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OSVALDO ARAUJO JUNIOR

NUTRIENTS AND AMINO ACIDS FOLIAR APPLICATION TO ALLEVIATE SUGARCANE DROUGHT STRESS

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To all my family,

All people who believe in the research,

All those who somehow supported me, I dedicate.

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ABSTRACT

Sugarcane is commonly exposed to extreme weather patterns, such as drought, which is the main abiotic factor responsible for limiting productivity in the crop. The effects of drought have as a consequence the production of reactive oxygen species, due to cellular oxidative stress, severely affecting plant metabolism. Foliar applications of nutritional complexes combined with amino acids can be used as an alternative to attenuate the negative responses of plants caused by drought conditions. However, all its effects such as foliar application in sugarcane fields, exposed to water stress, which allow increases in plant metabolism and productivity gains of stalks and sugar, and extraction of a better quality broth, have not yet been explored. The study aimed to evaluate the effectiveness of foliar application of two fertilizers of different nutritional values, associated with different amino acids in the composition, in June, under the influence of the driest period of the year, in ratoon cane, in four different regions. The treatments consisted of: control (without application of the complex) and with application of the drought protection complex (ASPC) in the cities of Pradópolis-SP and Motuca-SP. In the regions of Pereira Barreto and Nova Independência, the treatments were control and with application of the protective foliar complex (PFC). The hypothesis of Chapter 1 and Chapter 2, that the application of two nutritional complexes with different amino acids, in 4 different regions, could mitigate water stress in the sugarcane crop, and increase the production of antioxidant enzymes, accumulation of sucrose, enabling gains in stalk and sugar productivity and higher quality of the juice extracted, was confirmed in this work. The strategy of applying nutritional complexes associated with certain amino acids in the composition proved to be effective in lieving water stress and, at the same time, improving crop development, stalk yield, sugar production and biomass.

Keywords: *Saccharum spp.*; oxigen reactive species; antioxidant metabolism; drought stress; stress management strategies.

RESUMO

A cana-de-açúcar é comumente exposta às condições extremas de padrões climáticos, como a seca, que é o principal fator abiótico responsável por limitar a produtividade na cultura. Os efeitos da seca têm como consequência a produção de espécies reativas de oxigênio, devido ao estresse oxidativo celular, afetando severamente o metabolismo vegetal. Aplicações foliares de complexos nutricionais aliados à aminoácidos, podem ser empregados como alternativa para atenuar as respostas negativas das plantas causadas pelas condições de seca. No entanto, ainda não foram explorados todos seus efeitos como aplicação foliar em canaviais, expostos ao estresse hídrico, que possibilitam aumentos no metabolismo da planta e ganhos de produtividade de colmos e açúcar, e extração de um caldo de melhor qualidade. O estudo teve como objetivo avaliar a eficácia da aplicação foliar de dois fertilizantes de diferentes valores nutricionais, associados à diferentes aminoácidos na composição, no mês de junho, sob a influência do período mais seco do ano, em cana soca, em quatro regiões distintas. Os tratamentos consistiram de: controle (sem aplicação do complexo) e com aplicação do complexo protetor contra à seca (ASPC) nos municípios de Pradópolis-SP e Motuca-SP. Nas regiões de Pereira Barreto e Nova Independência, os tratamentos foram controle e com aplicação do complexo foliar protetor (PFC). A hipótese do Capítulo 1 e do Capítulo 2, de que a aplicação de dois complexos nutricionais com aminoácidos distintos, em 4 regiões diferentes, poderia mitigar o estresse hídrico na cultura da cana-de-açúcar, e elevar a produção de enzimas antioxidante, acúmulo de sacarose, possibilitando ganhos em produtividade de colmo e açúcar e maior qualidade do caldo extraído, foi confirmada neste trabalho. A estratégia da aplicação de complexos nutricionais associados à certos aminoácidos na composição, mostrou ser efetiva para aliviar o estresse hídrico e, ao mesmo tempo, melhorar o desenvolvimento da cultura, o rendimento do colmo, a produção de açúcar e a biomassa.

Palavras-chave: *Saccharum spp.*; espécies reativas de oxigênio; metabolismo antioxidante; estresse por seca; estratégias de gerenciamento de estresse.

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SUMMARY

GENERAL INTRODUCTION

Brazil is the world's largest producer of sugarcane, responsible for the production of about 630.7 million tons estimated in the 2020/2021 harvest, and 8.4 million hectares, corresponding to an average productivity of stalks of 75.02 Mg ha^{-1} (CONAB, 2020). Although this production is considered satisfactory, productivity is well below the crop's potential, exceeding 200 Mg ha^{-1} (ADORNA; CRUSCIOL; ROSSATO, 2013).

The great difficulty in achieving this productive potential is related to the different production environments (DIAS, 1997), as well as soil and climate interaction, crop management, the selected cultivar (CESAR *et al.*, 1987), water availability (REICHARDT, 1996), between others indicating the need for practices to increase productivity and profitability.

