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# EFFECT OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND GIBBERELLIC ACID ON SALT STRESS TOLERANCE IN TOMATO GENOTYPES

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## ABSTRACT

Salinity stress is a limiting factor that affects attainment of optimal yield of many vegetable crops at various growth stages in many arid and semi-arid parts of sub-Saharan Africa. The objective of this study was to explore salt tolerance of tomato (*Solanum lycopersicum* L.) genotypes under the influence of gibberellic acid ($GA_3$) and *Bacillus subtilis* under screen house conditions. Tomato seeds were pre-soaked with 0, 0.4, 0.5 or 0.6 mM concentrations of $GA_3$ and control in distilled water, respectively; for 12 hr at room temperature. The seeds were germinated in a screen house in 10 kg of soil contained 0, 100, or 200 mM NaCl treatment in polyethene bags. After two weeks of seed germination, the seedlings were inoculated with *B. subtilis* with the exception of controls. Results revealed that the single or combined treatments of $GA_3$ (at different concentrations) and *Bacillus subtilis* significantly ($P<0.05$) increased photosynthetic pigments, and enhanced the concentrations of potassium, calcium, magnesium and phosphorus ions in the salt-stressed tomato. Both tomato genotypes showed low concentrations of sodium ions at all levels of gibberellic acid with *Bacillus subtilis*. Also, there were significant ($P < 0.05$) increases in the compatible solutes, antioxidant enzymes activity and antioxidant potential of salt-stressed tomato genotypes, in the combined treatments of $GA_3$ and *Bacillus subtilis*. Tomato genotypes treated with $GA_3$ and *Bacillus subtilis,* showed greater salt-tolerance even at high levels of salinity, than single treatment of either $GA_3$ or *Bacillus subtilis*. Based on these findings, the genotypes are suitable for future breeding programmes to achieve optimal crop yield in saline conditions.

*Key Words*: Antioxidant enzymes, *Bacillus subtilis,* Gibberellic acid, *Solanum lycopersicum*

**RÉSUMÉ**

Le stress de salinité est un facteur limitant qui affecte la réalisation du rendement optimal de nombreuses cultures potagères dans de nombreuses régions arides et semi-arides de l'Afrique sub-saharienne. L'objectif de cette étude était d'explorer la tolérance au sel des génotypes de tomate (*Solanum lycopersicum* L.) sous l'influence de l'acide gibbérellique ($GA_3$) et de *Bacillus subtilis*. Les graines de tomate ont été préalablement trempées avec des concentrations de $GA_3$ de 0, 0,4, 0,5 ou 0,6 mM et du contrôle dans de l'eau distillée, respectivement; pendant 12 heures à température ambiante. Les graines ont germé dans un abri grillagé dans 10 kg de sol contenant 0, 100 ou 200 mM de traitement au NaCl dans des sacs en polyéthylène. Après deux semaines de germination des graines, les plants ont été inoculés avec B. *subtilis*. Les résultats ont révélé que des traitements uniques ou combinés de $GA_3$ (à différentes concentrations) et de *Bacillus subtilis* ($P < 0,05$) augmentaient considérablement les pigments photosynthétiques et augmentaient les concentrations d'ions potassium, calcium, magnésium et phosphore dans la tomate stressée par le sel. Les deux génotypes de tomates ont montré de faibles concentrations d'ions sodium à tous les niveaux d'acide gibbérellique avec *Bacillus subtilis*. En outre, il y a eu des augmentations significatives ($P < 0,05$) des solutés compatibles, de l'activité des enzymes antioxydantes et du potentiel antioxydant des génotypes de tomates stressés par le sel, dans les traitements combinés de $GA_3$ et de *Bacillus subtilis*. Les génotypes de tomates traités avec $GA_3$ et *Bacillus subtilis* ont montré une plus grande tolérance au sel même à des niveaux élevés de salinité. Sur la base de ces résultats, les génotypes conviennent aux futurs programmes de sélection pour obtenir un rendement optimal des cultures dans des conditions salines.

*Mots Clés*: Enzymes antioxydantes, *Bacillus subtilis,* l'acide gibbérellique, *Solanum lycopersicum*

## INTRODUCTION

Tomato (*Solanum lycopersicon* L.) is among the most important vegetable crops worldwide; with global production estimated at 200 million metric tones, with India and China as the leading producers (FOASAT, 2019). Tomato is rich in minerals, vitamins and lycopene; and is consumed raw or in processed forms. Lycopene, a major antioxidant in tomato prevents neurodegenerative diseases, diabetes and cancer. It also helps to lower the incidence of Ischemic heart disease (Mahdi *et al.,* 2011). The studies of Katerji *et al*. (2003) and Magan *et al*. (2008) have shown the sensitivity of a tomato plant to salinity stress; with major reductions traced particularly in the Mediterranean climatic conditions. Previous reports have demonstrated disastrous salinity stress effects on tomato production, manifested through mechanisms such as nutrient imbalance (Al-Karaki, 2000), ethylene explosion (Mayak *et al*., 2004), increased levels of $Na^+$ and Cl ions (Fan *et al*., 2012), and inhibition of photosynthetic potential (Dernetriou *et al*., 2007). Due to sea level rise, as a result of global climate change, as well as the decreasing availability of high quality irrigation water, salinity is of paramount concern to tomato growers world-wide. Therefore, strategies to improve tomato salt tolerance are increasingly imperative. Several reports have shown that exogenous application of phytohormones induces plant tolerance to various stresses (Pedranzani *et al*., 2003). Application of gibberellic acid ($GA_3$) to Arabidopsis seeds, for instance improved stress tolerance, reportedly through induction of salicylic acid (Alonso-Ramírez *et al*., 2009). Chakraborti and Mukherji (2003) and Turan *et al*. (2014) showed that $GA_3$ improves germination and seedling growth of the salt-stressed plants.

On the other hand, rhizospheric microorganisms of various plants have the ability to synthesize and release secondary

metabolites, like phytohormones under stress conditions (Egamberdieva, 2012). Among these microbes, *Bacillus subtilis* spp. are the major rhizobacteria involved in salt tolerance of plants. Saleh *et al*. (2005) observed inoculated *B. subtilis* on salt-stressed artichoke plants, which showed a reduction of the negative effects of salinity, and yet increased its productivity. The objective of this study was to explore the effects of giberalic acid ($GA_3$) and *B. subtilis* on some biochemical and nutritional constituents of salt-stressed tomato.

## MATERIALS AND METHODS

A screen house experiment was conducted in 2019 (April - August) at the Department of Biochemistry, University of Ibadan, Nigeria. Conditions in the screen house were *viz.* on average 27 °C during the day and 25 °C at night with relative humidity of 50%.

**Plant materials.** Tomato genotypes used in this study were BF1 and UC82B, which are genotypes widely used in Nigeria as a breeding lines, but with unknown ability to tolerate salinity stress. Seeds for the two genotypes were purchased from the Genetic Resources Laboratory unit at National Horticultural Research Institute, Ibadan, Nigeria.

**Treatments and design.** Treatments includded two tomato genotypes (BF1 and UC82B), gibberellic acid ($GA_3$) administered at 0.4, 0.5 or 0.6 mM concentrations; inoculation with *B. subtilis* and under either 0, 100 200 mM NaCl solution. Both $GA_3$ and *B. subtilis* were obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria and Agronomy Department, University of Ibadan, Nigeria, respectively. Gibberellic acid concentrations were used to pre-soak the seeds. Seeds of each genotype were sown in 10 kg polyethene bags of soil with analytical properties presented in Table 1.

Seeds were sown in soil that had been treated with a solution of NaCl based on allotted salinity concentration, in the factorial experiment and was laid out in a completely randomised designed, and treatments replicated three times.

After seed germination, three seedlings per pot were wet with equal volume of water thrice in a week until soil water attained field capacity. After 45 days of growth, all experimental plant leaves were harvested, air-dried for three weeks at room temperature and ground to powder using a mortar and a pestle before the biochemical assay.

***Bacillus subtilis* cell culture.** *Bacillus subtilis* was isolated from the rhizosphere soil of healthy tomato plants, at the Agronomy Department, University of Ibadan; and cultured on nutrient agar at the Pharmaceutical Microbiology Department, University of Ibadan, Nigeria. The culture was centrifuged at 6200 rpm for 10 min. at 4 °C. Distilled water was used to wash the pellets obtained and then re-suspended in sterilised distilled water to an optical density of 0.8 at 600 nm (approximate cell density of $1 \times 10^8$ CFUmL$^{-1}$). Two weeks after germination, each seedling was inoculated with 30 mL of cell suspensions of $1 \times 10^8$ CFU mL$^{-1}$.

