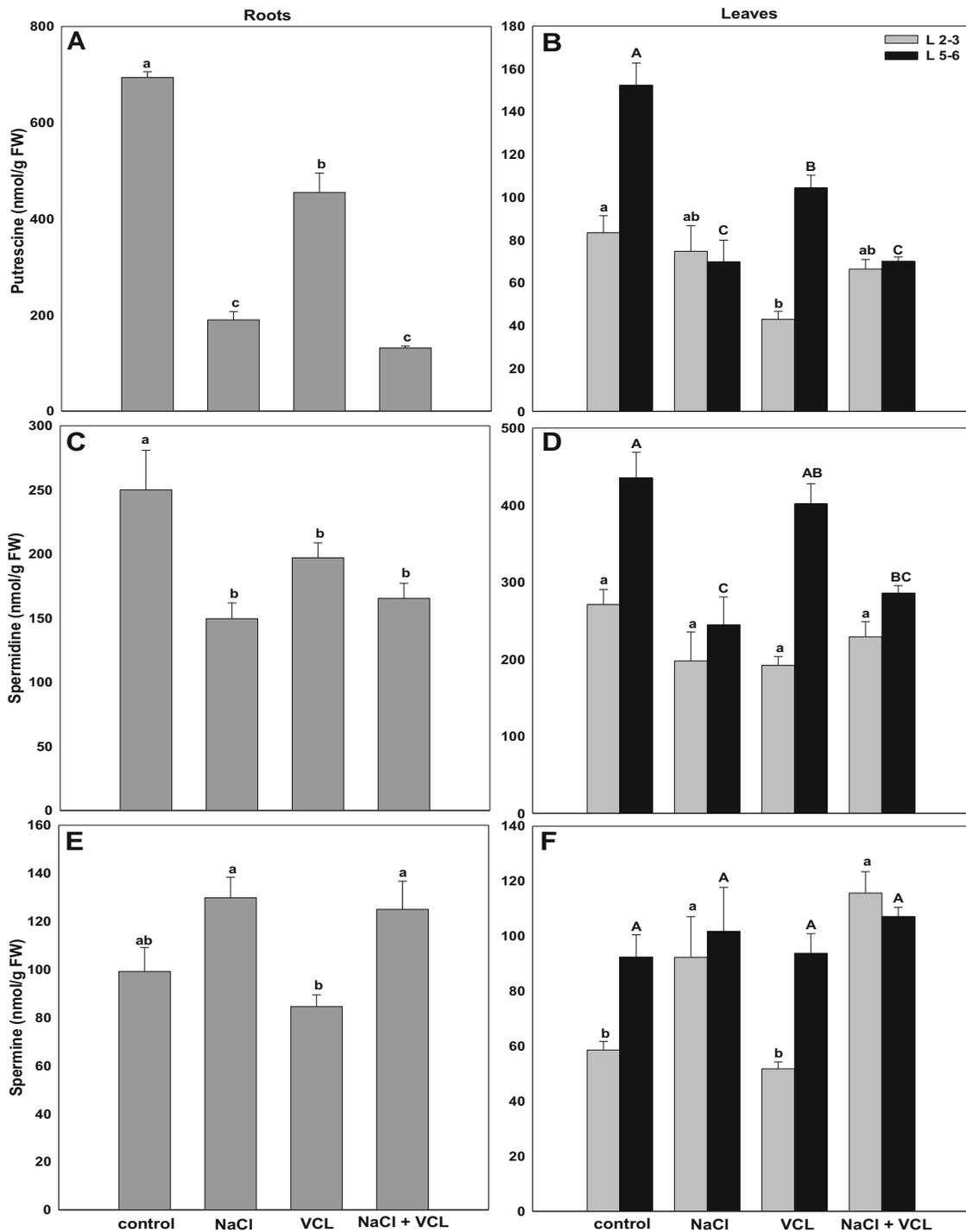
**Table 3** Aminocyclopropane carboxylic acid (ACC) content (in nmol.g<sup>-1</sup> FW) and ethylene biosynthesis (in nL.g<sup>-1</sup> FW h<sup>-1</sup>) in old (L2–3) and young leaves (L5–6) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) seedlings exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. Each value is the mean of six replicates  $\pm$  SE. For a given parameter and organ, different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at  $P < 0.05$

	ACC (nmol.g <sup>-1</sup> FW)		Ethylene (nL.g <sup>-1</sup> h <sup>-1</sup> )	
	L2-3	L5-6	L2-3	L5-6
Control	4.7 $\pm$ 0.4 a	3.5 $\pm$ 0.2 a	1.37 $\pm$ 0.12 a	0.97 $\pm$ 0.07 a
NaCl	91.7 $\pm$ 14.1 c	30 $\pm$ 1.4 c	6.21 $\pm$ 0.51 c	5.88 $\pm$ 0.11 c
VCL	3.1 $\pm$ 0.6 a	3.4 $\pm$ 1.3 a	1.25 $\pm$ 0.17 a	1.08 $\pm$ 0.17 a
NaCl+VCL	20.6 $\pm$ 2.3 b	18.6 $\pm$ 1.6 b	4.26 $\pm$ 0.21 b	2.01 $\pm$ 0.23 b



