Arbuscular mycorrhizal fungi (AMF) association increases plant stress tolerance. This study aimed to determine the mitigation effect of AMF on the growth and metabolic changes of cucumbers under adverse impact of salt stress. Salinity reduced the water content and synthesis of pigments. However, AMF inoculation ameliorated negative effects by enhancing the biomass, synthesis of pigments, activity of antioxidant enzymes, including superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, and the content of ascorbic acid, which might be the result of lower level lipid peroxidation and electrolyte leakage. An accumulation of phenols and proline in AMF-inoculated plants also mediated the elimination of superoxide radicals. In addition, jasmonic acid, salicylic acid and several important mineral elements (K, Ca, Mg, Zn, Fe, Mn and Cu) were enhanced with significant reductions in the uptake of deleterious ions like Na+. These results suggested that AMF can protect cucumber growth from salt stress.

1. Introduction

Plants often encounter several environmental stresses that result in significant unfavourable changes in growth and metabolism, which ultimately affects the yield of plants. Among these stress factors, soil salinity is a crucial damaging factor for plant growth and development (Hashem et al., 2014, 2016a). Particularly, high salinity in the arid and semiarid regions of the world is a major cause of crop damage and yield loss. Anthropogenic activities, including the excessive use of saline water for irrigation and low rainfall due to climatic changes, also convert fertile arable land into salt-affected waste lands, and nearly 7% of agricultural land area is affected by salinity (Ruiz-Lozano et al., 2012). An increasing rate of salinity creates osmotic and ionic stress in plants and hampers plant growth by affecting their physiological and biochemical homeostasis via negative impacts on photosynthesis, protein synthesis, enzyme activity and mineral nutrition (Iqbal et al., 2015; Hashem et al., 2016a), which retards both the growth and yield of many vegetables, including cucumbers (Tejera et al., 2004; Gamalero et al., 2010; Balliu et al., 2015). Additionally, it poses major threats to all metabolic pathways through excessive generation of toxic reactive oxygen species (ROS), which damage the structural and functional integrity of several key molecules, including proteins and nucleic acids. ROS (superoxide ions, hydrogen peroxide, hydroxyl and peroxide radicals) induce oxidative stress (Qun et al., 2007; Kohler et al., 2009), reduce membrane permeability by affecting the polyunsaturated lipid component and increase cellular electro-leakage (Alqarawi et al., 2014a, 2014b).

To overcome the deleterious effect of salinity on growth, a series of tolerance mechanisms are initiated to maintain the growth and development of plants. The up-regulation of the antioxidant system, greater accumulation of compatible osmolytes and the
efficient compartmentalization of excessive toxic ions into the vacuole are considered important tolerance strategies (Hashem et al., 2015, 2016a). The antioxidant defence system comprises both enzymatic and non-enzymatic components, which protect plants from salinity stress by eliminating excess accumulated ROS. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR), which are intricate electron donors during the enzymatic neutralization of ROS, while the non-enzymatic antioxidants include ascorbic acid and glutathione, which are involved in stress tolerance (Saqib et al., 2008; Velarde-Buendia et al., 2012; Abd_Allah et al., 2017). The greater synthesis and accumulation of compatible osmolytes like free proline, glycine betaine, soluble sugars and amino acids under stressed conditions stimulate osmoregulation to maintain the cellular tissue water content, thereby helping plants to maintain growth (Khan et al., 2014; Hashem et al., 2015; Mo et al., 2016). Plants exposed to salt stress usually take up ions like sodium, chloride and potassium from the growth media, and the excess sodium is compartmentalized into the vacuole, transported by the apoplastic pathway or excluded from the tissues (Khan et al., 2014).

Arbuscular mycorrhiza fungi (AMF) are beneficial fungal organisms that share symbiotic association with many land plants. AMF have the potential to improve soil characteristics, thereby promoting plant growth in normal and stressful environments (Gamalero et al., 2010; Navarro et al., 2014; Alqarawi et al., 2014a, 2014b). AMF colonization enhances plant growth and vigour (Tang et al., 2009; Yang et al., 2015; Mo et al., 2016) and changes the morphological, nutritional and physiological levels of plants to improve resistance against different abiotic stresses (Alqarawi et al., 2014a, 2014b; Hashem et al., 2015). AMF inoculation protects O. basilicum against salinity stress by improving mineral uptake, chlorophyll synthesis and water use efficiency (Shekooefeh et al., 2012). Tomato plants inoculated with AMF show an increase in the leaf area, nitrogen, potassium, calcium and phosphorous contents to enhance the plant growth rate compared to controls (Balliuet et al., 2015).

Cucumber (Cucumis sativus L.) belongs to the family Cucurbitaceae, which is an important cash crop worldwide and is mostly used in salad. The cucumber is an energetic vegetable and is a rich source of vitamin K. Cucumber cultivation is affected by soil salinity (Kirnak, 2006). The inoculation of AMF in cucumber plants can enhance antioxidant metabolism, nutrient uptake and osmotic regulation under saline conditions. Thus, the present study was conducted to analyse the impact of AMF on the growth, physiological and biochemical attributes of cucumber plants and their mitigating role against the deleterious effects of salt stress.

2. Materials and methods

2.1. Arbuscular mycorrhizal inoculum

The AMF (Claroideoglomus etunicatum [syn. Glomus etunicatum]; Rhizophagus intraradices [syn. Glomus intraradices]; and Funneliformis mosseae [syn. Glomus mosseae]) used in the current experiment were isolated previously (Hashem et al., 2016a, 2016b) from the rhizosphere of the Talh tree (Acacia gerrardii) grown in a salt marsh habitat in the Riyadh region of Saudi Arabia per the method of Daniels and Skipper (1982) and Utobo et al. (2011). The identification of AMF was carried out according to the description of subcellular asexual spore structures provided by the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (INVAM, 2014). The morphological characterizations of the experimental AMF used in the current study were described in detail with the help of illustrative pictures in our previous study (Hashem et al., 2016a, 2016b). The propagation of the AMF inoculum was carried out by the trap culture protocol using corn plants (Zea mays L.) as the host, and the infected roots, hyphae, spores, and substrates were collected. In this protocol, single spores of each AMF isolate were inoculated on autoclaved sand (121 °C for 3 separated times) as the culture bed in plastic pots (25 cm diameter), and sorghum seeds (0.5% [v/v] NaCl used for seed surface sterilization) were sown in the pots (five seeds/pot). The pots were incubated in a plant growth chamber at 27 ± 1 °C with an 18 h photoperiod, 750 μmol m⁻² s⁻¹ photosynthetic photon flux density, and 70–75% relative humidity for 3 months. Half-strength Hoagland’s solution was used to irrigate the pots. The trap culture was used as the mycorrhizal inoculum and was added to the experimental soil as 25 g of trap soil culture (approx. 150 spores/g trap soil)/pot. Soil that was not inoculated with mycorrhiza served as the control.

