ORIGINAL ARTICLE



Assessment of the preventive effect of vermicompost on salinity resistance in tomato (*Solanum lycopersicum* cv. Ailsa Craig)

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Abstract

To determine the effects of vermicompost leachate (VCL) on resistance to salt stress in plants, young tomato seedlings (*Solanum lycopersicum*, cv. Ailsa Craig) were exposed to salinity (150 mM NaCl addition to nutrient solution) for 7 days after or during 6 mL L⁻¹ VCL application. Salt stress significantly decreased leaf fresh and dry weights, reduced leaf water content, significantly increased root and leaf Na⁺ concentrations, and decreased K⁺ concentrations. Salt stress decreased stomatal conductance (g_s), net photosynthesis (A), instantaneous transpiration (E), maximal efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m), photochemical quenching (qP), and actual PSII photochemical efficiency (Φ PSII). VCL applied during salt stress increased leaf fresh weight and g_s , but did not reduce leaf osmotic potential, despite increased proline content in salt-treated plants. VCL reduced Na⁺ concentrations in leaves (by 21.4%), but increased them in roots (by 16.9%). VCL pre-treatment followed by salt stress was more efficient than VCL concomitant to salt stress, since VCL pre-treatment provided the greatest osmotic adjustment recorded, with maintenance of net photosynthesis and K⁺/Na⁺ ratios following salt stress. VCL pre-treatment also led to the highest proline content in leaves (50 µmol g⁻¹ FW) and the highest sugar content in roots (9.2 µmol g⁻¹ FW). Fluorescence-related parameters confirmed that VCL pre-treatment of salt-stressed plants showed higher PSII stability and efficiency compared to plants under concomitant VCL and salt stress. Therefore, VCL represents an efficient protective agent for improvement of salt-stress resistance in tomato.

Keywords NaCl · Osmotic adjustment · Priming · Salt · Sodium concentration

Introduction

Salinity is a major environmental constraint on agricultural production worldwide. Salt stress includes two major types of physiological stress in plants. First, increased NaCl can reduce water availability in plants, in terms of decreased external osmotic potential (Ashraf and Harris 2004; Acosta-Motos et al. 2017). Second, increased NaCl can induce ionic stress due to increased absorption of toxic ions, such as Na⁺,

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² Faculté des Sciences de la Nature et de la Vie, Université de Blida 1, Ouled yaich, Blida, Algeria and decreased absorption of essential elements, such as K^+ (Hamamoto et al. 2015) and P (Acosta-Motos et al. 2017). Salt-stress resistance in plants is consequently a direct function of the need to maintain water status through osmotic adjustment, thus allowing turgor maintenance, while also preserving K^+/Na^+ selectivity and ensuring vacuolar Na^+ sequestration, to avoid the interference of toxic ions in biochemical processes in the cell cytosol (Munns and Tester 2008).

In the plant kingdom, the resistance to salt stress varies greatly across species. Most cultivated plants are glycophyte species, and are thus relatively sensitive to salt stress (Ashraf and Harris 2004). This is especially true for tomato (*Solanum lycopersicum* L.), which is also one of the most consumed fruits worldwide (Romero-Aranda et al. 2001). Salt stress can have a large deleterious impact on tomato production. Some closely related plant species in the *Solanum* genus are halophyte plant species (e.g., *Solanum cheesmaniae, Solanum peruvianum, Solanum chilense*), and interspecific crosses might be an option to select for salt-stress-resistant hybrids (Cuartero et al. 2006). However, this is an expensive task that requires time and effort to select for promising material with the necessary agronomical properties (Flowers and Yeo 1995). Genetic engineering for the transfer of specific genes can also be considered, but this poses a wide range of societal problems and is not necessary realistic, considering that salt-stress resistance is a polygenic trait that relies on interactions between a large number of genes (Flowers 2004).