**Fig. 5** Concentration of indole-3-acetic acid (IAA; **a** and **b**), abscisic acid (ABA; **c** and **d**), salicylic acid (SA; **e** and **f**), and jasmonic acid (JA; **g** and **h**) in roots (**a**, **c**, **e** and **g**) and leaves (**b**, **d**, **f** and **h**) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined

treatments (NaCl+VCL) for 7 days. For each organ, values are means of three replicates ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at *P* < 0.05 (lower-case letter for old leaves (L2–3) and capital letter for young leaves (L5–6))



**Fig. 6** Concentration of putrescine (Put; **a** and **b**), spermidine (Spd; **c** and **d**) and spermine (Spm; **e** and **f**) in roots (**a**, **c**, **e**) and leaves (**b**, **d**, **f**) of tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants exposed to control conditions (Control), 125 mM NaCl (NaCl), 18 mL.L<sup>-1</sup> vermicompost leachate (VCL) and combined treatments (NaCl+VCL) for 7 days. For each organ, values

are means of three replicates  $\pm$  SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at  $P < 0.05$  (lower-case letter for old leaves (L2–3) and capital letter for young leaves (L5–6))

induced increase of *trans*ZOG content in the roots and a strong decrease of iPR level in young leaves.

Other phytohormones - auxin IAA, ABA and SA - were quantified in VCL at concentrations of 10.39 pmol.mL<sup>-1</sup>, 2.05 pmol.mL<sup>-1</sup> and 164 pmol.mL<sup>-1</sup>, respectively. JA was also present but at very low concentrations (0.06 pmol.mL<sup>-1</sup>).

Endogenous level of IAA remained constant in roots (Fig. 5a) and old leaves (Fig. 5b). It was always lower in old than young leaves where IAA concentration was increased by VCL treatment. ABA also remained constant in the roots (Fig. 5c) and was slightly lower in young leaves than in old ones being the lowest in plants exposed to VCL in the absence of NaCl (Fig. 5d). The root SA concentration significantly increased in NaCl- and in VCL-treated plants but remained similar to control in NaCl+VCL-treated plants (Fig. 5e). Leaf SA level remained low in plants maintained in the absence of NaCl (Fig. 5f). Salinity increased SA content but VCL reduced SA accumulation in old leaves of NaCl-treated plants in contrast to young leaves. Vermicompost increased JA concentration in plants cultivated in the absence of NaCl in both roots and leaves (Fig. 5g and h) while NaCl reduced JA content in VCL-treated plants.

The complete phytohormone profiles of auxins, ABA-related compounds, jasmonates and phenolic acids (BzA, SA and phenylacetic acid [PAA]) are shown in Tables S3 to S8. A strong increase is demonstrated for contents of IAA amino acid conjugate, IAA-aspartate (IAA-Asp), in response to NaCl (Tables S3 and S4). The full profile of ABA-related metabolites revealed an increase in concentrations of ABA catabolite PA in response to NaCl in old but not in young leaves (Tables S5 and S6). The JA precursor *cis*(+)-12-oxo-phytodienoic acid (*cis*OPDA) was found in high amounts in both roots and leaves and always exhibited the lowest values in VCL + NaCl-treated plants (Tables S7 and S8).

Putrescine (Put), spermidine (Spd) and spermine (Spm) were quantified in VCL at concentration of 8.83, 12.91 and 12.39 nmol.mL<sup>-1</sup>, respectively. VCL application reduced root Put content in the absence of NaCl (Fig. 6a) and NaCl also decreased Put concentration. Concentration of Put was higher in young than in old leaves in plants that were not exposed to NaCl (Fig. 6b). VCL decreased Put content in young and old leaves in the absence but not in the presence of NaCl. Salinity and VCL reduced the root Spd content (Fig. 6c). The concentration of Spd remained constant in old leaves but

decreased in response to salinity in young ones (Fig. 6d). Salinity increased Spm concentration in the roots (Fig. 6e) and in the old leaves (Fig. 6f) to a similar extent in the absence and the presence of VCL while Spm concentration remained unaffected by the treatment in young leaves.

