Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 08 | Issue : 01 | Jan.-Mar. | 2019
Pages:126-138

Sequenced vermicompost, glycine betaine, proline treatments elevate salinity tolerance in potatoes

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Received: 10 Jan. 2018 / Accepted 20 Mar. 2018 / Publication date: 10 Feb. 2019

ABSTRACT

During its life cycle, plant has to cope with a number of abiotic stresses including salinity stress. Salinity influences growth and entire metabolism. Antioxidants have to enable plant to beat such stresses. Therefore, effects of vermicompost (Vc), proline (Pro) and glycine betaine (GB) applied singly or in a sequence on potato growth, physio-biochemical attributes and antioxidant defense system activity were investigated under irrigated water saline (IWS) stress in sandy soil in the arid desert of Sinai region, during the growing summer seasons of 2016 and 2017. Water commonly used for irrigation was 2.85 dS m^{-1}. Adding IWS to potato plants retarded plant growth of both haulms and tubers, while increase the polyphenol oxidase PPO and physiological disorders (greening, secondary growth and cracks). Exogenous application of VC, GB and Pro applied singly or in in a sequence improved plant growth (e.g., plant length, leaf area, shoot fresh weight and shoot dry weight), photosynthetic efficiency (SPAD chlorophyll), plant health (i.e., increased leaf stability index of membrane MSI and relative content in water RWC), antioxidant defense systems activity (enzymatic, catalase CAT, ascorbate peroxidase APX) and tuber yield, yield components, and quality (Dry matter DM, tarch and specific gravity SG) over the control. Application of the VC-GB-Pro under IWS, growth parameters and tuber yields were increased by 14 and 12% over the control. Sequenced VC-GB-Prowas the best treatment of which this study recommends to use and improve salt tolerance of potatoes by increasing growth, bio-constituents and antioxidant enzymatic activity followed by VC-GB treatment, for growing potatoes under IWS stress.

Keywords: potatoes, saline water, irrigation, vermicompost, osmoprotectants

Introduction

In many countries, especially those in the arid climate zone with high rates of population growth, urbanization and industrialization, water is becoming a scarce natural resource. The increasing population will put an adverse impact on the fresh water allocation for irrigated agriculture in the years to come because of the competitive pressure. Due to this, low or marginal quality water will be used for irrigation in those areas where fresh water is becoming scarce. According to FAO (1995), under arid conditions, even with the availability of good water, irrigation projects lead to salinity problems (Guptaand Abrol, 1990). According to the study made by Katerji *et al*., (2003), there are two groups in which plants are classified. The first one consists of salt tolerant species, which maintain or improve their water use efficiency under salt stressed condition and the second one consists of the plants that are salt sensitive in which water use efficiency decline with increasing soil salinity. Based on this study, potato crop can be categorized under the first group. Furrow irrigation is a conventional practice for potato production in Egypt. Patell *et al*., (2001) reported the effect of initial soil salinity and irrigation water salinity on potato tuber yield and size in the field. They observed a decrease in these parameters with increasing salinity. Leaf water and osmotic potentials in potato declined significantly as salinity conditions intensified, with more accumulation of proline in the leaves (Heuerand Nadler, 1998). The negative effects of salinity on potato tuber formation are a consequence of the reduction in osmotic potential due to increasing salt levels in cells of tuber tissues which also leads to a reduction of water content and nutrient uptake in tubers (Momoh *et al*., 2002). The reduction in yield can also be ascribed to structural deterioration and poor permeability of soils irrigated with poor quality saline water (Minhas and Gupta, 1992).Drip irrigation has the potential to increase/sustain crop yields with less quantity of irrigation water. However, in areas where salt

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content of groundwater is increasing progressively, drip irrigation is a better alternative, to obtain an optimized and higher yield along with better quality, over the conventional irrigation method (Hanson and May, 2011).

Potato plants face a number of environmental adversities termed as abiotic stress which includes soil salinity, water deficit, extremely high or low temperatures, toxic metals, waterlogging, elevated ozone, and ultraviolet radiation, which all create a barrier for proper growth, metabolism, and productivity of crop plants (Romero *et al*., 2017). Among the environmental stresses soil salinity is a widespread environmental problem that has been found to affect more than 77 million hectares or 5% of the cultivable land of the universe (Athar and Ashraf, 2009).