Genetic improvement of cultivated material, simple and cheap procedures to optimize growing conditions for tomato have also been considered a good option to improve salt-stress resistance, at least on a short-term basis (Hou et al. 2016). Biostimulants are promising products for this purpose (Calvo et al. 2014). According to the European Biostimulants Industry Council, plant biostimulants contain substances and/or micro-organisms that when applied to plants or the rhizosphere, can stimulate natural processes to enhance or improve nutrient uptake and efficiency, plant stress tolerance, and hence crop quality. Numerous types of such products are available, and most of them are a complex mix of a multitude of active substances (Yakhin et al. 2017). Vermicompost is an environmentally friendly organic fertilizer that is produced by digestion of a variety of organic wastes by earthworms. It is naturally enriched with active humic substances, valuable nutrients, phenolic compounds, and hormones, which are known to have positive effects on plant physiology (Martinez-Balmori et al. 2014; Aremu et al. 2015; Zhang et al. 2015). The digestion of organic matter by earthworms produces the solid vermicompost along with a liquid phase that results from leaching processes. In tomato, vermicompost has been used to improve plant growth (Zucco et al. 2015) and fruit yield (Zaller 2007) and quality (Gutiérrez-Miceli et al. 2007). Vermicompost has also been shown to increase defense against the root-knot nematode (Xiao et al. 2016), and to reduce temperature and water stress in seedlings (Chinsamy et al. 2014). However, data regarding the impact of vermicompost on tomato resistance to salt stress remain scarce. Chinsamy et al. (2013) demonstrated that vermicompost leachate (VCL) can alleviate salt stress in tomato through more efficient accumulation of compatible solutes, such as proline and soluble sugar, and higher contents of photosynthetic pigments. In most of these studies, the product was applied at the same time as the environmental constraint. However, under natural field conditions, salt stress is not necessarily a permanent condition, but can instead occur as a consequence of inadequate irrigation practices (Acosta-Motos et al. 2017). It is, therefore, important to determine whether application of VCL before exposure to salt stress has a similar impact to that of vermicompost application concomitant with salt stress.

The research hypothesis is that VCL may afford protection against salt stress in tomato and that its effect varies depending on the timing of stress application. To analyze for potential 'protective' effects of vermicompost application on salt-stress resistance in *S. lycopersicum*, the present study investigated young plants cultivated in nutrient solution with VCL added before or during salt stress. Plant growth was analyzed in terms of regulation of plant water status, ion concentration, and the photosynthetic properties.

Materials and methods

Plant materials, vermicompost leachate, and growth conditions

Seeds of tomato S. lycopersicum L. (cv. Ailsa Craig) were germinated in a greenhouse in plastic jars containing loam as substrate. Fifteen-day-old seedlings were transferred to a hydroponic culture system in a growth chamber under a 16-h photoperiod, with light intensity of 150 μ mol m⁻² s⁻¹. The temperature was set to 25 °C during the day and 20 °C during the night, with corresponding relative humidities of 70 and 90%. The seedlings were distributed among 12 tanks (at six seedlings per tank) that contained 1.5 L aerated nutrient solution, in a completely randomized block design. The seedlings were fixed on polystyrene plates that floated in the aerated hydroponic nutrient solution, which containing: 3 mM KNO₃, 1 mM Ca(NO₃)₂, 2 mM KH₂PO₄, 0.5 mM MgSO₄, 32.9 µM Fe-EDTA, 30 µM H₃BO₄, 5 µM MnSO₄, 1 μ M CuSO₄, 1 μ M ZnSO₄, and 1 mM (NH₄)6Mo₇O. The pH was adjusted to 5.8 daily, and the solutions were renewed every 7 days.

The vermicompost used in this study was produced in the Biotechnology Laboratory in the University of Blida (Algeria) by vermicomposting food wastes using the earthworm *Eisinia fetida* and following the recommendations of Ndegwa and Thompson (2001). The collected VCL has previously been shown to contain 678 mg L⁻¹ humic acids, 41 mg L⁻¹ polyphenols, 1.4 mg L⁻¹ anthocyanins, 89 mg L⁻¹ C-glycosides, 4.7 mg L⁻¹ P, and 45 mg L⁻¹ K (Benhebil 2015).