The VCL contained high concentration of ACC (up to 197 pmol.mL<sup>-1</sup>). The root ACC content decreased from 346 nmol.g<sup>-1</sup> FW in control to 213 nmol.g<sup>-1</sup> FW in salt-treated plants. VCL surprisingly induced an additional decrease of ACC content in NaCl-treated roots (119 pmol.g<sup>-1</sup> FW). At the leaf level (Table 3), salinity increased ACC content in old leaves and to a lower extent in young ones. VCL significantly reduced ACC accumulation in salt-treated leaves. A similar pattern was observed for ethylene synthesis which was markedly reduced in young leaves of NaCl-treated plants (Table 3).

## Discussion

Vermicompost is commonly used as a tool in tomato production that improves plant growth and fruit yield and quality (Zaller 2007; Gutiérrez-Miceli et al. 2007; Zucco et al. 2015). It also contributes to improve tomato resistance to biotic and abiotic stresses (Chinsamy et al. 2014; Xiao et al. 2016; Benazzouk et al. 2018). The present work reports positive effects of VCL on salt-resistance in tomato in relation to an improvement of plant growth and an alleviation of salt-induced decrease in stomatal conductance and hastening of senescence processes (estimated on the basis of cell membrane permeability [RLR] and MDA content). It also demonstrates that NaCl induced a modification of hormonal profile in tomato roots and shoots as known from the literature (e.g. Ghanem et al. 2008; Žižková et al. 2015; Gharbi et al. 2017), but also that exogenous VCL modifies the phytohormone spectra when applied on plants. Moreover, the recorded modifications differed between non-stressed and NaCl-treated plants and varied depending on the considered organ. Phytohormones present in VCL may result from the biochemical degradation of organic matter by various microorganisms and the earthworm's gut acts as a natural bioreactor increasing microbial activities (Zhang et al. 2014). External biostimulants added to the nutrient solution as VCL may be absorbed by the plant and it is plausible that

NaCl has an impact on absorption and root-to-shoot translocation processes.

VCL reduced  $\text{Na}^+$  accumulation in young but not in old leaves. Preferential  $\text{Na}^+$  accumulation in old leaves and low accumulation in photosynthetically-active young ones has already been suggested as an efficient strategy of salt resistance at the whole plant level (Lutts et al. 1996, 1999). It may be hypothesized that VCL modulated contrastingly the transpiration in organs of different ages, but this explanation is probably not valid in the present case since young leaves of VCL-treated plants exhibited a higher stomatal conductance than old ones (Table 1). Moreover,  $\text{Mg}^{2+}$  exhibited a different behavior and was reduced in old leaves but not in young ones.

The fact that the VCL-induced decrease in leaf  $\text{Na}^+$  corresponded to a slight increase in root  $\text{Na}^+$  and to a decrease in xylem  $\text{Na}^+$  concentration suggests that VCL may to some extent, control retrieval of  $\text{Na}^+$  from the xylem which is an important component of salinity tolerance in glycophytes. Re-uptake of  $\text{Na}^+$  from the xylem occurs through HKT transporters such as HKT1;1 localized at the plasma membrane of xylem parenchyma cells (Davenport et al. 2007; Zhu et al. 2017) while loading of  $\text{Na}^+$  into the xylem is mainly regulated by the SOS1  $\text{Na}^+/\text{H}^+$  antiporter (Plett and Møller 2010). Our data lead us to hypothesize that VCL might differently affect these processes. Influence of phytohormones on  $\text{Na}^+$  retrieval however remains poorly documented. ABA was shown to stimulate  $\text{H}^+$  extrusion into xylem and so could potentially enhance xylem loading via stimulation of  $\text{Na}^+/\text{H}^+$  antiporter (Plett and Møller 2010). ABA was also found to induce the *ABSCISIC ACID INSENSITIVE (ABI) 4* gene coding for a transcription factor which downregulate *HKT1;1* gene expression and increase  $\text{Na}^+$  xylem content (Shkolnik-Inbar et al. 2013). ABA and auxin activate the expression of genes coding for vacuolar  $\text{H}^+$ -inorganic pyrophosphatase and vacuolar  $\text{H}^+$ -ATPase involved in vacuolar sequestration of toxic  $\text{Na}^+$  (Fukuda and Tanaka 2006). In our case, however, ABA concentration in VCL was rather low ( $2.05 \text{ pmol}\cdot\text{mL}^{-1}$ ) while concentration of its catabolic products (dihydrophaseic and phaseic acids) were quite higher. Bulk ABA content in the roots remained unaffected by VCL and the precise role of ABA on  $\text{Na}^+$  long distance transport thus require to also consider ABA distribution and sensitivities of ABA receptors. Beside ABA, sugars were also recently reported to regulate ABI4 (Zheng et al. 2019) and we

showed an obvious increase of soluble sugar accumulation in roots of salt-treated tomato cultivated in the presence of VCL (Benazzouk et al. 2018).