It is an urgent task of plant biologists to explore suitable mechanisms of developing salt tolerant crop plants that can produce sufficient yield under adverse condition. In recent decades, many researchers have been trying to find the ways to alleviate salt stress or to overcome salt injury in plants. Among them exogenous application of substances such as osmoprotectants, phytohormones, antioxidants, and trace elements came to attention in recent times (Hasanuzzaman *et al*., 2012; Noreen *et al*., 2013). Moreover, an intelligent and selective use of organic amendments like vermicompost in reclaimed sandy soils in this study, have proved effective in soil conditioning values and varying degrees of influence on soil properties. Organic amendments like vermicompost promote humification, increased microbial activity and enzyme production, which, in turn, increase the aggregate stability of soil particles, resulting in better aeration (Ansari, 2008). Organic matter has a property of binding mineral particles like calcium, magnesium and potassium in the form of colloids of humus and clay, facilitating stable aggregates of soil particles for desired porosity to sustain plant growth. Soil microbial biomass and enzyme activity are important indicators of soil improvement as a result of addition of organic matter (Ansari, 2008). Apart from these, earthworm castings are reported to contain plant growth promoters, such as auxins and cytokinins (Krishnamoorthy and Vajranabhaiah, 1986).Vermicompost is described as an excellent soil amendment and a biocontrol agent which make it the best organic fertilizer and more eco-friendly as compared to chemical fertilizers. Vermicompost is ideal organic manure for better growth and yield of many plants. It can increase the production of crops and prevent them from harmful pests without polluting the environment (Joshi *et al*., 2014).The use of vermicompost as a biological fertilizer under salinity stress conditions was studied by Akhzari *et al*. (2016). They found that application of vermicompost increased significantly of leaf relative water content, total chlorophyll content, leaf area and mineral content of NPK in *Medicago rigidula*.

In response to various environmental stresses, plants demonstrate a variety of adaptive mechanisms to counteract them. Since one of the primary responses under salt stress is osmotic adjustment, compatible solutes such as proline (Pro) and glycine betaine (GB) are very common to be accumulated during salt stress and play a fundamental role in osmotic adjustment in plants (Szabados and Savouré, 2010). These compatible solutes are accumulated in the cytosol without disturbing intracellular biochemistry, which ameliorate the detrimental effects of salinity (Ashraf and Foolad, 2007; Patade *et al*., 2014).

In many plant species increased accumulation of Pro and GB were observed as an indicator of salt stress tolerance (Ashraf and Harris, 2004; Ashraf and Foolad, 2007). However, most of the plants, especially under elevated levels of salt, cannot synthesize sufficient amount of these osmoregulators. In many recent reports exogenous applications of Pro and GB were found to act as protectants under salt stress (Raza *et al*., 2006; Patade *et al*., 2014). Besides osmoprotection, Pro and GB also showed their roles in elimination of oxidative stress by triggering the antioxidant defense and also glyoxalase system (Hoque *et al*., 2007; Banu *et al*., 2010; Patade *et al*., 2014).

Since, little information is available about the yield and quality of tubers under drip irrigation using saline water and application of vermicompost under these conditions. Although there are several reports on the role of Pro and GB in salt stress tolerance in terms of growth and physiology, few investigations have been done on the effects of exogenous Pro and GB on both antioxidant defense in potatoes. Enough literature is not available on quality parameters of the grade of potatoes as influenced by saline water or vermicompost application.

Therefore, we investigated the protective effects of these osmoprotectants on the growth, yield, quality and antioxidant defense in potatoes grown under irrigation with saline water.

Materials and Methods

Material of seed tubers and conditions of growing

Imported potato half seed tubers (weighing 30-40 g each), Spunta cultivar "Elite E" were cultivated at a private farm near New Ismailia, Sinai region, Ismailia Governorate during summer seasons of 2016 and 2017.Potato was hand planted on sandy soil with low organic matter content (<0.06%)with ECe2.4 dS m^{-1}. Some physical and chemical properties of the experimental soil at the depth of 0-30 cm were determined according to the standard procedures as described by Page (1982) (Table 1).The rainfall received during the cropping periods ranged from 27-32 mm. The total soil water, calculated between field capacity and witling point for an assumed potato root extracting depth of 0.60 m, was 75 mm. During the period from January to April, the mean daily maximum temperature was18°C to 32°C. Minimum temperatures average in night ranged to around4°C and 10°C.

Table 1: Some physical and chemical properties of the experimental soil.

Some Physical properties	Values		Some Chemical Properties	Values	
	1st season	2nd season		1st season	2nd season
Sand (%)	91.4	91.1	Wilting point (cm^3/cm^3)	0.05	0.05
Silt (%)	4.6	4.7	Available point (cm^3/cm^3)	0.15	0.16
Clay (%)	4.0	4.2	pH value	7.6	7.5
$CaCO_3$ (%)	0.45	0.50	EC dSm^{-1}	2.2	2.1
Organic matter (%)	0.04	0.05	Total N (%)	0.13	0.12
Saturation water content (cm^3/cm^3)	0.38	0.40	Available P (ppm)	0.89	12.0
Field capacity (cm^3/cm^3)	0.21	0.22	Available K (ppm)	44	40

Planting took place on 14 and 9January, for both seasons, respectively, in 75 cm rows with tubers spaced 25 cm apart, in a randomized complete block design with three replicates and eight treatments. The experimental area (22.5 m^2) consisted of 3 ridges with 10 m long. All plots were drip irrigated with saline water (ECw2.85 dS m^{-1}). Chemical characteristics of the used irrigation water were determined according to Jackson (1967) and outlined in Table 2.Each dripper had a 4 L h^{-1} flow rate. Water for each block passed through a water meter, gate valve, before passing through laterals placed in every potato row.