The occurrence of seasonal droughts in several producing regions has been one of the limiting factors in the production and longevity of sugarcane fields. According to the Union of sugarcane industries (UNICA), in the 2018/2019 harvest there was a 6.05% drop in sugarcane production due to water scarcity. This number is equivalent to 36 million tons of sugarcane that were no longer produced, matching the entire production of the state of Paraná, the fifth largest producer of sugarcane in the country (CONAB, 2020).

Sugarcane has C4 metabolism, characterized by being more efficient than C3 plants in fixing CO_2, more efficient use of water and transpiration rates to the environment, however, weather conditions are the main causes of the impact of the potential productive. Sugarcane requires favorable environmental conditions, such as water availability between 1200 and 2500 mm (DOORENBOS; KASSAM, 1979; SINGH; SHUKLA; BHATNAGAR, 2007) and temperature between 25°C and 35°C during the growth phase of the stalks (MANHÃES *et al.*, 2015).

The phenological cycle of sugarcane encompasses the tillering and intense growth phase of the stalks, also known as formation phases (60 to 150 days after planting), being identified as the most critical due to water demand (RAMESH, 2000; MACHADO *et al.*, 2009), mainly because in these phases about 70 to 80% of sugarcane productivity is defined (SINGH; RAO, 1987; SMIT; SINGELS, 2006; ZHAO; GLAZ; COMSTOCK, 2010).

The expansion of sugarcane cultivation in Brazil to non-traditional cultivation areas was accompanied by increased exposure to biotic and abiotic stresses, more often exposing the crop to stressful conditions. Stress is defined as intense pressure from some unfavorable factor, which tends to prevent the normal functioning of the plant's metabolism (JONES, 1990).

Intense climatic conditions, especially in unfavorable production environments, can lead to overproduction of reactive oxygen species (ROS), increasing to concentrations above the cell's antioxidant capacity, causing a redox imbalance, ceasing the electron transport chain and triggering oxidative stress (GRATÃO *et al.*, 2005). As plant responses to these stresses, we can mention changes in the content of chlorophyll pigments (BANERJEE; ROYCHOUDHURY, 2019; CHA-UM *et al.*, 2012); cellular osmotic adjustment (PATADE; BHARGAVA; SUPRASANNA, 2011); early closure of stomata (SILVA *et al.*, 2013; VAN HEERDEN *et al.*, 2004; ZHAO; GLAZ; COMSTOCK, 2013) and decreased photosynthetic capacity (BANERJEE; ROYCHOUDHURY, 2019; TAKAHASHI; BADGER, 2011).

ROS are molecules that cause severe damage to plant cells, causing damage to proteins, nucleic acids, photosynthetic pigments, in addition to activating programmed cell death and causing lipid peroxidation of cell membranes (CHOUDHURY *et al.*, 2017). Plants can fight these molecules through efficient defense systems, such as antioxidant systems in plant cells (PANDY *et al.*, 2016), enzymatic and non-enzymatic (MARQUEZ-GARCIA *et al.*, 2011; AZEVEDO *et al.*, 2012; GALLEGO *et al.*, 2012).

In the enzymatic defense system, catalases, superoxide dismutase (SOD), ascorbate peroxidase (APX) and antioxidant molecules (eg, ascorbate and glutathione) stand out (TAVANTI *et al.*, 2021). The non-enzymatic defense system encompasses compounds such as glutathione (GSH), ascorbate (AsA), alkaloids, phenols, tocopherols, carotenoids and proline (GRATÃO *et al.*, 2005).

Three classes of SOD's can be found in plants and classified according to their metallic cofactor : manganese (Mn), copper/zinc (Cu/Zn) or iron (Fe) (ALSCHER; ERTURK; HEATH, 2002).

Catalase (CAT) is an enzyme that catalyzes the conversion of H_2O_2 to water and O_2 released during the transformation of glycolate to glyoxalate during photorespiration (IGAMBERDIEV; LEA, 2002) or during the β-oxidation of fatty acids (HOLTMAN *et al.*, 1994). Ascorbate peroxidase (APX) is one of the most widely distributed antioxidant enzymes in plant cells and can be found in chloroplasts, cytosol, mitochondria and peroxisomes (ASADA, 1999), which, like CAT, also converts H_2O_2 into water and oxygen (CAO *et al.*, 2017).

Glutathione is a tripeptide that exists in the body in its reduced (GSH) and oxidized (GSSG) forms, acting directly or indirectly in protein synthesis, metabolism and cell protection (MEISTER *et al.*, 1983). Glutathione reductase (GR2) is a flavoprotein that catalyzes the reduction of GSSG to GSH, in a NADPH-dependent process. Under stress conditions, amino acid metabolism is greatly altered, with protein synthesis decreased and proteolysis increased (SODEK, 2004).

