Journal of the Saudi Society of Agricultural Sciences (2016) **15**, 145–154

King Saud University

Journal of the Saudi Society of Agricultural Sciences

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FULL LENGTH ARTICLE

Protective role of α-tocopherol on two *Vicia faba* cultivars against seawater-induced lipid peroxidation by enhancing capacity of anti-oxidative system

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Received 7 May 2014; revised 1 September 2014; accepted 2 September 2014
Available online 10 September 2014

KEYWORDS

Antioxidant enzymes;
Faba bean;
Ion content;
MDA;
Proline;
Yield;
α-Tocopherol

Abstract To examine the effect of seawater stress on growth, yield, physiological and antioxidant responses of faba bean plant and whether the exogenous application with vitamin E could mitigate the adverse effect of salinity stress or not, a pot experiment was carried out during 2011/12 winter season under green house of the National Research Centre, Dokki, Cairo, Egypt. Two faba bean cultivars (Giza 3 and Giza 843) irrigated with diluted seawater (Tap water, 3.13 or 6.25 dS m^{-1}) and α-tocopherol (0, 50 or 100 mg L^{-1}) were used. At 75 days after sowing, growth sample was taken for vegetative growth measurement, proline, carotenoids, antioxidant enzyme activities (SOD, CAT, POX and PAL), lipid peroxidation, and inorganic ions as well as seed yield and yield attributes were determined. The results revealed that seawater triggered significant inhibitory effects on faba bean growth and yield especially for Giza 3 cultivar with obvious increments in MDA and Na^+ ion contents. Foliar application with α-tocopherol at rate of 100 mg L^{-1} followed by 50 mg L^{-1} on faba bean plants exerted certain alleviative effects on these indices in particular on Giza 843. α-Tocopherol could play an important role in alleviation of injury of faba bean irrigated with diluted seawater through the enhancement of the protective parameters such as antioxidant enzymes, proline, carotenoids, and inorganic ions (K^+ and Ca^{2+}) to be effective in decreasing MDA content, lessening the harmful effect of salinity, and improving faba bean growth, seed yield and seed yield quality.

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Peer review under responsibility of King Saud University.

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1. Introduction

Salt tolerance in plants is a complex trait, which varies widely among closely related species and between different varieties (Ashraf, 2002). Differences between closely related plants are

http://dx.doi.org/10.1016/j.jssas.2014.09.001
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particularly interesting to identify a small number of factors responsible for salt tolerance (Gehlot et al., 2005). Salinity stress has been studied in relation to regulatory mechanisms of osmotic and ionic homeostasis (Ashraf and Harris, 2004). The response of plants to a salinity stress may vary with the genotype, nevertheless some general reactions occur in all genotypes. Salinity can affect plant physiological processes resulting in reduced growth and yield (Yamaguchi and Blumwald, 2005). Increased tolerance to salinity stress in crop plants is necessary in order to increase productivity with limited water supplies and high salinity.

Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel and Hirt, 2004). Chloroplasts are the major organelles producing the reactive oxygen species (ROS) such as, the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($O_1^{\cdot}$) during photosynthesis (Asada, 1992). The production of ROS can be particularly high, when plants are exposed to salinity stress (Ashraf, 2009). ROS cause chlorophyll degradation and membrane lipid peroxidation. So, malondialdehyde (MDA) accumulation is an oxidative stress indicator that is a tested tool for determining salt tolerance in plants (Yildrimin et al., 2008).

Removal of the toxic oxygen radicals rapidly is of prime importance in any defense mechanism. Plants protect cells and sub-cellular systems from the cytotoxic effects of these active oxygen radicals with both non-enzymatic and enzymatic antioxidant systems such as carotenoids, ascorbic acid, α-tocopherol, proline, SOD, peroxidase (POX) and catalase (CAT) (Munne-Bosch and Alegre, 2000; Sairam and Srivastava, 2001; Mishra et al., 2009). There are several reports that underline the intimate relationship between antioxidant enzyme activities and increased tolerance to environmental stress (Abd El-Motty and Orabi, 2013; Orabi et al., 2013). Differences in the accumulation patterns of Na^+ and K^+ were found under salinity stress. The salt tolerant plants maintained a high K^+ content and higher K^+:Na^+ ratio compared with the salt sensitivity plants (Azooz et al., 2004). High K^+:Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ (Cuin et al., 2003).

