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RESEARCH ARTICLE

Endophytic bacteria (*Sphingomonas* sp. LK11) and gibberellin can improve *Solanum lycopersicum* growth and oxidative stress under salinity

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This study aims to understand the effects of salinity on the growth and oxidative stress enzymes of endophytic bacteria (*Sphingomonas* sp. LK11) and tomato plants. In response to salinity and gibberellic acid (GA_4), catalase (CAT), superoxide dismutase, and reduced glutathione were significantly regulated in LK11 as compared to peroxidase (POD) and polyphenol oxidase (PPO). Salinity stress to tomato plants caused significant cessation in growth and biomass, which was accompanied by threefold increase in lipid peroxidation and decrease in glutathione, CAT, POD, and PPO activities. In contrast, sole and combined treatment of LK11 and GA_4 rescued plant growth and biomass production whilst exhibited lower lipid peroxidation and higher glutathione content under salinity stress. The activities of CAT, POD, and PPO were either lower or nonsignificant as compared to control. In conclusion, inoculation of bacterial endophytes offers a relative stress counteracting potentials as evidenced by the known plant growth regulators.

Keywords: endophytic bacteria; gibberellins; salinity stress; antioxidant enzymes

Introduction

Salinity has often known as a limiting factor for effecting crop growth and development. Exposure to salinity stress causes increase in water stress, ionic influx, oxidant imbalance, membrane disintegration, cell division impairment, and fruit development (Flowers 2004; Gill & Tuteja 2010; Shabala & Munns 2012). Plants respond to such stress conditions by transducing signaling pathways, which enable them to initiate defensive metabolism (Khan et al. 2012). This strategy includes the changes in the activities of ionic/osmotic carriers, stomatal closure, stress hormones, and secondary metabolites. Salinity induces ionic imbalances in cell and causes the production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, hydroxyl radicals, etc., which can suppress the cellular functions (Manai & Gouia 2014).

To counteract ROS production, plant recruits various antioxidants (like reduced glutathione) and enzymes (such as superoxide dismutase – SOD, peroxidase – POD, catalase – CAT, polyphenol oxidase – PPO, etc.), which buffer the cellular ionic efflux to minimize cellular toxicity (Mittler 2002; Türkan & Demiral 2009). Under stress conditions, superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) production are higher in plants, and self-defense mechanisms such as SOD and CAT are activated to prevent oxidative damage (Vranova et al. 2002). SOD converts O_2^- content to H_2O_2 and water, whereas CAT dismutates H_2O_2 to water and oxygen (Vranova et al. 1997; Mittler 2002). PPOs (EC 1.14.18.1) are involved in the oxidation of polyphenols into quinones using molecular oxygen as an electron acceptor, which can act as essential defense response arsenal. PODs (EC 1.11.1.7), on the other hand, oxido-reductive enzymes that participate in the wall-building processes such as oxidation of phenols, suberization, and lignification of host plant cells during the defense reaction against biotic and abiotic stresses (Mohammadi & Kazemi 2002). Similarly, glutathione involved in reducing H_2O_2 by donating electrons to encounter the stress-induced ROS attack.

The stress stimulates changes in plant growth activities, which drastically hinder crop yield. It is estimated that 45 million hectares of irrigated land have been damaged by salinity whilst approximately 1.5 million hectares are losing fertility for crop cultivation (Munns & Tester 2008). Though there are some physical and chemical ways to remediate arable land effects with salinity, whilst cultivation of varieties developed through conventional breading and transgenic approaches can also help in sustainable production. However, assessment and application of rhizospheric microbes can be more eco-friendly.

Up till now there are numerous studies performed on using plant growth promoting rhizobacteria (PGPRs) for improving the adverse effects of salinity on crops as shown by Mayak et al. (2004), Kang et al. (2014), Bashan et al. (2014), Egamberdieva and Lugtenberg (2014), etc. However, very few plant growth promoting endophytic bacteria (PGPEB) are known to confer ameliorative impacts during salinity. PGPEB are

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colonizing the healthy plant tissues without causing any symptoms of disease to the host plants. Endophytes have been regarded the most prolific bioperspective class of microorganisms, which can help the host plants in counteracting negative impacts of biotic and abiotic stress conditions (Khan et al. 2013). Endophytic symbiosis with host plants especially in roots can regulate and change the uptake of mineral nutrients, balance of plant hormones, exudation of defensive metabolites from root (Khan et al. 2013; Bashan et al. 2014). Very few endophytic bacterial strains have been reported to condone such beneficial effects.