After 7 days of acclimation, the plants in all of the 12 tanks were maintained for 7 days in nutrient solution, with four of these tanks also receiving addition of 6 mL L⁻¹ VCL, as the VCL pre-treatment. The plants without VCL pre-treatment then underwent one of the following treatments for the following 7 days: (1) nutrient solution alone (control); (2) nutrient solution plus 150 mM NaCl (salt-stress control); (3) nutrient solution plus 6 mL L⁻¹ VCL (VCL control); or (4) nutrient solution plus both 150 mM NaCl and 6 mL L⁻¹ VCL. At the same time, the plants under VCL pre-treatment underwent one of the following treatments for the following 7 days: (5) nutrient solution alone (VCL pre-treated control);

or (6) nutrient solution plus 150 mM NaCl. Each treatment was applied to 12 plants distributed across 2 tanks.

Plant growth, water status, and osmotic potential

Following each of these six treatments, the shoots and roots of four randomly chosen plants per treatment were separated. After determination of the fresh weight (FW), the dry weight (DW) of the roots and leaves were determined following incubation at 72 °C for 48 h. For the osmotic potential (Ψ_s), the samples were cut into small segments and placed into microcentrifuge tubes (Eppendorf, Germany) that were perforated with four small holes and put inside a second, intact, microcentrifuge tube. These samples were then exposed to three freeze/thaw cycles and then centrifuged at 15,000×g for 15 min at 4 °C. The sap from the plant tissue was analyzed for Ψ_s , whereby the osmolarity was assessed using a vapor pressure osmometer (Wescor 5520) and converted from mOsmol kg⁻¹ to MPa according to the Van't Hoff equation (Lutts et al. 1999).

Stomatal conductance, net photosynthesis, and chlorophyll fluorescence

Leaf stomatal conductance (g_s) was measured on the fourth fully expanded leaf of six plants per treatment, using a diffusion porometer (AP4; Delta-TDevices Ltd., Cambridge, UK). These measurements were performed between 14:00 and 16:00 h. The instantaneous transpiration rate (*E*) was measured using an infrared gas analyzer (LCA-2 8.7; ADC, Great Amwell, UK) and an air supply unit (ASU 10.87; ADC) mounted in series in an open system. The net carbon assimilation rate (*A*) under constant photosynthetic photon flux (500 µmol m⁻² s⁻¹) and intercellular CO₂ concentration (c_i) were measured using the same infrared gas analyzer system, according to Vandoorne et al. (2012). Measurements were made between 10:00 and 12:00 h on six leaves for each treatment.

Chlorophyll fluorescence measurements were carried out using a portable pulse-modulated chlorophyll fluorimeter (FMS2; Hansatech, King's Lynn, UK). The recordings were made on the fourth leaf. All of the measurements were performed on the middle of the leaflets. Leaflet portions were acclimated to darkness for 20 min. The minimal fluorescence level (F_0) was measured by measuring the modulated light (0.1 µmol m⁻² s⁻¹). The maximal fluorescence level (F_m) with all photosystem II (PSII) reaction centers open was determined with the dark-adapted leaves using a 0.8-s saturating pulse at 8500 µmol m⁻² s⁻¹. The leaves were then continuously illuminated for 3 min with white actinic light (320 µmol m⁻² s⁻¹), and the steady-state fluorescence (F_s) was recorded. A second saturating pulse at 8500 µmol m⁻² s⁻¹ was imposed to determine the maximal fluorescence for the light-adapted state $(F'_{\rm m})$. The actinic light was removed, and the minimal fluorescence level in the light-adapted state (F'_0) was determined by illuminating the leaf with a 3-s pulse of far-red light. The maximal efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m) , the photochemical quenching coefficient (qP), the non-photochemical quenching (NPQ) and the actual PSII photochemical efficiency (Φ PSII) were calculated according to Maxwell and Johnson (2000).

Determination of Na⁺ and K⁺ contents

The leaf and root tissues of four plants per treatment were oven dried at 72 °C for 2 days. They were then ground into powder in liquid nitrogen. These samples were digested in 4 mL 35% HNO₃ at 80 °C. The residues were redissolved in aqua regia (37% HCl: 65% HNO₃; 3:1) and filtered (Whatman, 11 mm). The ion contents were then quantified by flame atomic absorption spectrophotometry (ICE 3300; Thermo Scientific, Waltham, MA, USA).

Osmocompatible solute determination

The fresh leaves and roots of four plants per treatment were powdered in liquid N₂ and stored at -80 °C until analysis. Free proline was quantified according to the method of Bates et al. (1973).