Table 2: Chemical characteristics of irrigation water used in the experiment.

Seasons	EC (dSm^{-1})	pH	Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})				SAR
			Na^+	Ca^{++}	Mg^{++}	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	
1st	2.90	7.55	24.80	6.88	7.88	0.45	0.00	4.00	20.35	15.66	1.3
2nd	2.82	7.40	23.90	6.40	8.00	0.40	0.00	4.20	21.56	12.94	1.4

Treatments and crop management

Vermicompost VC: Cultures of *Pheretima californica* and *Pheretima elongata* (Annelida: Oligochaeta) species of Egyptian earthworms were set up in cement tanks of size 2 m x 1mx 1m (pH 9.5-10) and allowed to stabilize in sodic soilby the use of paddy straw and cattle dung at regular interval of 7 days over the soil bed and were used later in the field experiments. Vermicompost was produced by using the process of vermicomposting with the above cultures. Vermicompost was harvested every 45 days at the rate of 350 kg per tank. Vermicompost was added at a rate of 8.9 kg/plot (1.67 ton Fed.$^{-1}$). Vermicompost was placed in hills before planting seed tubers directly.The chemical analysis of the used vermicompost was determined using standard methods described by AOAC (1990) (Table 3).

Table 3: Chemical characteristics of vermicompost used in the experiment.

Seasons	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sodium (ppm)	Organic carbon (%)
1st	3.90	3.86	4.66	2.75	2.68	89.7	81.3
2nd	4.08	3.42	4.90	2.40	2.00	85.4	81.4

Glycine betaine (GB*)*: The GB (Sigma-Aldrich, 98%) perchloric acid titration, (carboxymethyl trimethyl ammonium inner salt, Oxyneurine, $C_5H_{11}NO_2$, MW: 117.15,was purchased from Sigma Aldrich Co., and used for the present study. Glycine betaine was applied at a rate of 25 mM. GB was applied manually over the top of the plant covering all over the plant's leaves, by using Pressurized Spray Bottle with 0.1%Tween 20as surface spreader and applied for three times on the plants at 30, 45, and 60 DAP.

Proline (Pro.): The Pro (Pyrrolidine-2-carboxylic acid), $C_5H_9NO_2$, MW: 115.13 g·mol^{-1},was purchased from Sigma Aldrich Co. Proline was applied at a rate of 5 mM with 0.1%Tween 20and applied for three times in conjunction with spraying with GB.

Treatments were used in a sequence; firstly in VC, then in GB, and finally in Pro. A sequence of VC-GB-Pro and their concentrations were selected due to that they conferred the best response according to our data (not shown) of preliminary studies.

Fertilizers were supplied for the cropping seasons in the same amounts. Nutrient supply included N, P and K at rates of 200, 75 and 96 kg Fed^{-1}, respectively, which were adopted from the local practices. All NPK fertilizers were injected directly into the irrigation water using venture-tube into the main line of drip systems at 6 days intervals in water soluble form. Fertigation was started two weeks after sowing and was stopped 30 days prior to the end of the crop period. Nitrogen was divided and delivered with the irrigation water in all treatments during early vegetative growth. After tubers initiation stage, 25 kg Fed^{-1}of potassium nitrate was applied. Calcium (Calcium nitrate) at 140 mg L-1 and magnesium (magnesium sulphate) at 24 mg L^{-1}, Fe (Fe-EDTA) at 5 mg L^{-1}, Zn (Zn-EDTA) at 0.8 mg L^{-1}, Mn (Mn-EDTA) at 0.5 mg L^{-1} were done by preparing a nutrient solution contains these nutrients for potato. The nutrients were supplied with irrigation water (fertigation system).

For irrigation scheduling, the method used was the water balance, by means of a spreadsheet program for Excel, developed according to the methodology formulated by Allen *et al.* (1998). The spreadsheet program estimates the day when the target soil water depletion (readily available water, RAW) for the treatment 100 L would be reached and the amount of irrigation water needed to replenish the soil profile to field capacity. The program calculates the soil water depletion on daily basis using the soil water balance and projects the next irrigation event based on the target depletion (35% of total available water in the root zone, 35% of TAW).

Measurements of growth attributes and yield parameters

Seventy DAP, plants (n = 9) were randomly chosen from each treatment and plant height, leaf area, shoot FW and shoot DW were recorded. At harvest time, 105 days after planting, the total tuber yield/feddan (ton) was recorded. Marketable yield/feddan was recorded using good shapes healthy tubers (30: 60 mm or more).Unmarketable yield was determined using off shape, rotten and less than 30 mm in diameter.