**Photosynthetic pigments estimation.** The Lichtenthaler and Wellburn (1983) method was applied for the photosynthetic pigments assay of the tomato leaves. Approximately 250 mg

TABLE 1. Analytical properties of the soil used

| | |
|---|---|
| pH | 7.10 |
| Exch. acidity (cmol. kg$^{-1}$) | 0.34 |
| Soil texture | Silt, sandy clay |
| Organic carbon (%) | 4.732 |
| N (%) | 0.25 |
| Avail. P (mg kg$^{-1}$) | 2.00 |
| Exchangeable $K^+$ (mol. kg$^{-1}$) | 1.33 |
| $Na^+$ (cmol. kg$^{-1}$) | 0.89 |
| $Ca^{2+}$ (cmol. kg$^{-1}$) | 45.65 |
| $Mg^{2+}$ (cmol. kg$^{-1}$) | 13.34 |

of tomato fresh leaves was homogenised with 85% acetone for about 5 min. The sample was mixed using a magnetic stirrer; centrifuged and the absorbance taken at 663, 646, and 470 nm. The concentrations of the pigments were estimated and expressed as mg $g^{-1}$ fresh weight.

**Extraction protocol for mineral elements.** The samples were digested in an oven at 600 °C for 4 hr, the ashes and the crucibles were previously decontaminated with a solution of 10% nitric acid for a night, and rinsed in the distilled water. Then, 10 mL of 5% nitric acid was added to the sample, and this mixture was heated until complete dissolution of the ash. This was filtered and the filtrate put into 25 mL volumetric flask, and then the volume made up to the required level using distilled water.

**Cationic contents.** The calcium, magnesium and potassium ion concentrations were determined in the samples according to AOAC (2005) method, using an atomic absorption spectrophotometer flame (BULKS SCIENTIFIC® model AA 240). The concentrations of $Na^+$ in the samples were determined by Flame Photometry according to the AOAC (2005) method. Total phosphorus content was determined as described by the spectrophotometry method.

**Free proline determination.** Proline concentration was estimated in the leaf tissues according to the method described by Bates *et al*. (1973). Proline was used as a standard (0–50 ìg $mL^{-1}$), and the reading of proline concentration was taken at 520 nm.

**Reducing sugar determination.** The method of El-Shihaby *et al*. (2002) was used to determine the reducing sugar concentration in the tomato plant samples. Briefly, 0.5 g of dried powder of tomato was extracted by using 80% ethanol (10 mL) at 50 °C, and a reaction was set up at about 15-30 min. of the extraction. Then, 1 mL of the extracted solution was estimated at 530 nm using UV/VIS Spectrophotometer. Reducing sugar concentration was expressed as mg $g^{-1}$ on dry weight. The glucose was applied as a standard.

**Total soluble protein determination.** The spectrophotometric technique described by Desingh and Kanagaraj (2007) was used to determine soluble proteins concentration in the plant samples, using Folin-Ciocalteau reagent. About 5 mL of 10% trichloroacetic acid was mixed with 0.5 g of dry leaf powdered sample. The mixture was centrifuged at 2000 rpm for 10 min. Then, about 5 mL of the supernatant was added to 0.1 M NaOH (5 mL), followed by the addition of 8 mL Biuret reagent. The standard used was BSA (Bovine Serum Albumin). The standard and the sample dilutions were incubated for 30 min at 25 °C before the reading was taken at 530 nm.

**Enzyme extractions and assays.** The Tejera *et al*. (2004) method was applied to prepare crude enzyme extract. A 1 g of plant sample was soaked in a buffer solution of potassium phosphate (0.1 M, pH 6) with 0.5 mM EDTA. The sample extract was centrifuged at 15000 rpm for 20 min. The enzymes content of the supernatant was then determined.

**Superoxide dismutase assay.** Superoxide dismutase activity was assayed using the procedure described by Kumar *et al*. (2012) (. A 1 mL of sample was taken at 25 to 500 ìg $mL^{-1}$ concentrations and mixed with 0.5 mL of buffer solution of potassium phosphate (50 mM, pH 7.6) with 0.1 mL of nitro blue-tetrazolium (NBT) (0.5 mM) and 0.3 mL of riboflavin (50 mM). A fluorescent lamp was used to initiate the reaction, following incubation for 20 min at 25 ºC. The activity of superoxide dismutase was measured at 560 nm using UV/VIS Spectrophotometer. The standard used was ascorbic acid (0.1mg/ml) . The enzyme activity was expressed as Units $mg^{-1}$ protein.

**Ascorbate peroxidase assay.** Ascorbate peroxidase activity was estimated using the method outlined by Yoshimura *et al*. (2000). The mixture of the reaction contained a buffer solution of potassium phosphate (50 mM, pH 7.0) with 0.1 mM of hydrogen peroxide, 0.5 mM ascorbic acid and 200 µL of enzyme extract. The mixture was incubated for 5 min at 25 ºC. The activity of ascorbate peroxidase was measured at 550 nm using UV/VIS Spectrophotometer. The enzyme activity was expressed as units $mg^{-1}$ protein.

**Polyphenol oxidase assay.** The Oktay *et al*. (1995) method was used to assay for polyphenol oxidase activity with slight modifications. The mixture of the reaction contained buffer solution of 0.1 M potassium phosphate at pH 6.0 with enzyme extract (0.5 mL) and 0.1 M catechol (1.0 mL). The reaction medium was incubated at 25 ºC for 5 min. After which, 1 mL of 2.5 N $H_2SO_4$ was added to stop the reaction. The reading was taken at 495 nm using UV/VIS Spectrophotometer.

**Total flavonoids determination.** The Park *et al.* (2008) method was applied to determine the total flavonoid concentration in the sample. The mixture of the reaction contained 0.15 mL of 0.3 M aluminum chloride ($AlCl_3$), 0.15 mL of 0.5 M sodium nitrite ($NaNO_2$), 3.4 mL of 30% methanol, and 0.3 mL of the sample extract. The addition of 1 M sodium hydroxide (1 mL) was done after 5 min. Incubation was donefor about half an hour at 25°C. Quercetin served as the standard by using its standard curve for determining the flavonoids concentration. Then, the reading was taken at 510 nm using UV/VIS Spectrophotometer.

**Total phenolic determination.** The spectrophotometric method was employed to determine total phenolic content in the sample (Kim *et al.,* 2003). Approximately 1 mL of Folin-ciocalteu's phenol reagent was added to 1 mL of the sample. A 10 mL of 7% $Na_2CO_3$ solution was added to the mixture after 5 min. and thoroughly mixed with distilled water (13 ml) using a magnetic stirrer. The reaction was stored for 90 min at 25 ºC in the dark. The reading was then taken at 750 nm using UV/VIS Spectrophotometer. Gallic acid was used as the standard by using its standard curve for determining the phenolic concentration.

**Statistical analysis.** The data collected were subjected to three-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS). Sample means were compared using Tukey-Kramer multiple comparison procedure at $P< 0.05$.

## RESULTS

**Photosynthetic pigments content.** Tables 2 and 3 show that single treatment of tomato seedlings with *B. subtilis* and gibberellic acid induced a rise in the concentrations of tissue chlorophyll a and b in all the NaCl-treated plants. Under the combined effects of gibberellic acid and *Bacillus subtilis,* tomato genotype UC82B responded more positively with increase contents of photosynthetic pigments than the genotype BF1.

**Minerals concentration.** Tables 4(a, b) and 5(a, b) show that the single treatment of *B. subtilis* or $GA_3$ at different concentrations, respectively; as well as the combined treatments of *B. subtilis* at different concentrations of $GA_3$ , enhanced the levels of potassium, calcium, magnesium and phosphorus ions in the two genotypes of salt-stressed tomato plants. Also, these were more significant at 0.5 and 0.6 mM concentrations of gibberellic acid combined with *B. subtilis* in the BF1 and UC82B genotypes than in the normal control and the two negative controls (100 and 200 mM of sodium chloride) groups. The individual concentration of gibberellic acid or B. *subtilis*, as well as the combined treatments of B. *subtilis* with different concentrations of gibberellic acid significantly reduced sodium ion concentration in the salt-stressed tomato plant of the two genotypes