Soluble sugars were extracted according to Vandoorne et al. (2012) and quantified by the anthrone method according to (Yemm and Willis 1954).

Statistical treatment

The data were analyzed using one-way analysis of variance (ANOVA). This was used to determine the main effects of the different treatments on the physiological and biochemical parameters of the plants. When ANOVA was significant at P < 0.05, the differences between the means were tested for significance according to Student–Newman–Keuls. The data were subjected to ANOVA tests using SYSTAT version 12, and are presented as means \pm standard errors.

Results

Compared to the untreated control (nutrient medium alone), salt stress (i.e., 150 mM NaCl) induced significant decreases in root (Fig. 1a) and leaf (Fig. 1b) FWs. The application of 6 mL L^{-1} VCL in the absence of salt stress had no impact on these parameters. In contrast, application of VCL before (pre-treatment) and during salt stress prevented the decrease in leaf FW caused by salt stress (Fig. 1b). Salt stress did not significantly affect root DW (Fig. 1c), although it





Fig. 1 Root (**a**) and leaf (**b**) fresh weights and root (**c**) and leaf (**d**) dry weights of *Solanum lycopersicum* (cv. Ailsa Craig) plants cultivated over 7 days under control (0 NaCl) and salt-stress (150 mM NaCl) conditions with vermicompost leachate (6 mL L^{-1} VCL) applied

for either 7 days before (VCL BEFORE) or 7 days during salt stress (VCL DURING). Data are means \pm SE (*n*=4). Data with different letters are significantly different (*P* < 0.05; Student–Newman–Keuls)

significantly decreased leaf DW (Fig. 1d). Again, when VCL was applied before or during salt stress, it suppressed salt-stress-induced inhibition of leaf growth.

Neither salt stress alone nor VCL alone had any significant effects on root WC (Table 1). Salt stress significantly reduced leaf WC, while plants exposed to VCL before or during salt stress showed similar leaf WC to unstressed plants. Salt stress reduced root and leaf osmotic potential (Ψ_s) (Table 1). VCL reduced root Ψ_s in salt-stressed plants, and to a greater extend in plants treated with VCL before salt stress, compared to those treated with VCL only during salt stress. Leaf Ψ_s was also lower in plants treated with VCL before salt stress, while no significant effect was seen on leaf Ψ_s for plants treated with VCL during salt stress. As expected, root and leaf Na⁺ contents remained low in the absence of salt stress, and VCL treatment had no effect on these parameters (Fig. 2). Salt stress increased root Na⁺ concentration (Fig. 2a), while VCL treatment before or during salt stress had contrasting effects: VCL pre-treatment significantly reduced root Na⁺ concentration, while concomitant VCL treatment significantly increased root Na⁺ concentration. In contrast, both of these VCL treatments significantly reduced Na⁺ accumulation in leaves of saltstressed plants (Fig. 2b). Salt stress slightly reduced K⁺ concentration in leaves but not in roots (Fig. 2c, d). In saltstressed plants, VCL pre-treatment increased K⁺ concentration in leaves of salt-stressed plants, while concomitant VCL treatment increased K⁺ concentration in roots.

Table 1 Water content and osmotic potential (Ψ_s) in roots and leaves of tomato (<i>Solanum</i> <i>lycopersicum</i> , cv. Ailsa Craig) plants cultivated over 7 days under control (0 NaCl) and salt-stress (150 mM NaCl) conditions with vermicompost leachate (6 mL L ⁻¹ VCL) applied for either 7 days before or 7 days during salt stress	Tissue	Condition	VCL addition	Water content (%)	Osmotic potential (Ψ_s ; MPa)
	Roots	Control	None	93.25±0.456 a	-0.447 ± 0.013 a
			Before	92.66±0.38 a	-0.417 ± 0.018 a
			During	91.97 ± 0.256 a	-0.460 ± 0.029 a
		Salt stress	None	91.56±0.406 a	-0.745 ± 0.020 b
			Before	92.90±0.158 a	-0.898 ± 0.021 d
			During	92.05±0.703 a	-0.822 ± 0.024 c
	Leaves	Control	None	91.27±0.598 a	-0.695 ± 0.019 a
			Before	91.63±0.498 a	-0.783 ± 0.022 b
			During	90.87±0.598 a	-0.668 ± 0.014 a
		Salt stress	None	88.30±0.745 b	-0.993 ± 0.029 c
			Before	92.32 ± 0.352 a	-1.215 ± 0.021 d
			During	90.85±0.512 a	-1.04 ± 0.025 c

