

LUCAS MORAES JACOMASSI

**SEAWEED EXTRACT- BASED BIOSTIMULANT AS DROUGHT MITIGATION IN
SUGARCANE**

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Dissertação apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Mestre em Agronomia (Energia na Agricultura).

Orientador: Prof. Dr. Carlos Alexandre Costa Crusciol

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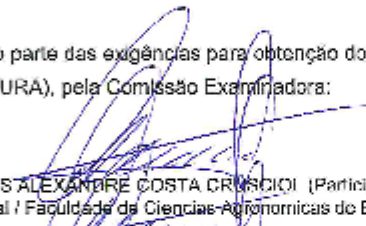
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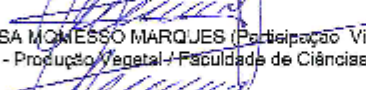
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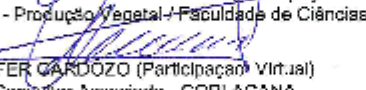
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A toda minha família,

A todas as pessoas que acreditam na ciência,

*A todos aqueles que não puderam ter a mesma
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Abstract

Drought is one of abiotic factors most inherent to the decrease in field yields, inducing the plant to morphological and physiological responses, severely affecting plant metabolism due to cellular oxidative stress, even in C4 crops species as sugarcane. Algae extracts based as biostimulants are agricultural practices used to mitigate negative plant responses caused by drought conditions. However, it remain unexplored its effects as foliar application in sugarcane field exposed to water stress that can promote increases in plant metabolism, stalk and sugar yields, as well as an extracted juice with greater technological quality. Thus, study aimed to evaluate the effectiveness of using an algae extract based foliar fertilizer at application timings under the influence of the driest period of the year in late harvest sugarcane. The commercial sugarcane fields consisted of three experiments carried out in harvest seasons of 2018 (site 1), 2019 (site 2) and 2020 (site 3) in Brazil, using RB85-5536 and SP80-3280 varieties in different ratoons (5th and 3rd). The treatments consisted of application and no application timings of foliar biostimulant in June (sites 2 and 3) and July (site 1). The dose used was 500 ml a.i ha⁻¹ in a 100 L ha⁻¹ water volume. The use of seaweed extract (SWE) mitigated the negative effects of drought, increasing the stalk yield per hectare by up to 3.08 Mg ha⁻¹, in addition to enabling greater accumulation of sucrose in the stalks by up to 2.8%, generating gains of 3.4 kg Mg⁻¹ of sugar per hectare, which raises the quality of the industrializable raw material. The Trolox-equivalent antioxidant capacity of treated plants was improved by up to 22%, increasing the activity of antioxidant enzymes in relation to the decrease of the metabolite 3-carbon dialdehyde MDA. Leaf analysis shows an efficient metabolic activity for SWE application, decreasing carbohydrate reserve levels in leaves while increasing total sugars by up to 34%. By positively stabilizing the cellular redox balance of plants, the action of SWE increases biomass production, resulting in greater energy generation up to 10.5%. Thus, the SWE strategy is a tool in alleviating drought stress while enhancing sugarcane development, stalk yield and sugar production, and improving plant physiological and enzymatic processes.

Keywords: drought; *Saccharum spp*; bio-stimulant; antioxidant metabolism; protection; yield.