In the present study, we isolated endophytic bacteria *Sphingomonas* sp. LK11 from a medicinal plant growing in arid environment. *Sphingomonas* belongs to a group of gram-negative bacteria producing yellow-pigmented colonies and exist in diverse range of environments. The bacterium is metabolically versatile, which means it can utilize a wide range of naturally occurring compounds as well as some types of environmental contaminants (Miyauchi et al. 1998; Aylward et al. 2013; Puskarova et al. 2013). *Sphingomonas* sp. has been recently shown to help in the degradation of persistent metabolites in environment (Puskarova et al. 2013). The genetic makeup of *Sphingomonas* sp. was shown to contain genes responsible for carbazole degradation. Additionally, it has the capability to regulate certain class of pesticides like dibenzo-p-dioxins and remediate heavy metals (Puskarova et al. 2013). It also possess the potential to accumulate the intracellular Zn^{2+}, reduction of Cd^{2+} uptake enhanced expression of the low-molecular weight cysteine rich protein metallothionein that sequesters heavy metals and their binding by associated proteins (Khan et al. 2014). Additionally, its whole genome has also been sequenced recently by Miller et al. (2010), Lee et al. (2012), Qu et al. (2013), and Tabata et al. (2013). However, its biological role in relation with salinity stress has not been explored.

Therefore, present study was planned to further explore its potential for application in improving crop production especially in saline environment. The study aimed to assess the growth dynamics and responses of various oxidative stress enzymes in *Sphingomonas* sp. LK11 in varying saline environment. Upon positive results, we inoculated this strain to tomato plants to evaluate the effects of *Sphingomonas* sp. LK11 application during salinity stress. Since, previously, it was found that *Sphingomonas* sp. LK11 also produce GA_4 (2.97 ng ml^{-1}; Khan et al. 2014) therefore, for comparative assessment, exogenous GA_4 was also applied to tomato plants.

Materials and methods

Endophytic bacterial growth

Bacterial endophyte, *Sphingomonas* sp. LK11 (accession number KF515708), was isolated from the leaves of *Tephrosia apollinea* (Papilionaceae), growing in the wild mountains of Jabal Al-Akhdar (23° 04′ 22.00 N″; 57° 40′ 07.00 E″), Sultanate of Oman. The endophytic bacteria were identified by PCR amplification and sequencing of 16S rDNAs using the 27F primer (5′-AGAGTTTGATC (AC) TGGCTCAG-3′) and 1492R primer (5′-CGG (CT) TACCTTGTTACGACTT-3′) as described by Khan et al. (2014). The LK11 was grown in nutrient broth (50 ml; autoclaved at 121°C for 20 min) for five days (shaking incubator = 200 rpm; temperature 28°C) and was centrifuged (5000 × *g* at 4°C for 15 min) to separate the liquid culture medium and bacterial cells.

Endophytic bacterial potential in saline environment

To know the potential of *Sphingomonas* sp. LK11 in saline environment, it was grown in varying concentrations of sodium chloride (NaCl; 100–500 mM) in nutrient broth on shaking incubator (200 rpm; temperature 28°C) for seven days. The potential of *Sphingomonas* sp. LK11 against exogenous gibberellin (GA_4) was also assessed to understand whether this is toxic or not to bacterial growth. *Sphingomonas* sp. LK11 was grown in GA_4 (10 and 100 µM) solely and in combination with NaCl in nutrient broth using same conditions. The bacterial growth was assessed by the bacterial cell density (OD_{600}) and biomass. The bacterial cells were harvested through centrifugation (10,000 × *g* at 2°C for 15 min). The bacterial cells were immediately stored at –80°C for further protein and antioxidant enzyme analysis.

Sphingomonas sp. LK11, GA_4, and NaCl application to tomato plants

The bacteria culture suspension was grown in nutrient broth for five days at 30°C on shaking incubator to obtain an estimated cell density of 10^8 CFU/mL. All materials, including seeds, pots, soil, and distilled water were sterilized by autoclaving at 121°C for 20 min prior to the experiment. Tomato (*Solanum lycopersicum*) seeds were surface sterilized with NaOCl (5% v/v) for 10 min and thoroughly rinsed with autoclaved distilled water. Seeds were sown in plastic pots containing horticulture soil (peat moss [13–18%], perlite [7–11%], coco-peat [63–68%], and zeolite [6–8%], while the macro-nutrients present were as follows: NH_4^+ ~90 mg/L, NO_3^- ~205 mg/L, P_2O_5 ~350 mg/L, and K_2O ~100 mg/L) under controlled greenhouse conditions at temperatures of 30 ± 2°C (Kang et al. 2014). Tomato seeds were primed with 50 mL of bacterial culture suspension at the time of sowing. Since the growth responses of *Sphingomonas* sp. LK11 were pronounced against salinity (NaCl; 250 mM) and GA_4 (100 µM), therefore, both the concentrations were applied to the tomato plants. Both, *Sphingomonas* sp. LK11 (50 mL with cell density of 10^8 CFU/mL) and GA_4 (50 mL of 100 µM solution) were applied to 14 days old tomato seedlings. NaCl induced salinity stress (50 ml of 250 mM solution) was applied to three weeks old tomato seedlings for seven days on daily basis. Upon completion of stress treatments, the representative tomato plants were harvested in

liquid nitrogen and immediately stored at –80°C for enzymatic analysis. Nutrient broth in the same amount was used as controls. The growth parameters, i.e. shoot and root length, shoot and root biomass and leaf numbers, were recorded. The experiment comprised of treatments with three replications. Each replication comprised of 21 plants.