2.2. Plant growth with salt and AMF treatments

Certified cucumber seeds (Cucumis sativus, cv. Dasher II), which were the product of Seminis Co, USA (http://www.seminis-us.com/product/dasher-ii/73), were germinated on a wetted blotter in Petri dishes at 26 °C in the dark for three days. Healthy and uniformly sized seedlings were selected and transplanted into plastic pots (25 cm diameter, one seedling/pot) containing autoclaved peat, perlite and sand (1:1:1, v/v/v). Plants were maintained one week after transplantation in the growth chamber (16 h light/8 h dark) at 25 ± 1 °C. The plants were irrigated daily (25 ml/pot) with Hoagland nutrient solution (Hoagland and Arnon 1950). The pots were divided into four groups: (A) plant control (no treatments); (B) plants sown in pots inoculated with AMF; (C) plants stressed with 200 mM NaCl in the absence of AMF; and (D) plants stressed with 200 mM NaCl in the presence of AMF. The salt concentration was increased gradually (25 mM NaCl/day) until 200 mM NaCl to adapt the plants before being salt treatment. The experiment was carried in three biological replicates with completely randomized design. The mycorrhizal inoculum was added to the experimental soil as 25 g of trap soil culture as described above (approx. 150 spores/g trap soil)/pot and without AMF used as the soil control. The plants were allowed to grow in the growth chamber for more two months, and root samples were collected to determine the mycorrhizal root colonization. Similarly, the third leaves of cucumber plants were collected and stored at −80 °C until further use for biochemical analysis.

2.3. Determination of arbuscular mycorrhizal colonization

The roots of the cucumber were gently separated and fixed in FAA (formalin/acetic acid/alcohol, v/v/v) solution until they were stained according to the protocol of Phillips and Hayman (1970) and Koske and Gemma (1989) using trypan blue in lactophenol. To determine the arbuscular mycorrhizal colonization of the cucumber roots, the stained root segments (one cm in length, 50 segments used as replicate for each sample) were observed under a digital computerized microscope (model DP-72, Olympus) at 20× magnification. The presence of mycelia, vesicles and arbuscules was recorded and analysed to assess the structural colonization as described by Giovannetti and Mosse (1980) according to the following formula:

\[ \% \text{AMF Colonization} = \frac{\text{Total numbers of AM positive segments}}{\text{Total number of segment studied}} \times 100 \]
2.4. Determination of photosynthetic pigments and stomatal conductance

The photosynthetic pigments were estimated in the leaves in dimethyl sulfoxide (DMSO) as described by Hiscox and Israelstam (1979). Absorbance was determined at 480, 510, 645, and 663 nm in a UV/VIS spectrophotometer (T80, PG Instruments Ltd, USA). Stomatal conductance in each treatment was recorded in fully expanded leaves using an infra-red gas analyser (CID-340, Photosynthesis system, Bio-Science, USA).

2.5. Determination of leaf water content

Leaf discs were punched from each treated plant, and their fresh weight was determined. The same leaf discs were floated on water for 4 h, and turgid weight was recorded after the samples were dried in an oven at 85 °C (Smart and Bingham 1974). Calculation of the leaf water content was done using the following formula:

\[
\text{RWC} = \frac{\text{Fresh weight – Dry weight}}{\text{Turgid weight – Dry weight}}
\]

2.6. Determination of membrane stability index, lipid peroxidation and hydrogen peroxide

The method of Sairam et al. (1997) was employed for determining the membrane stability index (MSI). With this method, 0.1 g of fresh leaf tissue was taken in two separate sets of test tubes containing 10 ml of double distilled water. One set was kept in a water bath for half an hour at 40 °C, and the electric conductivity (EC) was recorded (C1), while another set was kept in a water bath at boiling (100 °C), and the EC was recorded (C2). The MSI was calculated per the formula:

\[
\text{MSI} = \left[1 - \frac{C1}{C2}\right] \times 100
\]

2.7. Determination of proline and total phenols

For estimation of proline, 0.5 g of plant tissue was extracted in 3% (w/v) sulfosalicylic acid, and the homogenate was subjected to centrifugation at 12,000 g for 15 min. The supernatant (0.5 ml) was mixed with an equal volume of potassium phosphate buffer (pH 7.0) and potassium iodide. Samples were vortexed, and the absorbance was read at 390 nm to measure the hydrogen peroxide (Velikova et al., 2000).

For estimation of proline, 0.5 g of plant tissue was extracted in 80% aqueous acetone (4:1, v/v) containing butylated hydroxy toluene (10 mg/l) and were purified using EtOAc and NaHCO3 (Kusaba et al., 1998) to measure the endogenous plant growth regulators. Quantification of abscisic acid (ABA) was done as described by Kamboj et al. (1999) and calculated using a standard curve of ABA. For salicylic acid estimation, the extracts were vacuum dried at room temperature, and the concentration of SA was quantified per the method of Siegrist et al. (2000) using HPLC equipped with a fluorescence detector (LC-2010 AHT, SHIMADZU, Japan). The extraction and quantitative estimation of JA was carried out using an HPLC (Agilent 1100 HPLC system; Agilent Technologies, Böblingen, Germany) with a Dionex column as described by Kramell et al. (1999) and Pellegrini et al. (2013).JA was extracted from frozen fresh leaf samples (250 mg) using ethyl acetate at 4 °C, and the extract was kept at 4 °C overnight. The samples were centrifugation for 10 min at 10,000 rpm at 4 °C. The organic extract phase was shocked with acidified water, and the aqueous phase was separated using a separation funnel and analysed immediately using HPLC with a Dionex column (Acclaim 120, C18, 5 μm particle size, 46 mm internal diameter × 150 mm length). The detection of JA was carried out at 210 nm. The endogenous JA was quantified using the peak area of the standard JA.