RESUMO

A seca é um dos fatores abióticos mais inerentes à diminuição da produtividade das culturas no campo, induzindo a planta a respostas morfológicas e fisiológicas, afetando severamente o metabolismo vegetal devido ao estresse oxidativo celular, mesmo em espécies de cultivo C4 como a cana-de-açúcar. O uso de extratos de algas com ação bioestimulante é uma prática agrícola que vem sendo usada para mitigar as respostas negativas das plantas causadas pelas condições ambientais de seca. No entanto, ainda é pouco explorado seus efeitos na aplicação foliar em canaviais comerciais expostos ao estresse hídrico. Esses extratos podem promover aumentos no metabolismo da planta, produtividade de colmos e açúcar, bem como um caldo extraído com maior qualidade tecnológica. Assim, esse estudo teve como objetivo avaliar a eficácia da utilização de um fertilizante foliar à base de extrato de algas em épocas de aplicação sob a influência do período mais seco do ano na cana-de-açúcar de colheita tardia. O trabalho consistia em três experimentos realizados nas safras de 2018 (site 1), 2019 (site 2) e 2020 (site 3), utilizando as variedades RB85-5536 e SP80-3280 em diferentes soqueiras (5ª e 3ª soqueiras). Os tratamentos foram a aplicação ou não aplicação do bioestimulante foliar em junho (site 2 e 3) e julho (site 1). A dose utilizada foi de 500 ml a.i ha⁻¹ em um volume de água de 100 L ha⁻¹. O uso do extrato de algas marinhas (SWE – Seaweed extract) mitigou os efeitos negativos da seca, aumentando a produtividade de colmos por hectare em até 3,08 Mg ha⁻¹, além de possibilitar maior acúmulo de sacarose nos colmos em até 2,8%, gerando ganhos de 3,4 kg Mg⁻¹ de açúcar por hectare, o que eleva a qualidade da matéria-prima industrializável. A capacidade antioxidante equivalente a Trolox das plantas tratadas foi melhorada em até 22%, aumentando a atividade das enzimas antioxidantes em relação à diminuição do metabólito dialdeído 3-carbono MDA. A análise das folhas mostra uma atividade metabólica eficiente para aplicação do bioestimulante, diminuindo os níveis de reserva de carboidratos nas folhas enquanto aumenta os açúcares totais em até 34%. Ao estabilizar positivamente o balanço redox celular das plantas, a ação do SWE aumenta a produção de biomassa, resultando em maior geração de energia em até 10,5%. Assim, a estratégia de SWE é uma ferramenta para aliviar o estresse hídrico e, ao mesmo tempo, melhorar o desenvolvimento da cana-de-açúcar, o rendimento do colmo e a produção de açúcar, além de melhorar os processos fisiológicos e enzimáticos da planta.

Palavras-chave: seca; *Saccharum spp*; bioestimulante; metabolismo antioxidante; proteção; produtividade.

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1 INTRODUCTION

Globally, the crop migration to non-traditional areas of cultivation and climate change have increasingly put pressure on modern agriculture. As crop cultivation is more exposed to the inconsistencies of abiotic stress conditions, it is responsible for losses of up to 50% in the yield of the most crops (QIN; SHINOZAKI; YAMAGUCHI-SHINOZAKI, 2011; VIANNA; SENTELHAS, 2014; VINOCUR; ALTMAN, 2005; VOSSFELS; SNOWDON, 2016). Thus, examples of some of these stresses; drought, luminous intensity, high and low temperatures, among others, are limiting conditions to agricultural productivity (ASHRAF; FOOLAD, 2007).

For sugarcane, despite being a C4 species, the water availability in the soil is the abiotic factor that most interferes in its field yield, altering and/or inhibiting metabolic processes, and having negative effects on the plant's evapotranspiration rates, tillering, as well as on leaf area, inducing senescence. These negative effects affect growth rate of stalks and crop development (INMAN-BAMBER, 2004; INMAN-BAMBER; SMITH, 2005).

Among plant responses to stress conditions at cellular level, there are the changes in the content of chlorophyll pigments (BANERJEE; ROYCHOUDHURY, 2019; CHA-UM et al., 2012); cellular osmotic adjustment (MELO-ABREU; RIBEIRO, 2010; PATADE; BHARGAVA; SUPRASANNA, 2011); early stomata closure (DE ALMEIDA SILVA et al., 2013; VAN HEERDEN et al., 2004; ZHAO; GLAZ; COMSTOCK, 2013); decreased quantum efficiency of photosystem II (BANERJEE; ROYCHOUDHURY, 2019; TAKAHASHI; BADGER, 2011); production of reactive oxygen species (ROS's), weakening cellular redox homeostasis in favor of oxidizing molecules, which results in oxidative stress (PINCIROLI et al., 2019).