Determination of antioxidant-related enzymes

The LK11 was grown in different concentrations of sodium chloride (NaCl). After five to seven days of treatments, the bacterial cell proteins were extracted according to the method of Qin et al. (2006). Briefly, proteins were extracted with lysis buffer (0.5 M Tris-HCl, pH 8.3, 20 mM $MgCl_2$, 2% (v/v) – mercaptoethanol, and 1.0 mM phenylmethylsulfonyl fluoride) followed by sonication (and cooling on ice). The cell debris were removed by centrifugation (10,000 × *g* for 20 min at 2°C), while the supernatant was precipitated with ice-cold trichloro acetic acid (TCA, 10% w/v). The proteins were collected by centrifugation (10,000 × *g* for 20 min at 2°C) and washed three times with cold acetone to remove remaining TCA. The precipitate was finally solubilized in buffer (300 µl; 2 M thiourea, 7 M urea, 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (pH 8.0)) and either used immediately or stored at –80°C until use. Similarly, tomato plant's leaves were grinded in liquid nitrogen and powdered with pre-chilled mortar and pestle. The powder was homogenized in 50 mM Tris-HCl buffer (pH 7.0) containing 3 mM $MgCl_2$, 1 mM EDTA, and PVP (1.0% w/v). Both the homogenates of bacterial cells and plant tissues were centrifuged at 10,000 × *g* for 15 min at 2°C. The supernatant was used for total protein quantification according to the methods of Bradford (1976). All the enzymes activities were expressed as unit per mg protein.

CAT (E.C1.11.1.6) activity was assayed by the method of Aebi (1984). The crude enzyme extract was treated with 0.5 ml 0.2 M H_2O_2 in 10 mM potassium phosphate buffer (pH 7.0). CAT activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm and one unit of CAT was defined as µg of H_2O_2 released mg protein min^{-1}.

POD (E.C 1.11.1.7) and PPO (E.C 1.10.3.1) activities were measured as described by Kar and Mishra (1976) with some modifications**.** The samples were homogenized with potassium phosphate buffer pH 6.8 (0.1 M) and centrifuged at 2°C for 15 min at 5000 × *g* in a refrigerated centrifuge. The assay mixture for the POD activity comprised 0.1 M potassium phosphate buffer (pH 6.8), 50 µl pyrogallol (50 µM), 50 µl H_2O_2 (50 µM), and 0.1 ml enzyme extract. The mixture was incubated for 5 min at 25°C after which the reaction was stopped by adding 0.5 ml 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by the absorbance at 420 nm. The same assay mixture as that of POD without H_2O_2 was used to assay the activity of PPO. The absorbance of the purpurogallin formed was taken at 420 nm. One unit of POD and PPO was defined as an increase of 0.1 units of absorbance.

Reduced glutathione (GSH) content was measured according to the method of Ellman (1959). Briefly, samples were ground in a chilled mortar and pestle in 3 mL of 5% (v/v) TCA. The homogenates were centrifuged at 10,000 × *g* for 15 min at 4°C. Supernatant (0.1 mL) was added to 3.0 mL of 150 mM NaH_2PO_4 (pH 7.4). Five hundred microliters of 5,5′-dithio-bis(2-nitrobenzoic) (DTNB; 75.3 mg of DTNB was dissolved in 30 mL of 100 mM phosphate buffer, pH 6.8) was then added. The mixture was incubated at 30°C for 5 min. Absorbance was determined at 412 nm and the GSH concentration was calculated by comparison to a standard curve. All the experiments were repeated thrice.

The extent of MDA was determined by the method of Ohkawa et al. (1979). For this assay, 0.2 ml of 8.1% (w/v) sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (v/v; pH 3.5), and 1.5 ml of 0.81% (w/v) thiobarbituric acid aqueous solution were added in succession in a reaction tube. To this reaction mixture, 0.2 ml of one tissue homogenate extracted from 10 mM potassium phosphate buffer (pH 7.0) was added. The mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol:pyridine (15:1 v/v) solution were added. The upper organic layer was separated and the intensity of the resulting pink color was read at 532 nm using a spectrophotometer. Tetramethoxypropane was used as an external standard. The level of lipid peroxides was expressed as micro moles of malondialdehyde (MDA) formed/g fresh weight. The experiments were repeated thrice.

