Plant Growth Regul (2013) 71:41–47
DOI 10.1007/s10725-013-9807-6

ORIGINAL PAPER

Garden-waste-vermicompost leachate alleviates salinity stress in tomato seedlings by mobilizing salt tolerance mechanisms

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Received: 16 November 2012 / Accepted: 20 March 2013 / Published online: 28 March 2013
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Abstract The incidence of salinity-induced plant stress as a result of natural and anthropogenic factors in arid and semi-arid agricultural lands is great. In South Africa alone, 9 % of irrigated agricultural land is salt-affected. Commercial fertilizers used for improving soil nutrient levels are costly and affect the quality, lifespan and sustainability of soil and water resources. Organic farming practices are based on cost-effective and environmentally-aware management systems. Vermicompost leachate (VCL) is a vermicompost-derived liquid product that has become recognised as a suitable soil amendment product. Commercial tomato (*Lycopersicon esculentum* Mill var. Heinz-1370) seedlings were subjected to sodium chloride (NaCl) concentrations of 0, 25, 50 and 100 mM and were treated with 1:10 (v/v) WizzardWorms VCL prepared in Hoagland's nutrient solution under greenhouse conditions. Morphological characters of VCL-treated tomato seedlings showed improved root growth and stimulated overall aboveground growth with significantly higher numbers of leaves, greater stem thickness and increased leaf area, even at a high NaCl-tested concentration (100 mM). The accumulation of compatible solutes such as proline and total soluble sugars indicate an induced salt tolerance or adaptive mechanism in VCL-treated tomato seedlings. The current investigation demonstrates the potential of an organic liquid to maximise tomato productivity by improving seedling growth performance under salt stress conditions.

Keywords Tomato · Vermicompost leachate · Proline · Total soluble sugars · Salinity · Stress

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Abbreviations

ABA	Abscisic acid
NaCl	Sodium chloride
VCL	Vermicompost leachate

Introduction

The lack of arable land adversely affects crop production in many regions of the world. Furthermore, the agricultural sector in arid and semi-arid regions is faced with various environmental challenges which lead to accumulation of soluble salts in the soil. Fluctuating groundwater levels due to erratic and/or excessive rainfall, a combination of irrigation and poor drainage and deforestation contribute to soil salinity stress, which is characterised as an abiotic stress that may inhibit plant growth. The most common symptoms of salinity stressed plants include senescence, accelerated development and growth inhibition (Zhu 2007). The mechanisms of salt stress responses are generally related to oxidative and osmotic stress induced by abscisic acid (Zhu 2007).

Available agricultural land is routinely supplemented with commercial fertilizers to increase the nutrient levels of the soil. It is understood that the composition of runoff from such practices affects the quality, lifespan and sustainability of soil and water resources. Inorganic fertilizers also have two major drawbacks in that they are costly and generally inaccessible to small scale farmers (Welch and Graham 1999). Chaoui et al. (2003) have shown that inorganic fertilizers lead to salinity stress in plants. The use of organic and environmentally-friendly products is gaining popularity and vermicompost-derived products are reportedly more suitable for soil amendment (Chaoui et al. 2003).

Vermicompost and its liquid by-products may therefore be further defined as ecologically-aware nutrient resources (Tomati et al. 1990; Bachman and Metzger 2008; Wang et al. 2010).

Vermicompost leachate (VCL) is an organic liquid produced from earthworm digested material and earthworm casts during the vermicomposting process. It essentially improves agronomic and horticultural crop performance, increases soil plant growth hormone and enzyme content and microbial populations (Chaoui et al. 2003). The earthworm casts present in vermicompost also contain nitrogen, carbon, phosphorous, potassium, calcium and magnesium as available resources. Aremu et al. (2012) and Arthur et al. (2012) have demonstrated the beneficial effects of vermicompost leachate in commercially important crops such as banana and tomato respectively. Chaoui et al. (2003) reported decreased salinity stress with the addition of earthworm casts compared to conventional compost treatments. They concluded that earthworm-cast-treatment provides an efficient source of nutrients to plants.