Assessment of chlorophyll content (Chl.), relative content of water (RWC) and stability index of membrane (MSI)

Chlorophyll content of the top third and fourth leaves were measured using a chlorophyll meter (SPAD-502, Minolta, Japan).

Excluding the midrib, twenty discs (2 cm-diameters) of a fresh fully expanded leaf were used to assess RWC as detailed in Osman and Rady (2014) method. Once fresh discs weighed (FW) were saturated by floating on double distilled water in dark for 24 h. Adhering water drops were gently dried by placing discs on filter paper and a turgid weight (TW) was taken. Thereafter, discs were dehydrated at 70°C for 48 h to assess a dry weight (DW), and then the following formula was applied:

$$\text{RWC\%} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

Excluding the midrib, Premachandra *et al.* (1990) method modified by Rady (2011) was used to assess the MSI with duplicate 0.2 g fully expanded leaf. A sample (0.2 g) was placed in 10 ml of double-distilled water in a test-tube and heated using a water bath at 40°C for 30 min, and then a solution electrical conductivity (EC_1) was recorded. The second sample (0.2 g) was boiled at 100 °C for 10 min, and a solution electrical conductivity (EC_2) was taken, and then the following formula was applied:

$$\text{MSI\%} = [1 - (EC_1 / EC_2)] \times 100$$

Determinations of physiological disorders and tuber quality

Physiological disorders (greening, secondary growth and cracks) were assessed qualitatively at harvest; all tubers per a replicate were recorded and converted into percent attributed to total tuber yield. Percentage of tuber dry matter (calculated by drying 100 grams of fresh tubers in oven at 70° C tills a constant weight). Starch content was determined in tubers according the method described by AOAC (2000). Specific gravity (SG) is the weight of the tuber compared to the weight of the same volume of water. It is a measurement of density and can be calculated as follows:

SG = (Weight of tubers in air) / [(weight in air) - (weight in water)] (AOAC,2000).

Enzymatic antioxidants assays

Catalase (CAT) activity was assayed according to the method of Garcìa-Limones *et al.* (2002), with minor modifications. The reaction medium contained 150 µL of 500 mM potassium phosphate buffer (pH 7), 340 µL of 88 mM H_2O_2 and 200 µL of crude enzyme extract in a final volume of 1.5 mL. The reaction was initiated after the addition of H_2O_2, and the decrease in absorbance of peroxide was measured at 240 nm. The specific activity was expressed as µmol H_2O_2 /min/mg protein. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to Garcìa-Limones *et al.* (2002), with some modifications. The reaction mixture consisted of 300 µL of 500 mM potassium phosphate buffer (pH 7), 100 µL of 5 mM ascorbic acid, 6 µL of 88 mM H_2O_2, 100 µL of 10 mM sodium EDTA (pH 7) and 100 µL of crude enzyme extract in a final volume of 1.5 mL. The reaction was initiated after the addition of H_2O_2, and the oxidation of ascorbic acid was monitored at 290 nm. The specific activity was expressed as µmol ascorbate/min/mg protein. Polyphenoloxidase (PPO) activity was determined according to the method of Cao *et al.* (2009), with some modifications. A total of 2.5 g of fruit was homogenized in 5 mL of 200 mM sodium phosphate buffer (pH 6.5) containing 5% (w/w) PVPP. After incubation on ice for 1 h, the homogenate was centrifuged at 12,500× g for 10 min at 4 °C. The crude enzyme extract (100 µL) was subsequently incubated with 1.4 mL of 500 mM catechol in a final volume of 1.5 mL, and the increase in absorbance at 398 nm was monitored. The specific activity was expressed as µmol catechol/min/mg protein.

Statistical analysis

Data were analyzed using analysis of variance technique and the differences between individual pairs of treatment means were compared using Duncan Multiple Range Test at 5% according to Snedecor and Cochran (1989).

Results

Vegetative growth

Potato treating with VC, GB and Pro singly or in a sequence produced better healthy plants with significant improvements of all vegetative growth parameters (plant length, foliage F.W. and D.W. and leaf area) compared to the control. However, the sequenced application of VC-GB-Pro was the best treatment among all treatments including the control. The sequenced application of VC-GB-Pro significantly increased potato growth characteristics (by 14–35%, average two seasons) compared to the control (Table 4). The treatment of VC-GB occupied the second order increasing growth characteristics by 8 – 30% compared to the control.

Chlorophyll content and tissue health

Relative content of water in terms of RWC, SPAD chlorophyll and leaf MSI were significantly increased by the sequenced application of VC-GB-Pro (Table5). The increases were 12% for SPAD chlorophyll, 10% for RWC chlorophyll and 9% for leaf MSI compared to those of the control. They also increased by 12%, 7% and 7%, respectively by VC-GB treatment compared to the control.