Salinity induced a higher accumulation of proline in young leaves that in old ones and this tendency was reinforced by VCL application despite the recorded decrease in  $\text{Na}^+$  in young organs. This indicates that proline distribution did not correlate with  $\text{Na}^+$  distribution and does not support the classical view of cytosolic proline accumulating to osmotically equilibrate  $\text{Na}^+$  vacuolar sequestration. Similarly, Shkolnik-Inbar et al. (2013) reported that salt-treated *Arabidopsis abi4* mutant accumulated higher amounts of proline despite an important decrease in  $\text{Na}^+$  content. The fact that proline was not detected in VCL and that exogenous VCL did not induce proline accumulation in the absence of NaCl suggests that proline synthesis is an active process triggered by VCL in salt-treated plants only. Beside its role in osmotic adjustment, proline may assume a wide range of protective functions in salt-treated plants including free radical scavenging and protection of intracellular structures (see Kaur and Asthir 2015 for review). Proline accumulation was observed in the leaves and not in the roots of VCL-treated plants. Similarly, MDA decreased in the leaves but not in the roots in VCL + NaCl-treated plants comparatively to NaCl-treated ones and an obvious decrease for RLR also suggests that VCL-induced proline accumulation may afford protection against NaCl-induced oxidative stress.

Phenolic compounds are also involved in the management of oxidative stress. Aremu et al. (2015) identified major phenolic compounds such as protocatechic, *p*-hydroxybenzoic, *p*-coumaric and ferulic acid in VCL obtained from garden wastes. In our case, however, VCL slightly reduced total phenolics in NaCl-treated plants. Anthocyanins are known to assume important roles in salt-stressed plants, not only as antioxidants but also as modulators of nitrogen metabolism (Truong et al. 2018). Although anthocyanins were detected in VCL, the high level of dilution of VCL in nutrient solution could not explain VCL-induced anthocyanins accumulation in NaCl-treated young leaves. Two hormonal phenolic compounds, SA and BzA, were found at high concentrations in VCL where they constituted more than 80% of the total phytohormone pool. Exogenous SA was shown to improve salt resistance in tomato (Manaa et al. 2014; Mimouni et al. 2016) and concomitant application of NaCl and SA improved osmotic adjustment without any impact on ethylene synthesis

in stressed tomatoes (Gharbi et al. 2016). We found here an opposite trend: exogenous VCL did not lead to leaf osmotic adjustment (Table 1) but reduced ethylene synthesis in NaCl-treated plants (Table 3). This suggests that the positive impact of VCL could not be ascribed to exposure to exogenous SA, even if the studies using SA addition to nutrient solution commonly used higher SA concentration (frequently in the  $\mu\text{M}$  range) than SA concentration recorded in VCL. The precursor of SA, BzA was also present in VCL. High doses of BzA have been reported to exhibit toxic effects to *S. lycopersicum*: it could not thus be excluded that plants limited its absorption and IAA was recently demonstrated to play a key role in this respect (Singh 2015).

A stimulatory impact of VCL on root growth has already been attributed to an auxin effect of humic substances (Canellas et al. 2002; Trevisan et al. 2010). The VCL used in the present study contains up to  $700 \text{ mg.L}^{-1}$  humic acids (Chaichi 2018; Benazzouk, unpublished results). Canellas et al. (2002) demonstrated that humic acids may enhance root growth and stimulate  $\text{H}^+$ -ATPase activity in maize. Humic substances consist in supramolecular association of heterogenous molecules held together by hydrophobic interactions. Nevertheless, compounds with the lowest molecular size may flow into the apoplast and reach the plasma membrane where they can have a biochemical impact on the electrochemical gradients conditioning mineral nutrition. Trevisan et al. (2010) also demonstrated that humic substances may activate the expression of auxin-responsive genes. IAA may be clustered within the humic acid supramolecular arrangement (Canellas et al. 2002; Nardi et al. 2002). In our VCL sample, IAA concentration ( $10 \text{ pmol.mL}^{-1}$ ) was higher than values reported elsewhere (Aremu et al. 2015; Zhang et al. 2015). However, VCL did not stimulate root growth under our experimental conditions in the absence of NaCl and its positive effect on root-growth in NaCl-treated plants was not associated with an increase in IAA, which suggests that auxin was not directly involved in plant response to VCL.