Within oxidative stress, the main molecules characterized as oxidants are the superoxide anion (O_2^-), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-), which are produced in different cell compartments, mainly in mitochondria, chloroplasts and peroxisomes, caused by the dependence on O_2 by the metabolic processes of aerobic respiration, photosynthesis and photorespiration (BARBOSA et al., 2014). These molecules are highly harmful to plant cells, causing damage to proteins, nucleic acids, photosynthetic pigments, in addition to activating programmed cell death and causing lipid peroxidation of membranes (CHOUDHURY et al., 2017).

As an alternative to climate pressure patterns, products with bio-stimulant characteristics help to reduce the use of agrochemicals (SHUKLA et al., 2019; VAN OOSTEN et al., 2017; YAKHIN et al., 2017). According to the European Biostimulant Industry Consortium (EBIC), bio-stimulants are classified as substances that stimulate plant nutrition, improving the availability and absorption of nutrients from the soil, in addition to enabling greater tolerance to abiotic stresses (DU JARDIN, 2015). Therefore, they are molecules that work and improve plant physiology and metabolism (MARIANI; FERRANTE, 2017), used in several crops, with applications made via foliar or soil, including for organic agriculture (MÓGOR et al., 2008).

Within the category of bio-stimulants, algae extracts have great representation and are the fastest growing sector in this market (GOÑI; QUILLE; O'CONNELL, 2018). The bio-stimulant effects of products based on algae extracts over agricultural crops includes drought tolerance (CRAIGIE, 2011; SANGHA et al., 2014). Most of the algae species used for its extracts are those classified as brown algae, with greater representation for the specie *Ascophyllum nodosum* (CRAIGIE, 2011), however, it can also be used as a source of raw material algae of green or red species (GOÑI; QUILLE; O'CONNELL, 2018).

These algae extracts are important sources of polysaccharides, polyunsaturated fatty acids, enzymes, bioactive peptides, Lea proteins (Abundant Late embryogenesis), amino acids, plant hormones, macro- and micro-nutrients (DE ABREU; TALAMINI; STADNIK, 2008; OKOLIE; MASON; CRITCHLEY, 2018; SHUKLA et al., 2016). These extracts act in a specific or generalized way in the plant metabolism (CARMODY et al., 2020; ŁANGOWSKI et al., 2021). However, in general, these products stimulate the synthesis of pigments such as chlorophyll, optimizing photosynthesis, stimulating root growth and improve the water and nutrients uptake, with direct effect on crop's yields (BULGARI et al., 2015; YAKHIN et al., 2017).

On the other hand, brown algae extract (*Ascophyllum nodosum*) acts by stimulating the activity of antioxidant enzymes and in the cellular accumulation of defense metabolites. Usually, products with these characteristics show protection and response in the crop to water stress, mitigating yield losses under such circumstances (GOÑI; QUILLE; O'CONNELL, 2018; JITHESH et al., 2019).

Emphasizing the great importance of sugarcane, its scope in varied edaphoclimatic environments and the use of foliar fertilization as a management tool in situations of abiotic stress, the hypothesis of this study as follow: algae extract as

foliar fertilizer and biostimulant improves the activity of the antioxidant system of plants and promotes positive impacts on raw material quality and on biometric aspects in sugarcane under water stress.

This study aimed to evaluate the effectiveness of using a protective product based of algae extract based to sugarcane under water stress on physiological changes and its implications for quality and stalk yields.

2 LITERATURE REVIEW

2.1 Water stress in sugarcane crop (*Saccharum spp.*)

The scenario of increased commodities consumption from the sugarcane, such as sugar, ethanol and energy, makes its irrefutable importance for agricultural market. Thus, even with the inconsistencies, especially in the fuel market, sugarcane is a world reference in renewable energy (NEVES, 2014). Therefore, to meet these demands, there must be an increase in sugarcane average yield in Brazil, which according to (OLIVEIRA, 2019), its 76,4 t ha⁻¹ average.