TABLE 2. Gibberellic acid and *Bacillus subtilis* effects on photosynthetic pigments of salt-stressed tomato plant BF1 genotype

| Treatments | NaCl (mM) | Chlorophyll a (mg $g^{-1}$ fw) | Chlorophyll b (mg $g^{-1}$ fw) | Carotenoids (mg $g^{-1}$ fw) |
|---|---|---|---|---|
| 0 mM | 0 | 1.18 ± 0.28 | 1.10 ± 0.24 | 2.53 ± 0.42 |
| | 100 | 0.14 ± 0.16 | 1.61 ± 0.04 | 0.68 ± 0.56 |
| | 200 | 0.35 ± 0.01* | 0.96± 0.02* | 0.54 ± 0.14 |
| $GA_3$ (0.4 mM) | 0 | 1.53± 0.33 | 1.59 ± 0.28 | 3.34 ± 0.33 |
| | 100 | 2.38 ± 0.26** | 1.80 ± 0.22 | 2.35 ± 0.04 |
| | 200 | 0.40 ± 0.08 | 1.15 ± 0.16 | 1.40 ± 0.01 |
| $GA_3$ (0.5 mM) | 0 | 1.21 ± 0.14 | 1.91 ± 0.25 | 3.68 ± 0.32 |
| | 100 | 1.35 ± 0.28 | 1.15 ± 0.16 | 3.50 ± 0.25 |
| | 200 | 3.76 ± 0.02*** | 0.76 ± 0.02 | 2.13 ± 0.01 |
| $GA_3$ (0.6 mM) | 0 | 1.70 ± 0.02 | 1.89 ± 0.01 | 3.69 ± 0.02 |
| | 100 | 1.16 ± 0.28 | 1.23 ± 0.08 | 3.22 ± 0.16 |
| | 200 | 1.79 ± 0.31 | 0.99 ± 0.27 | 2.13 ± 0.28 |
| *B. subtilis* | 0 | 1.15 ± 0.01 | 1.14 ± 0.01 | 3.35 ± 0.01 |
| | 100 | 3.98 ± 0.02** | 1.20 ± 0.02 | 2.98 ± 0.01 |
| | 200 | 3.69 ± 0.04*** | 0.77 ± 0.04 | 2.40 ± 0.04 |
| $GA_3$ (0.4 mM) + *B. subtilis* | 0 | 1.54 ± 0.02 | 3.71 ± 0.02* | 5.89 ± 0.01* |
| | 100 | 3.10 ± 0.21** | 1.55 ± 0.16 | 3.75 ± 0.13 |
| | 200 | 3.36 ± 0.26*** | 0.33 ± 0.22 | 2.58 ± 0.22 |
| $GA_3$ (0.5 mM) + *B. subtilis* | 0 | 1.23 ± 0.16 | 1.13 ± 0.25 | 5.90 ± 0.16 * |
| | 100 | 3.59 ± 0.27** | 1.00 ± 0.31 | 4.28 ± 0.32** |
| | 200 | 3.96 ± 0.22*** | 0.90 ± 0.22 | 3.08 ± 0.32 |
| $GA_3$ (0.6 mM) + *B. subtilis* | 0 | 1.87 ± 0.04 | 1.54 ± 0.04 | 5.19 ± 0.05 |
| | 100 | 1.18 ± 0.25 | 1.62 ± 0.18 | 4.31 ± 0.22** |
| | 200 | 0.10 ± 0.14 | 1.18 ± 0.18 | 3.30 ± 0.14 |

Data are means ± SE (n = 3). *, ** and ***Significant differences at P<0.05 to the normal control, negative controls at 100 and 200 mM NaCl respectively determined by Tukey-Kramer multiple range test

TABLE 3. Gibberellic acid and *Bacillus subtilis* effects on photosynthetic pigments of salt-stressed tomato plant UC82B genotype

| Treatments | NaCl (mM) | Chlorophyll a (mg $g^{-1}$ fw) | Chlorophyll b (mg $g^{-1}$ fw) | Carotenoids (mg $g^{-1}$ fw) |
|---|---|---|---|---|
| 0 mM | 0 | 1.26 ± 0.02 | 1.30 ± 0.02 | 1.80 ± 0.02 |
| | 100 | 0.34 ± 0.20 | 1.65 ± 0.34 | 0.06 ± 0.29 |
| | 200 | 0.51 ± 0.34 | 0.86 ± 0.67* | 0.05 ± 0.65 |
| $GA_3$ (0.4 mM) | 0 | 3.26 ± 0.21 | 2.25 ± 0.17 | 1.50 ± 0.27 |
| | 100 | 2.25 ± 0.02 | 3.12 ± 0.02 | 1.18 ± 0.02 |
| | 200 | 1.19 ± 0.03 | 3.37 ± 0.03 | 0.87 ± 0.02 |
| $GA_3$ (0.5 mM) | 0 | 3.23 ± 0.29 | 3.59 ± 0.15*** | 1.41 ± 0.11 |
| | 100 | 2.13 ± 0.34 | 2.43 ± 0.33 | 0.82 ± 0.27 |
| | 200 | 1.12 ± 1.96 | 1.23 ± 0.18 | 0.49 ± 1.19 |
| $GA_3$ (0.6 mM) | 0 | 3.37 ± 0.94 | 2.91 ± 0.50 | 1.75 ± 0.37 |
| | 100 | 2.11 ± 0.56 | 1.31 ± 0.03 | 1.12 ± 0.03 |
| | 200 | 1.20 ± 0.11 | 1.95 ± 1.62 | 1.04 ± 0.20 |
| *B. subtilis* | 0 | 3.20 ± 0.07 | 3.43 ± 0.93 | 1.49 ± 0.36 |
| | 100 | 2.24± 0.56 | 2.48± 0.56 | 0.74± 0.75 |
| | 200 | 1.58 ± 0.88 | 1.26 ± 0.38 | 0.49 ± 0.52 |
| $GA_3$ (0.4 mM) + B. subtilis | 0 | 3.28 ± 0.12* | 1.55 ± 0.12 | 3.54 ± 0.50 |
| | 100 | 2.29 ± 0.75** | 2.95 ± 0.19 | 2.96 ± 0.74** |
| | 200 | 2.18 ± 0.24*** | 1.70 ± 0.17 | 2.29 ± 0.26 |
| $GA_3$ (0.5 mM) + *B. subtilis* | 0 | 3.50 ± 0.32* | 1.81 ± 0.34 | 3.50 ± 0.47 |
| | 100 | 2.21 ± 0.53** | 3.26 ± 0.51** | 2.43 ± 0.67** |
| | 200 | 1.14 ± 0.03 | 2.18 ± 0.03*** | 1.40 ± 0.01 |
| $GA_3$ (0.6 mM) + *B. subtilis* | 0 | 2.93 ± 0.32 | 1.13 ± 0.34 | 3.15 ± 0.24 |
| | 100 | 1.27 ± 0.38 | 2.9 ± 0.53 | 2.07 ± 0.23*** |
| | 200 | 3.16 ± 0.20*** | 3.12 ± 0.40*** | 1.56 ± 0.68 |

Data are means ± SE (n = 3). *, ** and ***Significant differences at P<0.05 to the normal control, negative controls at 100 and 200 mM NaCl respectively determined by Tukey-Kramer multiple range test

TABLE 4 A. The gibberellic acid or *Bacillus subtilis* effect on mineral (mg $g^{-1}$ dw) elements of salt-stressed tomato plant BF1 genotype

| Treatment | NaCl (mM) | Na | Mg | Ca | K | P |
|---|---|---|---|---|---|---|
| 0 (mM) | 0 | 106.27 ± 0.33 | 131.00 ± 3.70 | 125.73 ± 3.45 | 217.78 ± 4.10 | 262.11 ± 3.59 |
| | 100 | 211.87 ± 0.16 | 105.47 ± 3.80 | 105.34 ± 7.61 | 133.24 ± 6.83 | 181.60 ± 2.50 |
| | 200 | 291.51 ± 0.33 | 55.66 ± 5.00* | 82.85 ± 0.75* | 104.20 ± 3.00* | 103.60 ± 4.17* |
| $GA_3$ (0.4 mM) | 0 | 90.48 ± 0.26 | 155.69 ± 3.71 | 150.34 ± 3.52 | 364.64 ± 5.52 | 273.61 ± 2.00 |
| | 100 | 117.93 ± 0.17 | 132.06 ± 5.00 | 133.97 ± 0.56 | 290.10 ± 6.94 | 232.32 ± 2.99 |
| | 200 | 201.76 ± 0.24** | 115.39 ± 0.37 | 118.26 ± 0.74 | 360.20 ± 5.20 | 204.33 ± 2.13 |
| $GA_3$ (0.5 mM) | 0 | 21.08 ± 0.20 | 152.48 ± 1.20 | 139.22 ± 7.42 | 365.70 ± 4.90 | 281.69 ± 4.13 |
| | 100 | 17.47 ± 0.20 | 134.65 ± 1.20 | 128.10 ± 3.89 | 267.17 ± 3.49 | 240.22 ± 3.38 |
| | 200 | 22.87 ± 0.34 | 111.23 ± 5.00 | 114.47 ± 0.19 | 277.19 ± 4.90 | 209.75 ± 3.48** |
| $GA_3$ (0.6 mM) | 0 | 20.95 ± 0.29 | 155.39 ± 4.45 | 147.19 ± 4.27 | 367.82 ± 3.28 | 279.70 ± 3.09 |
| | 100 | 19.16 ± 0.15 | 134.62 ± 3.58 | 129.22 ± 0.75 | 329.85 ± 5.69 | 233.75 ± 2.56 |
| | 200 | 22.55 ± 0.11 | 110.54 ± 5.00 | 114.22 ± 0.75 | 359.23 ± 5.60 | 209.74 ± 1.91 |
| *B. subtilis* | 0 | 18.08 ± 0.27 | 152.98 ± 5.00 | 153.99 ± 3.52 | 350.20 ± 6.87 | 279.05 ± 4.05 |
| | 100 | 19.92 ± 0.33 | 137.83 ± 4.45 | 131.73 ± 0.19 | 321.21 ± 5.47 | 226.25 ± 3.25 |
| | 200 | 22.85 ± 0.34 | 109.85 ± 2.50 | 116.25 ± 0.56 | 309.21 ± 4.50 | 206.19 ± 3.34** |

Data are means ± SE (n = 3). *, ** and *** are significant differences at $P<0.05$ to the normal control and positive control groups ($GA_3$ or *B. subtilis*) respectively determined by Tukey-Kramer multiple range test

when compared to the normal control and the two negative controls (100 and 200 mM NaCl) groups.

