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CLEBER DE MORAIS HERVATIN

APPLICATIONS OF PLANT GROWTH PROMOTING REGULATORS AND NUTRIENTS IN THE ENZYME ACTIVITY OF SUGARCANE AND SUGAR-BIOENERGY PRODUCTION

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TÍTULO DA TESE: GROWTH-PROMOTING HORMONES AND NUTRIENTS APPLICATION IN SUGARCANE ENZIMATIC ACTIVITY AND SUGAR-BIOENERGY PRODUCTION

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—Carta de Issac Newton a Robert Hooke—

A ciência humana de maneira nenhuma nega a existência de Deus. Quando considero quantas e quão maravilhosas coisas o homem compreende, pesquisa e consegue realizar, então reconheço claramente que o espírito humano é obra de Deus, e a mais notável.

—Galileu Galilei—

Uma vez eliminado o impossível, o que resta por mais improvável que seja, deve ser a verdade.

—Arthur Conan Doyle—

ABSTRACT

The production of sugarcane is of global importance due to the high technology used and the supply of sugar, ethanol and biomass for energy production. One of the biggest challenges to reach high productivity is the unfavorable edaphoclimatic conditions, significantly reducing the productivity and quality of sugarcane. The expansion of sugarcane occurred in areas where the soils are limited in nutrients, as well as the predominance of adverse climates that negatively influence the culture to explore all its genetic potential. The foliar application of nutrients and plant regulators constitute an alternative to stimulate the primary and specialized metabolism of the plant, stimulating both the photosynthetic process and the defense system in mitigating possible biotic and abiotic stresses, consequently, a greater balance of photosynthesis, thus increasing productivity of sugar cane. The primary objective of these experiments was to understand how the supply of nutrients and plant regulators via foliar spraying could stimulate photosynthesis, the activity of antioxidant enzymes, greater productivity, quality and bioenergy. In this context, fifteen experiments were carried out with foliar applications containing in (mg l^{-1}) with 17 of GA_3, 820 of IAA and 43 of zeatin (Experiments RP) and seventeen experiments with foliar applications of fertilizers whose concentration in (g ha^{-1}) was 330.0 of N, 260.0 of B, 12.0 of Cu, and 300.0 of Zn associated with plant regulators containing in (mg ha^{-1}) 25 of indolebutyric acid, 25 of gibberellic acid and 45 of kinetin (FRP experiments) conducted during four consecutive harvests (2017-2018 , 2018-2019, 2019-2020 and 2020-2021), and in three sugarcane harvest seasons [beginning (May-July), middle (July-September) and end (November-December) of the harvest]. Foliar applications of plant regulators alone or associated with fertilizers were carried out in two stages, vegetative and maturation: Chapter 1 (RV) - (I) control, without foliar application of plant regulators (Control), (II) foliar application of regulators plants in the vegetative phase of sugarcane (V), (III) foliar application of plant regulators in the vegetative and maturation phase of sugarcane (VM). Chapter 2 (FRV) - (I) control, without foliar application of fertilizers associated with plant regulators (Control), (II) foliar application with fertilizer and plant regulators in the vegetative phase of sugarcane (V), (III) foliar application with fertilizer and plant regulators in the maturation phase of sugarcane (M), (IV) foliar application with fertilizer and plant regulators in the vegetative and maturation phase of sugarcane (VM). Both experimental models with foliar applications in (V and VM) promoted positive results for the photosynthetic enzyme phosphoenolpyruvate carboxylase (PEP; EC 4.1.1.31) and only (RV) resulted in increased activity of ribulose 1,5-bisphosphate carboxylase (Rubisco: EC4.1.1.39) when applied to V, M and VM, respectively. Lower values for malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) and increased activity of the enzyme peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC:1.11.1.11) and proline concentration were observed in VM for both experimental models and superoxide dismutase (SOD; EC 1.15.1.1) in (FRV). There were increases in stalk diameter and length in (FRV) and stalk yield in both experimental models at the three harvest times in VM. The use of plant regulators

alone or in combination with fertilizers in these studies promoted increases in the activity of photosynthetic and antioxidant enzymes, productivity and bioenergy, especially when performed in two seasons, in the vegetative stages and in maturation, confirming the proposed hypothesis.

Keywords: *Saccharum spp*. Growth regulator. Sucrose metabolism. Plant protection.