Humic substances may also exhibit a CK-like activity (Nardi et al. 2002). Pizzeghello et al. (2013) reported high levels of iPR in humic fractions extracted from earthworm faeces and the iPR concentration detected in our VCL material was even higher (Table S1). Exogenous treatment with VCL increased iPR concentrations in the young leaves of unstressed plants (Table S2). On the other hand, *transZ* that is considered as one of the most active CKs

produced in plants, was not detected in VCL in this study, as reported by Zhang et al. (2015) for vermicompost produced from fruit peel and water jacinth. It has to be noticed that other *transZ*-type derivatives, *transZOG* and *transZROG*, were detected at high concentrations in VCL in our work. The iP content reported by Zhang et al. (2014) for vermicompost tea was higher than in our VCL samples. At the whole plant level, the impact of NaCl on one hand, and VCL on the other, was completely different depending on the considered organ. Exogenous VCL increased contents of DHZ-type CKs in old leaves and iP-types in young ones but both effects were suppressed by the additional presence of NaCl. Salt stress drastically reduced CK concentration in young leaves, which remains puzzling considering the impact of CKs, and especially *transZ*, as antisenescent agent in NaCl-treated tomato leaves (Albacete et al. 2009; Ghanem et al. 2008, 2011). CKs of *cisZ*-types represented a major fraction detected in young leaves of NaCl-treated plants and increased as a consequence of VCL treatment, despite the fact that *transZ* and *cisZ*-type CKs concentrations in VCL were in the same range (Table S1). Although *cisZ*-type CKs are receiving more attention than in the past with assumed regulatory roles in plants under growth-limiting conditions (Gajdošová et al. 2011) and positive functions in pathogen and herbivore resistance (Schäfer et al. 2015), additional work is required to precisely assess the physiological significance of VCL-induced increase in contents of *cisZ*-types in young leaves of NaCl-treated plants.

Jasmonates which were present at extremely low concentrations (2500 times lower than SA and 7000 times lower than BzA) in VCL. Endogenous jasmonates strongly increased in root and leaves of plants exposed to exogenous VCL, which could hardly be explained by absorption of exogenous JA but rather by a VCL-induced stimulation in jasmonate endogenous biosynthesis. Salinity reduced accumulation of jasmonates in VCL-treated plants but it still remained slightly higher than in plants exposed to salinity in the absence of VCL. Jasmonates are now postulated to improve salinity resistance and to positively influence net photosynthesis and plasma membrane stability, including in halophyte plant species (Yuan et al. 2019). In contrast, ACC content was high in VCL (almost  $200 \text{ pmol.mL}^{-1}$ ) but it did not lead to ethylene overproduction: endogenous ACC concentration itself was not increased in leaves of plants exposed to VCL and was even strongly reduced in NaCl+VCL-treated leaves comparatively to NaCl-exposed ones.

Polyamines and ethylene share a common precursor (S-adenosylmethionine) and a VCL-induced modification in ethylene synthesis was therefore expected to impact polyamines status, especially considering that VCL itself contains consistent amounts of these compounds. The quantitative impact of VCL on endogenous polyamines titers nevertheless remained limited. In the presence of NaCl, exogenous VCL did not significantly modify the root and leaf contents of Put, Spd or Spm and VCL even reduced root and leaves Put concentrations in the absence of NaCl. Gharbi et al. (2016) reported that the salt-tolerant wild relative *Solanum chilense* had a distinct polyamines profile comparatively to the glycophyte *S. lycopersicum* and polyamines (especially Spd and Spm) played a major role in both plant development and protection against abiotic stresses (Quinet et al. 2014; Gharbi et al. 2016). The present study, however, suggests that they do not constitute an important target for VCL-induced improvement of salinity resistance in tomato.

Taken together, these data demonstrate a positive impact of VCL on salinity resistance in tomato *Solanum lycopersicum* in relation to an improved regulation of mineral nutrition possibly linked to Na<sup>+</sup> retrieval from the xylem, maintenance of stomatal conductance without significant decrease in water content, and delay of the salt-induced senescence processes. VCL contains a wide range of phytohormonal compounds and modifies the endogenous hormonal status of stressed organs. Most of the hormonal effects of VCL appeared however linked to a VCL-induced modification of endogenous biosynthesis rather than to a simple absorption from external VCL.

**Acknowledgements** The authors are grateful to Marie-Eve Renard, Brigitte Vanpee and Marie Korecka for invaluable technical support. This research was supported by the Czech Science Foundation (grant No. 19-12262S) and by the Ministry of Education, Youth and Sports of CR from European Regional Development Fund-Project “Centre for Experimental Plant Biology” (No. CZ.02.1.01/0.0/0.0/16\_019/0000738).