Plants, when exposed to water stress, may present accumulation of some amino acids, mainly proline, which may be correlated with drought tolerance (MOLINARI *et al.*, 2007), being widely used as a physiological-biochemical indicator of water stress (SHAO *et al.*, 2006).

The effectiveness of foliar fertilization with micronutrients can contribute to important metabolic processes, either as a substrate (organic compost) or as activators of enzymatic processes (MALAVOLTA; VITTI; OLIVEIRA, 1997). These metabolic functions can be part of the structure of some vital organic compound for the plant; enzyme constituent, participating in a specific structure, prosthetic/active group of enzymes; enzyme activator, affecting the speed of many reactions in plant metabolism.

Knowledge of the many metabolic and structural functions of nutrients and their requirements during the crop cycle can facilitate management and provide improvements in crop quality and productivity (EPSTEIN; BLOOM, 2005; MARSCHNER, 2012).

Adequate supply of macro and micronutrients at the right time is one of the most critical management practices for achieving satisfactory yields and sustainable income during all crop cycles (CRUSCIOL *et al.*, 2020). Foliar fertilizers can have a positive effect in situations of water stress, playing an important role in cellular activities, promoting both the control of stomatal opening and closing and osmotic adjustment of tissues, as well as acting on enzymatic activities (DOMINGOS; LIMA; BRACCINI, 2015; MANTOVANI *et al.*, 2017), minimizing the deleterious effects of water stress.

Macronutrients such as nitrogen, calcium, potassium and magnesium present a positive correlation with the increase in SOD, CAT and POD concentrations, while the micronutrients zinc, copper, manganese and iron act as cofactors of SOD in different cellular compartments (DINAKAR; DJILIANOV; BARTELS, 2012; WARAICH *et al.*, 2012).

Amino acids are constituents of proteins, and precursors of several substances that regulate plant metabolism (FLOSS; FLOSS, 2007), which can increase production and resistance to stresses caused by high temperatures and water stress (RUSSO; BERLYN, 1991). The use of amino acids via foliar is an interesting strategy to minimize the damage caused by water stress in order to improve the physiological characteristics of the plant, acting directly on the plant's metabolism, saving energy expenditure and assisting in the production of enzymes, proteins and hormones (COLLAÇO JUNIOR, 2019).

Amino acids act as biostimulants, attenuating the effects resulting from abiotic stresses, providing positive effects on plant growth and development, directly reflecting on productivity (HILDEBRANDT, 2018; KHAN *et al.*, 2019).

Much is discussed about the isomerism of amino acids and its effects on plant metabolism. In general, D-amino acids (D-AAs) and L-amino acids (L-AAs) stereoisomers (enantiomers) have the same chemical and physical properties, with the exception of rotating the plane of polarized light in different directions, i.e. left or right. right (FISCHER, 1909). Studies show that the types are synthesized by living organisms and have specific functions in plant metabolism, however D-AAs have more toxic tendencies to plant metabolism (KOLUKISAOGLU; SUAREZ, 2017).

Essential amino acids (leucine, isoleucine, methionine, phenylalanine, arginine, histidine, tryptophan, valine, threonine and lysine) are synthesized only by plants, while non-essential amino acids (alanine, asparagine, cysteine, glutamine, aspartic acid, glycine, proline, serine and tyrosine) are synthesized by plants and also by humans (KUMAR *et al.*, 2017). Amino acid synthesis is generated through substrates from three main pathways: glycolysis, the citric acid cycle, and the pentose phosphate pathway (TAIZ *et al.*, 2017).

The hypothesis that the application of these nutritional complexes (ASPC/PFC) may influence the plant's enzymatic antioxidant system as a response to alleviate water stress, improving biometric parameters, quality of raw material, yield of stalks and sugars, in addition to the production of biomass were accepted in Chapter 1 and 2 of this work.

The perception of isolated factors of nutrients and amino acids is known, however the idea of this work was to evaluate them together. Therefore, the objective of this work is to attenuate the effects of water stress in the sugarcane crop, using nutritional complexes composed of mineral nutrients and amino acids (ASPC/PFC), applied via foliar.

FINAL CONSIDERATIONS

This study investigated during 2 sugarcane harvests in 4 different regions the effects of water stress at the end of the sugarcane cycle (ratoon) and the relationship between the effects of applying 2 water protectors after different against water stress . Both products at the sites can mitigate water stress, representing gains in productivity of stalk and sugar, biomass and energy, increased levels of antioxidant enzymes, as well as a higher quality of the juice of the final industrializable product.

In addition, the drought protection of both complexes is a new option for managing sugarcane under stress, improving biometric parameters such as taller and thicker stalks and, consequently, higher yields of stalks and sugars and a plant metabolically stronger. Relevant questions about isolating the synergistic or antagonistic effect of certain nutrients and amino acids that make up a fertilizer, as well as concentrations and doses throughout a complete sugarcane cycle and in long-term cultivation, deserve further investigation.

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