**Free proline concentration.** As shown in Figure 1, in the two genotypes of tomato (BF1 and UC82B), *B. subtilis* or gibberellic acid, as well as the combined treatments at different concentrations of gibberellic acid under salinity stress increased proline accumulation in the tomato plant. But the significant increase in proline concentration was observed at 0.5 and 0.4 mM concentrations of gibberellic acid combined with *B. subtilis* in the BF1 or UC82B genotypes, when compared with the normal control and the NaCl-treated groups.

**Reducing sugar and soluble protein concentrations.** Results showed an increase reducing sugar concentrations in the two genotypes of tomato (BF1 and UC82B) under single treatment of *B. subtilis* or gibberellic acid, as well as co-treatments at different concentrations of gibberellic acid in the two tomato genotypes (Fig. 2). The increase in the concentrations of reducing sugar was higher at 0.5 and 0.4 mM concentrations of gibberellic acid combined with *B. subtilis* in the BF1 and UC82B genotypes, respectively when compared with the normal control and NaCl-treated groups (Fig. 2). A similar trend was observed for the total soluble protein concentrations in both BF1 and UC82B genotypes (Fig. 3).

**Ascorbate peroxidase activity.** Figure 4 shows an increase in the ascorbate peroxidase activity in salt-stressed tomato BF1 and UC82B genotypes in a single treatment of gibberellic acid or *B. subtilis*. But the steady increase in the ascorbate peroxidase activity was discovered in co-treatments of gibberellic acid and *B. subtilis* in salt-stressed tomato (BF1

TABLE 4 B. Gibberellic acid and *Bacillus subtilis* effects on mineral (mg/g dw) elements of salt-stressed tomato plant BF1 genotype

| Treatment | NaCl (mM) | Na | Mg | Ca | K | P |
|---|---|---|---|---|---|---|
| $GA_3$ (0.4 mM) ± B. subtilis | 0 | 93.85 ± 0.32 | 193.44 ± 5.65 | 268.45 ± 4.55 | 438.29 ± 7.01 | 347.32 ± 3.21 |
| | 100 | 75.05 ± 0.34 | 160.39 ± 7.79 | 180.80 ± 4.64 | 391.40 ± 5.31 | 245.82 ± 4.24 |
| | 200 | 56.3 ± 0.24 | 139.65 ± 1.20 | 151.27 ± 0.19** | 211.40 ± 5.31** | 218.35 ± 4.24 |
| $GA_3$ (0.5 mM) ± *B. subtilis* | 0 | 23.80 ± 0.28 | 182.81 ± 3.18 | 226.34 ± 4.51 | 432.80 ± 2.37 | 392.65 ± 5.01 |
| | 100 | 16.08 ± 0.28 | 150.57 ± 3.96 | 159.18 ± 3.35 | 383.25 ± 3.47 | 256.12 ± 4.28 |
| | 200 | 19.30 ± 0.33 | 135.35 ± 1.20 | 146.02 ± 0.38 | 283.25 ± 2.45** | 219.29 ± 4.20 |
| $GA_3$ (0.6 mM) ± *B. subtilis* | 0 | 23.55 ± 0.21 | 192.12 ± 2.23 | 233.21 ± 3.83 | 192.12 ± 2.23 | 353.48 ± 3.16 |
| | 100 | 17.20 ± 0.17 | 159.96 ± 4.21 | 180.34 ± 4.64 | 159.96 ± 4.21 | 262.15 ± 1.45 |
| | 200 | 18.39 ± 0.27 | 136.98 ± 5.00** | 135.57 ± 0.75** | 136.98 ± 5.00 | 230.27 ± 2.12 |

Data are means ± SE (n = 3). ** is a significant difference at P<0.05 to gibberellic acid or *B. subtilis* treated groups

and UC82B genotypes) plant as compared to the normal and NaCl-treated groups.

**Polyphenol oxidase activity.** Single treatment of gibberellic acid or *B. Subtilis* had little or no effect on the polyphenol oxidase activity of salt-stressed tomato (BF1 and UC82B genotypes) plant when compared to the normal control and NaCl-treated groups (Fig. 5). However, co-treatments with gibberellic acid and *B. subtilis* increased the polyphenol oxidase activity in salt-stressed tomato plant BF1 and UC82B genotypes, as compared to the single treatment of gibberellic acid or *B. subtilis*, normal control and NaCl-treated groups.

**Superoxide dismutase activity.** Figure 6 shows that superoxide dismutase activity was not improved in a single treatment of gibberellic acid or *B. subtilis* in salt-stressed tomato (BF1 and UC82B genotypes) plant when compared to the normal control and NaCl-treated groups. However, superoxide dismutase activity was significantly increased in the salt-stressed tomato plant BF1 and UC82B genotypes under the combined effects of gibberellic acid and *B. subtilis,* compared to a single treatment of gibberellic acid or *B. subtilis*, normal control and NaCl-treated groups.

**Total flavonoids and phenolic contents.** Figure 7 shows that pre-soaked seeds and seedling inoculated with *B. Subtilis* had no significant effect (P<0.05) on total flavonoids content in salt-stressed tomato (BF1 and UC82B genotypes), when compared with the normal control and NaCl-treated groups. However, total flavonoids content was significantly increased in the control group treated with combined effects of gibberellic acid and *B. subtilis* at all concentrations of gibberellic acid when compared with the normal and NaCl-treated groups in both genotypes. Figure 8 presented a similar trend for total phenolic contents in both BF1 and UC82B genotypes.

TABLE 5 A. Gibberellic acid or *Bacillus subtilis* effect on mineral (mg $g^{-1}$ dw) elements of salt-stressed tomato plant UC82B genotype

| Treatment | NaCl (mM) | Na | Mg | Ca | K | P |
|---|---|---|---|---|---|---|
| 0 mM | 0 | 16.34 ± 0.18 | 134.65 ± 1.20 | 135.73 ± 3.45 | 358.77 ± 3.21 | 181.78 ± 1.80 |
| | 100 | 220.17 ± 0.22* | 106.40 ± 9.30 | 115.98 ± 0.75 | 236.48 ± 8.50 | 126.42 ± 3.02 |
| | 200 | 302.6 ± 0.04* | 81.04 ± 0.50* | 86.78 ± 0.17* | 121.00 ± 3.70* | 113.05 ± 7.90 |
| $GA_3$ (0.4 mM) | 0 | 21.45 ± 0.04 | 161.85 ± 1.92 | 140.81 ± 0.11 | 408.00 ± 1.19 | 236.33 ± 1.80 |
| | 100 | 117.59 ± 0.04** | 152.22 ± 3.71 | 147.66 ± 1.62 | 360.33 ± 4.40 | 201.70 ± 2.17 |
| | 200 | 121.22 ± 0.04** | 121.23 ± 0.52 | 125.69 ± 0.20 | 237.85 ± 4.94 | 301.70 ± 8.90 |
| $GA_3$ (0.5 mM) | 0 | 42.96 ± 0.28 | 165.84 ± 0.88 | 160.39 ± 3.34 | 402.75 ± 3.39 | 244.41 ± 2.07 |
| | 100 | 17.88 ± 0.08** | 157.08 ± 3.80 | 149.88 ± 3.52 | 368.70 ± 5.80 | 212.10 ± 1.27 |
| | 200 | 22.13 ± 0.16 | 115.08 ± 3.71 | 128.98 ± 0.05 | 292.55 ± 4.09** | 235.56 ± 4.50 |
| $GA_3$ (0.6 mM) | 0 | 40.50 ± 0.24 | 161.65 ± 0.94 | 167.19 ± 3.90 | 404.00 ± 4.41 | 237.39 ± 2.69 |
| | 100 | 18.16 ± 0.28** | 150.98 ± 5.00 | 129.22 ± 0.75 | 339.20 ± 4.41 | 217.20 ± 3.52 |
| | 200 | 22.89 ± 0.16 | 125.65 ± 2.98 | 131.73 ± 0.19 | 271.85 ± 5.76 | 235.45 ± 8.90 |
| *B. subtilis* | 0 | 18.73 ± 0.22 | 156.33 ± 0.10 | 163.08 ± 4.64 | 407.75 ± 3.31 | 226.93 ± 2.77 |
| | 100 | 17.20 ± 0.16 | 139.78 ± 3.70 | 134.24 ± 0.03 | 364.15 ± 4.53 | 205.21 ± 2.02 |
| | 200 | 21.06 ± 0.22 | 114.24 ± 0.30 | 124.43 ± 0.56 | 252.24 ± 3.30 | 105.21 ± 7.80 |