Tomato is ranked the second-most important crop globally (FAO 2010). The agronomic importance of tomatoes is embodied in the nutritive and functional value of this crop. However, tomato is moderately sensitive to salinity (Ayers and Westcot 1985) and negative effects of salinity on seed germinability and vegetative growth have been reported (Cuartero and Fernández-Muñoz 1999). More specifically, root biomass and shoot growth were reduced. Similar reports on salinity stress responses in tomato show reduced stem and leaf dry weights, leaf area and stem thickness (Cruz and Cuartero 1990; Van Ieperen 1996). The beneficial effect of VCL under deficiency of macroelements on tomato seedlings was recently reported by Arthur et al. (2012). However, no studies have been reported so far on the effects of VCL on tomato seedlings under salinity stress, which is one of the major causes affecting its productivity. This study highlights the potential of VCL for enhancing the growth of tomato seedlings under salinity stress.

Materials and methods

Plant material and NaCl treatments

Commercial tomato (*Lycopersicon esculentum* Mill var. Heinz-1370) seeds purchased from McDonald Seeds, Pietermaritzburg, South Africa were used in salinity stress experiments. Vermicompost-leachate derived from garden-waste using red earthworm *Eisenia fetida* was purchased from WizzardWorms, Rietvlei, KwaZulu-Natal, South Africa. The average pH (7.82) and macro- and micronutrient content of the VCL as per dry matter basis (nitrogen: 2.26 %, phosphorus: 0.99 %, potassium: 0.64 %, calcium: 2.52 % and sodium: 631.03 mg kg^{-1}) was provided by the manufacturer. Hoagland's nutrient solution (50 %) was used as a nutrient medium. Three tomato seeds were sown in a 100 ml plastic pot at a depth of 1 cm in perlite. After 15 days, two seedlings were uprooted and a single seedling was retained in the pot. One-month-old seedlings were treated with or without VCL (1:10 v/v) at NaCl concentrations of 0, 25, 50 and 100 mM prepared using the Hoagland's nutrient solution. The experiment was conducted in a greenhouse with 60 ± 5 % relative humidity at 22/15 °C day/night temperature and average midday photosynthetic photon flux density of 450 μmol m^{-2} s^{-1}. Tomato seeds were treated once a week for the first 4 weeks. Thereafter, seedlings were treated twice weekly until termination of the experiment. The experiment had four replicates of five pots each. Morphological data of 10-week-old tomato seedlings, which included length and weight of shoot and root, stem thickness, number of leaves and leaf area were recorded. Harvested material was oven-dried at 70 ± 2 °C for 72 h to determine dry weights (shoot and root).

Determination of physiological parameters

Photosynthetic pigment (chlorophyll *a*, *b* and total chlorophyll) content was extracted by homogenising fresh leaf samples of treated and control seedlings (5 g) in 80 % acetone (20 ml) using a homogeniser (Jane and Kunkel IKA-Labortechnik Ultra-Turrax T25). The resultant solution was filtered through Whatman No. 1 filter paper (70 mm) using 80 % acetone (10 ml) to rinse the residue on the filter paper. The volume of filtrate was adjusted to 50 ml using 80 % acetone and the optical density was measured at 645 and 663 nm. The chlorophyll pigment content (mg g^{-1} tissue) was estimated using the formulae below:

$$\text{Chlorophyll}\,a(\text{C}_\text{a}) = [12.7(\text{A}_{663}) - 2.69(\text{A}_{645})] \times \text{Vol} \times (0.001) \times \text{W}$$

$$\text{Chlorophyll}\,b(\text{C}_\text{b}) = [22.9(\text{A}_{645}) - 4.68(\text{A}_{663})] \times \text{Vol} \times (0.001) \times \text{W}$$

$$\text{Total chlorophyll} = [20.2(\text{A}_{645}) + 8.02(\text{A}_{663})] \times \text{Vol} \times (0.001) \times \text{W}$$

where, A is the absorbance, Vol is the total volume of filtrate, and W is the weight of the leaf material.