Table 4: Effect of certain organic stimulators applied singly or in a sequence on vegetative growth characters of potato irrigated with saline water in 2016 and 2017 seasons.

Treatments:	**Plant length (cm)**		**Foliage F.W. /plant (g)**		**Foliage D.W. /plant (g)**		**Leaf area/plant (m^2)**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
1. Control (tap water)	41.00	38.33	289.22	280.56	32.78	32.10	3580	3420
2. Vermicompost (VC)	43.67	40.33	310.20	310.64	38.26	36.76	4272	4220
3. Glycine betaine (GB)	42.33	40.00	300.80	301.78	35.12	34.14	4320	4175
4. Proline (Pro.)	42.67	46.33	295.36	298.18	34.10	33.78	4215	4276
5.VC-GB	45.33	45.00	324.28	342.50	40.58	42.18	5050	5128
6. VC-Pro	45.00	44.00	302.45	320.08	36.18	38.36	4640	4840
7. Pro-GB	42.33	42.67	320.02	326.18	40.02	40.33	4528	4730
8. VC-GB-Pro	47.67	46.67	342.50	338.32	42.50	41.80	5438	5433
LSD 5%	2.02	2.34	18.18	20.02	2.15	2.18	260	308

Table 5: Effect of certain organic stimulators applied singly or in a sequence on chlorophyll content and tissue health of potato irrigated with saline water in 2016 and 2017 seasons.

Treatments	**Chlorophyll content (SPAD)**		**Relative content of water RWC (%)**		**Stability index of membrane MSI (%)**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
1. Control (tap water)	42.2	42.4	84.3	84.0	73.1	73.2
2. Vermicompost (VC)	45.6	45.8	87.4	87.4	77.0	77.4
3. Glycine betaine (GB)	45.0	45.2	87.0	86.9	75.4	75.6
4. Proline (Pro.)	44.2	44.4	86.8	86.2	75.0	75.4
5.VC-GB	47.6	48.0	90.7	90.8	78.1	78.8
6. VC-Pro	47.0	47.3	90.5	90.1	77.9	78.4
7. Pro-GB	46.1	46.5	88.4	88.2	77.2	78.2
8. VC-GB-Pro	48.1	48.4	93.4	92.8	78.4	80.6
LSD 5%	1.9	1.8	2.9	3.1	6.2	6.8

Total tuber yield, marketable and unmarketable yield

Total tuber yield, marketable and unmarketable yields were significantly affected by all treatment in both seasons (Table 6). Sequenced application of VC-GB-Prohad a positive effect on total tuber and marketable yields compared to the control (Table 6). Highest tuber yield (14.680 and 14.280 ton fed^{-1}, in both seasons, respectively) was obtained under the treatment received VC-GB-Pro. The second treatment regarding the increase in tuber yield (14.380 and 14.150 ton fed^{-1}) was VC-GB without significant differences between 1st and 2nd treatments, respectively. The percentage increases over the control reached to 13, 11% and 11, 10 % for both superiority treatments, in both seasons, respectively. On the other hand, the lowest tuber yield (12.860 and 12.700 ton fed^{-1}) was recorded under the check treatment, in both seasons.

Table 6: Effect of certain organic stimulators applied singly or in a sequence on total yield, marketable and unmarketable of potato irrigated with saline water in 2016 and 2017 seasons.

Treatments	**Total tuber yield (Ton/fed.)**		**Marketable yield (Ton/fed.)**		**Unmarketable yield (Ton/fed.)**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
1. Control (tap water)	12.860	12.700	12.000	11.880	0.860	0.920
2. Vermicompost (VC)	14.120	13.185	13.540	12.485	0.580	0.700
3. Glycine betaine (GB)	12.900	12.980	12.380	12.450	0.520	0.530
4. Proline (Pro.)	12.880	12.800	12.400	12.300	0.480	0.500
5.VC-GB	14.380	14.150	13.850	13.600	0.530	0.550
6. VC-Pro	12.920	12.880	12.120	12.000	0.800	0.880
7. Pro-GB	13.410	13.330	12.730	12.680	0.680	0.650
8. VC-GB-Pro	14.680	14.280	14.280	13.760	0.400	0.520
LSD 5%	0.320	0.380	0.310	0.670	0.152	0.198

Regarding, the marketable tuber yield (Table 6), it took the manner of total tuber yield as previously mentioned. Science, total yield increases were due to primarily the increase in tuber size in larger and medium grades (marketable) and decrease of the small grades (unmarketable). In this respect, the sequenced treatment of VC-GB-Pro and VC-GB decreased significantly unmarketable yield in both seasons.