Statistical analysis

All the experiments were repeated three times while each treatment had three replications (21 plants per replica). All the values were expressed as mean ± standard deviation. A completely randomized design was used with three treatments, i.e. (1) control, (2) endophyte, (3) gibberellins, (4) salinity, (5) salinity with gibberellins, (6) endophyte with salinity, and (7) endophyte with salinity and gibberellins. Data were presented by using Graph Pad Prism software (version 5.0, San Diego, California USA). The mean values were compared using Duncan's multiple range tests at $P < 0.05$ (SAS 9.1, Cary, NC, USA).

Results and discussion

Endophytic bacterial growth dynamics in salinity environment

Endophytic bacterial microbiome has been recently coined for their potential role in confronting abiotic stress situation (Brader et al. 2014). Although, rhizospheric bacteria are known regulators of soil ionic influx into the plant, but the potential of endophytic bacteria is least known. We have grown bacterial endophyte LK11 to understand the responses against the presence of sodium

chloride (NaCl) ions in the medium. Assessing the adaptation to stress environment of a particular bacterial strain is essential, which can envisage the survival of endophyte in natural environment, where salinity is higher. We had grown LK11 in 100, 250, and 500 mM NaCl stress and assess its growth dynamics. Under normal growth conditions, the LK11 growth was significantly high with 12.5×10^8 CFU mL^{-1}. Addition of NaCl to the growth media influenced the bacterial cell growth. At low concentrations (100 mM NaCl), LK11 growth was 9.84×10^8 CFU mL^{-1}, which was reduced to 8.37×10^8 CFU mL^{-1} at higher concentrations (500 mM NaCl). However, at moderate concentration (250 mM NaCl), the bacterial cell growth was not significantly different from the LK11 growing under normal growth conditions. Its growth was 11.96×10^8 CFU mL^{-1} (Figure 1). This suggests that the viability and cellular structures of bacterial cells have not been disturbed during moderate concentration. This regulatory networking extends ameliorative responses to the plants growing in the same niche as previously shown by Hoper et al. (2006) and Tsuzuki et al. (2011).

Co-synergism of salinity and gibberellin during bacterial growth

Gibberellin A_4 (GA_4) has been described as a potent regulator of plant growth and development. Its application to economically important crops has also been suggested to improve fruit yield (Hamayun et al. 2010). Additionally, it also extends stress tolerance to the crops after drought and salinity exposure (Philipson 1983). However, the interaction of endophytic bacteria with exogenous GA_4 application has been least understood. Previously, we reported that LK11 has the potential to secrete GA_4 (2.56 ± 0.06 ng mL^{-1}; Khan et al. 2014). To know the effects of exogenous GA_4 addition in bacterial growth medium, we have grown LK11 in different concentrations of NaCl and GA_4 (10 and 100 μM). The results showed that GA_4 application was inhibitory

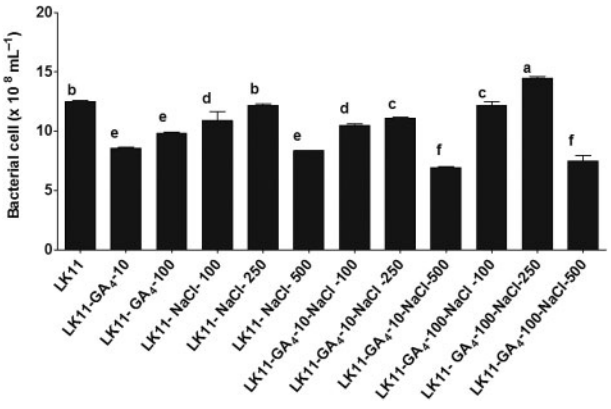

Figure 1. *Sphingomonas* sp. LK11 growth potentials in sodium chloride (NaCl) and gibberellin (GA_4) concentration.
Note: Different alphabetical letters show that the values are significantly different ($P < 0.05$) from each other as evaluated by Duncan multiple range test analysis.

to the growth of LK11 as compared to control LK11 growing under normal growth conditions. The lower concentration (10 μM) was more inhibitory than higher (100 μM) one. However, when NaCl and GA_4 (10 μM) were applied together, the 100 and 250 mM NaCl showed improvement in the bacterial cell density as compared to 500 mM NaCl concentration. Although, the values were significantly different than the untreated LK11 whilst the addition of NaCl reduced the toxicity of GA_4. This trend was more pronounced in higher concentration of GA_4 (100 μM) in 100 and 250 mM NaCl than 500 mM NaCl. This suggests that combination of NaCl (250 mM) and GA_4 (100 μM) treatments does not show inhibitory effects toward the growth of LK11, whilst such application might also be growth promoting for crops plants. This also shows that there are no or least damages to the bacterial cell structure and resultant oxidative stress develops by NaCl during growth.