Data are means \pm SE (n=4). For a given organ, data with different letters within each measure are significantly different (P<0.05; Student–Newman–Keuls)

With no salt stress, VCL treatments had no effects on root and leaf proline contents (Fig. 3a, b). Salt stress increased root and leaf proline contents and the recorded increase was greater for salt-stressed plants exposed to VCL treatment. VCL pre-treatment had greater impact on leaf proline content under salt stress than when applied concomitant to salt stress (Fig. 3b). For sugar content, with no salt stress, VCL treatment had no impact on the leaf sugar content, but increased root sugar content. Salt stress increased root sugar content, and especially with VCL pre-treatment (Fig. 3c). In contrast, VCL treatment had no effects on leaf sugar content in salt-stressed plants (Fig. 3d).

Salt stress reduced stomatal conductance (g_s) (Fig. 4a). Here, VCL treatment significantly increased g_s in saltstressed plants and the impact of VCL was greater for pretreatment than when applied concomitant to salt stress. Salt stress also reduced net photosynthesis (A) and instantaneous transpiration (E) (Fig. 4b, c) and only VCL pre-treatment significantly reduced these negative effects of salt stress. Salt stress had no significant impact on the intercellular CO₂ mole fraction (c_i) in plants cultivated in the absence of VCL, while it decreased C_i in VCL-treated plants (Fig. 4d).

The maximum quantum yield of dark acclimated leaves (F_v/F_m) and photochemical quenching (qP) were significantly reduced in response to salt stress (Table 2). VCL pretreatment protected against the deleterious impact of salt stress on these parameters, while salt-stressed plants under concomitant VCL treatment showed intermediate behavior. Salt stress alone increased non-photochemical quenching (NPQ), and VCL pre-treatment reduced this increase; VCL treatment concomitant with salt stress had a more limited protective effect. A similar situation was seen for photochemical efficiency of photosystem II (**PSII**), which was deceased by salt stress, and which remained higher in

VCL-pretreated plants compared to plants treated with VCL concomitant with salt stress.

Discussion

Increasing productivity of marginal lands is a major goal over the coming decades. This is especially the case for agricultural areas that are affected by salinity or drought, where the crop yields remain relatively low. Optimal fertilization might help plants to overcome some of the deleterious impacts of abiotic stresses. However, in several parts of the world that are affected by such adverse environmental conditions, chemical fertilizers are not available or they are too expensive for small farms. Moreover, intensive use of these products constitutes a risk for ecosystem stability. Vermicomposting processes are now frequently presented as attractive alternatives to chemical fertilizers (Bidabadi et al. 2016; Mengistu et al. 2017). Indeed, the post-thermophilic biodegradation of organic material through interactions between earthworms and micro-organisms provides an environmentally friendly product that is highly suitable for addition to soil (Martinez-Balmori et al. 2014; Joshi et al. 2014; Motallebi 2015). However, solid vermicompost often has high electrical conductivity that can result in increased soil salinity, which thus constitutes a major drawback for its use in salt-affected areas (Gutiérrez-Miceli et al. 2007; Bidabadi et al. 2016). In contrast, VCL has low electrical conductivity and provides the advantage of homogeneity, compared to application of the solid vermicompost fraction (Joshi et al. 2014). Indeed, vermicompost and VCL have been successfully used to improve yield and quality of tomato fruit under different systems, from fields to greenhouses and hydroponic systems (Gutiérrez-Miceli et al. 2007; Zaller 2007; Arancon et al. 2012; Haghighi et al. 2016; Xiao et al. 2016). Similar



Fig. 2 Root (**a**) and leaf (**b**) Na⁺ contents and root (**c**) and leaf (**d**) K⁺ contents of *Solanum lycopersicum* (cv. Ailsa Craig) plants cultivated over 7 days under control (0 NaCl) and salt-stress (150 mM NaCl) conditions with vermicompost leachate (6 mL L^{-1} VCL) applied

for either 7 days before (VCL BEFORE) or 7 days during salt stress (VCL DURING). Data are means \pm SE (n=4). Data with different letters are significantly different (P < 0.05; Student–Newman–Keuls)

data have been reported for a number of other plant species (Xu et al. 2016; Bidabadi et al. 2016).

