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ORIGINAL ARTICLE

Enhancing growth performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using arbuscular mycorrhizal fungi under salt stress

Elsayed Fathi Abd_Allah [a,*], Abeer Hashem [b,1], Abdulaziz Abdullah Alqarawi [a], Ali Hassan Bahkali [b], Mona S. Alwhibi [b]

[a] *Department of Plant Production, Faculty of Food & Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia*
[b] *Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh 11451, Saudi Arabia*

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Abstract Pot experiments were conducted to evaluate the damaging effects of salinity on *Sesbania sesban* plants in the presence and absence of arbuscular mycorrhizal fungi (AMF). The selected morphological, physiological and biochemical parameters of *S. sesban* were measured. Salinity reduced growth and chlorophyll content drastically while as AMF inoculated plants improved growth. A decrease in the number of nodules, nodule weight and nitrogenase activity was also evident due to salinity stress causing reduction in nitrogen fixation and assimilation potential. AMF inoculation increased these parameters and also ameliorated the salinity stress to some extent. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) as well as non enzymatic antioxidants (ascorbic acid and glutathione) also exhibited great variation with salinity treatment. Salinity caused great alterations in the endogenous levels of growth hormones with abscisic acid showing increment. AMF inoculated plants maintained higher levels of growth hormones and also allayed the negative impact of salinity.

* Corresponding author.
E-mail address: eabdallah@ksu.edu.sa (E.F. Abd_Allah).
[1] Permanent address: Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.
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1. Introduction

As sessile organisms plants are frequently encountered by many environmental stresses including abiotic as well as biotic resulting in altered plant growth and metabolism. Salinity is one of the important abiotic environmental factors having great effects on plant growth and development (Barnawal et al., 2014). Increased industrialization and use of saline water

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for irrigation purposes causes conversion of fertile soils into salt affected soils making the situation much graver. It has been estimated that around 5% to 7% of global land is salt affected (Ruiz-Lozano et al., 2012). This increase in soil salinity induces osmotic stress resulting in altered growth and physiology. Several physio-biochemical processes including photosynthesis, respiration, nitrogen metabolism and ion homeostasis are affected adversely by salinity (Tejera et al., 2004; Porcel et al., 2012).

Exposure of plants to stresses enhances the production as well as accumulation of toxic reactive oxygen species (ROS) including O_2^-, H_2O_2 and OH^- (Mittler, 2002). Increased production of ROS leads to oxidative damage and causes membrane leakage through lipid peroxidation (Shah et al., 2001). Moreover ROS induced effects are also obvious on the several other macromolecules including proteins, nucleic acids and photosynthetic pigments (Ahmad et al., 2010). In order to avoid salt stress induced oxidative damage plants have developed several protective mechanisms. Synthesis and accumulation of organic osmolytes, enhanced activities of antioxidant enzymes and efficient compartmentalization of toxic ions into other cellular compartments like vacuoles help plants to avert stress induced damage (Parvaiz and Satyawati, 2008; Tong et al., 2004; Liu et al., 2014). Both enzymatic as well as non-enzymatic antioxidants are involved in scavenging of toxic ROS. Enzymatic system comprises of superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) while as non enzymatic system includes ascorbic acid, glutathione, phenols, tocopherols etc are among the key antioxidants involved in scavenging of toxic ROS (Mittler, 2002; Ahmad et al., 2010).

Most plants form symbiotic associations with the arbuscular mycorrhizal fungi (AMF) and it has been well studied that AMF has the potential to enhance the rhizospheric soil characteristics considerably thereby affecting plant growth (Navarro et al., 2013; Ahanger et al., 2014). By acting as bio-ameliorators, AMF improves soil structure so as to promote plant growth under normal as well as stressed conditions (Rabie and Almadini, 2005; Cho et al., 2006). AMF enhances growth and mitigates stress by affecting both morpho-physiological and nutritional aspects. A direct beneficial effect of AMF on the plant growth and vigor is well documented (Asghari et al., 2005; Ahanger et al., 2014). In addition of affecting the plant physiological status AMF also alters root morphology so as to increase the absorption of water and nutrients (Aroca et al., 2013; Ahanger et al., 2014). AMF colonized plants show increased absorption as well as efficient utilization of essential mineral nutrients (Neumann and George, 2005; Hart and Forsythe, 2012).