2.8. Assay of antioxidant enzymes and ascorbic acid content

Fresh leaves (5 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polystyrene pyrrolidine. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C, and the supernatant was used to assay the enzyme activity. Protein in the enzyme extract was estimated according to Lowry et al. (1951). Superoxide dismutase (SOD, EC 1.15.1.1) was estimated according to Bayer and Fridovich (1987) following the photoreduction of nitroblue tetrazolium (NBT). The activity of SOD was expressed as enzyme units (EU) mg⁻¹ protein, and one unit of SOD was defined as the amount of protein causing a 50% decrease in the SOD-inhibitable NBT reduction. Catalase (CAT, EC 1.11.1.6) activity was assayed by the method described by Luck (1974). The CAT activity was calculated using an extinction coefficient of 36 × 10⁻³ M⁻¹ cm⁻¹ and expressed as EU mg⁻¹ protein. For determination of the ascorbate peroxidase (APX, EC 1.11.1.11) activity, the method of Nakano and Asada (1981) was followed. The assay mixture contained 0.1 ml of enzyme extract, 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H2O2 and 1 ml of potassium phosphate buffer (pH 7.0). The decrease in the absorbance of ascorbate was taken at 290 nm, and the activity was expressed as EU mg⁻¹ protein. The glutathione reductase (GR, EC 1.6.4.2) activity was assayed according to Carlberg and Mannervik (1985). Decreases in the absorbance were read at 340 nm for 2 min. The activity of GR was calculated using an extinction coefficient of 0.12 mM NADPH of 6.2 mM⁻¹ cm⁻¹ and expressed as the EU mg⁻¹ protein. Ascorbate was extracted from fresh leaves (0.8 g) in 3 ml ice-cold meta-phosphoric acid (5%) containing 1 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 20 min, and the supernatant was used for ascorbate analysis (Huang et al., 2005).

2.9. Extraction and quantification of plant growth regulators

The leaves were extracted in 80% aqueous acetone (4:1, v/v) containing butylated hydroxy toluene (10 mg/l) and were purified using EtOAc and NaHCO3 (Kusaba et al., 1998) to measure the endogenous plant growth regulators. Quantification of abscisic acid (ABA) was done as described by Kamboj et al. (1999) and calculated using a standard curve of ABA. For salicylic acid estimation, the extracts were vacuum dried at room temperature, and the concentration of SA was quantified per the method of Siegrist et al. (2000) using HPLC equipped with a fluorescence detector (LC-2010 AHT, SHIMADZU, Japan). The extraction and quantitative estimation of JA was carried out using an HPLC (Agilent 1100 HPLC system; Agilent Technologies, Böblingen, Germany) with a Dionex column as described by Kramell et al. (1999) and Pellegrini et al. (2013). JA was extracted from frozen fresh leaf samples (250 mg) using ethyl acetate at 4 °C, and the extract was kept at 4 °C overnight. The samples were centrifugation for 10 min at 10,000 rpm at 4 °C. The organic extract phase was shocked with acidified water, and the aqueous phase was separated using a separation funnel and analysed immediately using HPLC with a Dionex column (Acclaim 120, C18, 5 μm particle size, 46 mm internal diameter × 150 mm length). The detection of JA was carried out at 210 nm. The endogenous JA was quantified using the peak area of the standard JA.

2.10. Estimation of ions

Oven dried (110 °C) leaf and root samples were acid digested, and Na⁺, K⁺, Mg²⁺ and Ca²⁺ were estimated according to the method of Wolf (1982) using a flame photometer (Jenway Flame
Photometer, Bibby Scientific Ltd-Stone-Staffs-St15 UK. For estimation of Mn, Fe, Cu and Zn, 1 M hydrochloric acid was added, and the digested dried leaf powder and their elemental (Mn, Fe, Cu and Zn) concentration were determined by atomic absorption spectrophotometer.

2.11. Statistical analysis

The experiments were repeated three times and completely randomized. Statistical significance between the control and treatments were calculated by one-way ANOVA performed by Duncan’s Multiple Range Test (SPSS-21 software), and the differences in the means were determined by the least significant differences (LSD) (p = 0.05) test.

3. Results

Root colonization by the arbuscular mycorrhizal fungi was observed with most different structural colonizations in roots of cucumber at control treatment in high rates (Fig. 1A–D). Soil salinity (200 mM NaCl) reduced the AMF colonization in cucumber roots (Table 1). The number of mycelia, vesicles and arbuscule formation were reduced to 55.01%, 61.06% and 71.69%, respectively, due to the effect of salinity. The intensity of the structural colonization of AMF in cucumber plants was categorized as poor (P), medium (M) and abundant to determine the toxic effect of salinity on AMF growth (Table 2). Mycelial colonization of 17.21% to 56.77% was observed in the roots, while salt stress reduced the AMF growth, which resulted in a maximum of 5.21% colonization. Similar trends were noted in arbuscule formation, but development of the vesicle was higher (28.34%) in salt stress than in the control.

Plant growth is directly and indirectly regulated by photosynthetic pigments. AMF colonization supported and increased the pigment synthesis of chlorophyll and carotenoids. Salinity affected the cucumber plants, which showed less chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Table 3). However, the inoculation of AMF mitigated the negative effect by enhancing chlorophyll a (27.40%), chlorophyll b (17.51%), total chlorophyll (26.74%) and carotenoid (42.32%) compared to the NaCl-stressed seedlings. AMF association in cucumber plants altered the stomatal function. Salt stress decreased the stomatal conductance, while AMF significantly enhanced the stomatal conductance in leaves in the salinity condition.

Osmolyte production during stress conditions is a plant protective mechanism against stress. The accumulation of proline and total phenol was increased in the individual or combined effect of AMF and salt stress (Table 4). Salinity enhanced proline synthesis in the control and AMF treatment. However, AMF inoculation increased the proline by 24.54% and 83.01% in cucumber plants in the control and salt stress condition, respectively. A similar trend was observed in total phenol synthesis in plants exposed to salt and AMF treatments. NaCl caused an increase in phenol (59.58%) in plants, and this content was further enhanced.
AMF inoculation. The water content in plants during the salt stress condition was drastically reduced (50.03%), and AMF association was favourable in plants by enhancing the relative water content to mitigate the salt stress.