One of the factors that most contribute to this low productivity is the expansion of sugarcane cultivation in areas with low soil fertility, considered to be marginal areas, which often has a poor rainfall regime, or high annual variation in a specific season (BATTIE LACLAU; LACLAU, 2009; MELLIS & QUAGGIO, 2009; AZEVEDO et al., 2011; CARR; KNOX, 2021). Leading these sugarcane to be susceptible to abiotic stresses, such as drought and water deficit conditions. These abiotic limitations will promote stress response mechanisms in the plant that will be translated into three different sequential events, namely: signal perception, molecular level responses and morphophysiological responses (JOSEMARA; QUEIROZ, 2010).

The responses induced by abiotic stresses in plants are complex and show, at the cellular level, changes in the pigment content of chlorophyll (BANERJEE; ROYCHOUDHURY, 2019; CHA-UM et al., 2012), cell osmotic adjustment (PATADE; BHARGAVA; SUPRASANNA, 2011), early stomatal closure (DE ALMEIDA SILVA et al., 2013; VAN HEERDEN et al., 2004; ZHAO; GLAZ; COMSTOCK, 2013), decreased quantum efficiency of photosystem II (BANERJEE; ROYCHOUDHURY, 2019; TAKAHASHI; BADGER, 2011), in addition to activating antioxidant enzymes (ALMESELMANI et al., 2006; BANERJEE; ROYCHOUDHURY, 2019; DOS SANTOS et al., 2015).

Any of these processes, when altered by a biotic or abiotic factor, can result in a drop in the plant's growth rate and, consequently, in productivity. (SILVA et al., 2014). Due to the lower water content in the plant, the inhibition of leaf expansion is an early response to water deficit. Once there is less water in the cells, the walls contract and

loosen, the turgor pressure is lower and there is a higher concentration of cell solutes (TAIZ; ZEIGER, 2017).

In turn, the osmotic adjustment leads to the biosynthesis of compatible solutes, of protein nature and also sugars, accumulating in the vacuole or cytosol, causing an osmoprotective function in the cell. This physiological mechanism regulates the water potential, lowering it and allowing water to enter the cell, enabling the maintenance of water balance, in order to preserve the cellular integrity of membrane, proteins and enzymes (JALEEL et al., 2007).

Plants close their stomata to avoid losing water to facing the stress, due to excessive transpiration, which directly harms photosynthesis. Closed stomata does not permit atmospheric CO₂ freely to enter photosynthetically in active cells, reducing carbon assimilation (ENDRES, 2010; MACHADO et al., 2009; MEDEIROS et al., 2013; RIBEIRO et al., 2013; RODRIGUES GABRIEL SALES et al., 2012). However, in C₄ plants, such as sugarcane, stomatal conductance is less significantly affected by the deleterious effects of stresses, due to its internal mechanism of carbon dioxide concentration (HATCH, 1987).

Therefore, due to water deficit, negative effects to photosynthetic productivity in C₄ plants are more related to the decrease in the activity and quantity of the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo). As well as the proportions and the ability of the precursor ribulose-1,5-bisphosphate (RuBP) to regenerate (CARMO-SILVA et al., 2010; TEZARA et al., 2002).

Water stress can also seriously affect the apparatus of the PSII and PSI photosystems, which are essential for the electron transport chain. Thus, this damage can be interpreted as a result of the potential activity of PSII (Fv/Fo), which estimates the maximum productivity of the PSII, providing a good index of photosynthetic capacity at the time of measurement (PUTEH et al., 2013).

The responses to this physiological disorder are reduced carbon assimilation, resulting in limitations in the production of NADPH and ATP, the main carriers of metabolic energy. In addition, the excess of light energy, common to days that accompany dry periods, results in changes in the redox state of photosynthetic cells, leading to excessive production of reactive oxygen species (ROS) (ASADA, 2006).

2.2 Bioestimulant use in agriculture

The climate demand for viable alternatives to agricultural production under increasingly adverse weather conditions (REIS; LIMA; SOUZA, 2012), combined with the global search for a more sustainable agriculture that increasingly reduces the use of agrochemicals (ALI et al., 2016; YAKHIN et al., 2017). Moreover, currently world agriculture is looking for alternatives for biological management of abiotic stresses, such as the application of stimulating substances in sugarcane and other crops (PEREIRA et al., 2019; WATANABE et al., 2018).

