LIPID PEROXIDATION IN SUGARCANE YOUNG PLANTS UNDER SOIL SALINITY

PEROXIDAÇÃO LIPÍDICA EM PLANTAS JOVENS DE CANA-DE-AÇÚCAR SUBMETIDAS AO ESTRESSE SALINO

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ABSTRACT

Changes in the REDOX system of plant cells can trigger the production of compounds such as hydrogen peroxide (H_2O_2), leading to lipid peroxidation. Soil salinity contributes to oxidative stress and reactive oxegen species (ROS) production, causing lipid peroxidation and consequently membrane damage that leads to reduction of plant productivity. Sugarcane genotypes that maintain low lipid peroxidation and high membrane stability under salinity may be considered tolerant. The aim of this work was to verify the response of sugarcane young plants (SP81-3250 and IAC 87-3396) to lipid peroxidation and dry mass accumulation under salinity (0, 40, 80 and 160 mM NaCl). Both cultivar showed lipid peroxidation as measured by the production of MDA, however, cv. IAC 87-3396 had a higher accumulation of MDA, with an increase of 35% higher than in cv. SP 81-3250, while this last one presented higher biomass and H_2O_2 reduction at the highest salinity. Further IAC 87-3396 had 50% higher H_2O_2 indicating it was more sensitive to salinity.

Keywords: Hydrogen peroxide. Malondialdehyde. Plant physiology. *Saccharum* spp.

RESUMO

Alterações no sistema redoxde células vegetais podem desencadear a produção de compostos como o peróxido de hidrogênio (H_2O_2), levando à peroxidação lipídica. A salinidade contribui para o estresse oxidativo e a produção de espécies reativas de oxigênio (ERO), levando a peroxidação lipídica e, consequentemente, o estravasamento de eletrólitos, contribuindo com a redução da produtividade da planta. Os genótipos de cana-de-açúcar que mantêm baixa peroxidação lipídica e alta estabilidade de membrana sob salinidade podem ser considerados tolerantes. O objetivo deste trabalho foi verificar a resposta de plantas jovens de cana-de-açúcar (SP81-3250 e IAC 87-3396)quanto àperoxidação lipídica e acúmulo de massa seca sob alta salinidade (0, 40, 80 e 160 mM de NaCl). Ambos os genótipos apresentaram peroxidação lipídica, no entanto, a cv. IAC 87-3396 mostrou acúmulo de MDA 35% a mais que na cv. SP 81-3250, além da cv. SP 81-3250 ter apresentado produção de H_2O_2 reduzida e maior biomassa.

Palavras-chaves: Peróxido de hidrogênio. Malondialdeído. Fisiologia vegetal. *Saccharum* spp.

Changes in the redoxsystem of plant cells can trigger the compounds production such as hydrogen peroxide (H_2O_2), leading to lipid peroxidation. Oxidative stress is related to all plant stresses, developing from reactive oxygen species (ROS) accumulation, which will lead to chemical and physiological modifications (DEMIDCHIK, 2015) to altering several biomolecules, wherein the lipids are most impaired.ROS are produced by plants under normal growth conditions and are involved in cell signaling, once plants have mechanisms that keep these species under control (DAVEY et al., 2005). Several types of environmental stressors lead to oxidative stress, both abiotic, such as salinity, and biotic such as pathogens which initiate defense mechanisms through systemic signaling (GILL; TUTEJA, 2010).