The over-synthesis of ROS during stress conditions is toxic to other metabolic processes in plants. Salt-induced oxidative stress in cucumbers was measured by hydrogen peroxide production, electrolytic leakage and lipid peroxidation (Fig. 2A–C). The results of our study showed that there was a higher accumulation of hydrogen peroxide (56.04%), electrolytic leakage (47.18%) and lipid peroxidation (81.41%) in salt-affected plants when compared to the control. AMF helped to ameliorate the salt stress effects in cucumbers compared to NaCl-stressed plants. AMF helped to ameliorate the salt stress effects in plants by reducing AMF-acquired systemic resistance in C. sativus L. under salt stress, and the Pearson's correlation coefficients between AMF treatment and oxidative stress metabolism are presented in Table 5. The mycelium had a negative correlation with the vesicles, H₂O₂, EL, proline, MDA and TPC, while a positive correlation was recorded in arbuscules, StCond, RWC and AsA. The vesicles showed a significant negative correlation with arbuscules, StCond, RWC, and AsA, while there was a positive correlation with H₂O₂, EL, proline, MDA and TPC. Hydrogen peroxide was recorded as having a positive correlation with EL, proline content, MDA and TPC, while there was a negative correlation recorded with StCond, RWC and AsA. StCond showed a negative correlation with EL, proline, MDA and TPC, while a positive correlation was recorded with RWC and AsA. Electrical conductivity (EC) was noted as having a positive and significant correlation with proline, MDA and TPC, while a negative effect on RWC and AsA was shown. However, a positive correlation of RWC was observed in AsA, while a negative correlation was recorded in the proline results, MDA and TPC. The proline content had a significant positive effect with MDA and TPC, while a negative correlation was recorded for AsA. MDA had a negative correlation with AsA, while a positive response

Table 2

| Treatment   | Mycelium | Vesicles | Arbuscules |
|-------------|----------|----------|------------|
| AMF         | Poor     | Medium   | Abundant   |
|             | 17.21 ± 2.74⁴ | 13.61 ± 1.13³ | 56.77 ± 4.47⁴ |
| Salinity + AMF | 24.73 ± 3.32³ | 7.406 ± 1.13³ | 5.21 ± 0.53³ |
| LSD at: 0.05 | 5.026      | 3.772     | 34.816     |

Table 3

| Treatment   | Chl a (mg/g FW) | Chl b (mg/g FW) | Total Chl (mg/g FW) | Carotenoids (mg/g FW) | Stomatal conductance (mmol m⁻² s⁻¹) |
|-------------|-----------------|-----------------|---------------------|-----------------------|-------------------------------------|
| Control     | 0.873 ± 0.02b   | 0.438 ± 0.02b   | 1.606 ± 0.03b       | 0.2953 ± 0.01b        | 22.17 ± 0.63b                       |
| AMF         | 1.182 ± 0.02⁴   | 0.512 ± 0.00⁴   | 2.064 ± 0.01⁴       | 0.3693 ± 0.01d        | 39.08 ± 0.54d                       |
| Salinity    | 0.556 ± 0.01c   | 0.295 ± 0.00⁴   | 0.956 ± 0.02d       | 0.1046 ± 0.00d        | 6.94 ± 0.11c                        |
| Salinity + AMF | 0.766 ± 0.02⁴  | 0.358 ± 0.01⁴   | 1.305 ± 0.01c       | 0.1813 ± 0.01c        | 16.92 ± 0.36c                       |
| LSD at: 0.05 | 0.0504         | 0.0352          | 0.0544              | 0.0244                | 8.9451                              |

Table 4

| Treatment   | Proline (µmol/g FW) | Total phenols (mg/g FW) | RWC (%) |
|-------------|---------------------|-------------------------|---------|
| Control     | 3.75 ± 0.25⁴       | 1.991 ± 0.06⁴           | 92.99 ± 0.11a |
| AMF         | 4.97 ± 0.10c       | 2.304 ± 0.05c           | 95.70 ± 0.25⁴ |
| Salinity    | 17.09 ± 0.15b      | 4.926 ± 0.07⁴           | 46.46 ± 0.34⁴ |
| Salinity + AMF | 22.08 ± 0.40a     | 6.099 ± 0.06⁴           | 69.77 ± 0.61⁴ |
| LSD at: 0.05 | 0.5909           | 0.1511                  | 0.8721  |

Fig. 2. A-C: Effect of salinity (200 mM NaCl) on (A) hydrogen peroxide (µMol/ g DW), (B) electrolyte leakage (%) and (C) lipid peroxidation (malondialdehyde [MDA], µmol/g FW) in Cucumis sativus with and without AMF inoculation. Data presented are the means ± SE (n = 5). Data followed by different letters are significantly different at P < 0.05.
Table 5
Pearson's correlation coefficients between AMF colonization and oxidative stress attributes in cucumber plants.

|     | M     | V     | A     | H2O2  | StCond | EL    | RWC   | Proline | MDA   | AsA   | TPC   |
|-----|-------|-------|-------|-------|--------|-------|-------|---------|-------|-------|-------|
| M   | 1.00000 | -0.91030 | 0.91448 | 0.0117 | -0.97579 | 0.97116 | -0.96403 | 0.96260 | -0.96500 | -0.96139 | 0.95935 | -0.97674 |
|     |       |       |       |       |        |       |       |         |       |       |       |
| M   | 1.00000 | -0.93867 | 0.94101 | 0.0055 | -0.95737 | 0.97394 | 0.0009 | -0.99519 | 0.9604 | -0.99220 | 0.99304 | 0.98174 | -0.99707 | 0.99813 |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| V   | 1.00000 | -0.97579 | 0.94558 | 0.0044 | -0.97924 | 0.97882 | 0.0007 | 0.0010 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| A   | 1.00000 | -0.97537 | 0.97394 | 0.0009 | 0.0006 | 0.0007 | 0.0010 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| H2O2|       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| StCond | 1.00000 | -0.99857 | 0.99608 | -0.99596 | 0.9961 | 0.99395 | -0.99854 |       | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| EL  |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| RWC |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| Proline |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| MDA |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| AsA |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
|     |       |       |       |       |        |       |       |         |       |       |       |       |       |       |
| TPC |       |       |       |       |        |       |       |         |       |       |       |       |       |       |

M: Mycelium; V: Vesicles; A: Arbuscules; H2O2: Hydrogen peroxide; StCond: Stomatal conductance; EL: Electric conductivity; RWC: Relative water content; MDA: Malondialdehyde; AsA: Ascorbic acid; TPC: Total protein content.