Plant exposure to extreme weather events can be mitigated by the use of these biostimulant substances (VAN OOSTEN et al., 2017). Although there is still a lack of specific literature on how these molecules act in plants, its role in the increase of essential metabolites for the maintenance of plant physiology, through resistance to periods of stress, is well known (NGOROYEMOTO et al., 2019; SILVA et al., 2020)

Biostimulants are classified by the European Union Fertilizer Regulation as products that induce the plant to optimize its physiology and metabolic functions. Its use brings nutritional gains, stress tolerance and plant quality characteristics (DO ROSÁRIO ROSA et al., 2021; MARIANI; FERRANTE, 2017).

Therefore, these stimulant substances are from organic origin and are characterized as biostimulants, as they contain not only plant hormones, but also carbohydrates and amino acids (BRASIL et al., 2006). They work on the genetic potential of plants through changes in vital and structural mechanisms, in addition to establishing the hormonal balance, stimulating plant development (SILVA et al., 2008).

These stimulating substances can be divided into 8 categories: (1) humic substances; (2) complex organic materials; (3) beneficial chemical elements; (4) inorganic salts; (5) seaweed extracts; (6) chitin and chitosan derivatives; (7) antiperspirants and (8) amino acids (DU JARDIN, 2015a).

Algae extracts are the part of the biostimulant market that has had the greatest expansion nowadays (GOÑI; QUILLE; O'CONNELL, 2018b). Constituting more than 33% of the biostimulants word market, and could reach a value of 849 million EURO by 2022 (BOUKHARI et al., 2020).

The most used seaweeds in current agriculture are those from brown species, in especial *Ascophyllum nodosum*, which has a wide range of results in increasing

productivity of agricultural crops, being the dominant group in this market (CRAIGIE, 2011; DU JARDIN, 2015b).

The *Ascophyllum nodosum* species, in general, is found in northwestern Europe and northeastern North America (MOREIRA et al., 2017). These macroscopic organisms are exposed to extreme conditions of temperature, salinity and luminosity, therefore, naturally, throughout their evolution, they developed specific metabolic components to ensure their survival in these environmental conditions (OKOLIE; MASON; CRITCHLEY, 2018; SHUKLA et al., 2019). The algae extracts application can be performed to the leaves as a liquid substance, or powder/granulate, to the soil in drench, used as a conditioner and fertilizer (XU; LESKOVAR, 2015).

The extraction process must be done considering the characteristics of the biomass of the used species, the mode of application, and the intended physiological action on the plant (stress tolerance, improvement in soil fertility, crop quality, etc.). The ideal is the combination of techniques that optimize the extraction (BOUKHARI et al., 2020).

Although the most widely used method for extracting SWE consist in heating the algae with potassium hydroxide or sodium solvents in pressurized reaction vessels (CRAIGIE, 2011). There are different extraction modes that should be considered: Water-based extractions; acid hydrolysis; alkaline hydrolysis; microwave-assisted extraction; ultrasound-assisted extraction; enzyme-assisted extraction; super-critical fluid extraction; pressurized liquid extraction; etc (SHUKLA et al., 2019).

The increases in yield are related to stimulating the nutrient absorption from the soil, as well as the plant's growth, in addition, there is an evident greater resistance to biotic and abiotic stresses (BOUKHARI et al., 2020; KHOMPATARA et al., 2019; SALAH et al., 2018).

Possibly, the positive responses in productivity and resistance to stress are due to different bioactive substances present in the *Ascophyllum nodosum* extracts. These biostimulant molecules work together to create conditions for the biosynthesis of plant hormones, which restabilize the metabolism, influencing development and improving the photosynthetic process, in addition to delaying plant senescence (DI STASIO et al., 2018; SHUKLA et al., 2019).