RESUMO

A produção de cana-de-açúcar possui importância global devido à alta tecnologia empregada e pelo fornecimento de açúcar, etanol e de biomassa para produção de energia. Um dos maiores desafios para atingir altas produtividades são a condições edafoclimáticas desfavoráveis, reduzindo significativamente a produtividade e qualidade da cana-de-açúcar. A expansão da cana-de-açúcar ocorreu em áreas onde os solos são limitados em nutrientes, bem como predomínio de climas adversos que influenciam negativamente a cultura a explorar todo seu potencial genético. A aplicação foliar de nutrientes e reguladores vegetais perfazem uma alternativa para estimular o metabolismo primário e especializado da planta, estimulando tanto o processo fotossintético quanto o sistema de defesa na mitigação de possíveis estresses bióticos e abióticos, consequentemente, maior saldo de fotossintatos incrementando assim a produtividade da cana-de-açúcar. O objetivo primordial destes experimentos foi entender o que o fornecimento de nutrientes e reguladores vegetais via pulverização foliar poderia estimular a fotossíntese, a atividade de enzimas antioxidantes, maior produtividade, qualidade e bioenergia. Neste contexto quinze experimentos foram realizados aplicações foliares de reguladores vegetais contendo em (mg l^{-1}) 17 de GA_3, 820 de IAA e 43 de zeatina (Experimentos RP) e dezessete experimentos com aplicações foliar de fertilizantes cuja concentração em (g ha^{-1}) foi de 330.0 de N, 260.0 de B, 12.0 de Cu, e 300.0 de Zn associados a reguladores vegetais contendo em (mg ha^{-1}) 25 de ácido indolbutírico, 25 de ácido giberélico e 45 de cinetina (Experimentos FRP) conduzidos durante quatro safras consecutivas (2017- 2018, 2018-2019, 2019-2020 e 2020-2021), e em três épocas de colheita da cana-de- açúcar [início (maio-julho), meio (julho-setembro) e final (novembro-dezembro) de safra]. As aplicações foliares de reguladores vegetais isolados ou associados a fertilizantes foram realizados em dois estágios, vegetativo e de maturação: Capítulo 1 (RP) - (I) controle, sem aplicação foliar de reguladores vegetais (Controle), (II) aplicação foliar de reguladores vegetais na fase vegetativa da cana-de-açúcar (V), (III) aplicação foliar de reguladores vegetais na fase vegetativa e de maturação da cana-de-açúcar (VM). Capítulo 2 (FRP) - (I) controle, sem aplicação foliar de fertilizantes associados a reguladores vegetais (Controle), (II) aplicação foliar com fertilizante e reguladores vegetais na fase vegetativa da cana-de-açúcar (V), (III) aplicação foliar com fertilizante e reguladores vegetais na fase de maturação da cana-de-açúcar (M), (IV) aplicação foliar com fertilizante e reguladores vegetais na fase vegetativa e de maturação da cana-de-açúcar (VM). Ambos os modelos experimentais com aplicações foliares em (V e VM) promoveram resultados positivos para a enzima fotossintética fosfoenolpiruvato carboxilase (PEP; EC 4.1.1.31) e somente (RP) resultou em aumento da atividade da ribulose 1,5-bifosfato carboxilase (Rubisco: EC4.1.1.39) quando aplicadas em V, M e VM, respectivamente. Menores valores para malondialdeído (MDA) e peroxido de hidrogênio (H_2O_2) e aumento na atividade das enzimas peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), ascorbato peroxidase (APX; EC:1.11.1.11) e

concentração de prolina foram observados em VM para ambos os modelos experimentais e superoxide dismutase (SOD; EC 1.15.1.1) em (FRP). Houve incrementos de diâmetro e comprimento de colmos em (FRP) e rendimento de colmos em ambos os modelos experimentais nas três épocas de colheita em VM. A utilização dos reguladores vegetais isolados ou em associação aos fertilizantes nestes estudos promoveram incrementos na atividade das enzimas fotossintéticas e antioxidantes, de produtividade e bioenergia, principalmente quando realizados em duas épocas, nos estágios vegetativos e na maturação comprovando a hipótese proposta.

Palavras chave: *Saccharum spp*. Regulador de crescimento. Metabolismo da sacarose. Proteção de plantas.

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GENERAL INTRODUCTION

The growth of the world's population results in increased demand for food, fuel and electricity, and sugarcane can meet this demand by generating both sugar, ethanol and electricity through its products and by-products. The expansion of new areas of non-traditional sugarcane cultivation in Brazil has led crops to be exposed to adverse conditions, impacting the entire sugar and alcohol sector.

Currently, part of the areas occupied by sugarcane in Brazil are marginal areas with low soil nutrient content, high light intensity, low rainfall and altitude, being more susceptible to possible increasingly frequent and intense abiotic stresses, which which reduces productivity and in some cases, even the potential quality of sugarcane.

One of the main objectives of world agricultural production is to increase productivity, using fewer inputs and respecting environmental aspects. For this reason, a focus on stimulating the photosynthetic rate is crucial, as primary metabolism is the key to higher yields. To achieve this objective, new managements with new technologies must be adopted, with the use of plant regulators and nutrients via foliar application an alternative.

