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Research article

Treatment with spermidine protects chrysanthemum seedlings against salinity stress damage

Naiyuan Zhang, Xiaomeng Shi, Zhiyong Guan*, Shuang Zhao, Fei Zhang, Sumei Chen, Weiming Fang, Fadi Chen

College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

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ABSTRACT

Salinity-stressed plants of salinity sensitive ('Qx096') and tolerant ('Qx097') chrysanthemum cultivar were treated with a range of concentrations of spermidine (Spd). Plant performance, as indicated by various parameters associated with growth, was improved by the treatment, as was the tissue content of soluble protein and proline. The extent of both Na^+ accumulation and K^+ loss was reduced. Activity levels of the stress-related enzymes SOD, POD, APX and CAT were significantly increased and the production of malondialdehyde (MDA) decreased. The suggestion was that treatment with 1.5 mM Spd would be an effective means alleviating salinity-stress induced injury through its positive effect on photosynthetic efficiency, reactive oxygen species scavenging ability and the control of ionic balance and osmotic potential. Its protective capacity was more apparent in 'Qx096' than in 'Qx097'.

1. Introduction

Soil salinity represents a growing constraint to crop production, largely as a result of poor irrigation practice. High levels of salinity lead to ionic imbalance within the plant, inducing osmotic stress, which is then typically accompanied by oxidative damage (Zhu, 2001). In some regions of the world, soil salinity is considered to be the most important environmental factor limiting crop growth and productivity (Parida and Das, 2005).

In recent years, much attention has been devoted to the involvement of the polyamines (PAs) as second messengers in the context of a variety of environmental stresses (Roychoudhury et al., 2011). These low molecular weight, aliphatic nitrogenous compounds, which are protonated at physiological pH, were originally thought to bind to anionic macromolecules, including proteins and nucleic acids, and thus to perform a structural role (Alcazar et al., 2010). The three commonest plant PAs, namely putrescine (Put), spermidine (Spd) and spermine (Spm) are now known, which protect salinity-stressed plants by aiding the accumulation of sugars, proline (Pro) and other osmolytes, and in adjusting ion channels to maintain the plant's internal K^+/Na^+ balance (Shabala et al., 2007). They also serve to increase the activity of a range of antioxidant enzymes, thereby improving the plant's ability to control oxidative stress (Chattopadhayay et al., 2002).

The involvement of PAs in the response of higher plants to salinity stress has been widely reported. However, the change of endogenous PAs is different in plant species (Ikbal et al., 2014). Campestre et al (Campestre et al., 2011). have noted that salinity stress reduces the endogenous Put and Spd content of the soybean hypocotyl, while at the same time elevating that of Spm. In salinity tolerant cultivars of soybean, Spd and Spm are accumulated in the plasma membrane, while in salinity-sensitive ones, only Put is accumulated (Roy et al., 2005). One of the effects of salinity stress on the cucumber root is to depress the quantity of Put, Spd and Spm present (Janicka-Russak et al., 2010). However, in *Arabidopsis thaliana*, salinity stress increases the tissue content of Spd and Spm (Alet et al., 2012).

Supplying PAs has been suggested as a mean of alleviating salinity stress (Chattopadhayay et al., 2002), although the optimal concentration to give this effect varies considerably from species to species (Duan et al., 2008; Wen et al., 2011). Put is not generally considered to be beneficial (Parvin et al., 2014), although has been used to regulate ionic balance in barley (Velarde-Buendía et al., 2012). The potential benefit of exogenously supplying PAs to ornamentals subjected to salinity stress are rarely researched (Xu et al., 2011; Ding et al., 2010). Here, a description was given of the

* Corresponding author.
E-mail address: guanzhy@njau.edu.cn (Z. Guan).

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effect of supplying a range of concentrations of Spd on the growth, photosynthesis, osmotic adjustment, ionic balance and antioxidant enzyme activity to the popular ornamental species chrysanthemum (*Chrysanthemum morifolium*), which is rather sensitive to salinity stress (Song et al., 2014).

2. Materials and methods

2.1. Plant materials

Seedlings of the salinity sensitive chrysanthemum cultivar 'Qx096' and the tolerant one 'Qx097' (unpublished data) are maintained by the Chrysanthemum Germplasm Resource Preserving Centre (Nanjing Agricultural University, Nanjing, China). Cuttings were grown in a 1/1/1 (v/v/v) mixture of vermiculite, perlite and soil (Sun et al., 2013), and 20 day old seedlings of comparable size and bearing six or seven true leaves were cultured in 24 L of half strength Hoagland's nutrient solution (1/2HS). The solution was kept aerated (dissolved oxygen >8.0 mg L^{-1}) and within the temperature range 20–25 °C (Duan et al., 2008). Day and night air temperatures were maintained at, respectively, 25–30 °C and 15–18 °C, and the relative humidity fluctuated between 60% and 75%.

2.2. Exposure to salinity stress and the exogenous supply of Spd

Salinity stress was imposed by adding 100 mM NaCl to the 1/2HS, while the control treatment comprised 1/2HS. To deliver Spd, four foliar applications were given, following Kamiab et al. (Kamiab et al., 2014), Spd solution was made up in water (pH 9.20). The treatment was started on the first day of salinity stress and repeated every other day, the plants were harvested 2, 4, 6, and 8 days after treatment, and leaves were separated from the rest of the plant, frozen in liquid nitrogen and stored at −80 °C until further use. The concentration of Spd was varied from 0.5 mM to 2.0 mM, producing six distinct treatments of 1/2HS + (A) 0 mM NaCl/0 mM Spd, (B) 100 mM NaCl/0 mM Spd; (C) 100 mM NaCl/0.5 mM Spd; (D) 100 mM NaCl/1.0 mM Spd; (E) 100 mM NaCl/1.5 mM Spd or (F) 100 mM NaCl/2.0 mM Spd.