The biochemical content of biostimulants is described in the literature as a compound of hormones such as auxins, gibberilins, cytokinins, abscisic acid; besides of polysaccharides, sugar alcohols, betaine, oils, fats, vitamins, antioxidants, pigments

and phenolic compounds (DU JARDIN, 2015a; KHAN et al., 2009a; MICHALAK; CHOJNACKA, 2014).

It is also possible to find studies that indicate the action of the metabolic complex, present in algae extracts, that act directly or indirectly in the expression of genes linked to plant hormone biosynthesis, involved in plant development and growth (ALI; RAMSUBHAG; JAYARAMAN, 2019; ARIOLI; MATTNER; WINBERG, 2015).

Although studies show a positive response to the use of SWE in agriculture, for the cultivation of sugarcane, there is still lack of literature that proves its effectiveness, especially in conditions of water stress. Research carried out in India indicates that the use of SWE in sugarcane can raise productivity and sucrose content standards, as well as reduce fertilizer consumption (DESHMUKH; PHONDE, 2013; KARTHIKEYAN; SHANMUGAM, 2017). In addition, recent research reports the possibility that SWE can reduce carbon emissions, meeting the global demand to reduce the aggravating effects of climate change (SINGH et al., 2018). However, it is necessary to have studies that are compatible with the regional characteristics of climate and soil for the sugarcane producing regions in Brazil.

2.3 Reactive oxygen species (ROS)

The ROS accumulation has as a toxic consequence, these molecules act at the cellular level, promoting oxidative changes in polyunsaturated fatty acids, degradation of membranes, DNA, proteins and carbohydrates (DIETZ; PFANNSCHMIDT, 2011; MEYER; HELL, 2005). This occurs because under stress conditions the redox balance, which occurs as a function of the balance between the defense system and the concentrations of reactive oxygen species, is affected in favor of oxidizing molecules and initiates what is known as oxidative stress (HAYAT; AHMAD, 2007; YUN-HEE KIM; SANG-SOO KWAK, 2010).

Thereby, while O_2 is essential for the functioning of plant metabolism, this element also, in stress situations, leads to the production of superoxide anion (O_2^-), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^\cdot$). These molecules are produced in different cellular compartments, as mitochondrias, chloroplasts and peroxisomes. It is caused by the dependence on O_2 by the metabolic processes of aerobic respiration, photosynthesis and photorespiration (BARBOSA et al., 2014).

Photosynthetic cells, in the chloroplast portion, are the main producers of ROS. The low concentration of CO_2 , induced by stomatal closure in stressful situations, lowers CO_2 levels in the Calvin cycle, thus reducing NADPH oxidation. This leads to a lack of final acceptor (NADP) in the chloroplast electron transport chain (TAIZ; ZEIGER, 2017). Consequently, the electron that would be transferred to NADP, coming from the then Fe-S (reduced ferredoxin), is incorporated into the O_2 molecule, giving rise to ROS, more specifically O_2^- (TRIPATHY; OELMÜLLER, 2012). This process is even more intense due to the excess of light energy absorbed under a stress situation, producing a surplus of electrons in the cell.

In mitochondria there is a small loss of electrons from the complexes I and III of the electron transport chain, which will originate O_2^- molecules, as well as H_2O_2 and OH^- (MITTLER, 2002). Some authors also describe ubiquinone as being largely responsible for the production of H_2O_2 in the electron transport chain, and a super-reduction of ubiquinone can be the catalyst for the production of O_2^- and OH^- in mitochondria (BARBOSA et al., 2014; BHATTACHARJEE, 2010; HELDT, 2005).

Another site of ROS production in cells is the peroxisomes, which have the function of oxidizing the organic glycolate molecule, resulting from the competition between the substrates CO_2 and O_2 by the Rubisco enzyme in situations of low CO_2 concentration (BARBOSA et al., 2014; BHATTACHARJEE, 2010). In situations like this, due to photorespiration, O_2 has preference by the enzyme, resulting in the formation of glycolate, it will be oxidized to glyoxylate and H_2O_2 . H_2O_2 is produced 30 to 100 times faster in peroxisomes than in mitochondria (KARUPPANAPANDIAN et al., 2011; SHARMA et al., 2012).