Fig. 3. A-E: Effect of salinity (200 mM NaCl) on the activity of (A) SOD, (B) CAT (C) APX, (D) GR (U/mg protein) and the (E) AsA content (mg/g FW) in Cucumis sativus with and without AMF inoculation. Data presented are the means ± SE (n = 5). Data followed by different letters are significantly different at P < 0.05.
was recorded with TPC. The AsA content showed a negative correlation with TPC.

The expression of antioxidants in cucumber plants during the salt and AMF treatments are given in Fig. 3A–E. When compared to control plants, the AMF-treated plants showed higher SOD, CAT, APX and GR activity (6.87%, 28.83%, 34.10% and 26.17%, respectively). Salt stress also triggered these enzyme activities in plants. The combined effect of NaCl and AMF significantly accelerated the enzyme activities. AMF additionally induced an increase in SOD, CAT, APX and GR activities compared to salt-affected plants. AsA is a non-enzymatic antioxidant that significantly declined due to the effect of salt stress, and AMF helped to maintain the AsA content, which showed an increase of 32.27% over the control. The mitigation effect of AMF was expressed by enhancing the AsA content in salt-stressed plants.

The endogenous concentration of the plant stress hormones, such as abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA), were altered during salt stress and AMF treatment (Table 6).

Salt stress up-regulated the synthesis of ABA, JA and SA in plants, while AMF inoculation decreased the ABA and increased the JA and SA content in salt-affected plants.

Salt stress caused a significant reduction in the uptake of essential elements, such as potassium, calcium, magnesium, iron, zinc, manganese and copper and increased the sodium content in both leaf and root tissues (Tables 7 and 9). AMF inoculation increased the content of K, Ca, Mg, Fe, Zn, Mn and Cu by 23.09%, 36.92%, 23.11%, 5.85%, 18.96%, 6.77% and 25.32%, respectively, in leaves and by 29.77%, 48.93%, 30.97%, 7.9%, 28.10%, 14.98% and 30.99%, respectively, in root tissues. NaCl stressed plants showed declines in K, Ca, Mg, Fe, Zn, Mn and Cu compared to control plants. However, AMF ameliorated the stress on nutrition by improving the uptake of K (46.71%), Ca (49.50%), Mg (34.57%), Fe (19.29%), Zn (20.49%), Mn (33.28%) and Cu (24.71%) in salt-affected plants. A similar trend was observed in the nutritional uptake by roots under AMF and salt stress conditions. Specifically, AMF proved the mitigation effects by reducing the sodium (23%) content in leaves in NaCl-stressed plants.

The Pearson correlation coefficient between AMF colonization and the accumulation of elements in leaves are shown in Table 8. The mycelium showed a negative correlation for vesicles and Na+ ion concentration due to the effect of the significant positive correlation between arbuscules, K+, Ca2+, Mg2+, Fe2+, Zn2+, Mn2+ and Cu2+ ions in cucumber leaves. The statistical analysis showed a positive and significant correlation between vesicles and Na+ (0.938), while there was a negative correlation for arbuscules with the K+, Ca2+, Mg2+, Fe2+, Zn2+, Mn2+ and Cu2+ contents. Arbuscules showed a negative correlation with Na+, while there was a positive and significant correlation for K+, Ca2+, Mg2+, Fe2+, Zn2+, Mn2+ and Cu2+.

### Table 6

| Treatment     | ABA (ng/g FW) | JA (ng/g FW) | SA (ng/g FW) |
|---------------|---------------|--------------|--------------|
| Control       | 216.4 ± 2.99a | 1102.5 ± 7.73a | 136.1 ± 0.95a |
| AMF           | 133.9 ± 0.703a | 1552.1 ± 11.9a | 156.2 ± 3.02a |
| Salinity      | 779.7 ± 3.25a | 2494.0 ± 5.29b | 352.1 ± 3.24b |
| Salinity + AMF| 310.6 ± 1.06b | 2770.3 ± 33.6a | 470.2 ± 3.62a |
| LSD at: 0.05  | 53095         | 42553        | 67013        |

### Table 7

| Treatment     | Na+ (mg/g DW) | K+ (mg/g DW) | Ca2+ (mg/g DW) | Mg2+ (mg/g DW) | Fe2+ (mg/g DW) | Zn2+ (mg/g DW) | Mn2+ (mg/g DW) | Cu2+ (mg/g DW) |
|---------------|---------------|--------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Control       | 19.6 ± 0.75a  | 38.5 ± 0.92b | 1.933 ± 0.04a  | 1.533 ± 0.04a  | 114.2 ± 0.97b  | 39.4 ± 0.48b   | 187.8 ± 1.27b  | 8.52 ± 0.46b   |
| AMF           | 51.1 ± 0.43a  | 50.1 ± 0.58a | 0.606 ± 0.09a  | 0.203 ± 0.08a  | 121.3 ± 0.43a  | 48.6 ± 0.66a   | 201.4 ± 2.08a  | 11.41 ± 0.60a  |
| Salinity      | 44.3 ± 0.29a  | 15.9 ± 0.34a | 0.903 ± 0.01a  | 0.630 ± 0.02a  | 73.8 ± 0.81a   | 32.2 ± 0.59a   | 107.1 ± 4.41a  | 4.63 ± 0.27a   |
| Salinity + AMF| 25.5 ± 0.33a  | 29.8 ± 0.78a | 1.350 ± 0.07a  | 0.963 ± 0.05a  | 91.4 ± 0.36a   | 33.0 ± 0.95a   | 160.5 ± 1.44a  | 6.15 ± 0.14a   |
| LSD at: 0.05  | 1.1694        | 1.5997       | 0.1452         | 0.1356         | 1.6063         | 1.587          | 6.052          | 0.9478         |

