South African Journal of Botany 93 (2014) 54–63

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

The changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under seawater stress

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ARTICLE INFO

Article history:
Received 23 May 2013
Received in revised form 15 February 2014
Accepted 3 March 2014
Available online xxxx

Edited by PN Hills

Keywords:
Anatomical parameters
Antioxidant enzymes
Faba bean
Growth
Salinity
Seawater

ABSTRACT

The depletion of fresh water resources leads to the utilisation of various alternative sources of water, such as seawater. In this regard, the foliar application of proline is one of the alternative shotgun approaches to increase plant stress tolerance. A pot experiment was conducted in the wire house of the National Research Centre, Dokki, Cairo, Egypt, during the winter season of 2010–2011. The experiment consisted of three concentrations of proline (0, 25 and 50 mM) and two concentrations of diluted seawater (3.13 and 6.25 dS m^{-1}), whereas control plants were irrigated with tap water (0.23 dS m^{-1}). Diluted seawater caused significant reductions in growth parameters, photosynthetic pigments, some mineral contents (P, K, Ca^{+2}), the K^+:Na^+ ratio and the level of total carbohydrates. In contrast, N, Na^+, and Cl^- contents, osmoprotectants (soluble carbohydrates, total phenolic concentrations, free amino acids, proline), and activities of antioxidant enzymes (peroxidase and polyphenol oxidase) significantly increased with an increasing salinity level compared with control plants. The foliar application of 25 mM proline caused significant increases in growth parameters, photosynthetic pigments, N, P, K^+, and Ca^{+2} %, the K^+:Na^+ ratio, total carbohydrates, and soluble carbohydrates, accompanied by significant decreases in Na^+, Cl^-, phenolic contents, free amino acids, proline, and the activities of antioxidant enzymes compared with the control. In addition, 25 mM proline minimised the deleterious effect of salinity on the anatomical structure of the faba bean stem and leaf. The proline treatment at 50 mM was as essentially toxic to faba bean plants as to that of salinity stress. This toxicity was apparent by the reduction of growth parameters, photosynthetic pigments, N, P, K^+, and Ca^{+2}, K^+:Na^+ ratio and significant increases in Na^+ and Cl^- concentrations. Therefore, the exogenous application of proline at a concentration of 25 mM partially alleviated the toxicity of diluted seawater on faba bean plants, whereas the 50 mM proline treatment was toxic.

1. Introduction

Abiotic stresses are considered the most important factors for yield reductions in agricultural crops. It is estimated that yield losses in agricultural crops due to different abiotic stresses include 15% due to low temperature, 17% due to drought, 20% due to salinity, 40% due to high temperature, and 8% due to other environmental factors (Ashraf et al., 2008).

Water is an essential factor during the entire life of plant growth, from seed germination to the final growth stage. With increasing aridity, in conjunction with a fast increase in human population, water will become a scarce commodity soon, particularly in third-world countries. Hence, the depletion of fresh water resources has led to the utilisation of various alternative sources of water. The main problem in using different sources of water arises from salinity hazards.

Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes, such as photosynthesis, antioxidant phenomena, nitrogen metabolism, ion homeostasis, and osmolyte accumulation (Ashraf, 2004). Thus, salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity and by disturbing the uptake and translocation of nutritional ions (Misra and Dwivedi, 2004).

The rate of plant growth depends on several important events, such as cell division, cell enlargement and cell differentiation, as well as genetic, morphological, physiological, and ecological events and their complex interactions, which are severely affected by abiotic stress (Taize and Zeiger, 2006). When plants are exposed to harsh conditions

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http://dx.doi.org/10.1016/j.sajb.2014.03.002
0254-6299/

(e.g., salinity stress), plants exhibit certain adaptive features, which may be morphological, anatomical, physiological, or biochemical in nature to minimise the deleterious effects of unfavourable environmental conditions (Sakamoto and Murata, 2002) and to help plants to sustain and thrive under stress conditions. In this regard, plants perceive stress through their roots and send signals to change their metabolism for the activation/synthesis of defence mechanisms in different parts of the plant (Siopongco et al., 2008).

Plants must maintain their internal water potential below that of the soil to maintain turgor and water uptake for growth. This maintenance requires an increase in osmotica, either by the uptake of soil solutes or by the synthesis of compatible solutes (Tester and Davenport, 2003). These compatible organic solutes are of low molecular weight and are highly soluble compounds that are usually nontoxic at high cellular concentrations and that do not interfere with the plant's metabolism, even at molar concentrations (Alonso et al., 2001). The accumulation of such compatible osmolytes allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortage within the plant (Nanjo et al., 1999). Furthermore, because some of these solutes also protect cellular components from dehydration injury during stress, these solutes are commonly called osmoprotectants. The osmoprotectants synthesised in plants in response to adverse environmental conditions include a variety of proteins and amino acids (such as proline) and carbohydrates (Ashraf, 2010). Normally, these osmoprotectants protect plants from different abiotic stresses in several ways, including their role in adjusting cellular osmosis, scavenging reactive oxygen species (ROS), protecting cellular membranes, and stabilising proteins/enzymes and enzyme activities (Gill and Tuteja, 2010).