In the present study, VCL did not show any obvious advantages when the tomato plants were cultivated in the absence of salt stress. Here, the growth parameters, ion contents, and photosynthesis-related parameters were not affected by the VCL application, with only the root sugar content increased slightly in response to VCL in the unstressed plants. In contrast, other studies have reported that vermicompost or VCL can significantly stimulate tomato plant growth in the absence of salt stress, in terms of root and stem lengths, root and shoot FWs, and stem diameters (Chinsamy et al. 2013; Haghighi et al. 2016; Megistu et al. 2017). In several of these cases, the plants were cultivated in soil, and an improvement in the plant performance might be attributable to improved soil structure and soil microbial population (Azarmi et al. 2008). Zucco et al. (2015) demonstrated recently that the impact of VCL on tomato performance can differ greatly depending on the soil type considered. In the present study, the plants were maintained in nutrient solution, and thus any impacts of VCL on soil structure, size of aggregates, and substrate water retention capacity are not factors that need to be considered here. Arthur et al. (2012) demonstrated that VCL can alleviate P and K deficiencies for tomato seedlings. In the present study, the unstressed seedlings were maintained in an optimal nutrient situation that provided all of the elements required for plant growth and development. Consequently, these plants did not experience any nutrient deficiency. To consider the impact of VCL on fruit size and





Fig. 3 Root (**a**) and leaf (**b**) proline contents and root (**c**) and leaf (**d**) total soluble sugar contents of *Solanum lycopersicum* (cv. Ailsa Craig) plants cultivated over 7 days under control (0 NaCl) and salt-stress (150 mM NaCl) conditions with vermicompost leachate

(6 mL L⁻¹ VCL) applied for either 7 days before (VCL BEFORE) or 7 days during salt stress (VCL DURING). Data are means \pm SE (*n*=4). Data with different letters are significantly different (*P*<0.05; Student–Newman–Keuls)

quality, numerous studies have used vermicompost for long periods, from the seedling stage to the reproductive stage. In contract, the present study focused only on the seedling stage, and consequently used short duration of exposure to VCL (i.e., 7 days). Moreover, Zaller (2007) demonstrated that the impact of VCL can vary depending on the tomato cultivar considered, while Arancon et al. (2012) reported large variability in VCL composition that depended on the nature of the organic waste used for the composting processes. According to these previous studies, different VCL preparations might have different impacts on tomato plant growth. Hence, these differences in culture system, cultivar studied, and VCL composition might explain why in the present study there was no obvious impact of VCL alone on the seedlings maintained in the absence of salt stress. The situation appeared different here for plants exposed to salt stress, as the VCL applied either before or during the salt stress clearly mitigated some of the deleterious symptoms of salt stress. Salt stress significantly decreased leaf FW and DW, reduced the leaf WC, strongly increased root and leaf Na⁺ concentration and decreased K⁺ concentration. Salt stress also decreased g_s , A, E, F_v/F_m , qP, and Φ PSII, while it increased NPQ. Application of VCL during salt stress abolished the salt-stress-induced decrease in leaf FW, which confirms its positive impact on salt-stress resistance in *S. lycopersicum* (Chinsamy et al. 2013). A similar protective effect was also reported for sunflower (Jabeen and Ahmad 2017) and peppermint (Xu et al. 2016). The present study demonstrates that VCL applied during salt stress improves leaf WC, thus confirming that VCL can reduce the water

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b

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ab

0NaCl
NaCl



Fig. 4 Stomatal conductance (**a**), net photosynthesis (**b**), instantaneous transpiration (**c**), and intercellular c_i (**d**) in leaves of *Solanum lycopersicum* (cv. Ailsa Craig) plants cultivated over 7 days under control (0 NaCl) and salt-stress (150 mM NaCl) conditions with ver-

micompost leachate (6 mL L⁻¹ VCL) applied for either 7 days before (VCL BEFORE) or 7 days during salt stress (VCL DURING). Data are means \pm SE (*n*=4). Data with different letters are significantly different (*P* < 0.05; Student–Newman–Keuls)