### Table 8

Pearson’s correlation coefficients between AMF colonization and the accumulation of elements in cucumber leaves.

| M  | V  | A   | Na+ | K+  | Ca2+ | Mg2+ | Fe2+ | Zn2+ | Mn2+ | Cu2+ |
|----|----|-----|-----|-----|------|------|------|------|------|------|
| M  | 1.0000 | –0.91030 | 0.91448 | –0.97152 | 0.94855 | 0.97956 | 0.98779 | 0.96114 | 0.94761 | 0.93358 | 0.9605 |
| V  | 0.0117 | 0.0107 | 0.0012 | 0.0043 | 0.0006 | 0.0002 | 0.0002 | 0.0020 | 0.0065 | 0.0069 |
| A  | 0.0053 | 0.0056 | 0.0057 | 0.0017 | 0.0026 | 0.0038 | 0.0038 | 0.0003 | 0.0033 | 0.0020 |
| Na+| 1.0000 | 0.0015 | 0.0009 | 0.0025 | 0.94161 | 0.0003 | 0.0003 | 0.0002 | 0.0018 |
| K+ | 0.0000 | <0.0001 | 0.00012 | 0.00003 | 0.00001 | 0.00007 | 0.00007 | 0.000007 | 0.00005 |
| Ca2+| 1.0000 | 0.0004 | 0.0014 | <0.0001 | 0.00004 | 0.0001 | 0.00004 | 0.00004 | 0.00004 |
| Mg2+| 1.0000 | 0.0000 | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0000 | 0.00006 |
| Fe2+| 1.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Zn2+| 1.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Mn2+| 1.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Cu2+| 1.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

M: Mycelium; V: Vesicles; A: Arbuscules.
The Pearson's correlation coefficients between AMF colonization and the accumulation of elements in cucumber roots.

### Table 9

| Treatment | Na⁺ (mg/g DW) | K⁺ (mg/g DW) | Ca²⁺ (mg/g DW) | Mg²⁺ (mg/g DW) | Fe²⁺ (mg/g DW) | Zn²⁺ (mg/g DW) | Mn²⁺ (mg/g DW) | Cu²⁺ (mg/g DW) |
|-----------|---------------|--------------|----------------|---------------|---------------|---------------|---------------|---------------|
| Control   | 36.4 ± 0.37   | 51.8 ± 0.49  | 3.58 ± 0.19    | 2.786 ± 0.178 | 705.2 ± 2.679 | 21.23 ± 0.388 | 118.3 ± 1.098 | 6.01 ± 0.108  |
| AMF       | 41.3 ± 0.36   | 73.8 ± 0.75  | 7.01 ± 0.074   | 4.036 ± 0.044 | 765.7 ± 2.444 | 29.53 ± 0.322 | 139.1 ± 1.104 | 8.71 ± 0.19   |
| Salinity  | 68.7 ± 1.57   | 34.6 ± 1.24  | 1.78 ± 0.178   | 1.666 ± 0.076 | 113.8 ± 2.153 | 14.78 ± 0.468 | 61.2 ± 1.026  | 4.40 ± 0.32   |
| Salinity + AMF | 80.3 ± 0.74 | 41.5 ± 0.31  | 2.70 ± 0.084   | 1.996 ± 0.086 | 410.5 ± 0.894 | 17.93 ± 0.284 | 90.6 ± 1.38   | 5.01 ± 0.054  |

LSD at: 0.05 2.0967 1.8099 0.325 0.2363 4.9614 0.8542 2.6741 0.4618

Data presented are the means ± SE (n = 5). Data followed by different letters are significantly different at P < 0.05.

Similarly, Na⁺ had a negative correlation with other nutritional elements, including K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The quantity of K⁺ in leaves showed a positive correlation with Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The Ca²⁺ content was expressed as a positive relationship with Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. However, the accumulation of Mg²⁺ in leaves indicated a positive and significant correlation with Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The ion concentration of Fe²⁺ had a positive correlation with Zn²⁺, Mn²⁺ and Cu²⁺. The presence of Zn²⁺ in leaves showed a positive correlation with Mn²⁺ and Cu²⁺. In addition, the amount of Mn²⁺ showed a positive correlation with the concentration of Cu²⁺.

The Pearson’s correlation coefficients between AMF colonization and the accumulation of elements in the roots are described in Table 10. The growth of the mycelium had a negative correlation with vesicles and Na⁺, while there was a positive correlation with the formation of arbuscules and the concentration of K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The vesicles showed a positive and significant correlation with the accumulation of Na⁺ and Cu²⁺, while a negative effect was recorded for arbuscule formation and the concentration of K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Mn²⁺. In the statistical analysis, arbuscules showed a positive and significant correlation with the K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Mn²⁺ contents, while there was a negative correlation with the accumulation of Na⁺. The presence of Na⁺ in roots showed a negative and significant correlation with the K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺ contents. The accumulation of K⁺ in cucumber roots had a positive correlation with the concentration of Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The Ca²⁺ content in roots showed a significant positive correlation with Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The presence of Mg²⁺ in the roots indicated a positive correlation with the concentration of Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺. The Fe²⁺ ion concentration had a positive correlation with the accumulation of Zn²⁺, Mn²⁺ and Cu²⁺ in cucumber roots. However, the Zn²⁺ content showed significant and positive correlation with Mn²⁺ and Cu²⁺ concentration. In addition, the quantity of Mn²⁺ was recorded as a positive and significant correlation with the amount of Cu²⁺ in roots.