Faba beans (*Vicia faba* L.) are popular legume food with high yield capacity and high protein content (30% of their dry weight) which contain most of the necessary amino acids for human and animal nutrition and low sulfur amino acid concentrations (Gaber et al., 2000). In recent years, the importance of carotenoids and tocopherols has been increasingly recognized due to the emerging knowledge of their health benefits. Because humans can synthesize neither carotenoids nor tocopherols, they rely on their uptake through diet for the production of vitamin A and the supply with vitamin E (Fraser and Bramley, 2004). Tocopherols are a group of compounds synthesized only by photosynthetic organisms and are involved in the quenching and scavenging of $^1O_2$ (Neely et al., 1988) and act as highly efficient recyclable chain reaction terminators for the removal of polyunsaturated fatty acid (PUFA) radical species generated during Lipid peroxidation (Munne-Bosche and Falk, 2004). Furthermore, tocopherols contribute to membrane stability by influencing its fluidity and permeability (Fryer, 1999) and might participate in protection of the D1 protein against high light (Trebst et al., 2002). Tocopherols are believed to protect chloroplast membranes in plants from photo oxidation and help to provide an optimal environment for the photosynthetic machinery, their accumulations also occur as a response to variety of abiotic stress including high light, drought, salt and cold and may provide an additional line of protection from oxidative damage (Munné-Bosch and Algere, 2002). The major tocochromanol in leaves is α-tocopherol, whereas seeds accumulate higher levels of tocotrienols (Grusak and Dellapenna, 1999).

Comparing the response among genotypes of the same species to salinity provides a convenient and useful tool for un-veiling basic mechanisms involved in salt tolerance. The mechanism of salt tolerance is still not fully understood (Gharsa et al., 2008). Therefore, this work was conducted to compare the effect of salinity stress on growth, yield parameters, physiological and antioxidant responses of two faba bean (*V. faba* L.) genotypes differing in salt tolerance and to examine whether exogenous application with vitamin E could mitigate the adverse effect of salinity stress.

2. Materials and methods

2.1. Experimental procedures

A pot experiment was conducted at the green house of the National Research Centre, Dokki, Cairo, Egypt during the winter season of 2011/12 to study the effect of foliar spray of α-tocopherol (Vitamin E) on faba bean grown under salinity conditions. Daytime temperatures ranged from 14.5 to 30.2 °C with an average of 23.2 ± 3.8 °C whereas temperatures at night were 12.4 ± 1.8 °C, with minimum and maximum of 8.0 and 17.0 °C, respectively. Daily relative humidity averaged 57.7 ± 9.6% in a range from 38.1% to 78.7%.

Two Faba bean (*V. faba* L.) cultivars were used in this experiment, namely Giza 3 (G3, *Orobanche*-susceptible) and Giza 843 (G843, *Orobanche*-tolerant) were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Faba bean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Ten seeds were sown on November 22, 2011 along a centre row in each pot at 30-mm depth in plastic pots, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1 (V:V), respectively in order to reduce compaction and improve drainage. Saline water was prepared by mixing fresh water (0.23 dS m^{-1}) with seawater (51.2 dS m^{-1}). Concentration of EC, pH, cations and anions of irrigation water and soils used on the pot experiment are shown in Table 1. At sowing, granular commercial rhizobia were incorporated into the top 30-mm of the soil in each pot with the seeds. Granular ammonium sulfate 20.5% N at a rate of 40 kg N ha^{-1}, and single super phosphate (15% P_2O_5) a rate of 60 kg P_2O_5 ha^{-1} were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

The experiment was laid out in factorial design using three factors (cultivars, seawater, and α-tocopherol) with five replications. Seedlings were thinned after 10 days after sowing (DAS) to leave four seedlings per pot till harvest and irrigated with equal volumes of tap water until 15 DAS. Starting from 16th day, all pots were irrigated either with tap water (S0) or different dilutions of seawater namely 3.13 or