## References

- Alam MZ, Braun G, Norrie J, Hodges DM (2013) Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry. *Can J Plant Sci* 93:23–36
- Albacete A, Martinez-Andujar C, Ghanem M, Acosta M, Sanchez-Bravo J, Asins MJ, Cuartero J, Lutts S, Dodd IC, Pérez-Alfocea F (2009) Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence and increased leaf area and crop productivity in salinized tomato. *Plant Cell Environ* 32:928–9238
- Aremu AO, Masondo NA, Van Staden J (2014) Physiological and phytochemical responses of three nutrient-stressed bulbous plants subjected to vermicompost leachate treatment. *Acta Physiol Plant* 36:721–731
- Aremu AO, Stirk WA, Kulkarni MG, Tarkowská D, Turečková V, Gruz J, Bílba O, Novák O, Stirk WA, Doležal K (2015) Evidence of phytohormones and phenolic acids variability in garden-waste-derived vermicompost leachate, a well-known plant growth stimulant. *Plant Growth Regul* 75: 483–492
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207
- Benazzouk S, Djazouli ZE, Lutts S (2018) Assessment of the preventive effect of vermicompost on salinity resistance in tomato (*Solanum lycopersicum* cv. Ailsa Craig). *Acta Physiol Plant* 40:121
- Bui EN (2013) Soil salinity: a neglected factor in plant ecology and biogeography. *J Arid Environ* 92:14–25
- Calvo P, Nelson L, Klopper JW (2014) Agricultural uses of plant biostimulants. *Plant Soil* 383:3–41
- Canellas LP, Olivares FL, Okorokova-Façanha AL, Façanha AR (2002) Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H<sup>+</sup>-ATPase activity in maize roots. *Plant Physiol* 130:1951–1957
- Chaichi W (2018) Effets des Biofertilisants sur la Bio-fourmiture et la Correction du Stress. PhD Thesis. Université de Blida 1, Algeria, 324 p
- Chinsamy M, Kulkarni MG, Van Staden J (2013) Garden-waste-vermicompost leachate alleviates salinity stress in tomato seedlings by mobilizing salt tolerance mechanisms. *Plant Growth Regul* 71:41–47
- Chinsamy M, Kulkarni MG, Van Staden J (2014) Vermicompost leachate reduces temperature and water stress effects in tomato seedlings. *HortScience* 49:1183–1187
- Chmielowska-Bak J, Lefèvre I, Lutts S, Deckert J (2013) Short term signaling responses in roots of young soybean seedlings exposed to cadmium stress. *J Plant Physiol* 170:1585–1594
- Ciura J, Kruk F (2018) Phytohormones as targets for improving plant productivity and stress tolerance. *J Plant Physiol* 229: 32–40
- Cristescu SM, De Martinis D, Te Lintel HS, Parker DH, Harren FJM (2002) Ethylene production by *Botrytis cinerea* in vitro and in tomatoes. *Appl Environ Microbiol* 68:5342–5350
- Davenport RJ, Muñoz-Mayor A, Jha D, Essah PA, Rus A, Tester M (2007) The Na<sup>+</sup> transporter AtHKT1;1 controls retrieval of Na<sup>+</sup> from the xylem in Arabidopsis. *Plant Cell Environ* 30: 497–507
- Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem* 50:3010–3014
- Dhindsa RS, Matowe W (1981) Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. *J Exp Bot* 32:79–91