### 4. Discussion

The unfavourable climate changes in the contemporary era are a major challenge to improving crop growth and yield despite environmental stresses including soil salinity. The exploitation of the desirable genetic potential of plant species for crop cultivation without proper management techniques is difficult under unfavourable conditions (Hosseini et al., 2017; Egamberdieva et al., 2017). In the present study, AMF was tested for its salt stress amelioration potential in cucumber plants. Although salt stress inhibited the mycelial growth and formation of arbuscules in cucumber roots, it stimulated vesicle formation. Shekoofeh et al. (2012) and Alqarawi et al. (2014) reported that AMF colonization and spore populations declined due to the salinity in soil and suggested that the greater accumulation of toxic sodium ions in AMF reduced their growth and survival. The uptake of excess toxic ion transport into roots resulted in a decreased rate of cell division and cell elongation (Hasanuzzaman et al., 2013). Reduced cell growth under stress probably occurs due to the reduced uptake of water, and AMF colonization significantly increased water...
uptake by the roots, improving root attributes. The plant growth-promoting effect of AMF and their subsequent mitigation effects against salinity stress have been studied by others (Tang et al., 2009; Gamalerio et al., 2010; Alqarawi et al., 2014a). Aroca et al. (2013) demonstrated the amelioration effect of AMF against the deleterious effects of salinity in lettuce. The evidence for enhanced nutrient uptake in plants through the actions of AMF confirms the resilience of plants to abiotic stress conditions (Kohler et al., 2009; Tang et al., 2009). AMF triggered the growth promotion of *C. sativus*, which was correlated with the increased synthesis of photosynthetic pigments and a possible impact of AMF in protecting the structure and function of the pigment-protein complex (Hajiboland et al., 2010; Porcel et al., 2015; Yang et al., 2015) which is, however, drastically affected by salinity (Chaves et al. 2009; Aroca et al., 2013). High salinity hampers the *de novo* synthesis of proteins and chlorophyll components (El-Tayeb 2005). Several studies have documented reduced chlorophyll pigments due to salinity (Sudhir et al., 2005; Neelam and Subramanyam, 2013; Khan et al., 2014; Hashem et al., 2015, Yang et al., 2015). In the present study, AMF inoculation mitigated the effects on photosynthetic pigments in salt-stressed *C. sativus*. Our results corroborate with other crop plants, such as *Solanum lycopersicum* L., lettuce and *Panicum turgidum* subjected to salt and AMF treatments (Hajiboland et al., 2010; Aroca et al., 2013; Hashem et al., 2015).

Many are of the opinion that the AMF-triggered synthesis of chlorophyll is due to greater uptake of Mg, which forms the central component of the chlorophyll pigment (Sheng et al., 2008). The higher concentration of pigments in AMF-inoculated plants might be due to the up-regulation of the enzymes involved in the synthesis of chlorophylls concomitant with the reduction of chlorophyllase activity. AMF increases the activity of Rubisco and the associated carbon-metabolizing enzymes leading to the positive impact on the growth under normal as well as salinity stress conditions (Hashem et al., 2015). The positive influence of AMF on the stomatal conductance indicates their contribution to growth regulation in *C. sativus* under stress conditions. Recently, Yang et al. (2015) observed that AMF colonization of the black locust (*Robinia pseudocacia* L) enhanced root growth, morphology, hydraulic conductivity, photosynthetic attributes like stomatal conductance and net photosynthesis, water use efficiency and nutrient uptake. AMF can protect the PSII activity in chloroplasts and thus improve the water use efficiency under metal stress (Lu and Vonskak 2002; Allakherdiev and Murata 2008; Chaves et al., 2009; Yang et al., 2015).

It was interesting to observe that *C. sativus* seedlings colonized with AMF exhibited an apparent decline in the accumulation of ROS and prevented oxidative damage to cellular structures and their functional integrity (Kohler et al., 2009; Velarde-Buendia et al., 2012; Hashem et al., 2015). The results of NaCl-induced damage to the structure and stability of cellular membranes was described by Ruiz-Lozano et al. (2012); He et al. (2017) and Yang et al. (2015) who observed that *Oryza sativa*, *Lycium barbarum* and *Robinia pseudocacia*, respectively, exposed to increased salinity showed membrane leakage resulting from damage to their plasma membranes; however, greater maintenance of water balance in tissues due to AMF colonization may have protected their membrane structures. The stress-induced reduction in the structural and functional stability of the membranes mainly results from the rapid increase in the lipid peroxidation (Fouda et al., 2014; Abd_Allah et al., 2015a; Nath et al., 2016). Salinity stress reduces membrane permeability by affecting the polyunsaturated lipids of the membranes, resulting in considerable leakage of the cellular components (Sánchez-Rodríguez et al., 2010; Alqarawi et al., 2014b). Earlier, we reported greater protection of the membrane lipids and functional stability of the membranes due to AMF inoculation (Abd_Allah et al., 2015a; Mo et al., 2016). Additionally, the reduced production of hydrogen peroxide in AMF-colonized plants prevents membrane dysfunction (Andrio et al., 2013; Puppo et al., 2013). ROS primarily affects membrane lipids and proteins leading to the loss of membrane integrity, thereby causing the leakage of electrolytes. However, AMF inoculation mitigated stress effects via substantial increases in the antioxidant activity and the accumulation of osmolytes (Qun et al., 2007). The association of AMF with *Solanum lycopersicum* significantly improved the lipid and fatty acid content in cadmium-affected plants to reduce membrane damage and leakage (Fouda et al., 2014; Hashem et al., 2016b; Nath et al., 2016).

*C. sativus* exposed to salinity stress exhibited a significant decline in the leaf relative water content, and our results concur with the results of Arulbalachandran et al. (2009), Hashem et al. (2015) and Yang et al. (2015). AMF application regulates root phenotypic characteristics causing greater uptake of water during stress (Augé, 2001). However, the inoculation of AMF enhanced the uptake of water in root cells under normal conditions, as well as during salinity stress. The increase of water levels in stressed plants maintains the cell turgor, photosynthesis, enzyme activity, cellular integrity and growth, all of which ameliorate the deleterious effect of salinity (Sade et al., 2010). Proline is one of the key osmoprotectants preventing cellular oxidative damage by maintaining tissue water potential and protein integrity and functioning (Mansour and Ali 2017). The current study showed that AMF inoculation improved the synthesis of proline, and it has been demonstrated that proline synthesis is up-regulated in stressful environments with a significant decline in its catabolism (Mo et al., 2016; Iqbal et al., 2015). This result supported the findings of Yooiyongwech et al. (2013), who reported that proline was enhanced in response to AM treatment. Shekoofeh et al. (2012) and Wu et al. (2016) also reported that AMF triggered the accumulation of proline, which leads to improved water transport that improves the metabolic efficiency of plants.

The enhancement of proline accumulation caused by AMF symbiosis might be due to the up-regulation of the delta1-pyrroline-5-carboxylate synthetase gene (the rate-limiting enzyme in proline biosynthesis, MeP5CS) as described by Huang et al. (2013).