Data are means ± SE (n = 3). *, ** and *** are significant differences at $P<0.05$ to the normal control and positive control groups ($GA_3$ or *B. subtilis*) respectively determined by Tukey-Kramer multiple range test

## DISCUSSION

The higher photosynthetic pigments content of groups treated with $GA_3$ and *B. subtilis* (Tables 2 and 3) than in the salt-stressed groups may be due to the growth promoting effects of $GA_3$ and or *B. subtilis*, which increases photosynthetic efficiency, and in turn improves plant biomass. Mohamed and Gomaa (2012) observed increases in chlorophyll *a*, *b* and carotenoid contents in leaves of salt-stressed radish plants, following inoculation with strains of *B. subtilis* and *Pseudomonas fluorescens.* Studies of salinity stress in plants have shown its inhibition of photosynthesis and protein synthesis, which in turn serve as barriers to horticultural production (Hashem *et al*., 2014). It is clear from this study that salinity stress increases the concentrations of tissue sodium, with a reduction in the concentrations of some essential mineral elements (Mg, Ca, K, and P) in the tomato genotypes, which in turn leads to nutrient deficiency (Tables 4 and 5). The implication of salinity stress on the nutrient compositions of plant tissues, especially calcium and potassium ion contents have been studied widely, and affirm the detrimental effects of salinity on nutrient availability (Tester, 2003; Hussain *et al*. 2015). The phosphorus, potassium, calcium, and magnesium ions concentrations were found to be higher than sodium ion in the groups treated with gibberellic acid and *B. subtilis* in BF1 and UC82B genotypes (Tables 4a, b and 5a, b). This support the observation of Dodd and

TABLE 5 B. Gibberellic acid and *Bacillus subtilis* effects on mineral (mg $g^{-1}$ dw) elements of salt-stressed tomato plant UC82B genotype

| Treatment | NaCl (mM) | Na | Mg | Ca | K | P |
|---|---|---|---|---|---|---|
| $GA_3$ (0.4 mM) ± B. subtilis | 0 | 82.30 ± 0.25 | 202.24 ± 1.85 | 241.18 ± 1.34 | 456.32 ± 2.82 | 328.98 ± 1.93 |
| | 100 | 114.76 ± 0.18 | 181.29 ± 2.13 | 194.77 ± 1.85 | 437.40 ± 4.65 | 297.90 ± 0.36 |
| | 200 | 116.36 ± 0.22** | 147.65 ± 3.70 | 154.88 ± 3.52** | 396.31 ± 2.97 | 197.90 ± 7.89 |
| $GA_3$ (0.5 mM) ± *B. subtilis* | 0 | 22.98 ± 0.14 | 193.77 ± 2.27 | 245.55 ± 2.22 | 459.53 ± 2.11 | 324.71 ± 0.93 |
| | 100 | 15.72 ± 0.18 | 174.23 ± 3.12 | 175.20 ± 1.20 | 437.85 ± 7.60 | 290.12 ± 1.70 |
| | 200 | 19.25 ± 0.14 | 156.76 ± 3.58 | 150.05 ± 0.24 | 362.85 ± 3.55 | 190.12 ± 4.70 |
| $GA_3$ (0.6 mM) ± *B. subtilis* | 0 | 22.12 ± 0.21 | 204.17 ± 4.45 | 270.38 ± 3.68 | 204.17 ± 4.45 | 321.32 ± 1.58 |
| | 100 | 16.50 ± 0.16 | 178.44 ± 4.23 | 195.51 ± 3.52 | 178.44 ± 4.23 | 287.24 ± 2.84 |
| | 200 | 18.35 ± 0.13 | 169.89 ± 0.29 | 156.23 ± 0.56** | 169.89 ± 0.29 | 267.14 ± 7.84 |

Data are means ± SE (n = 3). ** is a significant difference at $P \leq 0.05$ to gibberellic acid or *B. subtilis* treated groups

Perez-Alfocea (2012) that plant growth-promoting rhizobacteria (PGPR) could decrease toxic ions acquisition and maintain the intracellular ionic equilibrium, and thus increase nutrients availability in plants.

The lower level of sodium ion observed in the treatments at different concentrations support the growth of tomato by protecting its tissues from toxic effects of salinity stress, and thus ensure ionic homeostasis in the two tomato genotypes. Esan *et al*. (2017) observed improvement in okra plants under saline conditions, following seeds pre-treatment with indole acetic acid and salicylic acid. Salt stress increased proline concentration in BF1 and UC82B tomato genotypes, which in turn upregulates the enzymes activity and down regulates the catabolising enzymes activity for proline synthesis (Moxley *et al*., 2011). Accumulation of proline is an indicator of stress tolerance in plants (Parviz and Satyawat, 2008). Results obtained were similar to the one reported by Ahmad *et al*. (2016), who observed increased proline concentration, which in turn improved the fresh and dry weights of chickpea plants growing under high-salinity conditions by inoculation with *Bacillus subtilis*.

The increase in reducing sugar content of salt-stressed tomato (BF1 and UC82B genotypes) treated with gibberellic acid and *B. subtilis* (Fig. 2), is responsible for the osmotic potential, which ensure water uptake in the tissue of salt-stressed tomato. A similar report was published by Amin *et al*. (2009), who observed increased reducing sugar concentrations in okra under drought stress, which is a signal for water deficiency tolerance, it also reduces water potential to prevent oxidative losses and protein structure maintenance during water shortage. The significant increase in soluble protein content of salt-stressed tomato (BF1 and UC82B genotypes) treated with gibberellic acid and *B. subtilis* (Fig. 3) may be attributed to the production of some proteins in response to salt stress, which is deducted by gibberellic acid. Metwali *et al*. (2015) reported a similar

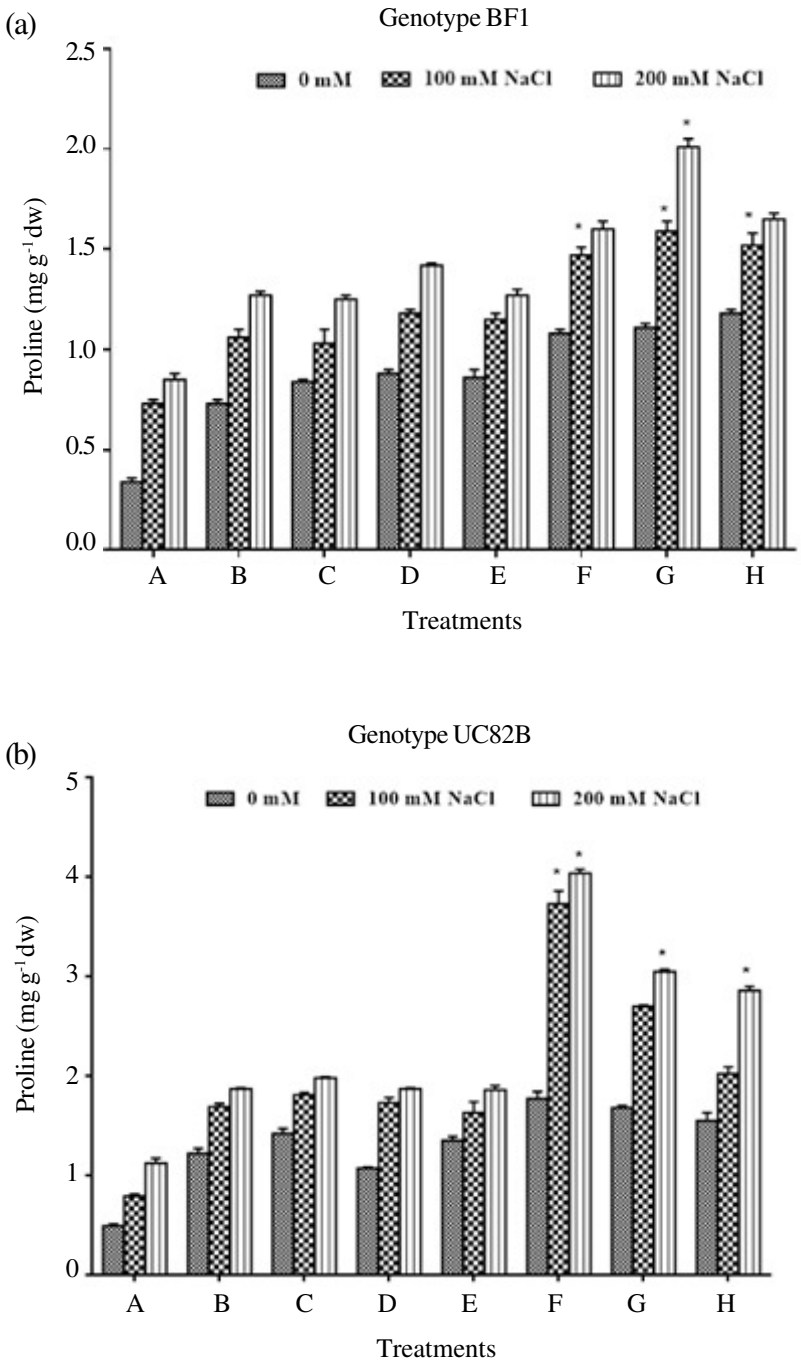


Figure 1. Proline accumulation of salt-stressed tomato genotypes (a) BF1and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