Proline content was determined using the sulfosalicylic acid method (Bates et al. 1973). Fresh leaf samples (0.4 g) were homogenised with 3 % sulfosalicylic acid (5 ml) and boiled for 10 min. An aliquot of the resultant supernatant (2 ml) together with distilled water (2 ml), glacial acetic acid (2 ml) and 4 ml of acid-ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) was boiled for 60 min and thereafter cooled on ice to stop the reaction. Toluene (4 ml) was added to the cooled solution, which was vortexed and allowed to stand for 30 min at room temperature (≈23 °C). The optical density of the upper phase was measured at 520 nm, using 1 ml toluene as blank.

Total soluble sugar content was determined using the anthrone method, where leaf sugars were extracted with 80 % ethanol (4 ml) at 80 °C for 40 min followed by centrifugation at 2,000×*g* for 15 min (Men and Liu 1995). The leaf sugar ethanol extract (0.1 ml) was reacted with 3 ml anthrone reagent [0.2 g anthrone in 100 ml H_2SO_4 (1:0.4 v/v)] in a boiling water bath for 10 min. Total soluble sugar content was estimated by measuring the optical density of cooled samples at 625 nm.

Statistical analysis

One-way analysis of variance (ANOVA) was performed on all data sets using GenStat statistical package for Windows (GenStat® 14th, VSN International, Hemel Hempstead, UK). The mean values were separated using Duncan's multiple range test ($P = 0.05$), as indicated by letters in the table and figures.

Results

Morphological parameters

Ten-week-old tomato seedlings were significantly improved in VCL-supplemented salt solutions prepared with Hoagland's nutrient solution (Table 1; Fig. 1). With the exception of 50 mM NaCl treatment, control, 25 and 100 mM salt solutions supplemented with VCL produced morphologically superior tomato seedlings in terms of number of leaves, stem thickness and leaf area when compared to the respective controls (Table 1). Shoot length was significantly increased in all VCL-treated seedlings across all tested salt concentrations (Fig. 1a). Shoot fresh weight of tomato seedlings was significantly higher in VCL supplemented salt solutions (Fig. 1c), with the most significant increase in shoot dry weight (432 mg) at 25 mM NaCl (Fig. 1e). Addition of VCL significantly increased shoot fresh (4.7 ± 0.26 g) and dry weights (711 ± 53 g) at 100 mM NaCl compared to the controls (Fig. 1c, e). The only significant increase in tomato seedling root length was recorded at 100 mM salt concentration (Fig. 1b).

Physiological parameters

Vermicompost leachate-treated seedlings had a significantly higher photosynthetic pigment content with the exception of chlorophyll *a* content at 100 mM NaCl (Fig. 2a–c). There was a general decline in chlorophyll *a* and total chlorophyll content in control and VCL-treated seedlings between 25 and 100 mM NaCl (Fig. 2a, c). At 50 mM NaCl, chlorophyll *b* content of VCL-treated seedlings was increased significantly to 1.315 ± 0.01 mg g^{-1} in comparison to all other treatments.

In comparison to the respective controls, there was significantly greater accumulation of low-molecular compatible solutes such as proline and total sugars in VCL-treated seedlings between 25 and 100 mM NaCl. Increasing salt concentrations increased accumulation of total sugars in VCL-treated seedlings (Fig. 2d). A similar trend was observed for proline content (Fig. 2e). The greatest significant increase in proline (Fig. 2e) and total sugars (Fig. 2d) between control and treated tomato seedlings was at 100 mM NaCl.

Discussion

In this study, the adverse effects of salt stress on root development were evident (Fig. 1d, f). The application of VCL facilitated recovery, so that at 100 mM NaCl root dry weight was similar to that of control seedlings, experiencing no salt stress (80 ± 5 mg) (Fig. 1f). There was increased root density (indicated by root fresh and dry weight) (Fig. 1d, f) in VCL-treated tomato seedlings as compared to the control seedlings. This implies an increase in root surface area, which would have enhanced nutrient uptake thereby stimulating seedling growth and development. Osmotic stress as a result of high salinity has a greater effect on leaf growth than on root growth (FAO Land and Nutrition Management Service 2008). Mahajan and Tuteja (2005) interpret the adaptive mechanism as a response to reduce transpiration where processes such as leaf senescence and abscission are accelerated under such stresses. Tomato shoot dry weight increased remarkably at all tested salt concentrations with the addition of VCL (Fig. 1e). The improved root capacity in VCL-treated seedlings may have led to the enhanced aboveground growth parameters (Fig. 1a; Table 1). Common problems associated with inorganic fertilizers such as loss of soil nutrients due to leaching and salinity-induced stress can be averted by using earthworm casts as a soil amendment alternative. In closed systems, earthworm cast treatments improved soil porosity which allowed for improved root growth and development (Chaoui et al. 2003). The growth potential of VCL-treated tomato seedlings was increased with a significantly higher number of leaves, improved stem thickness and increased leaf area at a higher concentration of NaCl (100 mM). Vermicompost leachate-