To neutralize ROS and prevent oxidative damage to cells, plants up-regulate the antioxidant defence system, which is an intriguing protective system. SOD is a key enzyme mediating the scavenging of toxic superoxide radicals for protection of electron transport. SOD activity was increased in response to salinity and has been reported in cowpea, sorghum, cotton and *Populus cathayana* (Freitas et al., 2011; Wu et al., 2016). SOD modulates the Haber-Weiss reaction substrates, superoxide and H2O2, thereby reducing the formation of more toxic hydroxyl (OH-) radicals. Greater SOD activity in AMF-inoculated plants might be due to the increased uptake of Zn, Cu, Mn and Fe, which form co-factors for its isozymes (Ghorbanli et al., 2004; Qun et al., 2007; Kohler et al., 2009). In stressed plants, the generation of hydrogen peroxide after SOD-mediated neutralization of superoxide is eliminated by either CAT or APX via the ascorbate-glutathione cycle, in which GR, AsA and GSH are the key components controlling ROS neutralization (Velarde-Buendia et al., 2012). Greater APX and CAT activity due to AMF inoculation in *C. sativus* correlates with the results of Ghorbanli et al. (2004), Hashem et al. (2015) and Wu et al. (2016). It revealed that AMF inoculation improves the ROS efflux activity of tap and lateral root results, with considerable reductions in their accumulation, and prevents cellular oxidative damage (Huang et al., 2017). An increase in CAT and APX activity due to AMF inoculation-mediated fast breakdown of H2O2 (Foyer et al., 1994; Abd_Allah et al., 2015a, 2015b) protects against cellular damage. Non-enzymatic antioxidants are also involved in the declining accumulation of ROS (Huang et al., 2017). APX and CAT play an indispensable role in stress amelioration, and the activity...
of APX is coupled to GR, AsA and GSH. The ascorbate–glutathione cycle, which contains a series of redox reactions, maintains the NADP/NADPH ratio and the cellular redox state to prevent the formation of ROS and thereby protect photosynthetic electron transport. The activity of APX and GR and the content of AsA and GSH increased due to AMF in salt-affected C. sativus (Hasheem et al., 2016a; Abd_Allah et al., 2015a, 2015b). Similarly, Cekic et al. (2012) observed enhanced GR activity in AMF-inoculated Capsicum annum under salt stress to maintain plant growth. The strengthening of the antioxidant system in AMF plants further boosted the synthesis of polyphenols. In our previous study, we demonstrated that greater accumulation of phenols in AMF-inoculated plants confers stress tolerance (Abd_Allah et al., 2015a, 2015b). Polyphenols regulate cellular functioning by modulating nitric oxide and the functioning of voltage-gated ion channels for integrating different signalling pathways (Upadhyay and Dixit 2015). AMF inoculation promoted the accumulation of several important polyphenols, contributing to the growth of marjoram, lemon balm and marigold (Engel et al., 2016). In addition, the priming of seeds with AMF can enhance the expression of enzymes involved in phenol biosynthetic pathways (Song et al., 2015).

AMF association with plant roots regulates stress hormone signalling during unfavourable environmental conditions. The inoculation of AMF stimulated the endogenous concentration of JA and SA. The synthesis and transport of SA and JA involve several cellular processes for plant growth development. In the present study, AMF inoculation reprogrammed their concentrations to enhance stress tolerance. Plant growth-promoting fungi cause an increase in the endogenous levels of phytohormones (Waqs et al., 2012, 2014; Abd_Allah et al., 2015a, 2015b). ABA acts as an antitranspirant leading to a reduction in water loss through the modification of stomatal functioning. However, AMF significantly reduced ABA production in cucumber plants and thereby regulating the rate of transpiration. JA and its subsequent derivatives are an important group in oxylipin signalling molecules, which regulate several key physiological processes and metabolite synthesis under stress conditions. JA signalling in plants is an important process that integrates different mechanisms against several biotic and abiotic stresses, while SA signalling is widely accepted as a biotroph immunity indicator (Wasternack and Hause 2013; De-Vleesschauwer et al., 2014). The up-regulation of JA and SA in AMF-inoculated plants confers their potential role in the amelioration of salinity via improvement in the synthesis of secondary metabolites (Qun et al., 2007; Arulbalachandran et al., 2009; Yuan et al., 2010). The accumulation of ABA in plant cells has negative effects on stomatal conductance under salinity stress. The biosynthesis of ABA in guard cells in stressed plants triggers the redistribution and accumulation of ABA within the cells and induces the release of water and ions leading guard cells to turn flaccid and stomata to close (Bray, 1997). However, AMF reduced the synthesis of ABA to regulate stomatal functioning. Previous reports also showed an increase in ABA in soybean cultivars subjected to salt stress (Hamayun et al., 2010). ABA acts as a signal for regulating stomatal closure, plant growth and development under stressed conditions (Wasilewska et al., 2008). The enhancement of ABA in plants during stress conditions is an early response mechanism against stress (Xiong et al., 2002). AMF inoculation stimulated greater uptake of nutrients, including potassium, calcium, and magnesium to promote the synthesis of various important metabolites and enzymes (Yuan et al., 2010). In the present study, AMF triggered the production of SA, thereby reducing oxidative damage (Conrath, 2006) through the induction of systemic acquired resistance, which depends on the endogenous concentrations of SA and ROS and the expression of specific resistance genes (Durrant and Dong, 2004).

The results of the present study indicate that high salinity treatment restricted the uptake of key essential macronutrients, including potassium, calcium, and magnesium, as well as other microelements like zinc, iron and copper. Sodium shows an antagonistic relationship with important mineral ions such as potassium and calcium. AMF protected plants by inhibiting sodium uptake and regulating the ionic balance in cells during soil salinity. Several...
reports have revealed that salinity affects mineral uptake by plant roots, including studies by Kohler et al., (2009) and Hashem et al. (2015). For example, the growth of cucumber subjected to salinity stress exhibited a considerable adverse impact of salt in Vigna radiata (L.) Decne. Acta Biol. Hung. 65 (1), 61–71. https://doi.org/10.1556/ Afoi.65.2014.1.6.

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