Lipid peroxidation products are highly reactive, and are able to react with biomolecules such as DNA and proteins, irreversibly damaging them and causing damage to cell membrane functions (DEL RIO; STEWART; PELLEGRINI, 2005).Several side products that aggravate oxidative damage are produced by lipid peroxidation, including malondialdehyde (MDA), which is the main and most studied product of lipid peroxidation (DEL RIO; STEWART; PELLEGRINI, 2005). MDA is known as a molecular marker to designate lipid peroxidation in plant cells under different abiotic stresses, such as salinity (DAVEY, 2005). ROS species including H_2O_2 can occur in chloroplasts where the O_2 produced reacts with excess electrons (GILL; TUTEJA, 2010). H_2O_2 is a stable compound with relatively long half-life and can cause enzyme inactivation (GILL; TUTEJA, 2010) although being relatively unreactive compared to other ROS (PETROV, 2015). Studies of this compound have allowed an understanding of oxidative stress (DEMIDCHIK, 2015). The concentration of H_2O_2 is from 0.03 - 1μM (no stress) to 0.1 - 10μM (under stress), depending on the period of stress and other variables (DEMIDCHIK, 2015).Cell death process under salinity may be associated with ionic imbalance due to Na^+ increase and K^+ decrease (GRATAO, 2005). Sugarcane genotypes that maintain low lipid peroxidation and high membrane stability under salinity may be considered tolerant plants (GOMATHI; RAKKIYAPAN, 2011).

To detect lipid peroxidation in young sugarcane plants under salt stress, two cultivars (SP81-3250 and IAC87-3396) were submitted to salinity treatments. The experiment was conducted at Sao Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal. Experimental design was completely randomized in a 2x4 factorial scheme (two cultivars and four treatments: 0, 40, 80 and 160 mM NaCl), with four replicates per treatment. Vessels were conducted in a greenhouse under 30°C temperature and 40% of relative humidity. Fertilization was carried out according to the crop recommendation and irrigation done daily in order to restore water loss through evapotranspiration.Soil salinization was performed according to the method of Raij et al. (2001). Thirty-days-old plants were exposed to salt stress and the evaluations were performed on

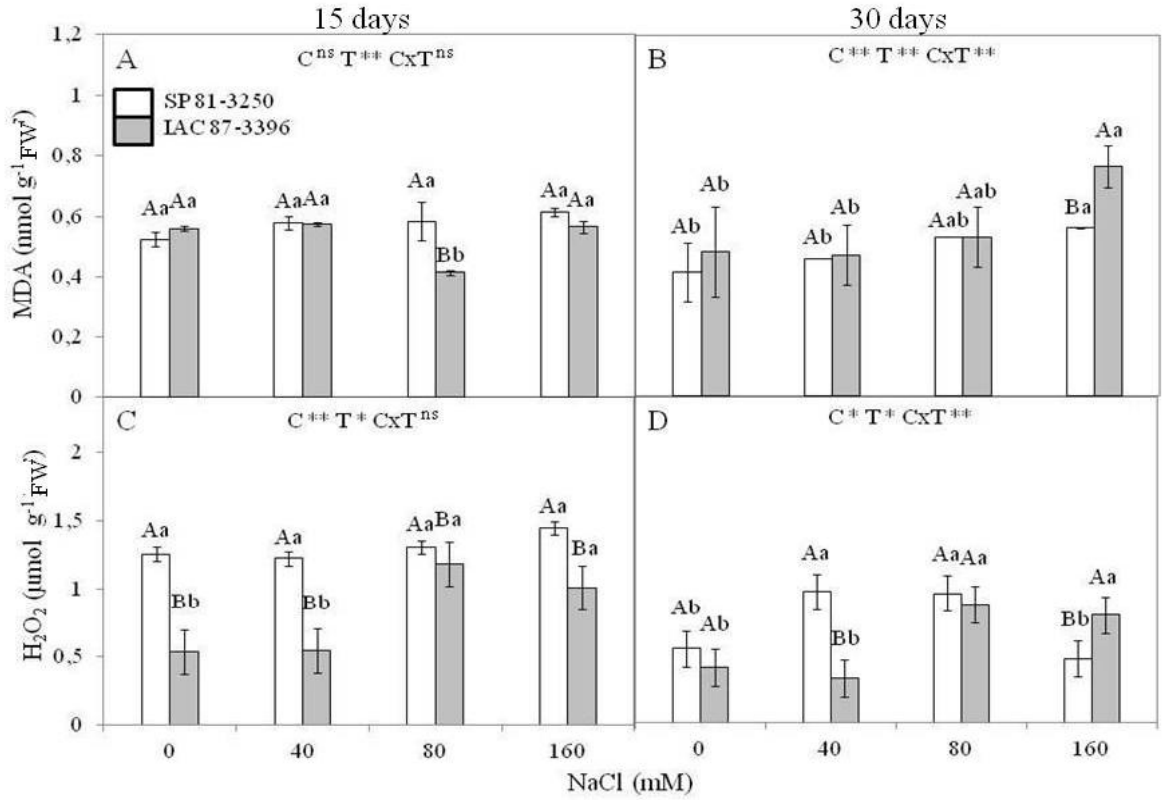
15 and 30 days (45 and 60 days of plant development, respectively). The content of $H_2O_2$was determined according to Gay et al. (1999), wherein leaves were homogenized in methanol (5:1, buffer: fresh mass), centrifuged at 10,000 rpm for 5 min and absorbance reading at 560 nm.Leaf tissue (0.25 g) was macerated in liquid nitrogen and homogenized with 0.1% (w/v) trichloroacetic acid (TCA) and20% insoluble polyvinylpyrrolidone. Then it was centrifuged at 15,000 x g for 15 min at 4 °C and an supernatant aliquot of 0.5 ml was added to 1.5 ml of 0.5% 2-thiobarbituric acid (TBA) (prepared in 20% TCA). Samples were homogenized and the colorimetric reaction conducted at 90 °C for 20 min after incubation in a water bath. After immersed and clarified by centrifugation at 15,000 x g for 15 min at 4 °C, samples absorbance was read at 532 nm and the non-specific absorbance at 660 nm in a Spectrophotometer (model Beckman DU 640, USA). Lipid peroxidation was estimated as total content of TBA-reactive substances and expressed as MDA equivalents (CAKMAK; HORST, 1991). Dry biomass of shoot was performed by drying at 65ºC.