- Djilianov DL, Dobrev PI, Moyankova DP, Vankova R, Georgieva DT, Gadjošová S, Motyka V (2013) Dynamics of endogenous phytohormones during desiccation and recovery of the resurrection, plant species *Haberlea rhodopensis*. *J Plant Growth Regul* 32:564–574
- Dobrev P, Kamínek M (2002) Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid phase extraction. *J Chrom A* 950: 21–29
- Dobrev PI, Vankova R (2012) Quantification of abscisic acid, cytokinin, and auxin content in salt-stressed plant tissues. In *Plant salt tolerance: methods and protocols, methods in molecular biology*. Vol. 913, Springer science + business media, eds. Shabala S., and Ann Cuin T; 17; 251–261
- Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. *Sci Hort* 196:3–14
- Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O (2015) Breeding and domesticated crops adapted to drought and salinity : a new paradigm for increasing food production. *Front Plant Sci* 6:978
- Fukuda A, Tanaka Y (2006) Effects of ABA, auxin, and gibberellin on the expression of genes for vacuolar H<sup>+</sup>-inorganic pyrophosphatase, H<sup>+</sup>-ATPase subunit A, and Na<sup>+</sup>/H<sup>+</sup> antiporter in barley. *Plant Physiol Biochem* 44:351–358
- Gajdošová S, Spíchal L, Kamínek M, Hoyerová K, Novák O, Dobrev PI, Galuszka P, Klíma P, Gaudinová A, Žižková E, Hanuš J, Dančák M, Trávníček B, Pešek B, Krupička M, Vaňková R, Strnad M, Motyka V (2011) Distribution, biological activities, metabolism, and the conceivable function of *cis*-zeatin-type cytokinins in plants. *J Exp Bot* 62:2827–2840
- Ghanem ME, Albacete A, Martínez-Andujar C, Acosta M, Romero-Aranda R, Dodd IC, Lutts S, Pérez-Alfocea F (2008) Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). *J Exp Bot* 59: 3039–3050
- Ghanem ME, Albacete A, Smigocki AC, Frebort I, Pospisilova H, Martínez-Andujar C, Acosta M, Sanchez-Bravo J, Lutts S, Dodd IC, Pérez-Alfocea F (2011) Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato (*Solanum lycopersicum* L.) plants. *J Exp Bot* 62:124–140
- Gharbi E, Martínez JP, Benahmed H, Fauconnier ML, Lutts S, Quinet M (2016) Salicylic acid differently impacts ethylene and polyamine synthesis in the glycophyte *Solanum lycopersicum* and the wild-related halophyte *Solanum chilense* exposed to mild salt stress. *Physiol Plant* 158:152–167
- Gharbi E, Martínez JP, Benahmed H, Hichri I, Dobrev PI, Motyka V, Quinet M, Lutts S (2017) Phytohormone profiling in relation to osmotic adjustment in NaCl-treated plants of the halophyte tomato wild relative species *Solanum chilense* comparatively to the cultivated glycophyte *Solanum lycopersicum*. *Plant Sci* 258:77–89
- Gharbi E, Lutts S, Dailly H, Quinet M (2018) Before or during the stress? Comparison between the impacts of two different modes of salicylic acid application on tomato (*Solanum lycopersicum*) responses to salinity. *Plant Signal Behav* 13(5):e1469361
- Gurmani AR, Bano A, Ullah N, Khan H, Jahangir M, Flowers TJ (2013) Exogenous abscisic acid (ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na<sup>+</sup>) transport and bypass flow in rice (*Oryza sativa* indica). *Aust J Crop Sci* 7:1219–1226
- Gutiérrez-Miceli FA, Santiago-Borraz J, Montes Molina JA, Nafate CC, Abud-Archila M, Oliva Llaven MA, Rincón-Rosales R, Dendooven L (2007) Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (*Lycopersicon esculentum*). *Bioresour Technol* 98: 2781–2786
- Hernandez OL, Calderín A, Huelva R, Martínez-Balmori D, Guridi F, Aguiar NO, Olivares LP, Canellas LP (2015) Humic substances from vermicompost enhance urban lettuce production. *Agron Sust Dev* 35:225–232
- Kamínek M, Brezinova A, Gaudinova A, Motyka V, Vankova R, Zazimalova E (2000) Purine cytokinins: a proposal of abbreviations. *Plant Growth Regul* 32:253–256
- Kaur G, Asthir B (2015) Proline: a key player in plant abiotic stress tolerance. *Biol Plant* 59:609–619
- Khan MN, Siddiqui MH, Mohammad F, Naeem M, Khan MMA (2010) Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing anti-oxidative defence system and osmoprotectant accumulation. *Acta Physiol Plant* 32:121
- Lutts S, Kinet JM, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann Bot* 78:389–398
- Lutts S, Bouharmont J, Kinet JM (1999) Physiological characterization of salt-resistant rice somaclones. *Aust J Bot* 47:835–849
- Manaa A, Gharbi E, Mimouni H, Wasti S, Aschi-Smiti S, Lutts S, Ahmed HB (2014) Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars. *South Afr J Bot* 95:32–39
- Mancinelli AL (1984) Red-far red reversibility of anthocyanin synthesis in dark-grown and light-pretreated seedlings. *Plant Physiol* 76:281–283
- Mimouni H, Wasti S, Manaa A, Gharbi E, Chalh A, Vandoorne B, Lutts S, Ahmed HB (2016) Does salicylic acid (SA) improve tolerance to salt stress in plants? A study of SA effects on tomato plant growth, water dynamics, photosynthesis, and biochemical parameters. *Omic* 20:180–190
- Nardi S, Pizzeghello D, Muscolo A, Vianello A (2002) Physiological effects of humic substances on higher plants. *Soil Biol Biochem* 34:1527–1536
- Pizzeghello D, Francioso O, Ertani A, Muscolo A, Nardi S (2013) Isopentenyladenosine and cytokinin-like activity of different humic substances. *J Geochim Expl* 129:70–75
- Plett DC, Möller IS (2010) Na<sup>+</sup> transport in glycophytic plants: what we know and would like to know. *Plant Cell Environ* 33:612–626
- Quinet M, Bataille G, Dobrev PI, Capel C, Gómez P, Capel J, Lutts S, Motyka V, Angosto T, Lozano R (2014) Transcriptional and hormonal regulation of petal and stamen development by *STAMENLESS*, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class *APETALA3* gene. *J Exp Bot* 65: 2243–2256
- Rady MM, Mohamed GF (2015) Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Scientia Hort* 193:105–113