Table 1 Effect of vermicompost leachate (1:10 v/v) on aboveground growth parameters of tomato seedlings (10-weeks-old) under salinity stress

Treatment	Leaves (no)	Stem thickness (mm)	Leaf area (cm^2)
Control	7.4 ± 0.21bc	3.13 ± 0.15cd	81 ± 7bc
25 mM NaCl	7.3 ± 0.10c	3.19 ± 0.11c	82 ± 5bc
50 mM NaCl	7.9 ± 0.20ab	3.17 ± 0.13cd	81 ± 7bc
100 mM NaCl	6.9 ± 0.16c	2.83 ± 0.08d	61 ± 4c
Control + VCL (1:10 v/v)	8.3 ± 0.16a	3.67 ± 0.12ab	116 ± 11a
25 mM NaCl + VCL (1:10 v/v)	8.3 ± 0.13a	3.79 ± 0.08a	117 ± 6a
50 mM NaCl + VCL (1:10 v/v)	8.1 ± 0.22a	3.45 ± 0.13abc	100 ± 10ab
100 mM NaCl + VCL (1:10 v/v)	8.1 ± 0.19a	3.35 ± 0.08bc	88 ± 4b

Mean values (±SE) in a column with different letters are significantly different according to Duncan's multiple range test ($P = 0.05$)