Effect of salinity and GA_4 on bacterial oxidative apparatus

Excess of sodium chloride (NaCl) in any medium can be toxic to the living organism, since, it creates an ionic flux in cell by further invigorating the production of ROS such as hydrogen peroxide and superoxide anion (Vranova et al. 2002). To avoid massive disruption of cellular function due to ROS attack, the oxidative stress enzyme production is activated (Mittler 2002; Tsuzuki et al. 2011). Antioxidants and related enzymes such as CAT, POD, polyphenol peroxidase, superoxide dismutase, and glutathione are activated to convert the reactive species in harmless constituents (Vranova et al. 1997; Halverson et al. 2000; Torres-Barceló et al. 2013).

Our preliminary results revealed that 250 mM NaCl and 100 μM GA_4 treatments in bacterial medium were not harmful for LK11 growth. We applied the same environmental conditions to bacterial growth to assess the regulation of various essential oxidative stress enzymes. The results showed that bacterial proteins were significantly (~1.2 to 1.8-fold) activated in sole NaCl and NaCl with GA_4 treatments as compared to LK11 without any treatment. The CAT activity was pronounced by the combined treatment of NaCl and GA_4. This stimulation was in gradient manner but the level of CAT activated was not significantly different between sole NaCl and combined treatment with GA_4 (Figure 2). In response to NaCl (250 mM) and GA_4 applications, the POD and PPO activities were almost similar in sole and combined treatments. Only NaCl (250 mM) showed a little increase in POD activity as compared to other treatments; however, this was statistically not significant. The superoxide dismutase activity was significantly higher in LK11 + NaCl treatment as compared to normal growth conditions. Combined application of GA_4 and NaCl showed significant superoxide dismutase activity in comparison to LK11 and sole GA_4 growing in normal conditions (Figure 2). In case of bacterial glutathione contents, the combined treatment of

