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Short-term physiological changes in roots and leaves of sugarcane varieties exposed to H_2O_2 in root medium

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ABSTRACT

The aim of this study was to evaluate the differential sensitivity of sugarcane genotypes to H_2O_2 in root medium. As a hypothesis, the drought tolerant genotype would be able to minimize the oxidative damage and maintain the water transport from roots to shoots, reducing the negative effects on photosynthesis. The sugarcane genotypes IACSP94-2094 (drought tolerant) and IACSP94-2101 (drought sensitive) were grown in a growth chamber and exposed to three levels of H_2O_2 in nutrient solution: control; 3 mmol L^{-1} and 80 mmol L^{-1}. Leaf gas exchange, photochemical activity, root hydraulic conductance (L_r) and antioxidant metabolism in both roots and leaves were evaluated after 15 min of treatment with H_2O_2. Although, root hydraulic conductance, stomatal aperture, apparent electron transport rate and instantaneous carboxylation efficiency have been reduced by H_2O_2 in both genotypes, IACSP94-2094 presented higher values of those variables as compared to IACSP94-2101. There was a significant genotypic variation in relation to the physiological responses of sugarcane to increasing H_2O_2 in root tissues, being root changes associated with modifications in plant shoots. IACSP94-2094 presented a root antioxidant system more effective against H_2O_2 in root medium, regardless H_2O_2 concentration. Under low H_2O_2 concentration, water transport and leaf gas exchange of IACSP94-2094 were less affected as compared to IACSP94-2101. Under high H_2O_2 concentration, the lower sensitivity of IACSP94-2094 was associated with increases in superoxide dismutase activity in roots and leaves and increases in catalase activity in roots. In conclusion, we propose a general model of sugarcane reaction to H_2O_2, linking root and shoot physiological responses.

Introduction

Root system is the first plant organ affected by low soil water availability, with sugarcane plants showing reduction in shoot hydration and decreases in stomatal conductance, transpiration and CO_2 assimilation (Ribeiro et al., 2013; Sales et al., 2013). Regarding the causes of low photosynthesis, the following can be noted, low mesophyll CO_2 conductance, reductions in chlorophyll content and impairment of photochemical and biochemical reactions under severe water deficit (Ghannoum, 2009; Machado et al., 2009), when plants are likely under oxidative stress. The reduced consumption of photochemical products due to decreases in carboxylation reactions generates excess energy at photosystem level that can lead to photoinhibition and further decreases in quantum efficiency of photosynthesis (Chagas et al., 2008; Ribeiro et al., 2013).

Under cellular redox imbalance conditions due to excessive energetic pressure, reactive oxygen species (ROS) are formed, such as superoxide anion and hydrogen peroxide (H_2O_2). In C_4 plants, H_2O_2 is generated in chloroplasts and is harmful to cell organelles and membranes. It also acts as a secondary messenger in signal transduction due to its relative long half-life and high membrane permeability (Hung et al., 2005; Cheeseman, 2007; Quan et al., 2008). In addition, H_2O_2 is involved in the coordination of root-shoot metabolism and in stomatal control of leaf gas exchange

Abbreviations: A_N, leaf CO_2 assimilation; APX, ascorbate peroxidase; CAT, catalase; ETR, apparent electron transport rate; EXC, relative energy excess at PSII level; F_V/F_M, potential quantum efficiency of photosystem II; g_S, stomatal conductance; IWUE, intrinsic water use efficiency; k, instantaneous carboxylation efficiency; L_r, root hydraulic conductance; PSII, photosystem II; Q, photosynthetically active radiation; ROS, reactive oxygen species; SOD, superoxide dismutase.

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(Desikan et al., 2004). This role is based on the inhibitory effect of H_2O_2 on aquaporin activity, affecting water transport (Boursiac et al., 2008). Under stressful conditions, H_2O_2 accumulation may decrease root hydraulic conductance (L_r) by forming an oxidative gate that closes aquaporin channels and reduces water transport through plant membranes (Ye and Steudle, 2006; Ehlert et al., 2009).

Plants have evolved an antioxidant system to avoid damaging effects of ROS, eliminating such molecules through reactions mediated mainly by superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Bienert et al., 2007; Gill and Tuteja, 2010). Genotypic variation is found when we consider the physiological responses of sugarcane to water deficit, with drought tolerance being associated with increased activity of antioxidant enzymes. While SOD is the main enzyme for $O_2^{\bullet-}$ degradation, CAT acts in reducing H_2O_2 concentration in sugarcane plants under stressful conditions (Mitler, 2002; Cia et al., 2012; Sales et al., 2013). On the other hand, drought-sensitive sugarcane genotypes present high lipid peroxidation and increased H_2O_2 concentration in leaves (Boaretto et al., 2014).

Studies relating the antioxidant system to water transport are scarce and they mostly focus only on leaves or roots separately without establishing a connection between both organs. This fact limits our overall understanding of how plants respond to stressful situations and interactions between water relations, antioxidant system and photosynthetic metabolism remain unknown. As plants face soil water deficiency, an increase in ROS production is expected in their roots (Hung et al., 2005). In such situation, the drought-tolerant genotype should present rapid antioxidant response in roots, preserving the water transport and the photosynthetic apparatus. Taking into account the genotypic variation found in sugarcane, this study aims to test the hypothesis that the drought-tolerant genotype would present a more effective root antioxidant system response against exogenous H_2O_2, thus, reducing the negative impact of H_2O_2 on root hydraulic conductance and leaf gas exchange when compared to the drought-sensitive genotype.