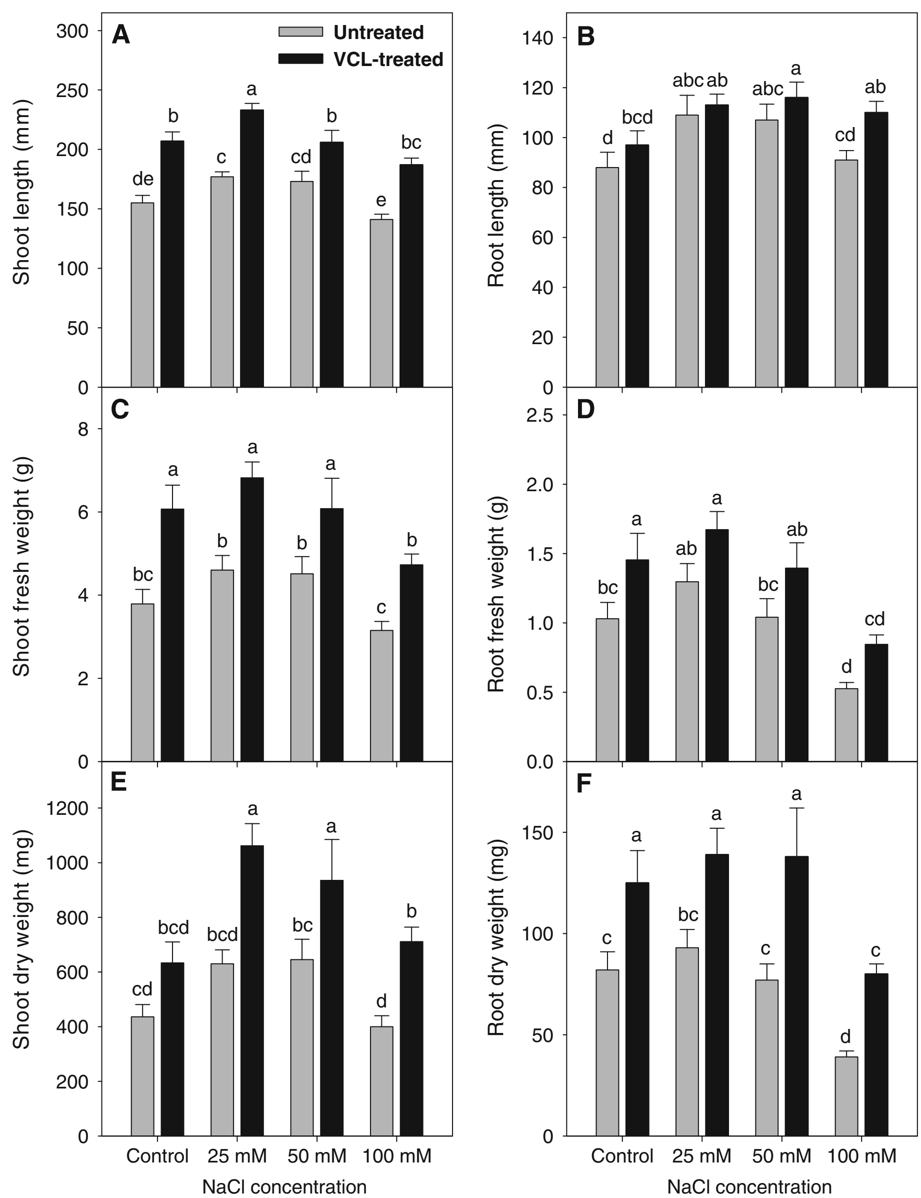

Fig. 1 Effect of vermicompost leachate (1:10 v/v) on shoot and root growth parameters of tomato seedlings (10-weeks-old) under salinity stress. *Bars* (±SE) with different *letters* are significantly different according to Duncan's multiple range test ($P = 0.05$)

