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Some synthetic cyclitol derivatives alleviate the effect of water deficit in cultivated and wild-type chickpea species

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ABSTRACT

Cyclitols were prepared from corresponding allylic hydroperoxides, synthesized by photooxygenation of the appropriate cyclic alkenes. These hydroperoxides were then separately treated with a catalytic amount of OsO_4. Synthesized *dl*-cyclopentane-1,2,3-triol **9** (A), *dl*-cyclohexane-1,2,3-triol **12** (B), and *dl*-cycloheptane-1,2,3-triol **15** (C) were used in the investigation of plant stress. Antioxidants, lipid peroxidation, and water status of chickpea species exposed to synthetic cyclitols under water deficit were examined. Cyclitol derivatives significantly decreased leaf water potential, lipid peroxidation and H_2O_2 levels of wild and cultivated species under water deficit. Cyclitol treatments affected antioxidant enzyme activities differently in both species under water deficit. The highest SOD activity was found in A10-treated *Cicer arietinum* (cultivar) and C10-treated *Cicer reticulatum* (wild type) under water deficit. CAT activity increased in *C. arietinum* exposed to A cyclitols, while it increased slightly and then decreased in cyclitol-treated *C. reticulatum* under stress conditions. AP and GR activities were significantly increased in *C. arietinum* under water deficit. AP activity increased in C derivatives-treated *C. arietinum*, while it remained unchanged in *C. reticulatum* on day 1 of water deficit. GR activity was increased in A derivaties-treated *C. arietinum* and C derivatives-treated *C. reticulatum* on day 1 of water deficit and decreased with severity of stress (except for B10-treated *C. arietinum*). The level of AsA in C treatments and GSH in A treatments increased in *C. arietinum* on day 1 of water deficit, while in *C. reticulatum*, AsA and GSH levels decreased under stress conditions. We conclude that exogenous synthetic cyclitol derivatives are biologically active and noncytotoxic, resulting in higher antioxidant activities and lower water potential, thus increasing the water deficit tolerance of chickpea under water deficit, especially of cultivated chickpea. We also propose that synthetic cyclitol derivatives can reduce reactive oxygen species and membrane damage and are beneficial for stress adaptation.

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Introduction

Polyols are either open chain (acyclic) compounds with the general formula $HOCH_2$ $[CH(OH)]n$ CH_2OH or cyclic compounds (cyclohexan hexols or pentols), usually termed cyclitols or inositols. The cyclitol derivatives confer some intriguing biological activities to plants, such as glycosidase inhibition. The natural and synthetic cyclitol derivatives are widely used in pharmaceutical and food industries owing to their high solubility in water, antibiotic and antioxidant activities (Hudlicky et al., 1990; Gültekin et al., 2004). Cyclitols such as pinitol, quebrachitol, and quercitol can accumulate in relatively large amounts, up to several percent of dry matter on a whole-plant basis during photosynthesis (Merchant et al., 2006a,b; Arndt et al., 2008). Many eukaryotes use inositol-based cytosolic solutes as protective compounds under stress conditions (Valluru and Van den Ende, 2011). Cyclitols are found in all plants and the O-methyl cyclitols are especially common in legumes (Streeter et al., 2001). They have been reported to accumulate in response to water deficit (Nguyen and Lamant, 1988) and salinity (Borland et al., 2006). Cyclitol and its derivatives are an emerging family

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of compounds that are crucial for development and signaling in plants. They essentially play a role as either metabolic mediators or contribute in various signaling pathways in response to stress (Hudlicky et al., 1990; Valluru and Van den Ende, 2011).

Accumulation of cyclic carbohydrates (cyclitols) during water deficit leads to osmotic adjustments of plants (Irvine and Schell, 2001; Piotrowicz-Cieslak et al., 2007). The accumulation of high amounts of osmoprotectants that form upon water deficit and salinity stress is common in plants (Ahn et al., 2011). Osmoprotectants stabilize proteins and membranes and lower the osmotic potential of membranes to prevent the loss of water and photorespiration (Piotrowicz-Cieslak et al., 2007; Ahn et al., 2011; Aroca, 2012). Osmotic adjustment occurs when plants accumulate solute for the purpose of maintaining positive cell turgor (Arndt et al., 2008). Inositols are also essential for growth in plants (Valluru and Van den Ende, 2011; Ahn et al., 2011).

Cicer arietinum is an important legume cultivated around the world. It is susceptible to drought stress. *Cicer reticulatum* is wild type chickpea and is more tolerant to drought stress (Toker et al., 2007). Many studies have reported that plants actively produce reactive oxygen species (ROS), which may control many different physiological processes such as biotic and abiotic stress responses (Gong et al., 2005; Ünyayar et al., 2005; Sankar et al., 2008; Gill and Tuteja, 2010). The equilibrium between the production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as drought, salinity, and pathogen attacks (Gill and Tuteja, 2010). ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Fridovich, 1986; Davies, 1987; Ünyayar et al., 2010). Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR), and catalase (CAT), as well as nonenzymatic low molecular metabolites, such as ascorbate (AsA) and glutathione (GSH) (Davies, 1987; Baek and Skinner, 2003). The ability of various cyclitols to scavenge hydroxyl radicals *in vitro* has been shown (Orthen et al., 1994; Merchant et al., 2011). Other researchers have reported that cyclitols are known to accumulate to high levels in *Mesembryanthemum crystallinum* in response to salinity and have been proposed to act not only as compatible solutes for maintaining osmotic balance, but also to curtail oxidative damage in saline conditions by scavenging ROS (Borland et al., 2006).