The superoxide anion (O_2^-) is the first molecule generated during O_2 reduction reactions, it has a very slow diffusion, and is considered moderately reactive and unstable. This molecule triggers even more harmful reactive species processes such as OH^- and $^1\text{O}_2$, which can cause lipid peroxidation and amino acid oxidation in cells (BARBOSA et al., 2014).

Dismutation of O_2^- is fast and can occur spontaneously or through the enzyme superoxide dismutase, generating H_2O_2 and H_2O . Furthermore, O_2^- can interact with Fe^{3+} , donating electrons and giving rise to Fe^{2+} . In turn it reduces H_2O_2 generating OH^- , in a process known as the Haber-Weiss reaction. The last part of this process, in which the oxidation of H_2O_2 generates OH^- , is called Fenton reaction, and it can occur

in the presence of iron or copper metals.(BHATTACHARJEE, 2010; GILL; TUTEJA, 2010).

Hydrogen peroxide (H_2O_2), is a very low reactive ROS molecule, the smallest of all in both reactivity and size, and this allows it to diffuse much more and faster beyond its formation site (BHATTACHARJEE, 2005). H_2O_2 causes damage to membranes, but it also works as a messenger of biotic and abiotic stresses and, in high concentrations, is involved in programmed cell death (GADJEV; STONE; GECHEV, 2008). Although it has the ability to oxidize groups of some enzymes, deactivating them, its most unwanted effect is to participate in the reactions that lead to the origin of OH^- , the most reactive ROS in the group; occurring in the presence of metals iron (Fe) or copper (Cu), through the Haber-Weiss and Fenton reactions (KARUPPANAPANDIAN et al., 2011; QUAN et al., 2008).

The hydroxyl radical (OH^-) is the most reactive ROS molecule of the entire family and has no antioxidant mechanism that can eliminate it (BARREIROS; DAVID; DAVID, 2006). Controlling the processes that lead to its formation is the only way to avoid its damage (BLOKHINA; VIROLAINEN; FAGERSTEDT, 2003). This molecule can modify nitrogenous bases, inactivating or causing DNA mutations, it can also oxidize sulfhydryl groups and disulfide bonds, denature proteins, damage carbohydrate molecules and give rise to lipid peroxidation. Thus, excess production can lead to cell death (PHOTINI V. MYLONA; ALEXIOS N. POLIDOROS, 2010; VRANOVA, 2002).

Finally, singlet oxygen ($^1\text{O}_2$), less reactive than OH^- , but more reactive than H_2O_2 and O_2^- , diffuses from its site of origin (BARBOSA et al., 2014). Its reaction occurs due to insufficient energy dissipation during photosynthesis, departing from the excited electron from chlorophyll to molecular oxygen, causing direct damage to photosystems I and II (KRIEGER-LISZKAY; FUFEZAN; TREBST, 2008).

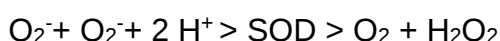
Abiotic stress factors, which stimulate stomatal closure, lead to a decrease in intracellular CO_2 levels and also favor the formation of $^1\text{O}_2$, which will react with biological molecules ranging from proteins, pigments, nucleic acids to lipids (TRANTAPHYLIDÈS; HAVAUX, 2009; WAGNER et al., 2004).

Likewise OH^- , the $^1\text{O}_2$ molecule cannot be eliminated via enzymatic reactions; therefore, for its elimination, its excitation energy must be transferred to other molecules, or through oxidation reactions with organic molecules, including lipids, proteins, nucleic acids and carbohydrates (RONSEIN et al., 2006; TRIANTAPHYLIDÈS; HAVAUX, 2009).