Materials and methods

Plant material and experimental conditions

We tested our hypothesis with sugarcane (*Saccharum* spp.) genotypes developed by the Sugarcane Breeding Program of the Agronomic Institute (ProCana, IAC, Brazil). IACSP94-2094 is a drought-tolerant genotype, whereas IACSP94-2101 is sensitive to water deficit (Landell et al., 2005; Ribeiro et al., 2013). Thirty days-old plants grown in commercial substrate (Carolina Soil of Brazil, Vera Cruz SC, Brazil) with three to four leaves were transferred to a nutrient solution adapted from Sarruge (1975). Such solution was composed by potassium nitrate (0.31 g L^{-1}), calcium nitrate (1.20 g L^{-1}), magnesium sulfate (0.50 g L^{-1}), ammonium nitrate (0.08 g L^{-1}), di-hydrogen potassium phosphate (0.14 g L^{-1}), potassium chlorate (0.06 g L^{-1}), EDDHMA iron (0.07 g L^{-1}), boric acid (1.69 mg L^{-1}), zinc sulfate (1.10 mg L^{-1}), copper sulfate (0.16 mg L^{-1}), manganese sulfate (0.92 mg L^{-1}) and ammonium molybdate (2.32 mg L^{-1}).

The experiment was carried out in a growth chamber (model PGR15, Conviron, Winnipeg MB, Canada), with a 12-h photoperiod, air temperature of 30/20 °C (day/night), air relative humidity of 80% and photosynthetically active radiation (Q) of 800 μmol m^{-2} s^{-1}. In such environment, plants were subjected to two exogenous application of H_2O_2 in nutrient solution, which reached H_2O_2 concentrations of 3 and 80 mmol L^{-1}. Plants grown in nutrient solution without exogenous H_2O_2 application were considered as reference. The H_2O_2 treatments were chosen in previous experiments in which the H_2O_2 concentration in the nutrient solution varied from 0.25 to 80 mmol L^{-1}. 3 mmol L^{-1} was the lowest concentration, which caused changes in leaf gas exchange whereas 80 mmol L^{-1} was the highest concentration tested, inducing severe reduction in stomatal conductance. The H_2O_2 concentration in nutrient solution was determined according to Baccan et al. (2001).

Physiological evaluations and samplings were taken 15 min after H_2O_2 treatment, on the first fully expanded leaf with apparent ligule. The exposure time was based on previous experiments, in which we noticed significant changes in the leaf gas exchange just after 15 min of treatment with H_2O_2 in nutrient solution. The leaf and root samples were collected, immediately immersed in liquid nitrogen and then stored at −80 °C for further biochemical analyses.

Leaf gas exchange and photochemical activity

Leaf gas exchange was measured with an infrared gas analyzer (model Li-6400, Licor, Lincoln NE, USA), coupled to a modulated fluorometer (6400-40 LCF, Licor, Lincoln NE, USA). Leaf CO_2 assimilation (A_N), stomatal conductance (g_S) and intercellular CO_2 concentration (C_I) were measured under Q of 2000 μmol m^{-2} s^{-1} and air CO_2 concentration of 380 μmol mol^{-1}. The vapor pressure difference between leaf and air (VPD_L) was 2.2 ± 0.3 kPa and leaf temperature (T_F) was 29.1 ± 0.3 °C during the evaluations. The intrinsic water use efficiency (IWUE = A_N/g_S) and the instantaneous carboxylation efficiency ($k = A_N/C_I$) were calculated according to Machado et al. (2009).

Simultaneous measurements of leaf gas exchange and chlorophyll fluorescence were taken and the apparent electron transport rate estimated as ETR = $\phi_{PSII} \times Q \times 0.85 \times 0.4$, in which ϕ_{PSII} is the effective quantum efficiency of photosystem II (PSII), 0.85 is the light absorption and 0.4 is the fraction of light energy partitioned to PSII (Edwards and Baker, 1993; Baker, 2008). In leaf tissues adapted to darkness (30 min), the potential quantum efficiency of photosystem II (F_V/F_M) was estimated according to Rohácek (2002). The relative energy excess at PSII level was calculated as EXC = $[(F_V/F_M) - (\phi_{PSII})]/(F_V/F_M)$, as suggested by Bilger et al. (1995).

Root hydraulic conductance

Root hydraulic conductance (L_r) was estimated according to the procedure described by Aroca et al. (2001). The procedure was based on the shoot cutting at the plant base, with root system in nutrient solution being placed into a Scholander pressure chamber (model 3005, Soil Moisture Equipment Corp., Santa Barbara CA, USA). Steady-state exudation rates were measured sequentially under varying pressures (from 0.2 to 0.8 MPa) in periods that ranged from 8 to 12 min. Afterwards, roots were stored in 80% ethanol solution and root area was measured with the Safira software v. 1.1 (Stonway, São Carlos SP, Brazil). L_r was calculated as the slope of the relation between the exudation flow and pressure, corrected for the root area and it was expressed as mmol m^{-2} s^{-1} MPa^{-1}. Measurements were carried out around noon to avoid differences in L_r due to circadian rhythm (Hachez et al., 2012).

Hydrogen peroxide content and lipid peroxidation

The quantification of H_2O_2 in plant material was performed following the method of Alexieva et al. (2001). We collected the homogenate obtained from 0.1 g of fresh tissue ground in liquid nitrogen with the addition of polyvinylpolypyrrolidone (PVPP) and 0.1% of trichloroacetic acid (TCA) solution (w/v). The extract was centrifuged at 10,000 rpm and 4 °C for 15 min. The reaction medium consisted of 1.2 mL of KI 1 mol L^{-1}, potassium phosphate buffer (pH 7.5 and 0.1 mol L^{-1}) and crude extract. The microtubes were incubated on ice under dark for 1 h. After this period, the absorbance