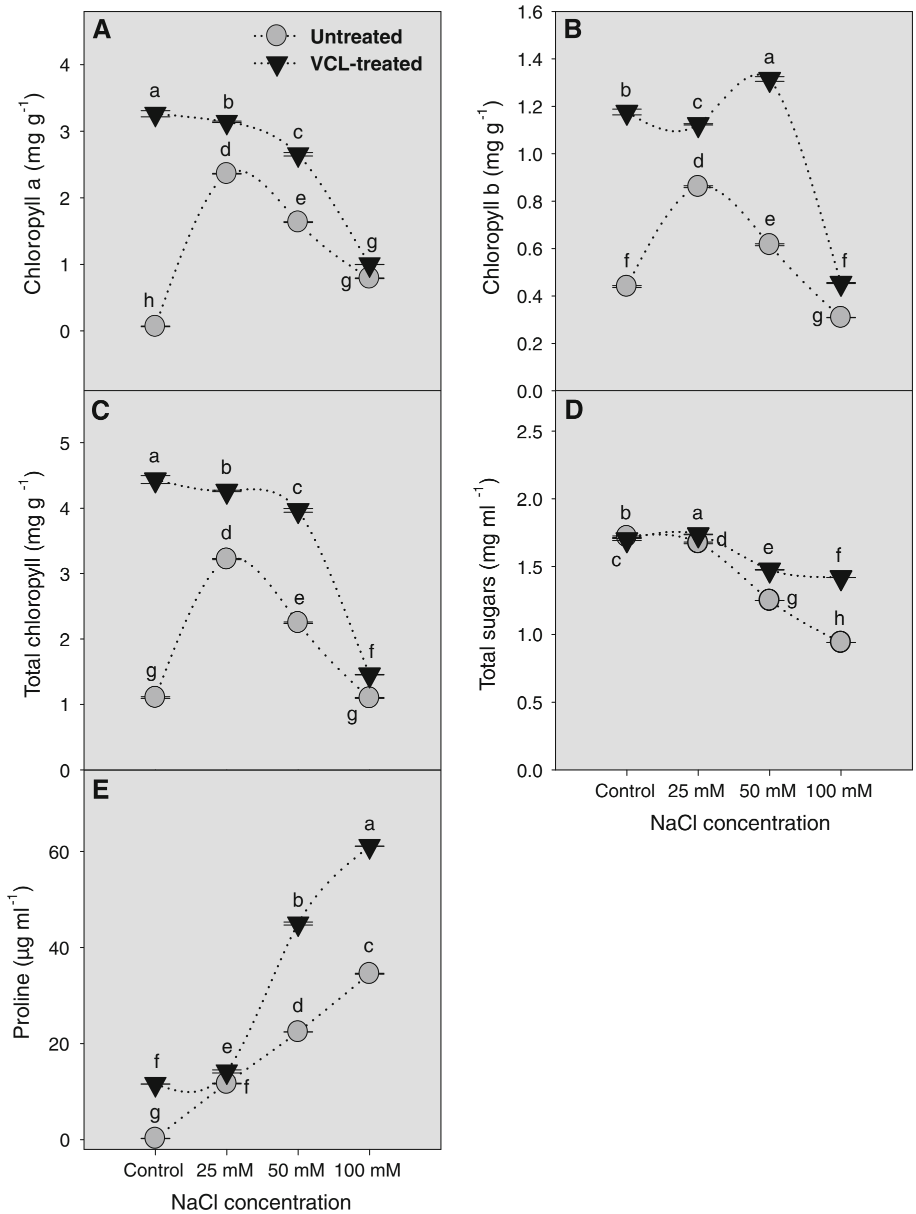

Fig. 2 Effect of vermicompost leachate (1:10 v/v) on physiological parameters of tomato seedlings (10-weeks-old) under salinity stress. *Symbols* (±SE) with different *letters* are significantly different according to Duncan's multiple range test ($P = 0.05$)

treated tomato seedlings performed better under salinity stress with well developed vegetative structures.

Plant uptake of water and nutrients is compromised due to the low water potential created in regions with accumulated soluble salts resulting in salinity and drought stress. The resultant osmotic stress causes accumulation of Na^+ ions which disrupt ionic equilibrium of the cells; affect enzyme function, inhibit cell division and growth, and decreases rate of photosynthesis (Mahajan and Tuteja 2005). This effect is countered by the presence of ions such as K^+ and Ca^{2+}. Increased cytosolic Ca^{2+} concentration induced by salinity stress signals, salt tolerance or adaptive mechanisms in plants. Earthworm casts present in vermicompost are sources of essential ions such as K^+ and Ca^{2+}. The comparable calcium content to nitrogen content may have contributed to the increased cytosolic concentration in treated tomato seedlings; and could explain the higher salt tolerance in these samples. Calcium ions are known to stabilise cell membranes and improve nutrient uptake in plants under salinity stress, which suggests an induced salt tolerance mechanism. Previously hypersensitive conditions of some salt-stressed plants were corrected by adding Ca^{2+} (Liu and Zhu 1998).

Cellular metabolic processes induced by salinity stress, stimulate the synthesis of compounds such as proline which is involved in protecting and maintaining cell membranes and osmotic potential (Mahajan and Tuteja 2005). Proline accumulation in response to salt stress has previously been documented (Hernandez et al. 2000). However, there is a large ongoing debate on the role of proline in salt tolerance in plants (Chen et al. 2007). Under normal conditions the amino acid is synthesised from available glutamine in plant leaves. Osmotic adjustment is a key phase when referring to the function of osmolytes or compatible solutes such as proline. Compatible solutes also protect against oxidative stress. A positive correlation between proline content and salt tolerance in sugar beet was reported by Ghoulam et al. (2002). This was attributed to the osmotic adjustment and protective function of proline. The amino acid also served as a source of energy and nitrogen during salinity stress. Alia et al. (1997) postulated that proline functioned as a protective agent of thylakoids from photoinhibitory damage. Proline levels in treated tomato seedlings increased with increasing salinity stress. The accumulation of proline as a salt tolerance mechanism to achieve osmotic adjustment under increasing salinity has been reported for certain cultivars and varieties of *Perilla frutescens* (Zhang et al. 2012). The mechanism of accumulation of proline may be a co-ordinated interplay of increased synthesis and inhibited catabolism to effect stress tolerance (Lee and Liu 1999; Zhang et al. 2012). The amount of proline accumulation under salinity stress varies from species to species. Tissue-cultured tomato shoots showed significant increases in proline levels when incubated under high salinity (Aziz et al. 1999). The greatest difference in proline content between control and VCL-treated tomato seedlings was noted at 100 mM of NaCl (Fig. 2e). This shows that tomato seedlings treated with VCL were more tolerant to osmotic stress induced by increased salinity. The rate of proline biosynthesis and mobilisation is upregulated in plants which are less susceptible to osmotic stress (Nayyar and Walia 2003).