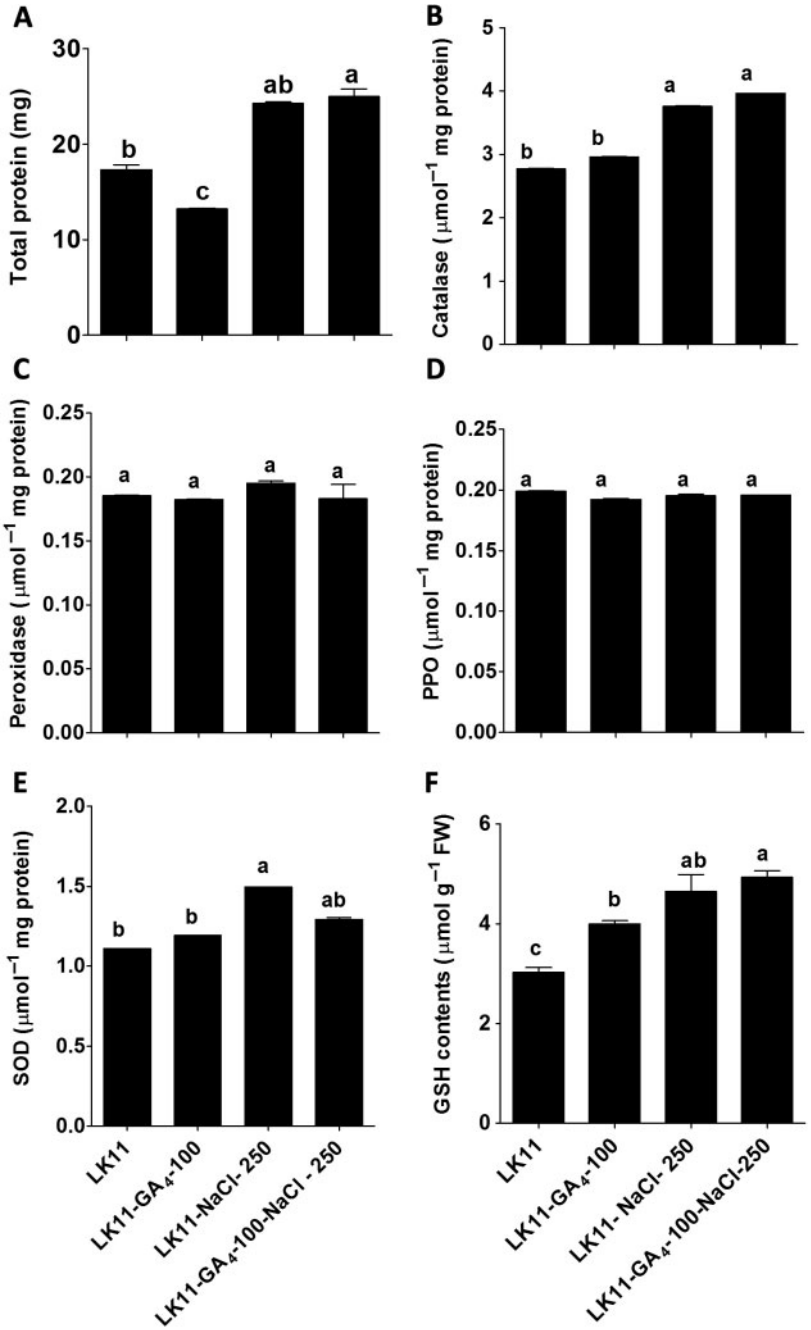

Figure 2. Effects of NaCl (250 mM) and gibberellin (GA_4; 100 µM) on the antioxidant apparatus of *Sphingomonas* sp. LK11. The figure shows the effects of NaCl and GA_4 on total protein (A), catalase – CAT (B), peroxidase (C), polyphenol peroxidase – PPO (D), superoxide dismutase – SOD (E), and reduced glutathione – GSH (F).
Note: Different alphabetical letters show that the values are significantly different ($P < 0.05$) from each other as evaluated by Duncan multiple range test analysis.

GA_4 and NaCl has significantly increased its synthesis as compared to other treatments and LK11 growing under normal conditions. This suggests the active participation of glutathione in ionic influx resulted from salinity especially Na^+.

Excessive ionic flow can influence the equilibrium in the cell. Though bacterial cells have energy-dependent active transport system to maintain the influx of such ions, however, during such continued exposure the radical/ion scavenging enzymes can help in arresting the adverse effects (Halverson et al. 2000; Ali et al. 2014). Besides ions, some molecules such as H_2O_2 can be converted through catalytic reaction ($2H_2O_2 \rightarrow O_2 + 2H_2O$) by oxidative stress enzymes such as CAT. The current results also support the regulation of excessive salt ions by activating CAT, POX, and glutathione, whilst the GA_4 did not offers inhibitory responses in growth media. This could be contributed to the potentials of endophytes in counteracting oxidative stress either by producing extra or intra cellular enzymes such as CAT (White & Monica 2010; Mercado-Blanco & Lugtenberg 2014).

Effect of LK11 inoculation on growth of tomato plants during salinity

Regular exposure to salinity hinders crop biomass and production. In current experiments, the LK11 inoculated plants exhibited significantly higher shoot length (~40%) under normal growth conditions. Similarly, GA_4 application also showed significantly higher shoot length (~55%) than non-inoculated control tomato plants. Overall, GA_4 application showed pronounced shoot elongation as compared to LK11 and control (Table 1). Almost same beneficial effects were also observed for shoot biomass, root length, and biomass in GA_4 and LK11 application under normal growth conditions as compared to non-inoculated control plants. The number of leaves and internodes was significantly higher in GA_4 and LK11 plants than in control. However, when the tomato plants were exposed to NaCl (250 mM), the shoot/root length, shoot/root biomass, and leaf number were significantly retarded. The sole NaCl treated showed stunted and abnormally lower growth potential. Previously, such negative and harmful effects were also reported by Wang et al. (2003), Hasegawa et al. (2000), Munns and Tester (2008), Khan et al. (2011, 2012, 2013), and Kang et al. (2014) in various crop plants such as rice, soybean, cucumber, pepper, etc. These showed that the toxicity of NaCl caused reduced plant growth and biomass which was also noted in our experiments. Plant growth parameters were significantly ameliorated when tomato plants were treated with endophytic bacteria LK11 and GA_4 as compared to sole NaCl treatments. The LK11 inoculation significantly increased (~35 to 100%) the plant growth parameters (shoot/root length, shoot/root biomass, and leaf number) as compared to only NaCl application (Table 1). However, this increase in tomato plant growth was more pronounced when treated with GA_4, which is a commercially known growth regulator for crops. In comparative assessment of GA_4 and LK11, only shoot length was significantly higher in GA_4 treatments but other parameters such as root length and biomass were either significantly higher or nonsignificant in LK11 inoculation under salinity stress (Table 1). This suggests the regulative role of endophytic bacteria in salinity stress. A combined application of both GA_4 and LK11 showed more vigor and tolerance to the salinity stress, resulting in significantly higher plant growth parameters among all sole treatments (Table 1). Tomato plants are very sensitive to salinity stress. Most of the previous studies showed an application of 50–200 mM (Zhang & Blumwald 2001; Santa-Cruz et al. 2002; Mayak et al. 2004; Tuna et al. 2008; Kang et al. 2014). However, current study shows that combine and sole application of endophytic bacteria and GA_4 can extend the salinity tolerance to tomato crops. On the other hand, exogenous GA_4 application to crops can not only increase crop growth and yield but also increase plant's fitness against abiotic limiting factors such as salinity as shown by Maggio et al. (2010), Tuna et al. (2008), Zhang et al. (2014), etc. Endophytic microbes residing in the host tissues can

Table 1. Effects of endophytic bacteria and GA_4 application on the growth parameters of tomato plants under salinity stress.

