



Exogenous putrescine reduces flooding-induced oxidative damage by increasing the antioxidant properties of Welsh onion

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ABSTRACT

Soil flooding is an environmental factor of seasonal occurrence that negatively affects plant performance. Polyamines play an important role in the plant response to adverse environmental conditions including flooding stress. The objective of the present study was to assess the comparative oxidative damage to Welsh onion plants caused by flooding stress and to examine the role of putrescine (Put) in this response. Welsh onion plants were treated with Put prior to 10 d flooding. A positive effect was observed when treating with 2 mM Put. Exogenous application of Put resulted in alleviation of flooding-induced reduced relative water content, plant growth and chlorophyll fluorescence. Superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) contents were also reduced in stressed plants after Put pre-treatment and thereby the oxidative stress in plant cells was lowered. The antioxidant system, as an important component of the waterlogging-stress-protective mechanism including α,α -diphenyl- β -picrylhydrazyl (DPPH)-radical scavenging activity, superoxide anion radical scavenging, metal chelating activities and reducing power, can be upgraded by Put, which is therefore able to moderate the radical scavenging system and to lessen oxidative stress. Under non-flooding conditions, the anti-oxidative activity of Welsh onion was regulated and elevated by Put pre-treatment. These results suggest that Put confers flooding tolerance to Welsh onion, probably through inducing the activities of various anti-oxidative systems. Thus, exogenous 2 mM per plant of Put 24 h prior to flooding could alleviate flooding stress.

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1. Introduction

Polyamines (PAs) are small aliphatic amines that are ubiquitous in plants, animals and microorganisms. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the major PAs in plants and they are involved in various processes such as cell proliferation, growth, morphogenesis, differentiation and programmed cell death (Serafini-Fracassini et al., 2002; Kusano et al., 2007). Because of their polycationic nature at physiological pH, PAs are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby activating or stabilizing these molecules.

Recent studies have focused on the involvement of PAs in the defense reaction of higher plants to various environmental stresses (Kusano et al., 2007). The link between PAs and abiotic stress was

first documented through Put accumulation in response to suboptimal potassium levels in barley (Richards and Coleman, 1952). The physiological significance of Put in plants has focused on various types of abiotic stress responses, such as high osmotic pressure (Flores and Galston, 1982), low pH (Young and Galston, 1983), potassium deficiency (Watson and Malmberg, 1996) and cadmium toxicity (Weinstein et al., 1986).

Exogenous PA application is a convenient and effective approach for enhancing the stress tolerance of crops and eventually improving crop productivity. Exogenous Put increases the relative importance of the alternative respiratory pathway. The utilization of this pathway could provide a mechanism whereby respiratory flux is maintained under conditions in which the availability of ADP and Pi is restrictive (Parsons et al., 1999), and it could also stabilize the reduction state of the ubiquinone pool thus attenuating the formation of reactive oxygen species (Hilal et al., 1997). Indeed, exogenous Put application has been successfully used to enhance salinity (Verma and Mishra, 2005; Ndayiragije and Lutts, 2006a), cold (Nayyar and Chander, 2004; Nayyar, 2005), drought (Zeid and Shedeed, 2006) and waterlogging tolerance of plants (Arbona et al., 2008). The physiological role of Put in abiotic

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stress responses is a matter of controversy. It has been very difficult to establish a direct cause-and-effect relationship between increased Put levels in plants and abiotic stress (Krishnamurthy and Bhagwat, 1989; Ndayiragije and Lutts, 2006b). In mango fruit, chilling stress promoted Put accumulation in the early stages of ripening (Nair and Singh, 2004). However, Put increase did not prevent chilling injury. Elevated Put might be the cause of stress-induced injury or, alternatively, a protective response resulting from stress (Reggiani et al., 1993).

Usually, high light levels following heavy rainfall exert a deleterious effect on Welsh onion during the summer in Taiwan. Therefore, rapid senescence, such as chlorosis, is seen when Welsh onion is subjected to waterlogging (Yiu et al., 2008). Waterlogging induces a progressive reduction in soil O₂ concentration and redox potential (Ruiz-Sánchez et al., 1996), which contributes to the appearance of several reduced compounds of either chemical or biochemical origin (Kozłowski, 1997). Flooding stress also contributes to the accumulation of acetaldehyde and other compounds derived from anaerobic metabolism, which could be susceptible to degradation yielding hydrogen peroxide (H₂O₂) as an end product (Blokhina et al., 2003).

Polyamines are now regarded as a new class of growth substances (Bouchereau et al., 1999), and are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as for their membrane and cell wall stabilizing abilities (Berta et al., 1997; Zhao and Yang, 2008). Scarce data, however, are available on the production of active oxygen species and antioxidant systems in plants subjected to flooding, or on the involvement of PAs as protectants against this type of stress. The objectives of the present study were therefore to determine whether Put is involved in flooding tolerance and whether exogenous Put affects antioxidant properties and increases long-term flooding tolerance in Welsh onion.