Physiological disorders

There were slightly significant differences in physiological disorders of potato tuber parameters, in both seasons (Table 7). Application of VC-GB-Pro in sequence significantly decreased secondary growth (0.450 and 0.600 kg plot^{-1}, in both season, respectively) and cracks (0.000 kg plot^{-1}, 1st season), in comparison with other treatments. Pro-GB also had a significant decrease in secondary growth in 1st season only. However, there were no significant differences among all tested treatments on tuber greening or cracks in 2nd season of this study.

Table 7: Effect of certain organic stimulators applied singly or in a sequence on physiological disorders of potato irrigated with saline water in 2016 and 2017 seasons.

Treatments	Greening (Kg/plot)		Secondary growth (Kg/plot)		Cracks (Kg/plot)	
	2016	2017	2016	2017	2016	2017
1. Control (tap water)	0.580	0.570	1.100	1.200	0.470	0.530
2. Vermicompost (VC)	0.410	0.600	0.590	0.680	0.450	0.450
3. Glycine betaine (GB)	0.380	0.560	0.820	0.600	0.000	0.440
4. Proline (Pro.)	0.420	0.600	0.740	0.730	0.000	0.380
5.VC-GB	0.350	0.480	1.000	0.950	0.000	0.540
6. VC-Pro	0.490	0.450	0.900	1.150	0.610	0.600
7. Pro-GB	1.000	0.440	0.480	1.050	0.250	0.335
8. VC-GB-Pro	0.520	0.580	0.450	0.600	0.000	0.300
LSD 5%	0.420	NS	0.440	0.520	0.380	NS

Dry matter, Starch and Specific gravity

All of bio-osmoregulators gave higher bio-constituent of Spunta tubers, compared with those of untreated tubers in average the two seasons (Table 8). Application of VC-GB-Pro to Spunta plants gave the highest significant dry matter (both seasons), starch content in the tubers (1st season) and specific gravity (1st season).These constituents preserved stored tubers, keeping the internal biochemical enzymatic activities in minimum levels. The increases were 11% for tuber DM, 19% for starch content and 2% for specific gravity compared to those of the control. They also increased by 11%, 17% and 2%, respectively by VC-GB treatment compared to the control.

Table 8: Effect of certain organic stimulators applied singly or in a sequence on quality of potato tubers irrigated with saline water in 2016 and 2017 seasons.

Treatments	Dry matter (%)		Starch (%)		Specific gravity	
	2016	2017	2016	2017	2016	2017
1. Control (tap water)	19.432	19.640	12.90	14.75	1.0780	1.0818
2. Vermicompost (VC)	20.841	20.200	14.65	14.80	1.0885	1.0898
3. Glycine betaine (GB)	21.185	21.280	14.70	14.73	1.0894	1.0823
4. Proline (Pro.)	21.820	20.840	14.40	14.82	1.0855	1.0830
5.VC-GB	22.434	21.184	15.63	15.10	1.0992	1.0833
6. VC-Pro	21.400	21.190	15.24	15.00	1.0962	1.0842
7. Pro-GB	21.650	21.635	15.14	14.93	1.0918	1.0815
8. VC-GB-Pro	21.850	21.374	15.92	15.03	1.0989	1.0905
LSD 5%	0.263	0.550	0.02	NS	0.0045	NS

Catalase (CAT), Ascorbate peroxidase (APX) and Polyphenol oxidase (PPO)

Catalase CAT, APX and PPO are physiological parameter reflecting the health condition of plant. Data of physiological profile of potato tubers as affected by the tested treatments are presented in Table9.Enzymatic activity of CAT and APX was significantly enhanced by the sequenced application of VC-GB-Pro (Table 9). The increases were 50% and 76% respectively compared to the control (average two seasons). In this regards, the second and third treatments were VC-GB and VC-Pro, respectively. In contrast, PPO was significantly reduced by the sequenced application of VC-GB-Pro (Table 9). The reduction was 48% compared to the control. The treatment of VC-GB occupied the second order reducing PPO by 38% compared to the control.

Table 9: Effect of certain organic stimulators applied singly or in a sequence on enzymatic antioxidant systems of potato tubers irrigated with saline water in 2016 and 2017 seasons

Treatments	**Catalase** (µ mol H_2O_2/min./mg Protein)		**Ascorbateperoxidase** (Ascorbate/min./mg Protein)		**Polyphenol oxidase** (µ mol Catechol/min./mg Protein)	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
1. Control (tap water)	0.30	0.28	0.10	0.08	0.42	0.46
2. Vermicompost (VC)	0.50	0.48	0.28	0.28	0.30	0.30
3. Glycine betaine (GB)	0.44	0.44	0.20	0.18	0.34	0.38
4. Proline (Pro.)	0.38	0.36	0.14	0.13	0.36	0.38
5.VC-GB	0.55	0.52	0.38	0.36	0.26	0.26
6. VC-Pro	0.54	0.50	0.33	0.32	0.28	0.30
7. Pro-GB	0.48	0.44	0.24	0.22	0.32	0.34
8. VC-GB-Pro	0.60	0.55	0.42	0.40	0.22	0.24
LSD 5%	0.04	0.05	0.04	0.03	0.04	0.03