	Normal conditions			Salinity stress			
Growth attributes	Control	LK11	GA_4	NaCl	LK11 + NaCl	NaCl + GA_4	LK11 + NaCl + GA_4
Shoot length (cm)	20.67 ± 1.15c	28.33 ± 3.51b	31.67 ± 1.53a	11.07 ± 0.29d	15.33 ± 3.69c	17.04 ± 1.32b	18.50 ± 2.18a
Shoot weight (g)	2.29 ± 0.84c	3.07 ± 0.21b	4.43 ± 2.11a	1.25 ± 0.10c	2.03 ± 0.12a	1.87 ± 0.45b	1.90 ± 0.10b
Root length (cm)	5.83 ± 1.61c	6.17 ± 1.61b	8.00 ± 1.32a	2.17 ± 1.04c	6.67 ± 2.25b	6.34 ± 0.87b	7.50 ± 1.32a
Root weight (g)	0.23 ± 0.05c	0.37 ± 0.18a	0.40 ± 0.00a	0.13 ± 0.06c	0.28 ± 0.05a	0.23 ± 0.06b	0.29 ± 0.06a
Leaf number	11.67 ± 1.53c	15.33 ± 3.06b	19.67 ± 3.79a	13.67 ± 1.15b	17.33 ± 0.58a	17.67 ± 2.31a	17.67 ± 4.16a
Lipid peroxidation (μM mg^{-1} FW)	0.82 ± 0.091a	0.91 ± 0.21a	0.88 ± 0.11a	3.39 ± 0.69a	2.81 ± 0.13b	2.94 ± 0.61b	2.71 ± 0.21c
Glutathione (μM mg^{-1} FW)	61.8 ± 1.54b	79.1 ± 1.21a	81.4 ± 2.1a	67.1 ± 2.12c	134.1 ± 2.20a	133.9 ± 1.12a	91.9 ± 1.61b

Note: Different alphabetical letters in 'normal conditions' and 'salinity' show that the values are significantly different ($P < 0.05$) from each other as evaluated by Duncan multiple range test analysis.

regulate the ionic influx (Khan et al. 2014; Mercado-Blanco & Lugtenberg 2014) which enters through passive transport via roots (Sattelmacher 2001). Since the endophyte was growing proficiently in NaCl media with lesser damage to the cellular oxidative apparatus (Figure 2), therefore, during salinity stress, the tomato plants inoculated with endophyte resulted in increased biomass and growth. Similar regulatory and stress modulatory behavior of bacterial endophyte was also observed by Ali et al. (2014), Mercado-Blanco and Lugtenberg (2014), and Egamberdieva and Lugtenberg (2014).

Regulation of oxidative stress in tomato plants

NaCl induced ROS development damages the function of cellular organelles by peroxiding the essential lipid layer (Munns & Tester 2008). ROS are removed from the cell directly (CAT and POD) or indirectly (redox molecules like glutathione). In present study, the tomato plant grown under normal growth conditions did not showed any significant sign of lipid peroxidation as the level of MDA formation was not significant (Table 1) in LK11, GA_4, and control plants. However, exposure to NaCl stress caused an increased rate of lipid peroxidation in sole NaCl treated plants. The sole LK11 and GA_4 application counteracted the NaCl toxicity by lesser effecting the lipid peroxidation. The combined application of LK11 + GA_4 resulted in significantly reduced level of lipid peroxidation (Table 1). Since membrane bounded lipid hydroperoxides are difficult to measure due to their instability, therefore we measured the degree of lipid peroxidation to quantify secondary breakdown products like MDA. Higher ROS, on the other hand, autocatalyze peroxidation of lipid membrane and affect membrane semi-permeability under high stress (Khan et al. 2013). Activation of antioxidant scavengers can enhance membrane stability against ROS attack while MDA content can be used to assess the stress injury of plants (Rivero et al. 2014). Thus our result suggests a lesser membrane injury to the LK11, GA_4, and LK11 + GA_4 plants than sole NaCl plants. Both endophytic bacteria and fungi have been recently shown to ameliorate adverse effects of stress by reducing the level of lipid oxidation (Kang et al. 2014), which is in compliance with our findings as well.

To minimize the responses of salinity induced stress, the plant cell recruits glutathione to mitigate the oxidative imbalance. The major role of glutathione is to protect cell from the toxic burst of H_2O_2 and its pre-requisites such OH radicals (Ruiz & Blumwald 2002). Our results showed that endophyte and GA_4 treatment induced the production of glutathione as compared to control plants under normal growth conditions (Table 1). The glutathione levels were significantly higher in LK11 and GA_4 as compared to sole NaCl treatment under salinity stress. However, glutathione level in tomato plants got reduced when a combined treatment of LK11 and GA_4 was given under salinity. This suggests a stress aversion by endophytes during salinity. While in case of sole application, both LK11 and GA_4 treated plants has to activate the synthesis of glutathione to incinerate the toxic effects of NaCl. However, these levels were significantly lower in sole NaCl applied tomato plants (Table 1).