The generation of reactive oxygen species (ROS) is a common phenomenon in plants under normal growth conditions. However, their production increases under adverse environmental conditions, including salinity. The production of these ROS under stress conditions is highly dangerous because ROS impair the normal functions of cells due to their oxidative reaction with membrane proteins, lipids, and deoxyribonucleic acid, as well as the inactivation of enzymes (Ashraf, 2009). The detoxification of ROS in plant cells can be categorised as enzymatic and non-enzymatic in almost all plants. The non-enzymatic antioxidants include ascorbic acid, tocopherols, flavonoids, phenolics and carotenoids. The important anti-oxidant enzymes include peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) (Ashraf, 2010; Ali and Ashraf, 2011).

The use of osmoprotectants as seed priming or as a foliar spray can be an economically viable strategy to enhance stress tolerance under adverse environmental conditions (Ali et al., 2007; Ashraf and Foolad, 2007; Ali and Ashraf, 2011). Proline is one of the compatible osmolytes induced by salinity stress in plants. Several functions are proposed for the accumulation of proline in tissues exposed to salinity stress: C and N reserves for growth after stress relief (Hayashi et al., 2000), the stabilisation of proteins and membranes (Mansour, 1998), the protection of macromolecules from denaturation (Hamilton and Heckathorn, 2001), osmoprotection (Kishor et al., 1995), free radical scavenging (Chen and Dickman, 2005), anti-oxidation (Hoque et al., 2007), and as a readily available source of energy and reducing power (Stewart et al., 1974).

The exogenous application of proline is known to induce abiotic stress tolerance in various plant species (Ali et al., 2007, 2008; Ashraf and Foolad, 2007; Abdelhamid et al., 2013). Ali et al. (2007) reported that the exogenous application of 30 mM proline at all growth stages of maize was found to be most effective in inducing drought tolerance, enhancing the biomass production, and increasing the photosynthetic rate, stomatal conductance, and internal CO_2 concentration. In another study of maize, Ali et al. (2008) reported that the exogenous application of proline enhanced the nutrient uptake in roots and shoots under water deficit conditions and correlated the results with an enhanced plant transpiration rate. In contrast, the effect of proline is dependent on its concentration, as mentioned by Ashraf and Foolad (2007), because an excessive amount of free proline has negative or side effects on cell growth or on protein functions. The over-accumulation of intracellular proline significantly represses several genes involved in the synthesis of other amino acids or normal morphogenesis in *Arabidopsis* plants (Nanjo et al., 2003). Proline overaccumulation at a concentration as low as 100 mM suppresses the activity of the major chloroplastic enzyme ribulose 1,5-bis-phosphate carboxylase in higher plants (Sivakumar et al., 1998); therefore, intracellular proline must be present at an appropriate level to confer stress tolerance. The effectiveness of proline applied as a foliar spray depends on the type of species, plant developmental stage, time of application and on the concentration (Ashraf and Foolad, 2007). Therefore, it is necessary to determine the optimal concentrations of exogenously applied proline that can provide beneficial effects in economically important crop plants, such as *Vicia faba*, when exposed to abiotic stress.

The faba bean (*V. faba* L.) plant is one of the most important crops in Egypt due to its high nutritive value in terms of both energy and protein contents. Therefore, increasing faba bean production is one of the most important targets of agricultural policy in Egypt.

This study aimed to measure the potential effects of the exogenous application of proline on some physiological, biochemical and anatomical parameters at the vegetative growth stage of faba bean plants irrigated with diluted seawater.

2. Materials and methods

2.1. Plant materials and growth conditions

A pot experiment was conducted in a wire-house at the National Research Centre, Dokki, Cairo, Egypt (30°20′ N; 31°53′ E) from 6 December 2010 to 10 February 2011. During this period, daytime temperatures ranged from 14.5 to 30.2 °C, with an average of 23.2 ± 3.8 °C. Night temperatures ranged from 8.0 to 17.6 °C, with an average of 12.4 ± 1.8 °C. The daily relative humidity averaged 57.7 ± 9.6% and ranged from 38.1 to 78.7%.

Seeds of faba bean (*V. faba* L.; cv. Giza 843) were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Healthy faba bean seeds (n = 10) were selected for uniformity by choosing those seeds of equal size and of identical colour. The selected seeds were washed in distilled water, sterilised in 1% (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again in distilled water, and left to dry at room temperature (25 °C) for approx. 1 h.

Ten uniform, air-dried faba bean seeds were sown along a centre row in each plastic pot (30 cm in diameter) at a depth of 30 mm, in approx. 7.0 kg of clay soil. To reduce compaction and to improve drainage, the soil was mixed with yellow sand in a proportion of 3:1 (v:v).

A granular commercial *Rhizobium leguminosarum* (obtained from the Biofertilizer Inoculum Production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Centre, Giza, Egypt) was incorporated into the top 30 mm of soil in each pot with the seeds at the time of sowing. Granular ammonium sulphate [20.5 (w/w) % N] was applied at a rate of 40 kg N ha^{-1}, and single superphosphate [15% P_2O_5] was added at a rate of 60 kg P_2O_5 ha^{-1} to each pot. These N and P fertilisers were mixed into the soil in each pot immediately before sowing.

The experiment was arranged in a factorial arrangement, with three levels of seawater (S0, S1, or S2) and three levels of proline (P0, P1, or P2). Four replicates were used. To induce salt stress, seawater was dissolved in fresh water, and the plants were watered with an equal volume of 0.23, 3.13 and 6.25 dS m^{-1} 3 weeks after sowing (treatments S0, S1, and S2, respectively). Saline water was prepared by mixing fresh water (0.23 dS m^{-1}) with seawater (51.2 dS m^{-1}) to achieve salinity levels of 3.13 and 6.25 dS m^{-1}. Electrical conductivity (EC), pH,