Saudi Journal of Biological Sciences xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L.

Abeer Hashem [a,b,*], Abdulaziz A. Alqarawi [c], Ramalingam Radhakrishnan [d], Al-Bandari Fahad Al-Arjani [a], Horiah Abdulaziz Aldehaish [a], Dilfuza Egamberdieva [e], Elsayed Fathi Abd_Allah [c]

[a] Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
[b] Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza 12511, Egypt
[c] Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
[d] Department of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea
[e] Faculty of Biology, National University of Uzbekistan, Tashkent, Uzbekistan

ARTICLE INFO

Article history:
Received 19 January 2018
Revised 28 February 2018
Accepted 18 March 2018
Available online xxxx

Keywords:
AMF
Lipid peroxidation
Antioxidant enzymes
Proline
Growth hormones
NaCl

ABSTRACT

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 macromolecules, 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

* Corresponding author at: Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia.
E-mail address: habeer@ksu.edu.sa (A. Hashem).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.03.009
1319-562X/

Please cite this article in press as: Hashem, A., et al. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L.. Saudi Journal of Biological Sciences (2018), https://doi.org/10.1016/j.sjbs.2018.03.009

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-Buendía 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 apoplast 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 *Ocimum basilicum* against salinity stress by improving mineral uptake, chlorophyll synthesis and water use efficiency (Shekoofeh 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 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] NaOCl 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:

$$\% \text{ AMF Colonization} = \frac{\text{Total numbers of AM positive segments}}{\text{Total number of segment studied}} \times 100$$

Please cite this article in press as: Hashem, A., et al. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L.. Saudi Journal of Biological Sciences (2018), https://doi.org/10.1016/j.sjbs.2018.03.009