The results showed that at 15 days there was no significant statistical difference in the MDA accumulation between treatments in both sugarcane cultivar (Figure 1A), however, H_2O_2 accumulation increased in the more severe treatments for cv. IAC 87-3396 and despite the higher accumulation of this ROS, cv. SP 81-3250 plants showed no increase under salinity (Figure 1B). At 30 days, MDA accumulation increases according to salinity treatments, markedly in cv. IAC 87-3396 plants (Figure 1C). H_2O_2 content increased according to the treatments for cv. IAC 87-3396, whereas cv. SP 81-3250 showed a marked reduction of these ROS in the most severe salinity treatment (Figure 1D). Both cultivar showed lipid peroxidation as measured by MDA production, however, cv. IAC87-3396 had a higher accumulation of this substance, with an increase of 35% higher than cv. SP81-3250. The cv. SP81-3250 showed a decrease in dry biomass of shoot ($P < 0.05$), but it only occurred at 30 days of salinity, in 160 mM~ NaCl and in a maximum of 25% of loss. The cv. IAC87-3396 presented a marked biomass decrease under salinity ($P < 0.05$) and the productivity reduction reached 46%, twice as showed by cv. SP 81-3250 (CHICONATO, 2016).

This result indicates that cv. SP81-3250 plants developed a mechanism to avoid a worst damage under severe salinity, controlling the ROS production and avoiding lipid peroxidation. Sugarcane cultivar that maintain membrane stability under salt stress may be considered tolerant plants (GOMATHI; RAKKIYAPAN, 2011), therefore, this result leads to the conclusion that cv. IAC87-3396 is more sensitive to salinity than cv. SP81-3250 sugarcane plants.

This study shows that MDA provides a useful and cost-effective biomarker for assessment of salt tolerance, provided that the higher level of salinity (160 mM NaCl) and longer time (30d) is used.

Figure 1. Lipid peroxidation in sugarcane young plants under salinity. A: MDA content at 15 days; B: MDA content at 30 days; C: H_2O_2 content at 15 days; D: H_2O_2 content at 30 days. (SP 81-3250, IAC 87-3396).



C: sugarcane cultivars; T: salinity treatments; CxT: interaction; Capital letters (between cultivars) and small letters (between treatments) show significant differences ($p \leq 0.05$) according to Tukey test. Values are means ± SD (n = 4).

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