VCL BEFORE VCL DURING

Table 2Chlorophyllfluorescence parametersmeasured for tomato (Solanumlycopersicum, cv Ailsa Craig)plant leaves cultivated over7 days under control (0 NaCl)and salt-stress (150 mM NaCl)conditions with vermicompostleachate (6 mL L⁻¹ VCL)applied for either 7 days beforeor 7 days during salt stress

Condition	VCL addition	Chlorophyll fluorescence parameter					
		$F_{\rm v}/F_{\rm m}$	qP	NPQ	φSII		
Control	None	0.854 ± 0.002 a	0.981 ± 0.009 ab	0.229 ± 0.028 c	0.858±0.013 b		
	Before	0.857 ± 0.003 a	0.996±0.003 a	0.191 ± 0.017 c	0.898 ± 0.007 a		
	During	0.856 ± 0.005 a	0.995 ± 0.003 ab	0.243 ± 0.009 c	0.880 ± 0.004 ab		
Salt stress	None	0.804 ± 0.003 c	0.938±0.001 c	0.557 ± 0.030 a	$0.793 \pm 0.008 \text{ d}$		
	Before	0.859 ± 0.002 a	0.985 ± 0.004 ab	0.241 ± 0.011 c	0.857 ± 0.008 b		
	During	0.823 ± 0.002 b	0.971 ± 0.001 b	0.452 ± 0.029 b	0.822 ± 0.002 c		

The parameters given include: maximum quantum yield of dark acclimated leaves $(F_{\sqrt{F_m}})$; photochemical quenching (qP); non-photochemical quenching (NPQ); and photochemical efficiency of photosystem II (ϕ PSII). Data are means ± SE (n=4). Data with different letters within each measure are significantly different at (P < 0.05; Student–Newman–Keuls)

stress component of salt stress. This effect, however, was not due to stomatal closure, as in response to VCL application, g_s increased, rather than decreased. Chinsamy et al. (2014) suggested that VCL can improve osmotic adjustment under water-stressed conditions, in terms of proline and soluble sugar accumulation. Although VCL applied here during salt stress induced an increase in proline compared to plants under salt stress in the absence of VCL, this increase was not sufficient to significantly reduce leaf Ψ_s . In contrast, VCLtreated roots that were concomitantly exposed to salt stress showed lower $\Psi_{\rm s}$ than plants exposed to salt stress in the absence of VCL, which might be due, at least in part, to soluble sugar accumulation. The biochemical basis of VCLinduced increases in proline content remains to be determined but has also been recorded in the absence of stress in tomato (Motallebi 2015). Although increases in glutamate dehydrogenase (EC 1.4.1.2) and glutamine synthase (EC 6.3.1.2) activities have key roles in the synthesis of proline precursors in salt-stressed plants, Jabeen and Ahmad (2017) recently demonstrated that vermicompost has no impact on these enzyme activities in salt-stressed Helianthus annuus. Moreover, proline concentration does not always increase in response to VCL application; indeed, Xu et al. (2016) reported that vermicompost protected Silybum marianum and Mentha haplocalyx against salt stress, where the proline content decreased due to the global reduction of the intensity of the salt stress.

Monovalent cation absorption and translocation from roots to shoots might be another target for the beneficial effects of VCL in salt-stressed plants. VCL applied here to salt-stressed plants reduced leaf Na⁺ content but increased root Na⁺ content. This suggests that VCL reduces Na⁺ translocation to the aerial plant parts, which is an efficient strategy for salt-stress resistance in the tomato cv. Ailsa Craig (Gharbi et al. 2017). In contrast, root K⁺ increased in VCL-treated plants exposed to salt stress, while it slightly decreased in salt-stressed plants in the absence of VCL. VCL also contains K, and hence it is commonly used as a 'NPK' fertilizer (Singh et al. 2010). However, root K⁺ content in VCL-treated plants increased here with salt stress, but not in the absence of VCL, which thus suggests that the salt stress interacts with VCL to improve K⁺ nutrition. The humic substances in VCL might indeed interact with membrane transporters, as previously shown by Canellas et al. (2002) for H⁺-ATPases and by Quaggiotti et al. (2004) for nitrate. With Quaggiotti et al. (2004), humic substances even stimulated the expression of genes encoding two putative maize nitrate transporters. Hence, interactions of VCL with K^+ transporters cannot be ruled out, although the specific impact of the NaCl-induced salt stress on this interaction still requires further study.