Sesbania sesban Linn, a plant within family Fabaceae is an important medicinal plant and is commonly known as Egyptian sesban and is well widely distributed in several tropical countries (Gomase et al., 2012). According to World Health Organization about 80% of people living in developing countries rely exclusively on traditional medicines for their primary health care needs. Different parts of the *Sesbania sesban* including leaves, pods and seeds are known for their medicinal value (Mittal et al., 2012). Traditionally leaves of *S. sesban* have been used as purgative, demulcent, maturant, anthelmintic, inflammation and all pains (Gomase et al., 2012; Mythili and Ravindhran, 2012). Present study was carried out with the aim to evaluate the role of AMF in ameliorating the salinity induced changes in growth and biochemical attributes in *S. sesban*.

2. Material and methods

2.1. Pot experiment and treatment

Seeds of sesbania (*S. sesban* [L.] Merr; Syn *Sesban aegyptiaca* Poiret) were obtained from farms of Faculty of Agriculture, Cairo University, Giza, Egypt. Present study was conducted at the Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The soil used for the experiment has the following properties (%): sand (83.5); clay (8.7); silt (7.8); organic carbon, 0.15; total nitrogen, 0.0062; EC, 7.14 dS/m; and pH 7.5. Equal quantity (450 g) of autoclaved soil was divided among plastic pots. The seeds were surface sterilized with sodium hypochlorite (0.5%, v/v) for 3 min, washed thoroughly with distilled water before germination on blotter. Healthy germinating seeds were transferred to pots (1 plant/pot) with normal soil in one set of experiments and soil of second set of experiment was amended with Arbuscular Mycorrhizal Fungi (AMF). The biofertilizer (*Rhizobium leguminosarum* bv. *viciae* Frank) was added to the germinated seeds as thin film of Peat Inoculant (2×10^8 CFU/g) at rate of 4 g inoculant/kg seed. Hoagland's solution (Hoagland and Arnon, 1950) was used for irrigation with different concentrations of NaCl to get concentration of 0, 75 and 150 mM. The irrigation was carried out every alternate day. The seedlings were grown for eight weeks at 27 ± 1 °C with 12 h light (750 μmol m^{-2} S^{-1}) and 12 h dark photo-cycle and relative humidity of 70–75% after transplantation. The experiment was laid out in a completely randomized block design with five replications. At the end of pot experiment (8 weeks), the plants were harvested carefully, washed in distilled water, separated into shoots and roots. The samples were dried at 70 °C for 48 h and dry weight was recorded. Leaf samples were used for estimation of photosynthetic pigments, antioxidative enzymes, non enzymatic antioxidants and growth regulators. Fresh root samples were used for observing rhizobial nodules and related mycorrhizal studies.

2.2. The mycorrhizal inoculums

The selected mycorrhizal fungi [*Funneliformis mosseae* (syn. *Glomus mosseae*); *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*)] used in the present experiment were isolated as described by Hashem et al. (2014). Fungal inoculums potential was determined by the most probable numbers method (Alexander, 1982) and each trap culture contained 10.2×10^3 propagules per pot (1 Kg capacity). Fungal inoculums consisted of AM fungal spores, hyphae and colonized root fragments. 10 g of trap soil culture (approx. 100 spores/g trap soil, $M = 80\%$)/ pot (1 Kg) was added to the experimental soil as mycorrhizal inoculum.

2.3. Nodulation and nodules activity

Nodules were speared from roots and counted instantly. Fresh weight of nodule was taken instantly after harvesting, whereas