- Rupani PDF, Embrandiri A, Ibrahim MH, Ghole W, Lee CT, Abbaspour M (2018) Effects of different vermicompost extracts of palm oil effluent and palm-pressed fiber mixture on seed germination of mung bean and its relative toxicity. *Environ Sci Poll Res* 25:35805–35810
- Schäfer M, Brütting C, Meza-Canales ID, Großkisky DK, Vankova R, Baldwin IT, Meldau S (2015) The role of *cis*-zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. *J Exp Bot* 66:4873–4884
- Shkolnik-Inbar D, Adler G, Bar-Zvi D (2013) *ABI4* downregulates expression of the sodium transporter *HKT1;1* in Arabidopsis roots and affects salt tolerance. *Plant J* 73:993–1005
- Singh SNB (2015) Alleviation of allelopathic stress of benzoic acid by indole acetic acid in *Solanum lycopersicum*. *Scientia Hort* 192:211–217
- Singh R, Sharma R, Kumar S, Gupta R, Patil R (2008) Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa* Duch.). *Bioress Technol* 99:8507–8511
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics and phosphomolybdic-phosphotungstic reagents. *Am J Enol Vitic* 16:144–158
- Trevisan S, Pizzeghello S, Ruperti B, Fransisco O, Sassi A, Palme K, Quaggiotti S, Nardi S (2010) Humic substances induce lateral root formation and expression of the early auxin-responsive IAA19 gene and DR5 synthetic element of Arabidopsis. *Plant Biol* 12:604–614
- Truong HA, Lee WJ, Jeong CY, Trinh CS, Lee S, Kang CS, Cheong YK, Hong SW, Lee H (2018) Enhanced anthocyanin accumulation confers increased growth performance in plants under low nitrate and high salt stress conditions owing to active modulation of nitrate metabolism. *J Plant Physiol* 231:41–48
- Wassman R, Hien NX, Hoang CT, Tuong TP (2004) Sea level rise affecting the vietnamienese Mekong Delta: water elevation in the flood season and implication for rice production. *Climate Change* 66:89–107
- Wu X, He J, Chen J, Yang S, Zha D (2014) Alleviation of exogenous 6-benzyladenine on two genotypes of eggplant (*Solanum melongena* Mill.) growth under salt stress. *Protoplasma* 251:169–176
- Xiao Z, Liu M, Jiang L, Chen X, Griffiths BS, Li H, Hu F (2016) Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants. *Appl Soil Ecol* 105:177–186
- Yang L, Zhao F, Chang Q, Li T, Li F (2015) Effects of vermicomposts on tomato yield and quality and soil fertility in greenhouse under different soil water regimes. *Agric Water Manag* 160:98–105
- Yuan F, Liang X, Li Y, Yin S, Wang B (2019) Methyl jasmonate improves tolerance to high salt stress in the recretohalophyte *Limonium bicolor*. *Funct Plant Biol* 46:82–92
- Zaller JG (2007) Vermicompost as a substitute for peat in potting media: effects on germination, biomass allocation, yields and fruit quality of three tomato varieties. *Sci Hort* 112:191–199
- Zarei M, Abadi VAJM, Moridi A (2018) Ciomparison of vermiwash and vermicompost tea properties produced from different organic beds under greenhouse conditions. *Int J Recycl Org Waste Agric* 7:25–32
- Zhang H, Tan SN, Wong WS, Ng CYL, Teo CH, Ge L, Chen X, Yong JWH (2014) Mass spectrometric evidence for the occurrence of plant growth promoting cytokinins in vermicompost tea. *Biol Fert Soils* 50:401–403
- Zhang H, Tan SN, Teo CH, Yew YR, Ge L, Chen X, Yong JWH (2015) Analysis of phytohormones in vermicompost using a novel combinative sample preparation strategy of ultrasound-assisted extraction and solid-phase extraction coupled with liquid chromatography–tandem mass spectrometry. *Talanta* 139:189–197
- Zhang Y, Tian Y, Hu D, Fan J, Shen M, Zeng G (2019) Is vermicompost the possible *in situ* sorbent? Immobilization of Pb, Cd and Cr in sediment with sludge derived vermicompost, a column study. *J Hazard Mat* 367:83–90
- Zheng M, Yang T, Peng T, Zhu T, Zhu G, Fu Y, Hsu YF (2019) *Arabidopsis* GSM1 is involved in ABI4-regulated ABA signaling under high-glucose condition in early seedling growth. *Plant Sci* 287:110183
- Zhu M, Zhou M, Shabala L, Shabala S (2017) Physiological and molecular mechanisms mediating xylem Na<sup>+</sup> loading in barley in the context of salinity tolerance. *Plant Cell Physiol* 40:1009–1020
- Žižková E, Dobrev PI, Muhovski Y, Hošek P, Hoyerová K, Haisel D, Procházková D, Lutts S, Motyka V, Hichri I (2015) Tomato (*Solanum lycopersicum* L.) SIPT3 and SIPT4 isopentenyltransferases mediate salt stress response in tomato. *BMC Plant Biol* 15:85
- Zucco MA, Walters SA, Chong SK, Klubek BP, Masabni JG (2015) Effect of soil type and vermicompost applications on tomato growth. *Int J Recycl Org Was Agric* 4:135–141

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.