Discussion

Dry land cultivation of potato (*Solanum tuberosum* L.) is expanding worldwide (FAO, 1995). Saline water is an important resource in arid areas and areas with poor quality groundwater resources. Use of poor quality water poses serious loss in yield and plant growth. Potato is one of the most important crop which can tolerate salinity of irrigation water up to a threshold value of 2.5 dSm^{-1}. In such areas drip irrigation proves to be a boon for tuber formation. In traditional furrow irrigation method tuber numbers and yield widely gets affected due to osmotic effects by the salts in the rooting zone. Drip irrigation forms a wetting front that reduces the salinity around the root and hence optimizing the conditions suitable for growth (Jha, 2016).

From our results obtained under irrigation with saline water, it has been observed that osmoprotectants or antioxidants applications (i.e., vermicompost VC, glycine betaine GB and proline Pro, applied singly or in a sequence) had positive effects that were more pronounced under IWS stress condition. The pronounced positive effects of the sequenced application of VC-GB-Prounder IWS stress conditions explain the significant differences in growth characteristics of potato plants (Table 4). SPAD chlorophyll content, RWC and MSI values were coincided with plant growth values (Table 5). However, all total tuber yield parameters and marketable tuber yield under IWS (Table6) were significantly lower, while the activity of PPO was significantly higher (Table 9).The RWC, MSI and chlorophyll SPAD content in potato plants pretreated with sequenced antioxidants under IWS stress showed also significant improvements. These improvements in RWC, MSI and chlorophyll were occurred due to membrane integrity maintenance under IWS stress and prevention of plasma membrane damage (Zouari *et al*., 2016). This may be explained on the basis of the role of antioxidants as a main mechanism in alleviating the salinity stress effects on plants (Murmu *et al*., 2017). In addition, Salinity affects almost every aspect of the physiology and biochemistry of plants and leads to water deficit, and causes ion imbalance of the cellular ions resulting in osmotic stress and ion toxicity which, significantly reduces membrane permeability, plant growth and yield (Tuteja *et al*., 2012).

Exogenous VC, GB and Pro, applied singly or in a sequence, enhanced the activities of ROS scavenging and metal ion chelating, forming a crucial part of abiotic stress responses in plant cells. Generally, the sequenced VC-GB-Pro application generated better results followed by VC-GB application, ameliorating the deleterious effects of IWS stress. Exogenously-applied antioxidants have been observed to ameliorate the injurious effects of salinity on plant growth and metabolic processes (Rasheed *et al*., 2014; Rady and Hemida, 2016).

The better finding of the sequenced VC-GB-Pro application than their single applications may be attributed to the integrative mode of actions of these organic stimulators and antioxidants. The beneficial effect of vermicompost on growth and yield characteristics may be due to improving the soil structure conditions, which encouraged the plant to have a good root development by improving soil water holding capacity and this permitted favorable plant supply with water and nutrients which in turn, increases the amount of plant biomass produced (Ansari, 2008; Joshi *et al*., 2014). Vermicompost is ideal organic manure for better growth and yield of potato plants. It can increase the production of crops and prevent them from harmful adverse impact without polluting the environment. Application of vermicompost increased seed germination, stem height, number of leaves, leaf area, leaf dry weight, root length, root number, total yield, number of tubers/plant, chlorophyll content, pH of juice, TSS of juice, micro and macro nutrients, carbohydrate and protein content and improved the quality of the tubers. VC contains of humic acids, plant growth promoting bacteria and vermicomposts can be used for a sustainable agriculture discouraging the use of chemical fertilizers (Joshi *et al*., 2014; Akbasova *et al*., 2015). Vermicompost is ideal organic manure for better growth, yield and quality of many plants due to following reasons: Vermicompost has higher nutritional value than traditional composts. This is due to increased rate of mineralization and degree of humification by the action of earthworms (Azarmi *et al*., 2009). Vermicompost has high porosity, aeration, drainage, and water-holding capacity (Jak and Thies, 2006). Presence of microbiota particularly fungi, bacteria and actinomycetes makes it suitable for plant growth (Tomati *et al*.,1987). Nutrients such as nitrates, phosphates, and exchangeable calcium and soluble potassium in plant-available forms are present in vermicompost (Orozco *et al*., 1996). Plant growth regulators and other plant growth influencing materials produced by microorganisms are also present in vermicompost (Tomati *et al*., 1988). Production of cytokinins and auxins was found in organic wastes that were processed by earthworms (Krishnamoorthy and Vajrabhiah, 1986). Earthworms release certain metabolites, such as vitamin B, vitamin D and similar substances into the soil (Akbasova *et al*., 2015). In addition to increased N availability, C, P, K, Ca and Mg availability in the casts are found (Orozco *et al*. 1996). Our results in Tables5, 6, 8in the came in this context and confirmed with previous trends.