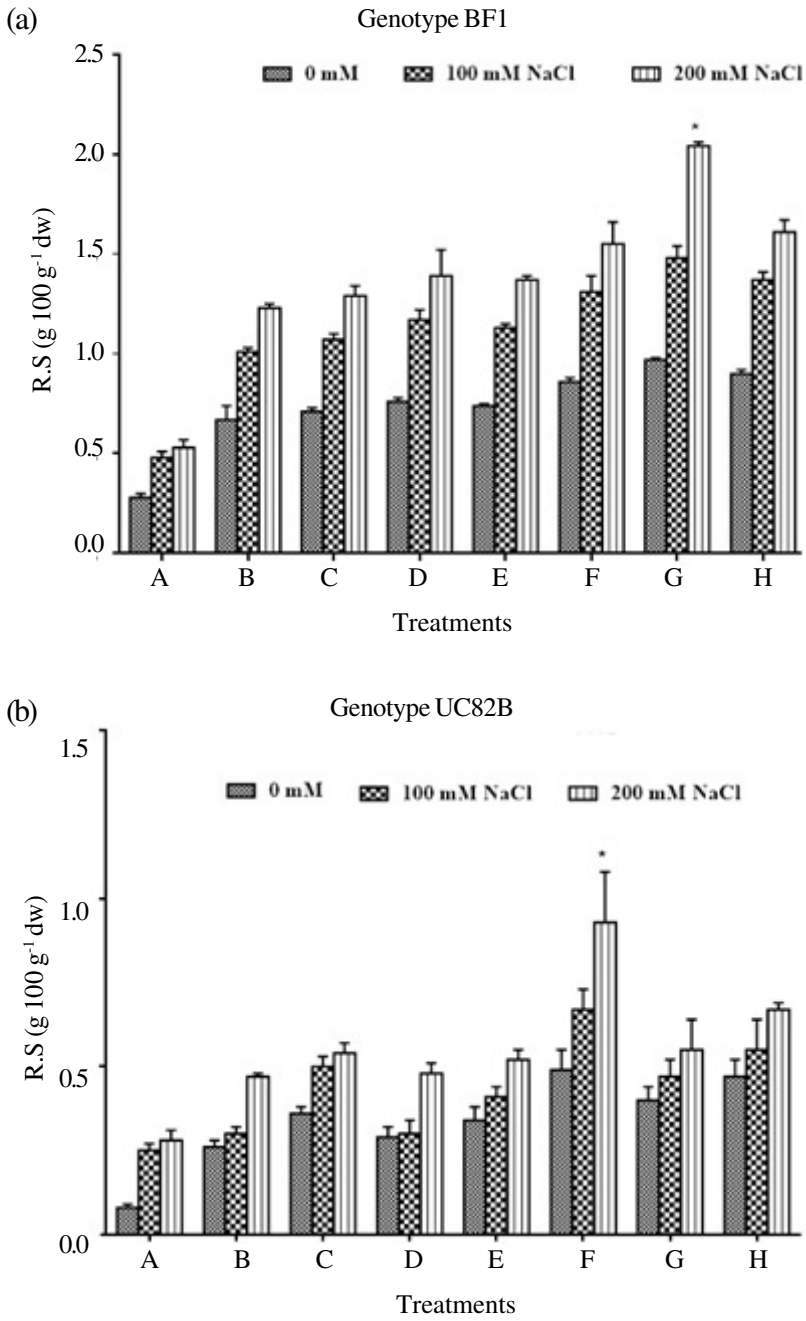


Figure 2. Reducing sugar accumulation of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

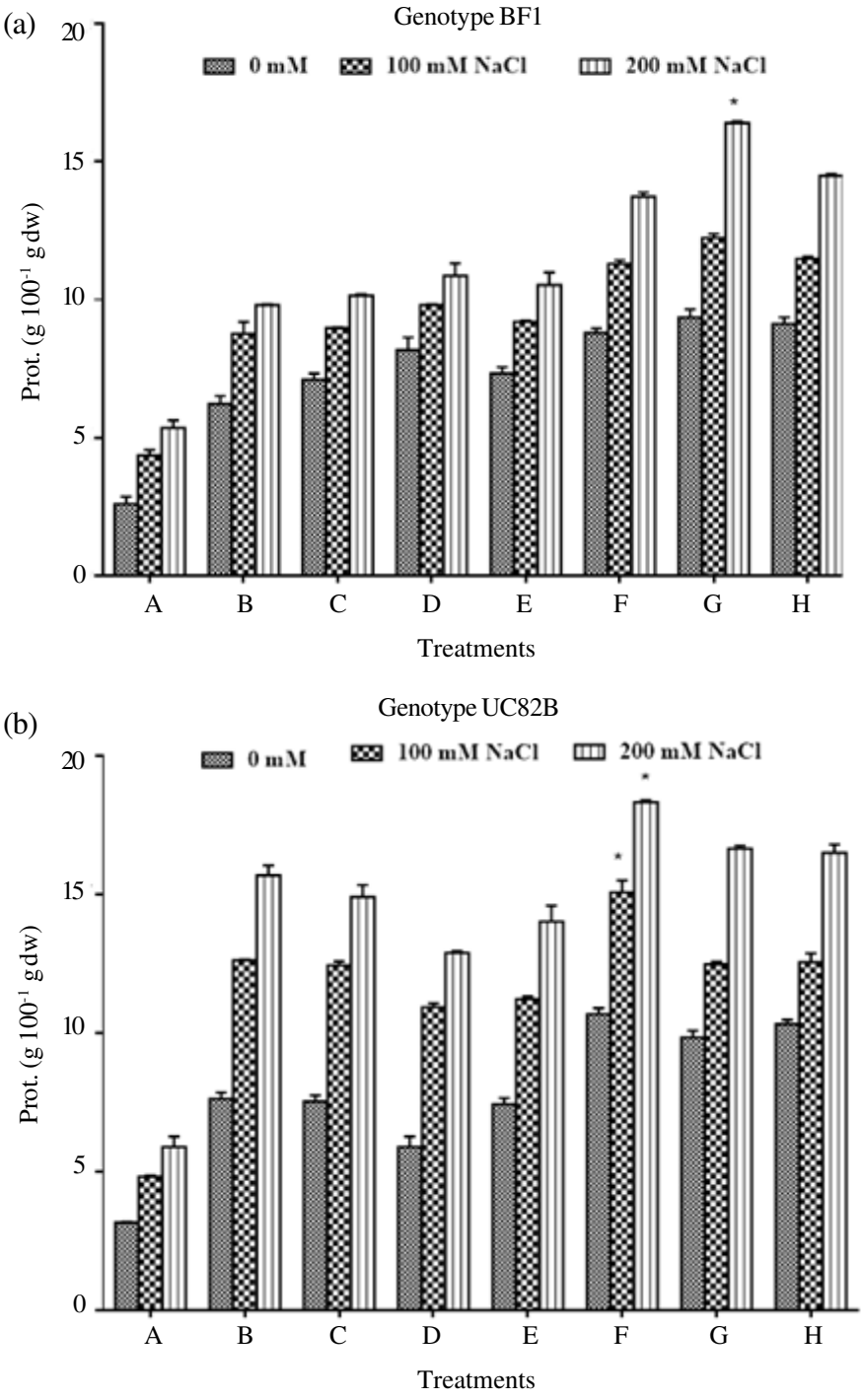


Figure 3. Soluble protein accumulation of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) relatives to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

