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Research article

Changes in content of free, conjugated and bound polyamines and osmotic adjustment in adaptation of vetiver grass to water deficit

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ABSTRACT

Osmotic adjustment and alteration of polyamines (PAs) have been suggested to play roles in plant adaptation to water deficit/drought stress. In this study, the changes in cell intactness, photosynthesis, compatible solutes and PAs [including putrescine (Put), spermidine (Spd) and spermine (Spm) each in free, conjugated and bound forms] were investigated in leaves of vetiver grass exposed to different intensity of water deficit stress and subsequent rewatering. The results showed that, when vetiver grass was exposed to the moderate (20% and 40% PEG-6000 solutions) and severe (60% PEG solution) water deficit for 6 days, the plant injury degree (expressed as the parameters of plant growth, cell membrane integrity, water relations and photosynthesis) increased and contents of free and conjugated Put decreased with the rise of PEG concentration. Under the moderate water deficit, the plants could survive by the reduced osmotic potential (ψ_s), increased free and conjugated Spd and Spm in leaves. After subsequent rewatering, the osmotic balance was re-established, most of the above investigated physiological parameters were fully or partly recovered to the control levels. However, it was not the case for the severely-stressed and rewatering plants. It indicates that, vetiver grass can cope well with the moderate water deficit/drought stress by using the strategies of osmotic adjustment and maintenance of total contents of free, conjugated and bound PAs in leaves.

1. Introduction

Approximately one-third of the world's arable land suffers from chronically inadequate supplies of water for agriculture [1]. Water deficit/drought stress is one of the main abiotic stresses subjected to plants during their whole growth and development [2], and is also one of the major causes of osmotic stress to plants under natural conditions [3]. In addition, drought stress also induces oxidative stress because of inhibition of photosynthetic activity due to imbalance between light capture and its utilization [4]. Under drought/water deficit stress condition, plants exhibit a wide range of biochemical and physiological responses at the molecular, cellular and whole plant levels, such as altered water status and stomatal closure, repression of cell growth and photosynthesis, over-production of phytohormone abscisic acid (ABA), accumulation of metabolites (including soluble sugars, such as sucrose, trehalose and sorbitol; free amino acids, such as proline; and amines, such as glycine betaine and polyamines, etc.), and alteration in gene expression and efficiency of antioxidative enzymes. The accumulated metabolites under drought stress may function as compatible osmolytes by maintaining cell turgor and water relations with the decrease of water potential (ψ_w), or as cell antioxidants, reactive oxygen species (ROS) scavengers and protein stabilizer, which above all help plants to avoid and/or tolerate the environmental water deficit stress [2,4–7].

Polyamines (PAs) are low molecular and aliphatic nitrogen organic cations, the diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm) are the main PAs found in all living cells, and occur as free molecular bases (free forms), or are often conjugated to small molecules like phenolic acids (conjugated forms) and to various macromolecules like proteins (bound forms) [8]. Many researchers have shown the relationships of PAs metabolism (including changes in forms, contents, biosynthetic and catabolic enzymes of PAs) with plant responses to various abiotic stress conditions, and PAs may stabilize the membranes, scavenge free-radical, modulate the activities of certain ion channels and control many aspects of DNA, RNA and protein turnover under drought stress [8–10].

Abbreviations: Ci, internal CO_2 concentration; Fv/Fm, chlorophyll fluorescence; GB, glycinebetaine; Gs, stomatal conductance; MDA, malondialdehyde; PAR, photosynthetically active radiation; PAs, polyamines; PCA, perchloric acid; PEG, polyethylene glycol; Pn, net photosynthesis rate; PPFD, photosynthetic photon flux density; Put, putrescine; REL, relative electrolyte leakage; ROS, reactive oxygen species; RWC, relative water content; ψ_s, osmotic potential; Spd, spermidine; Spm, spermine; Tr, transpiration rate; WUE, water use efficiency.

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Vetiver grass (*Vetiveria zizanioides*), a kind of sterile and perennial grass, is a graminaceous plant native to tropical and subtropical areas. It can tolerate many kinds of extreme environments, such as drought, hot, acid, salt, alkali, and heavy metal, etc., and has been successfully used in about 100 countries as a low-cost, low technology and effective means for soil and water conservation, land rehabilitation, wastewater treatment, pollution control, and many other environmental applications [11,12]. In addition, vetiver grass is now considered to have future potential as bio-fuel for power generation and cellulosic ethanol [13]. Our previous work has demonstrated for the first time that the osmotic adjustment was effective for vetiver grass seedlings under moderate saline stress (less than 200 mmol L^{-1} NaCl) [14]. Up to date, the detailed and comprehensive studies on physiological adaptation of vetiver grass to water deficit/drought stress are still lacking. In the present study, the changes in cell intactness, photosynthesis, compatible solutes and PAs [including putrescine (Put), spermidine (Spd) and spermine (Spm) each in free, conjugated and bound forms] were examined in leaves of vetiver grass during the moderate and severe water deficit stress and subsequent rewatering.

2. Materials and methods

2.1. Plant materials and treatments of water deficit and rewatering

Vetiver grass plants were picked from hypaethral experimental garden which lay in the city of Nanjing, PR China. Some 9-month-old vetiver grass plants were individually fixed in the sheet of foam board and whose roots were dipped into Hoagland solution in plastic square boxes. They were cultured at a 26 ± 2°C day/20 ± 2°C night under light irradiation of 500 μmol $m^{-2}s^{-1}$ (16 h per day) in the growth chamber. When the plants renewed their growth, the plants were subjected to the following treatments: Hoagland solution (control, solution water potential: ∼−0.01 MPa), Hoagland solution +20% PEG −6000 (∼−0.52 MPa), Hoagland solution +40% PEG −6000 (∼−1.86 MPa), and Hoagland solution +60% PEG-6000 (∼−4.04 MPa). After water deficit treatment for 6 days, part of the plants were sampled, the residual plants were rewatered in Hoagland solution for another 6 days, and then were sampled. All the solutions (pH = 6.0) were renewed every 2 days.