Although a great deal is known about the function of cyclitols in plants, the effect of synthetic derivatives of cyclitols for plant management remains unexplored. We hypothesized that exogenous treated synthetic cyclitol derivatives could result in higher activities of antioxidant enzymes and antioxidant levels. As a consequence, exogenous treated cyclitols could contribute to mitigation of damage to membrane lipids in cultivated and wild chickpea species subjected to water deficit. To test this hypothesis, leaves of chickpea plants sprayed with three different concentrations of synthetic cyclitol derivatives were subjected to full irrigation and water deficit conditions. Plant comparisons were performed to test the effect of some synthetic cyclitol derivatives treatments on growth, lipid peroxidation, SOD, CAT, GR, AP, GSH, AsA, and H_2O_2 levels in cultivated *C. arietinum* and wild-type *C. reticulatum* subjected to full irrigation and water deficit conditions.

Materials and methods

Plant material and experimental setup

Cyclitols, which have skeleton structure as 1/2,3-trihydroxy-cyclopentane **9**, 1/2,3-trihydroxy-cyclohexane **12**, 1/2,3-trihydroxy-cycloheptane **15**, were prepared from corresponding allylic hydroperoxides **8**, **11**, **14**, respectively. These hydroperoxides **8**, **11**, **14** were synthesized by photooxygenation reaction of cyclopentane **7**, cyclohexane **10** and cycloheptane **13** and then separately treated with a catalytic amount of OsO_4 in water–acetone (1:9) solution at room temperature. The cyclic allylic hydroperoxides were successfully converted to the corresponding 1,2/3-triols **9**, **12**, **15** with high chemical yields and selectivity.

The effects of the above-mentioned synthetic cyclitols on the growth of *Cicer arietinum* Küsmen 99 (cultivated chickpea) and *Cicer reticulatum* AWC 611 (wild type) were evaluated. Seeds were imbibed in aerated water for 1 day at 22 °C. Then they were transferred to plastic pots (2 L) filled with a soil:peat:manure mixture (2/1/1, 2000 g). All seedlings were grown at 23 ± 2 °C, 16/8 h photoperiod, irradiance 480 $\mu mol\, m^{-2}\, s^{-1}$, 65 ± 5 relative humidity up to 35 days by irrigating. Then half of the pots were well-watered (WW, control) and other pots were non-watered until wilted leaves of untreated plants. Leaves of half of the seedlings in each group (well-watered and non-watered) were sprayed with 50 mL cyclitol solution (10 μM, 20 μM, and 30 μM for each cyclitol) for 3 days, while the controls were sprayed with distilled water. A, B and C letters for *dl*-cyclopentane-1,2,3-triol, *dl*-cyclohexane-1,2,3-triol, *dl*-cycloheptane-1,2,3-triol were used, respectively (Fig. 1) (Alp et al., 2009). Cyclitol concentrations were stated as A10, A20 and A30 for *dl*-cyclopentane-1,2,3-triol, B10, B20 and B30 for *dl*-cyclohexane-1,2,3-triol, C10, C20 and C30 for *dl*-cycloheptane-1,2,3-triol. Treatments were repeated three times with different sets of plants. Leaves were rinsed in distilled water before they were used for analysis. 24 h (day 1) and day 7 we stopped watering and expanded leaves were harvested for analysis.

Plant–water relations

Leaf water potential (Ψ_{leaf}) was measured with a pressure chamber (PMS Instrument Co., Model 1000).

H_2O_2 level measurement

Hydrogen peroxide (H_2O_2) levels were measured according to Velikova et al. (2000) with minor modifications. Leaf tissues (500 mg) were homogenized in an ice bath with 5 mL of 2% (w:v) TCA. The homogenate was centrifuged at 12,000 × *g* for 15 min and the supernatant was used for the H_2O_2 assay. H_2O_2 was quantified with a Bioxytech H_2O_2-560 assay kit (OXIS International, Inc., USA).

Malonyldialdehyde (MDA) assay

The level of lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) according to Ohkawa et al. (1979).

Assay of antioxidant enzymes

Fresh leaves from each treatment were homogenized with a pestle and mortar with 5 mL of 0.1 M potassium phosphate buffer (pH 6.8) containing 100 mg of PVP and 0.1 mM EDTA. The homogenate was centrifuged at 16,000 × *g* for 5 min and the supernatant was immediately used for analyzing SOD, CAT, and GR. SOD activity (superoxide dismutase; EC 1.15.1.1) was determined as the amount of enzyme that was required to cause 50% inhibition of the reduction of NBT by measuring at 560 nm as described in detail by Beyer and Fridowich (1987). CAT (catalase; EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 at 240 nm for 30 s as described by Aebi (1983). GR activity (glutathione reductase; EC 1.6.4.2) activity was determining by following the oxidation of NADPH at 340 nm as described by Carlberg and Mannervik (1985). AP (ascorbate peroxidase; EC 1.11.1.11) activity was detected at

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