Increases in proline and total sugar content in hydroponically grown mangroves was recorded as salinity levels increased (100, 200 and 400 mM NaCl) (Parida et al. 2002). The functions of other compatible solutes such as glucose and sucrose include osmotic adjustment, carbon storage and radicle scavenging (Parida et al. 2002). The levels of total soluble sugar content were higher in all VCL-treated tomato seedlings. Fairly low NaCl concentrations can stimulate changes in total sugar content. This may be a result of conversion from starch to sugars or reduced use of stored starch in plant tissue. Total soluble sugar content generally increases in salt tolerant plant species (Zhang et al. 2012).

A noticeable decline in total chlorophyll content with an increase in salinity stress in both control and treated seedlings were observed (Fig. 2c). Interestingly, photosynthetic capacity of VCL-treated tomato seedlings was markedly improved with significantly higher total chlorophyll content than in corresponding control seedlings. At 100 mM NaCl, total chlorophyll content of VCL-treated seedlings was significantly better than the control (Fig. 2c). Decrease in total chlorophyll at higher concentrations of salt (200–400 mM) can be attributed to damage of chloroplasts and/or heightened chlorophyllase activity. Changes in the ratio between lipids and proteins in pigment-protein complexes may also contribute to the decrease (Iyengar and Reddy 1996). In this study, vermicompost leachate may have helped to alleviate damage to the chloroplasts and complexes by reducing chlorophyllase activity.

The role of microorganisms, present in vermicompost and vermicompost by-products such as VCL, in plant growth hormone production was extensively reviewed by Sinha et al. (2009). The authors cite substantial evidence to support the view that earthworms improve microbial populations in soils that show enhanced plant growth hormone (auxins, gibberellins, cytokinins and ethylene) content. The presence and function of plant hormones in salinity-induced plant stress was earlier described by Vaidyanathan et al. (1999). Plant hormones most commonly associated with stresses are ABA, cytokinins and ethylene. It would appear that ABA content in roots and leaves of salt-stressed plants is mediated by pH levels. The root-to-shoot communication as described by Blackman and Davies (1985) may explain how ABA can stimulate stomatal closure in response to water deficiencies in the soil as a result of salinity-stress. The growth limiting effects of NaCl on certain plants is essentially alleviated by ABA (Popova et al. 1995). Romero-Aranda et al. (2001) reported that in tomato plants stomatal density was reduced in response to increased salinity. Abscisic acid increases proline biosynthesis, which would have accounted for increase in proline levels in VCL-treated tomato seedlings. Similarly, trace amounts of growth promoting hormones such as auxin and gibberellic acid may have stimulated root and shoot growth in VCL-treated tomato seedlings. Results of this study indicate that salt stress tolerance mechanisms of tomato seedlings may have been physiologically stimulated with the addition of VCL.

Conclusions

Natural and anthropogenic-induced soil salinity stress in agricultural land poses a major threat to crop production. Constraints imposed by soil salinity on agricultural productivity are predicted to increase in coming years. Osmotic and oxidative stress as a result of soil-salinity stress has a greater effect on leaf growth than on root growth. Vermicompost leachate was a suitable soil amendment alternative as it improved root, shoot, stem and leaf growth and development under elevated saline conditions. Tomato seedlings treated with VCL were more tolerant to osmotic and oxidative stress due to the accumulation of compatible solutes such as proline and total soluble sugars and increased total chlorophyll content. Findings of this study show the potential of VCL for managing crops that are subjected to salt stresses.

Acknowledgments The University of KwaZulu-Natal is thanked for the award of Postdoctoral Fellowships to first two authors. We thank Mr Don Blacklaw of Wizzard Worms, Rietvlei, KwaZulu-Natal, South Africa, for providing vermicompost leachate analytical data.

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