2.2. Assay of plant height growth rate, relative water content (RWC), malondialdehyde (MDA) contents and relative electrolyte leakage (REL)

Plant height was directly measured using a centimeter ruler at 0th, 6th, and 12th day treatments, respectively, and the plant height growth rates under water deficit and after rewatering were expressed as cm d^{-1}.

RWC was measured with the method described by Coleman [15]. The leaves were weighed immediately to obtain the fresh weight (FW), then were subsequently rehydrated in distilled water for 24 h at 4 °C in the dark to obtain the turgid weight (TW), and dried for 48 h at 80 °C to obtain the dry weight (DW). RWC in leaf was determined using the following formula: RWC = (FW − DW)/(TW − DW) × 100%.

MDA content in leaves was measured according to the method described by Jouve et al. [16]. A total of 0.5 g of fresh tissue was ground in 5 mL 5% (w/v) trichloroacetic acid (TCA), 2 mL of supernatant mixed with 2 mL of 0.67% (w/v) thiobarbituric acid (TBA) in 5% (w/v) TCA, and the samples incubated at 100 °C for 30 min. After centrifuging, the optical density was measured at 450, 532 and 600 nm, respectively. The amount of MDA was calculated from the following formula: $C = 6.45(A_{532} - A_{600}) - 0.56A_{450}$, C represented the concentration of MDA in supernatant and expressed as μmol L^{-1}, A_{532}, A_{600} and A_{450} represented the absorbance values at 532, 600 and 450 nm, respectively, the MDA content was finally expressed as nmol g^{-1} FW.

REL in leaves was assayed as the method described by Vannini et al. [17] with minor modifications. The percentage of electrolytic leakage was calculated as: C_1-C_w/C_2-C_w, where C_1 is the conductance of leaf tissue before boiling, C_2 is the conductance after boiling, and C_w is the deionized water conductance.

2.3. Measurement of photosynthesis and chlorophyll fluorescence (Fv/Fm)

The portable photosynthesis meter (LI-6400, LI-COR Inc., USA) was used to measure the values of Pn, Gs, Ci, Tr in leaf. The photosynthetic photon flux density (PPFD), temperature and CO_2 concentration were 1000 μmol m^{-2} s^{-1}, 26 °C and ∼390 μmol mol^{-1}, respectively. Pn/Gs were taken as an estimate of intrinsic water use efficiency (WUE) [18].

Fv/Fm was measured at room temperature with a plant efficiency analyzer (Handy-PEA Fluorometer, Hansatech Instruments, UK). The first pair of unifoliolate leaves was dark-adapted for 30 min using Handy-PEA leaf clips. The flux density of incident photosynthetically active radiation (PAR) was 3000 μmol m^{-2} s^{-1}. Fv/Fm was read directly.

2.4. Determination of osmotic potential (ψ_s), contents of soluble sugars, free amino acids and glycinebetaine (GB)

The leaf pieces were placed in centrifuge tubes that were sealed and frozen in liquid nitrogen, the ψ_s was determined by an automatic freezing-point depression osmometer (FM-8P type, Shanghai, China) on sap extracted from the frozen plant samples by pressing in the injector. $\Psi_s = -$ nRT (n = mosmol L^{-1}, the value was read directly from the osmometer; R = 0.008314 L MPa mol^{-1} K^{-1}, and $T(K)$ = ambient temperature) [19].

Soluble sugars were extracted and determined by the anthrone method [18]. Water extract of fresh leaves were added to 0.5 mL 0.1 mol L^{-1} anthrone-ethyl acetate, 5 mL 1.08 mol L^{-1} H_2SO_4. The mixture was heated at 100 °C for 10 min, and its absorbance at 620 nm was read after cooling to room temperature. A calibration curve with sucrose was done as a standard.

Total free amino acids were extracted and determined following the method of Lee et al. [20] with slight modifications. A total of 0.5 g fresh tissue was homogenized in 5 mL 10% (w/v) acetic acid, extracts were added with 1 mL of distilled water and 3 mL of ninhydrin reagent, then boiled for 15 min and fast cooled, and the volume was made up to 5 mL with 60% (v/v) ethanol. Absorbance was read at 570 nm. The content of total free amino acids was calculated from a standard curve prepared against leucine.

GB were extracted and determined following the method of Moghaieb et al. [21] with slight modifications. The samples of dried leaf powder were transferred to vials subjected to methanol extraction, and stored in the dark at 4 °C. The methanol extracts were passed through Dowex-OH^- and Dowex-50-H^+columns, and then GB was eluted with 6 mol L^{-1} NH_4OH and analyzed by Waters HPLC System (USA) with a reverse phase C_{18} column. Acetonitrile/H_2O (8:92) (v/v) was used as an isocratic eluting solvent at a flow rate of 0.4 mL min^{-1}. GB peak was detected by a Perkin–Elmer LC-95 absorbance detector at 195 nm.

2.5. Extraction and analysis of PAs

Free, conjugated and bound PAs were quantified according to Kotzabasis et al. [22] with minor modifications. Samples of fresh leaves (0.5 g) were homogenized in 5 mL of 5% (v/v) perchloric acid