2. Materials and methods

2.1. Plant material and cultivation conditions

Single tillers of Welsh onion, *Allium fistulosum* L. cv. erhan, were grown in pots 35 cm high and measuring 10 cm × 10 cm at the top and 9 cm × 9 cm at the base. The most homogeneous selection of single tillers practicable (64–67 cm in height with 3–4 leaves) was made avoiding, to the greatest extent possible, variations resulting from cultivation. The pots were filled with a substrate of peat moss, vermiculite and perlite (1:2:2, v/v) (Yiu et al., 2008). Specimens were planted during July and August 2007 in the greenhouse of Ilan University. Plants were evenly spaced at intervals of 30 cm to encourage similar growth rate and size. Plants were watered with half-strength Hoagland solution every other day to maintain optimal irrigation, and were allowed to grow for 40 d before flooding stress imposition. The Put treatments were carried out in experiments of completely randomized design with three replicates. During the period of study, average day/night temperatures were 34/27 °C, respectively, and the average day length was 12 h with an irradiance of 900–1100 μmole m⁻² s⁻¹ and a relative humidity (RH) of 70%.

2.2. Treatments and growth and development measurements

In the first experiment, each plant was treated with a single 120 mL dose of an aqueous solution of Put (0, 1, 2 or 3 mM, Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany). After 24-h Put or water (control) treated plants were subjected to flooding conditions for 10 d. For each treatment replicate, samples were placed in a 60 cm × 40 cm × 40 cm plastic bucket containing water to a level of 5 cm above the substrate surface. Put-untreated

plants were considered to be a control to provide a basis to compare the effects of Put under flooding conditions. Each treatment was performed in triplicate. After 10 d of treatment, 50 plants per treatment were collected for determination of relative water content (RWC). For determination of fresh weight, shoots and roots were separated and weighed after being washed with sterile distilled water. The dry weight was obtained after drying at 75 °C in an oven for 72 h. RWC, indicating the level of water stress in leaves, was estimated according to Weatherley (1950), and calculated according to the formula:

$$\text{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{fresh weight at full turgor} - \text{dry weight})} \times 100\%$$

In the second experiment, 0 or 2 mM Put were applied to the substrate surface as a single 120 mL liquid drench. Twenty-four hours after Put treatment, the plants were subjected to one of two water conditions (non-flooding and flooding) for 0, 1, 5 or 10 d, followed by 5 d drainage. For each different flooding time treatment replicate, samples were placed in a 60 cm × 40 cm × 40 cm plastic bucket containing water to a level of 5 cm above the substrate surface. Put-untreated plants under non-flooding conditions were considered to be negative controls. Each treatment consisted of 50 plants distributed inside the greenhouse at random. During the assay, plant height, chlorophyll content and chlorophyll fluorescence were regularly evaluated by considering 30 randomly distributed plants per treatment.

2.3. Chlorophyll analysis

Chlorophyll was extracted with dimethyl sulphoxide (DMSO) (Merck, Germany), using a method adapted from Barnes et al. (1992). The third leaves of 30 plants randomly selected from each replicate set were cut into standard 1 cm lengths. These were placed in 50 mL glass tubes with 25 mL DMSO. The tubes were sealed and placed in a 60 °C oven for 12 h. Shoot tips were then removed and placed in a drying oven for 24 h at 80 °C and were individually weighed. Absorbance was measured at wavelengths of 648.2 and 664.9 nm on a spectrophotometer in a 1 cm cell. Equations used to calculate chlorophyll *a* and *b* concentrations are found in Barnes et al. (1992) $\text{Chl } a + b = (7.49 \times A_{664.9} + 20.34 \times A_{648.2}) \times [V/1000 \times (1/W)]$. The unit is defined as mg per g dry weights (DW). *V* = extract volume (mL). *W* = leaf DW (g).

2.4. Chlorophyll *a* fluorescence measurements

Chlorophyll fluorescence at room temperature was performed on three leaves (fully expanded) using a portable pulse-modulated fluorimeter (PAM-2000, Walz, Germany). Samples were kept in the dark for 30 min prior to the measurements. The minimum (dark) fluorescence yield (*F*₀) was obtained upon excitation of leaves with a weak measuring beam (14 μmol photon m⁻² s⁻¹). The maximum fluorescence yield (*F*_m) was determined with a 600 ms pulse of white light (4000 μmol photon m⁻² s⁻¹). Variable fluorescence (*F*_v) was calculated as *F*_m – *F*₀. The quantum efficiency of electron flux through photosystem II (ΦPSII) was estimated according to Genty et al. (1989) from the ratio of *F*_v/*F*_m.

2.5. Superoxide anion (O₂⁻)

The detection of O₂⁻ was based on its ability to reduce nitro blue tetrazolium (NBT) (Doke, 1983). Fully expanded leaves of Welsh onion (100 mg) were cut into 1 mm × 1 mm fragments and immersed in 10 mM potassium phosphate buffer, pH 7.8, containing 0.05% (w/v) NBT and 10 mM Na₂S₂O₃, and incubated for 1 h at room temperature. After incubation, 2 mL of the reaction solution was heated at 85 °C for 15 min and cooled rapidly. Optical density

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