Glycine betaine and prolineare of the important antioxidants in the antioxidative defense system in plants. It reacts with many ROS such as H_2O_2, $O_2^{-\bullet}$, OH^- and liquid hydro peroxidases. It also involved in several types of biological activities in plants (e.g., an enzyme co-factor, an antioxidant and a donor/acceptor in electron transport either at plasma membrane or in chloroplasts), which are related to tolerance to oxidative stress (Rady and Hemida, 2016; Murmu *et al*., 2017). In integration with the role of GB, Pro is a common physiological response established in many plants grown in various abiotic stresses, and thus it has been suggested to serve as a tolerance index to stress (Dar *et al*., 2016).

It mitigates salinity stress damage by detoxifying the ROS and increasing the contents of GB and Pro along with the increased activities of catalase; CAT and APX (Table 9), all of these by acting at the transcription level and/or translation and the endogenous level of Pro (Murmu *et al*., 2017). Additionally, it supplies energy for plant growth and survival, helping it to tolerate stress. It also protects plants from salinity-induced degradation of membranes and leakage of electrolytes (Kavi Kishor *et al*., 2005), and improves leaf water potential (Table5) by protecting cell membranes from metal-induced oxidative damage (Okuma *et al*., 2004).

The positive influence of these materials may be due proline and glycine betaine has antioxidant and neurotrophic effects (Murmu *et al*., 2017). As a result of climate changes in recent times and that led to the high temperatures, which could cause stresses developing plants in the summer season lead to the develop of physiological disorders (Jovovic *et al*., 2016). Salinity leads to physiological changes in tubers, especially osmotic and oxidative stress and may be reelecting on tuber off-shape (Gao *et al*., 2014). The accumulation of osmoprotectants is important for plant to adapt to osmotic stress (Murmu *et al*., 2017). Proline, an important compatible osmolyte in plants, could maintain cell turgor and function in osmotic adjustment to improve plant tolerance to osmotic stress (Huang *et al*., 2013). It has been demonstrated, through studies of both plant physiology and genetics, that the level of accumulated GB is correlated with the degree of salt tolerance (Rhodes et al., 1989; Saneoka *et al.,* 1995). Moreover, an exogenous supply of GB also increases the salt tolerance of some plants that are otherwise unable to accumulate GB (Murmu *et al*., 2017). To obtain direct proof that the accumulation of GB in vivo enhances the ability of plants to tolerate high concentrations of salt, studies were made of the physiological consequences of the transgenic engineering of GB synthesis via overexpression of two choline-oxidizing enzymes, namely COD and CDH (Hayashi *et al*., 1998; Holmström *et al*., 2000). Transgenic Arabidopsis plants that produced COD in their chloroplasts not only acquired resistance to high concentrations of NaCl during germination but also were able to tolerate high levels of salt during the subsequent growth of seedlings and mature plants (Hayashi *et al*., 1998). The addition of bio-osmoregulators such as glycine betaine and proline which contains the anti-oxidant reduces free radicals (O_2^-, OH^- and ROO^-) and other harmful compounds, thereby reducing the physiological disorders (Table 7; Akpinar-Bayizit *et al*., 2016). Oxidative stress generated by free radicals can disturb DNA, proteins and lipids. Antioxidants inhibit or restrain the oxidation of molecules in the cells and are considered to confer positive effects against physiological disorders.

Phenolic compounds are the most abundant antioxidants in potatoes, and their levels in tubers are affected by growing (Reyes *et al*., 2004).The poly phenol oxidase PPO was significantly induced in the tubers of control plants compared with other treatments (Table 9). PPO is activated in case of control treatment due to the oxidative stress occurred due to salinity. Although high PPO is associated with high potential of stresses (Külen *et al*., 2013), they also have positive benefits such as enhancing the antioxidant capacity of plant tissue, mainly related to its role of eliminating reactive oxygen species (ROS) and free radicals (Akpinar-Bayizit *et al*., 2016).

Conclusion

Exogenous VC (soil amendment), GB and Pro (foliar spray) applied singly or in a sequence to potatoes, improved plant growth, photosynthetic activity, antioxidant defense system and plant health assessed as increased RWC and MSI and decreased PPO activity under 2.5 dS m^{-1} stresscondition. The improving effect of sequenced VC+GB+Pro treatment was more pronounced under IWS stress followed by VC+GB treatment than other single applications of VC,GB and proline, therefore increasing the tolerance of potato plants to saline water stress and enhancing their growth and health. The sequenced VC-GB-Pro was more effective treatment followed by VC-GB treatment than other single; VC, GB and proline ones.

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