To further regulate the NaCl induced oxidative stress, antioxidant enzymes such as CAT, POD, and PPO are also activated. Our results showed the CAT activity was significantly higher in LK11 + GA_4 and control treatments whilst significantly lower in LK11 + GA_4 + NaCl, GA_4 and LK11 (Figure 3). The activity of POD was significantly higher in LK11 inoculation while in other treatments, its activity was significantly lower. The control and other treatments had a similar response (nonsignificant) except LK11 inoculation. In case of PPO, the enzyme activity was significantly higher in LK11 and LK11 +

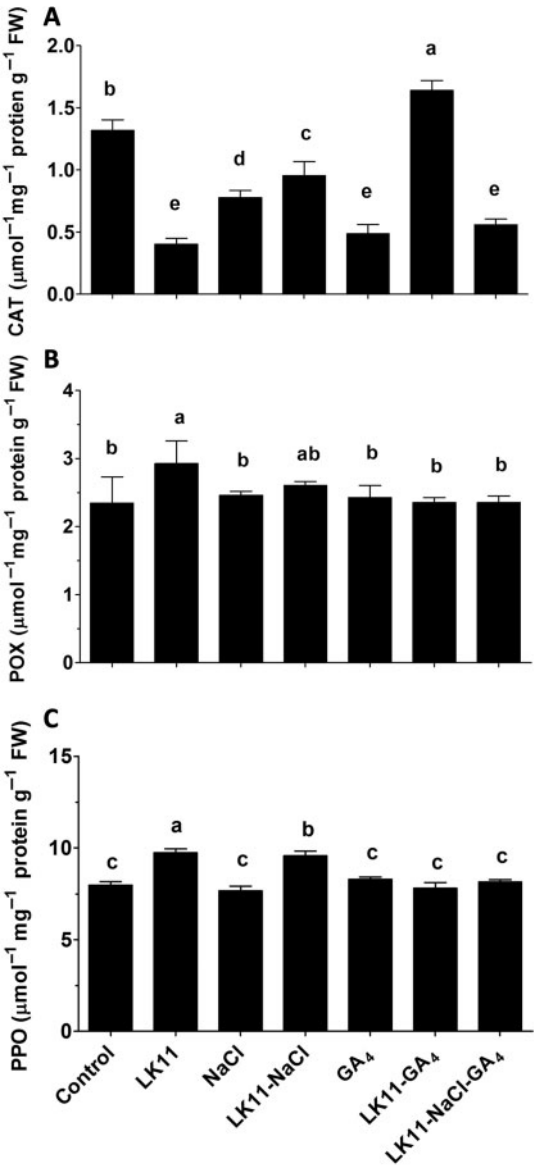

Figure 3. Antioxidant enzyme regulation of tomato plants by *Sphingomonas* sp. LK11 and GA_4 under salinity stress. The figure shows the effects of NaCl and GA_4 on (A) catalase, (B) peroxidase, and (C) polyphenol peroxidase – PPO.
Note: Different alphabetical letters show that the values are significantly different ($P < 0.05$) from each other as evaluated by Duncan multiple range test analysis.

NaCl treated plants. In rest of the other treatments, the activity was significantly lower and was similar to that of control plants growing under normal conditions. This inactivation behavior of enzymes against stress was also previously described by Cavalcanti et al. (2004) and Khan et al. (2012).

Conclusion

The current study supports the use of endophytic bacteria during saline environmental conditions. The endophyte had the potential to grow prolifically in sodium chloride induced salinity stress without compromising its vital cellular assets for production of antioxidant and related enzymes as was noted for the activities of PODs, PPO, and CAT. The bacterial cells were synergistically growing in 250 mM of NaCl and known growth regulator GA_4, thus showing little or no signs of toxicity against growth. The comparison of sole and combine endophyte and GA_4 treatment on tomato plants showed a significant increase in plant growth and biomass. Additionally, the inoculation and PGR treatment also modulated the oxidative stress and lipid peroxidation during salinity stress. Continued NaCl toxicity becomes lethal to plants because it excites the generation of ROS inside tissues. These along with other species, such as superoxide anion, hydrogen peroxide, etc., imitate the role and function of cellular organelles by creating an imbalance of oxidation/reduction cycle. To rescue cellular function, plant cell synthesizes a wide array of antioxidants and related enzymes. Since, the endophytic bacterial inoculation helped the plants to restore the tissues from peroxidizing the essential lipid membrane, therefore, it is revealed that LK11 might also counteract ROS induced toxicity through regulation of antioxidants and related enzymes response. The present results conclude that application of endophytic bacteria shows similar growth-promoting effects, which are known for PGPR and exogenous gibberellic acid application to crop plants during salinity stress.

Disclosure statement

No potential conflict of interest was reported by the authors.

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