Plant regulators are artificially produced synthetic compounds with effects similar to phytohormones that are organic and naturally produced by plants, such as auxins, cytokinins, gibberellins, ethylene, abscisic acid, brassinosteroids, jasmonates and salicylates, but in modern agriculture, the exogenous application of substances with hormonal characteristics in plants are widely used and can be applied directly to plants, whether of natural or synthetic origin.

Plant regulators have essential functions from a physiological, biochemical and anatomical point of view, as growth promoters, modulate photosynthesis, stimulating the production of enzymatic and non-enzymatic antioxidants through signaling and promotion of metabolic balance. However, even at low doses, the foliar application of these substances can lead the plant to promote or inhibit physiological and morphological processes, such as stimulating growth and vegetative development in response to these changes in metabolism. Therefore, these

substances are related to biological mechanisms ranging from cell division within a tissue to stalk elongation, flowering, fruiting and maturation.

On the other side, nutrients act at various stages of the plant development process, as constituents of proteins and enzymes, as enzymatic co-factors or as part of the plant's structure. Besides, mineral nutrition plays an important role in effecting stress tolerance in plants. It induces the regulation of the osmotic potential of plant cells, providing amino acid synthesis, greater mechanical strength and cell wall structure; besides of regulating the opening and closing of stomata, enzymes activation, including those antioxidants.

Thus, it is essential to maintain a balance nutrition, keeping plants structurally integrated and physiologically active, for better tolerate biotic and abiotic adversities. Examples of some of the main nutrients acting in this context are nitrogen and magnesium, part intrinsic to chlorophylls, primordial in photosynthesis; phosphorus, providing nucleic acid energy and structure; just as potassium works in favor of cellular osmoregulation, enzyme activation and control of the opening and closing of stomata.

Likewise, nutrition attenuates the deleterious effects of stress by stimulating the defense system in relation to antioxidant enzymes, since nutrients such as nitrogen, calcium, potassium and magnesium increase the concentrations of SOD, CAT and POD, whereas zinc, copper, manganese and iron are SOD cofactors in different cell compartments.

The efficiency of nutrients and plant regulators, both in the initial and intermediate stages of the development of the sugarcane crop, is closely linked to its biostimulant action on plants, specially the exogenous application of plant regulators. Its effect on plants acts on metabolism, growth and morphogenesis, working as a chemical messenger that influences the various responses of vegetative development. Thus, the initial stages of sugarcane crop are benefited, increasing its growth rate; however, there is still a lack of information regarding the late vegetative and maturation stage, which we intend to elucidate in this research.

The objective of these studies was to evaluate the effect of foliar application of plant regulators containing GA_3, IAA and zeatin or GA_3, IAA and kinetin associated with the nutrients N, B, Cu and Zn in the vegetative and maturation stages of

sugarcane in stimulating the highest productivity and consequently the amount of biomass for energy production.

Our hypothesis is that through the exogenous use of plant regulators alone or in association with nutrients applied via the foliar route in the vegetative and maturation stages, it can stimulate the photosynthetic apparatus, promoting greater photosynthesis and, consequently, greater production of photosynthates.

The products of photosynthesis are directed both to growth in the vegetative stage and to storage in the maturation stage, in addition to stimulating greater production and activity of enzymatic and non-enzymatic antioxidants that will protect the photosynthetic apparatus against reactive oxygen species (ROS) resulting in less stress and greater stability and photosynthetic efficiency.

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FINAL CONSIDERATIONS

This study investigated, during four sugarcane harvests in 32 different locations, the effects of the use of plant growth regulators alone or in combination with nutrients applied via foliar application, in the vegetative and maturation stages in three different sugarcane harvest times and the effect provided on enzymatic, biometric, qualitative and bioenergetic parameters.

Through the application of the respective products, there was an increase in the activity of the photosynthetic enzyme phosphoenolpyruvate carboxylase (PEP; EC 4.1.1.31) and ribulose 1,5-bisphosphate carboxylase (Rubisco: EC4.1.1.39), consequently greater production of photosynthate, which may this being directed to both growth and storage in parenchyma cells.

There was a reduction in the activity of the enzymes malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) due to the increase in the activity of the antioxidant enzymes superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC:1.11.1.11) and proline concentration.

There were increases in the biometric parameters of diameter, length of stalks and productivity of stalks in both protocols at the three harvest times, as well as a significant increase in energy production.

Briefly, experimental models in their respective locations stimulated enzymatic activity, productivity and consequently biomass for energy production, with the best results when performed in two seasons, in the vegetative and in maturation stages, proving the proposed hypothesis.

Relevant questions about the effectiveness of the plant regulators GA_3, IAA, IBA, zeatin and kinetin and the nutrients N, B, Cu and Zn, throughout the complete cycle of sugarcane, deserve further investigation.