Of note, in terms of improvement of salt-stress resistance, VCL applied here before salt stress was more efficient than

VCL applied concomitant to salt stress. Indeed, this preventive VCL application led to the best osmotic adjustment recorded at the end of the stress period (Table 1). It also reduced Na⁺ concentration in both roots and shoots, which suggests that Na⁺ absorption rather than Na⁺ translocation was modified under these circumstances. This protective application of VCL also increased K⁺ concentration and led to the highest proline concentration in the leaves, as well as the highest sugar concentration in the roots. The impact of this protective VCL application on the fluorescence-related parameters confirmed that salt-stressed plants previously treated with VCL had greater PSII stability and efficiency when exposed to salt stress than plants that received VCL and were exposed to salt stress concomitantly. Moreover, VCL application before salt stress contributed to stomatal opening during the salt stress; this will allow maintenance of gas exchange and net photosynthesis without any deleterious impact on plant water status, which appeared to be efficiently regulated by osmotic adjustment. While VCL application in the absence of salt stress did not induce any morphological modifications, it appears that it modified the physiological status of the plants in such a way that the plants coped with the salt stress more efficiently. VCL might thus be regarded as an efficient protective agent that can reinforce plant resistance to salt stress.

The underlying mechanisms that sustain such adaptations might be related to the hormonal properties of VCL. It is well-established that humic acids isolated from earthworm compost have auxin properties, and that bioactive molecules such as indole acetic acid cluster with the humic acid supramolecular arrangement (Canellas et al. 2002; Pizzeghello et al. 2013). Recent studies have also demonstrated that VCL contains a wide range of phytohormones (Zhang et al. 2015; Aremu et al. 2015). Hormonal status has a key role in plant responses to salt stress, and especially for the Solanum genus (Gharbi et al. 2017). In particular, this relates to the cytokinins that act as anti-senescent hormones. Cytokinins accumulation significantly improves salt-stress resistance in tomato (Žižková et al. 2015). Using an enzyme-linked immunosorbent assay, Pizzeghello et al. (2013) detected the cytokinin isopentenyladenosine in humic fractions extracted from earthworm feces. More recently, Aremu et al. (2015) used ultra-high performance liquid chromatography-tandem mass spectrometry to quantify a wide range of cytokinins and gibberellins in various sources of VCL. Similarly, Zhang et al. (2015) detected high levels of the most active cytokinin, trans-zeatin, in vermicompost produced from organic waste. Although some studies have reported that vermicompost can also contain abscisic acid (ABA), Calderín García et al. (2014) reported that humic acids might reduce these ABA levels, or prevent stress-induced increases in endogenous ABA levels. Increased cytokinins in association with decreased ABA might also explain the maintenance of high g_s in salt-stressed tissues. Indeed, a tempting hypothesis here is that exposure to VCL before salt stress leads to high stomatal conductance, which allows maintenance of the transpiration stream. As a consequence, the plants can accumulate protective phytohormones or antioxidants from VCL, and these protective compounds are thus already present within the tissues when the salt stress is applied. In contrast, exposure to concomitant salt stress and VCL does not allow maintenance of stomatal conductance, and the stomata close as a consequence of low external osmotic potential. This will thus reduce the transpiration stream and the translocation of putative protective compounds.

Conclusion

Application of VCL to these tomato plants partly abolished the detrimental impact of salt stress on growth and photosynthesis, and this was related to decreased Na⁺ accumulation. VCL applied before salt stress was more efficient than VCL applied concomitant to salt stress. VCL might thus be used as an efficient protective agent to improve saltstress resistance in tomato. Further studies are in progress to test this hypothesis through complete hormonal profiling of VCL and of VCL-treated plants under control and saltstress conditions.

Author contribution statement SB performed the analysis and all aspects related to the experimental work. ZED provided the vermicompost leachate and help in the interpretation of collected data. SL supervised the experimental work and wrote the protocol. All authors wrote the manuscript.

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