2.3. PA extraction and tissue content determination

Free PA was extracted from 0.5 g lyophilized leaves by homogenizing the tissue in 5% (v/v) perchloric acid at 4 °C for 30 min, after which the homogenate was centrifuged (15,000 × g, 15 min) and the supernatant held at −20 °C (Campestre et al., 2011). The subsequent dansylation step was performed by adding a 1.5 mL volume of carbonate buffer (pH 9.2) to a 1 mL aliquot of the supernatant, followed by the addition of 1 mL 5 mg/mL dansyl chloride dissolved in acetone. After a 1 h incubation at 50 °C in the dark (Dadáková et al., 2009), 100 μL 100 mg mL^{-1} Pro was added to stop the reaction and the reaction was extracted with 3 mL n-heptane. A 1 mL aliquot of the organic phase was evaporated and re-suspended in 1.5 mL acetonitrile, and this solution was subjected to HPLC using a L2000 device equipped with a reverse phase C18 column (250 nm × 4.6 nm) (Agilent, USA). The eluate was monitored using a UV-detector. The flow rate was 1.0 mL/min, the column temperature was 25 °C and the detection wavelength was 225 nm (Dadáková et al., 2009). The separation was based on a solute gradient involving 100% acetonitrile (A) and 50% acetonitrile (B) as follows: 0 min: 60% A/40% B, 5 min: 20% A, 80% B, 10 min: 10% A, 90% B, 15 min: 5% A, 95% B, 20 min: 60% A, 40% B.

2.4. Measurement of leaf area (LA)

Fully expanded leaves, sampled from the first to the fifth positions from the apex, were scanned using an Expression 1680 desktop scanner (EPSON EXPERSSION 1680). LA was calculated from the scanned images using the image analysis software package WinRHIZO (Regent Instruments, Canada).

2.5. Photosynthetic gas exchange parameters

A LI-6400 portable optical instrument (LICOR, USA) was used to measure the following photosynthetic gas exchange parameters: leaf net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r). Fully expanded leaves of the third or fourth positions from the seedling apex were used for these determinations. Within the leaf chamber, the temperature was 25 °C and the CO_2 concentration was 400 ± 10 μmol mol^{-1}. The leaves were illuminated with a photosynthetic photo flux density of 1000 μmol $m^{-2} \cdot s^{-1}$ provided by an LED red-blue light source.

2.6. Physiological parameters

The relative water content of the leaves was given by the ratio between (fresh weight [FW] - dry weight [DW]) and (saturated FW - DW) and electrolyte leakage was determined using an electrical conductivity meter, following Saleethong (Saleethong et al., 2011). The MDA (malondialdehyde) content was obtained via the thiobarbituric acid reaction (Liu et al., 2006). Pro content based on a method modified from Chandler and Thorpe (Chandler and Thorpe, 1987) by X.-P. Wen (Wen et al., 2011). Briefly, about 50 mg FW of leaf tissue was homogenized in 4 ml of methanol: chloroform: water (12:5:1, v/v/v). After addition of 1.5 ml water and 1 ml chloroform, the solution was mixed and centrifuged. An aliquot (0.2 ml) of the upper phase was diluted with 0.8 ml water, 2.5 ml of a 3:2 (v/v) mixture of 4 μmol^{-1} ml glycine in acetic acid and 6 M phosphoric acid, and 2.5 ml 40 mg^{-1} ml ninhydrin. The solution (6.0 ml) was boiled at 95 °C in a water bath for 40 min, 5 ml of toluene were added, and the supernate was measured at the absorbance of 520 nm. The proline content was calculated using a standard curve (Wen et al., 2011).

Soluble protein was quantified following the Bradford (Bradford, 1976) method, using bovine serum albumin as the standard. The proteins for the enzyme assays were extracted from 0.5 g lyophilized leaves with the grinding medium consisting of 0.1 M K-phosphate and 0.1 mM EDTA (pH 7.8), and the homogenate was centrifuged twice at 13,000 g for 10 min (Giannopolitis and Ries, 1977). Superoxide dismutase (SOD) activity was assayed as described by Giannopolitis and Ries (Giannopolitis and Ries, 1977), that of peroxidase (POD) following Kara and Mishra (Kar and Mishra, 1976), that of catalase (CAT) following Cakmak and Marschner (Cakmak and Marschner, 1992) and that of ascorbate peroxidase (APX) following Nakano and Asada (Nakano and Asada, 1981).

2.7. Tissue Na^+ and K^+ content

Seedlings were rinsed at least three times with deionized water, after which the material was oven-dried at 70 °C for 48 h (Quinet et al., 2010). A 50 mg aliquot of dry material was digested in 1 mL 35% HNO_3 for 35 min. Na^+ and K^+ were resuspended in 10 mL HNO_3 (10%). The Na^+ and K^+ content was quantified by emission spectrometry using an Optima 8000 ICP-OES spectrometer (PerkinElmer, Waltham, MA, USA).

2.8. Statistical analysis

Three biological replicates were included. For each parameter determination, three technical replicates were included. The