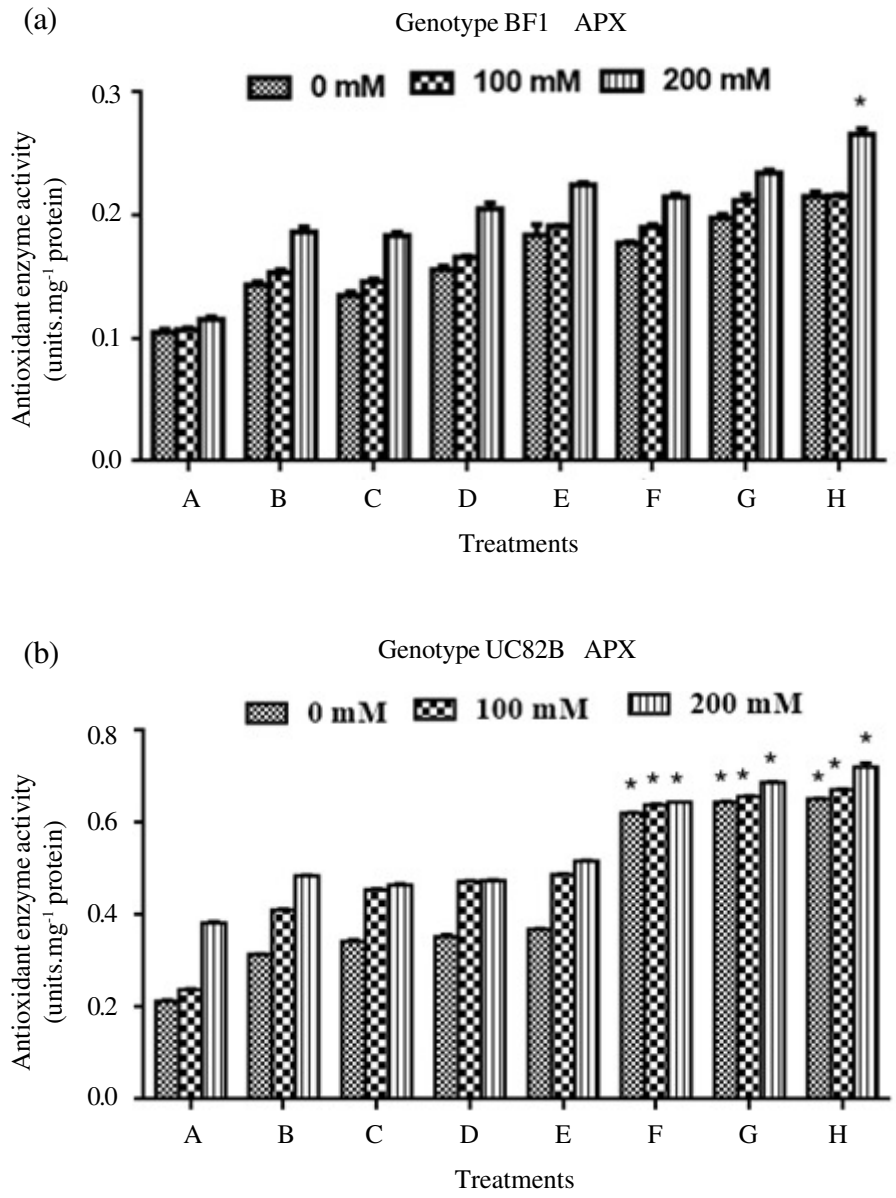


Figure 4. Ascorbate peroxide (APX) activity of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

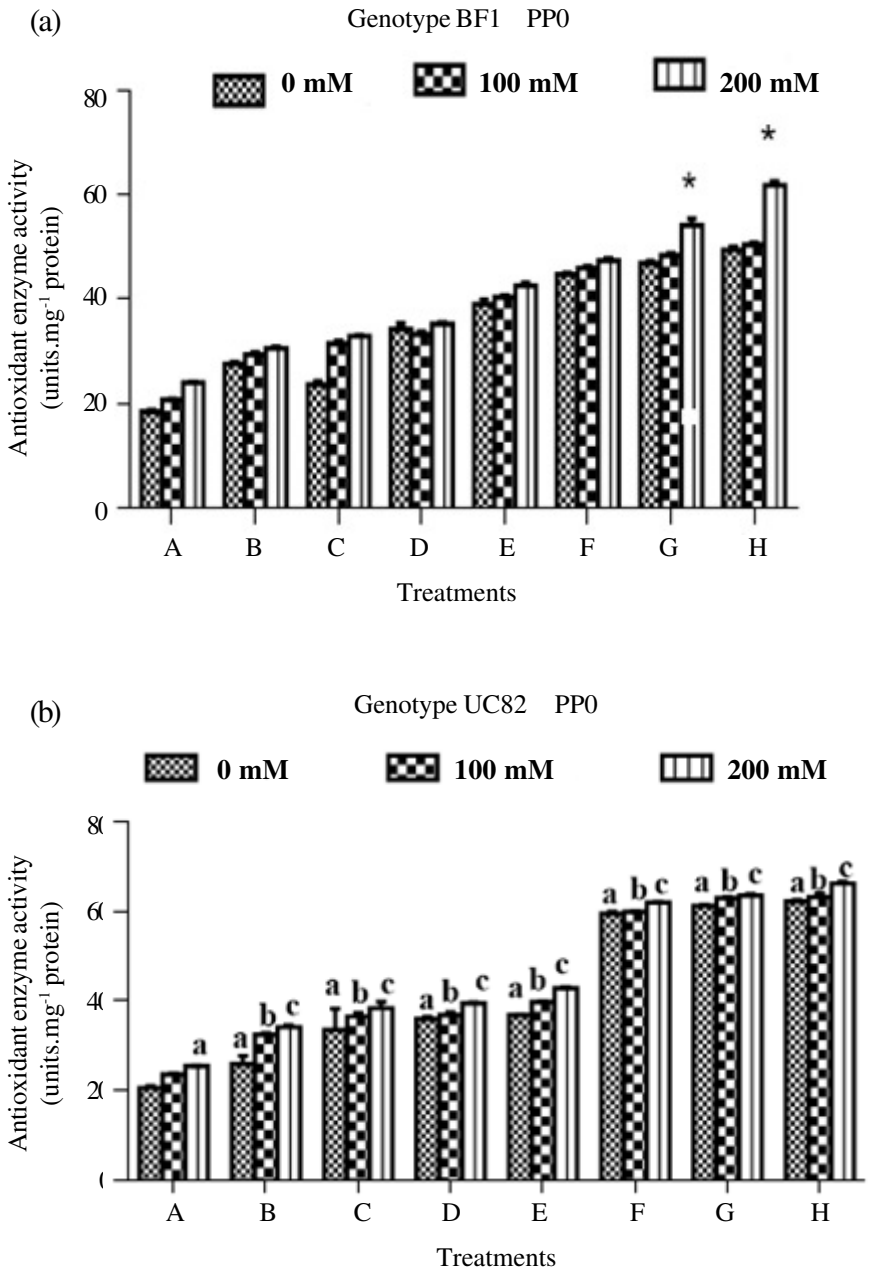


Figure 5. Polyphenol oxidase (PPO) activity of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) relatives to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

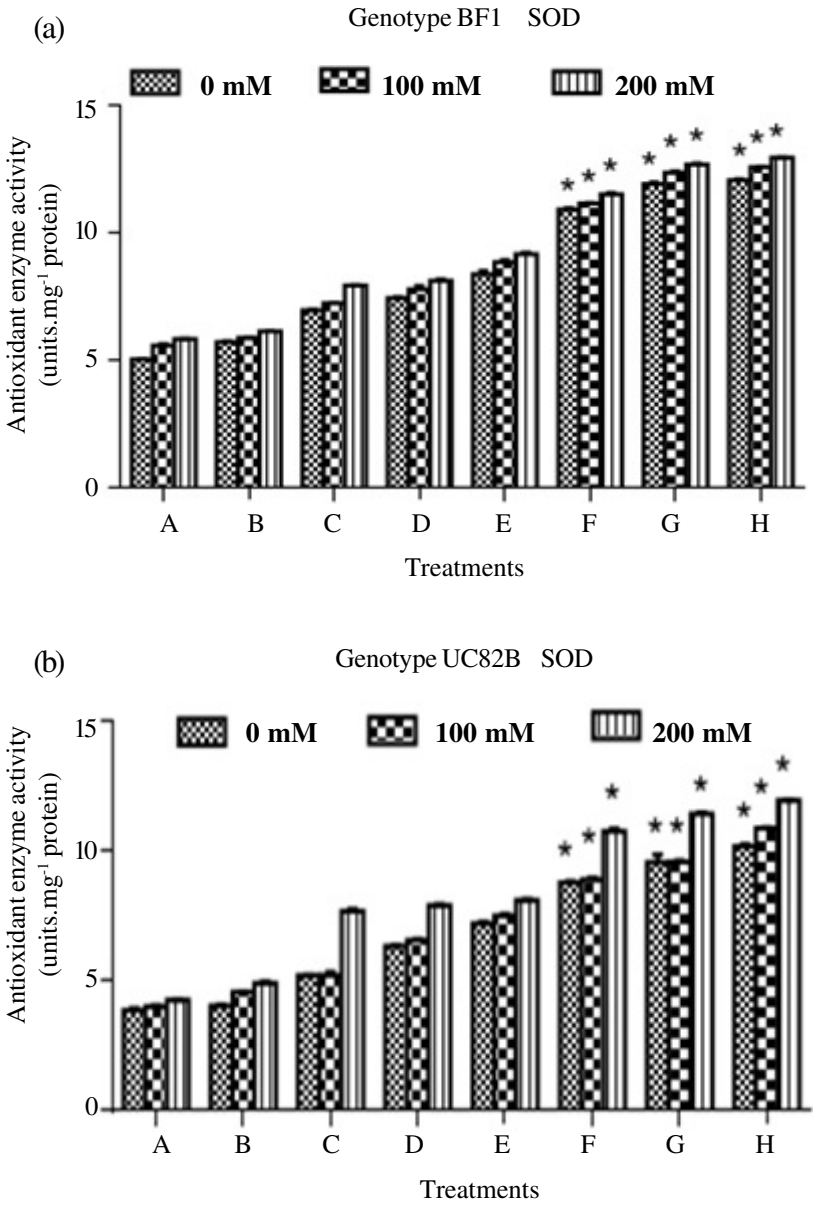


Figure 6. Superoxide dismutase (SOD) activity of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

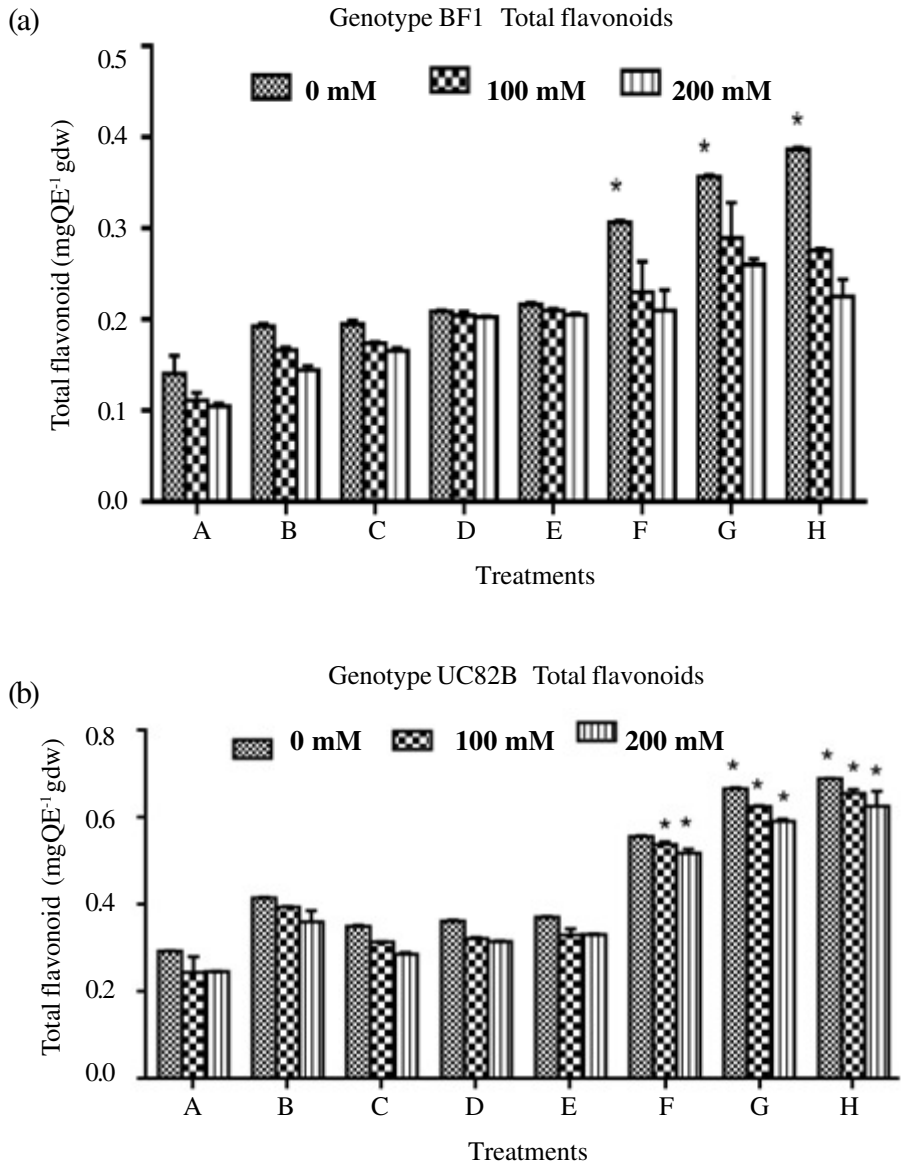


Figure 7. Total flavonoids content of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) relatives to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

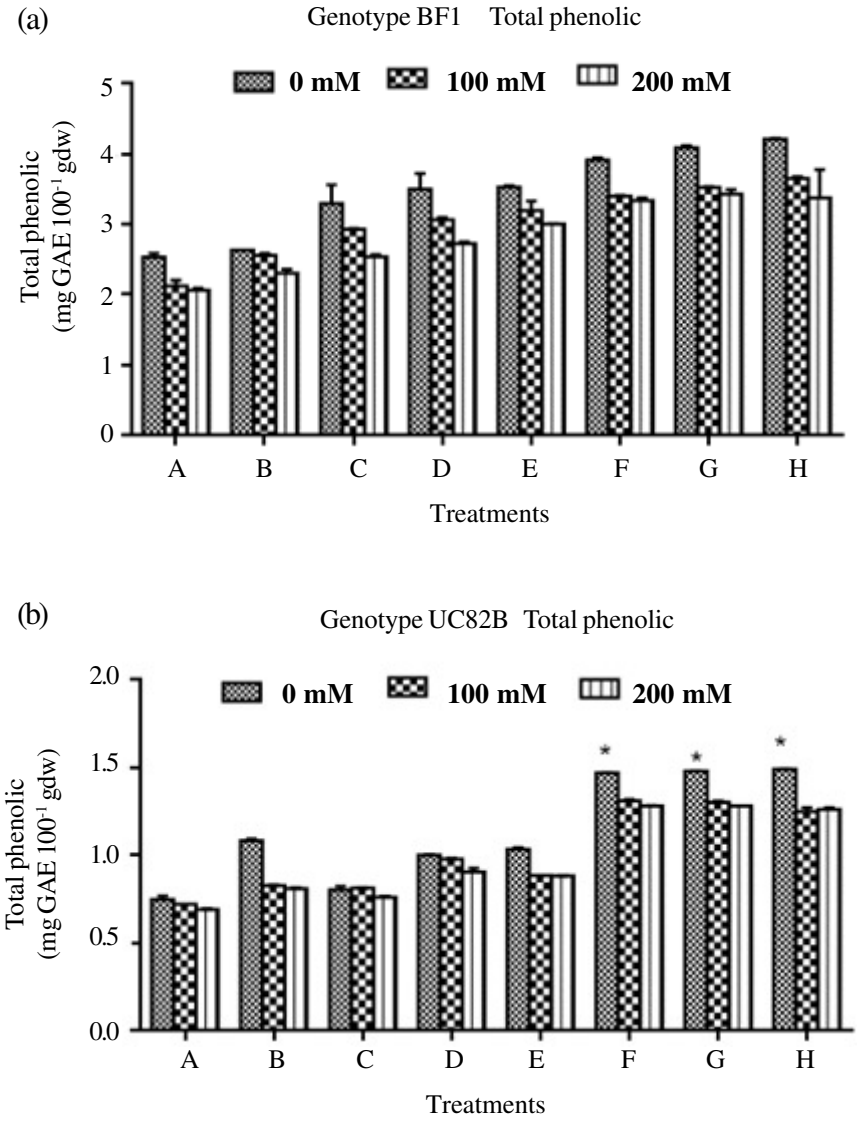


Figure 8. Total phenolic content of salt-stressed tomato genotypes (a) BF1 and (b) UC82B under the influence of gibberellic acid and *B. subtilis*. Values represent means ± standard error (n = 3). *Significant difference ($P < 0.05$) relatives to the salt-stressed control and the normal control groups. Where A = Controls (Normal control and Negative control groups), B, C, and D = 0.4, 0.5, and 0.6 mM of gibberellic acid respectively, E = Bacillus subtilis, F, G, and H = 0.4, 0.5, and 0.6 mM of gibberellic acid and Bacillus subtilis respectively.

increase in soluble protein content in faba bean plant growing under high-saline conditions.

The increased activity levels of superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) in salt-stressed tomato (BF1 and UC82B genotypes) under the combined effects of gibberellic acid and *B. subtilis* (Figs. 4,5, and 6) may be as a result of mechanisms of actions of both gibberellic acid and *B. subtilis*, that modulate reactive oxygen species through signal transduction. This corroborated with the findings of Mittova *et al*. (2003), who observed that plants defend themselves against reactive oxygen species by induction of activities of certain anti-oxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, with phytohormones application in respect to salt tolerance.

## CONCLUSION

Pretreatment of tomato seeds with gibberellic acid ($GA_3$) and seedlings inoculation with *B. subtilis* individually or in combination contribute to salt stress tolerance in the two tomato (BF1 and UC82B) genotypes. This is evident with increase photosynthetic pigments, mineral concentrations, and enhance synthesis of proline, reducing sugar, soluble protein and modulation of antioxidant enzymes defense component and antioxidant potential of tomato plant. Gibberellic acid and *B. subtilis* reduce the effects of salinity stress on the two (BF1 and UC82B) genotypes of the tomato plant. Hence, they serve as alternatives, methods for averting food insecurity worldwide. Therefore, the antioxidative defence system in the salt-stressed tomato genotypes improved by the synergistic effects of gibberellic acid and *B. subtilis*.

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