2.4 Plant antioxidant system

In order to eliminate ROS, plants have a defense system to maintain the cellular redox balance, either enzymatic or non-enzymatic, in which molecules such as carotenoids, ascorbic acid, vitamin E, flavonoids, proline, glutathione, among others stand out molecules (DAVAR; DARVISHZADEH; MAJD, 2013). Likewise, the enzymes of the antioxidant system can be found in different sites of the cellular structures, being crucial for the control of the unrestrained production of ROS, contributing to the recovery of the organism's redox homeostasis. Representing this system are the enzymes Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Peroxidases (PODs) (Barbosa et al., 2014). Although there are other enzymes in the system such as Glutathione Reductase (GR) and Polyphenoloxidase (PPO).

SODs are the enzymes that stand out for being the first in the line of defense against oxidative stress and catalyze the dismutation of two O_2^- radicals, where one is reduced to H_2O_2 and the other is oxidized to O_2 . Therefore, by removing the O_2^- this enzyme reduces the risk of originating cellular $OH^\cdot$, following the reaction (BHATTACHARJEE, 2010; DINAKAR; DJILIANOV; BARTELS, 2012):



These enzymes are classified according to their metallic cofactors copper and zinc (Cu/Zn-SOD), manganese (Mn-SOD) and iron (Fe-SOD) and are located in different cellular sites, including chloroplasts, mitochondria, peroxisomes and in the cytosol itself (DUBEY; PANDEY, 2011; GILL; TUTEJA, 2010; MITTLER, 2002).

CATs are essential for detoxifying the plant organism through high concentrations of H_2O_2 , when they are more efficient. Therefore, soon after SODs reduce O_2^- to hydrogen peroxide, catalases, among other enzymes, begin to work on the reduction of this molecule into water and oxygen, following the reaction



the ascorbate-glutathione cycle being responsible for the process both in the cytoplasm and in other organelles (CATHERINE J. HOWARTH, 2005).

Thus, although it can also be found in mitochondria, this enzyme acts mainly on peroxisomes and glyoxisomes (DUBEY; PANDEY, 2011). As it does not have a reducing agent, this is an enzyme that, in terms of energy expenditure, is more convenient for plants, lowering the levels of H_2O_2 and preventing the high

concentration of this molecule from inactivating the activity of SODs, thus having a fundamental role in defense from plant to varied stresses (NASCIMENTO; BARRIGOSI, 2014; SHARMA; AHMAD, 2001).

APXs, unlike CATs, require a reducing molecule to act as a cofactor preventing regeneration to O_2 . Since the final product is water, it uses ascorbic acid as a substrate to detoxify the cell through the presence of H_2O_2 (BLOKHINA; VIROLAINEN; FAGERSTEDT, 2003). These two molecules have high affinity, even at low concentrations the hydrogen peroxide can be eliminated, following the reaction $H_2O_2 > APX > H_2O$. This enzyme has different isoforms that can be found in chloroplasts, mitochondria, peroxisomes and in the cytosol itself (MHAMDI; NOCTOR; BAKER, 2012; SHARMA et al., 2012).

PODs have a wide range of isoforms in plants and while some are expressed in a way that constitutes metabolism, others are induced by abiotic stresses. (LOCATO, 2010). These enzymes are located mainly in the cell wall and vacuoles and are important in the detoxification of ERO's in cells because it uses H_2O_2 as an oxidant, eliminating the excess generated by the action of SOD (NASCIMENTO; BARRIGOSI, 2014).

PPO's (polyphenoloxidases) are present in quantity and high activity in many sugarcane varieties. In the quality of the juice, it suggests a negative effect, since this same enzyme is linked to the browning of fruits and vegetables, hindering the production of white sugar (DE AZEVEDO et al., 2019).

As it is an oxidoreductase, when accompanied by O_2 , phenolic compounds are oxidized, and their catalysis is governed by two distinct reactions: the hydroxylation of monophenol to o-dihydroxyphenol and the oxidation of o-dihydroxyphenol to o-quinone, originating melanins (BUCHELI; ROBINSON, 1994), giving the darkened aspect to the middle. According to Tomás-Barberán; Espín (2001), PPO promotes POD, due to the oxidation of phenolic compounds to generate H_2O_2 .

3 MATERIAL AND METHODS

3.1 Experimental area description

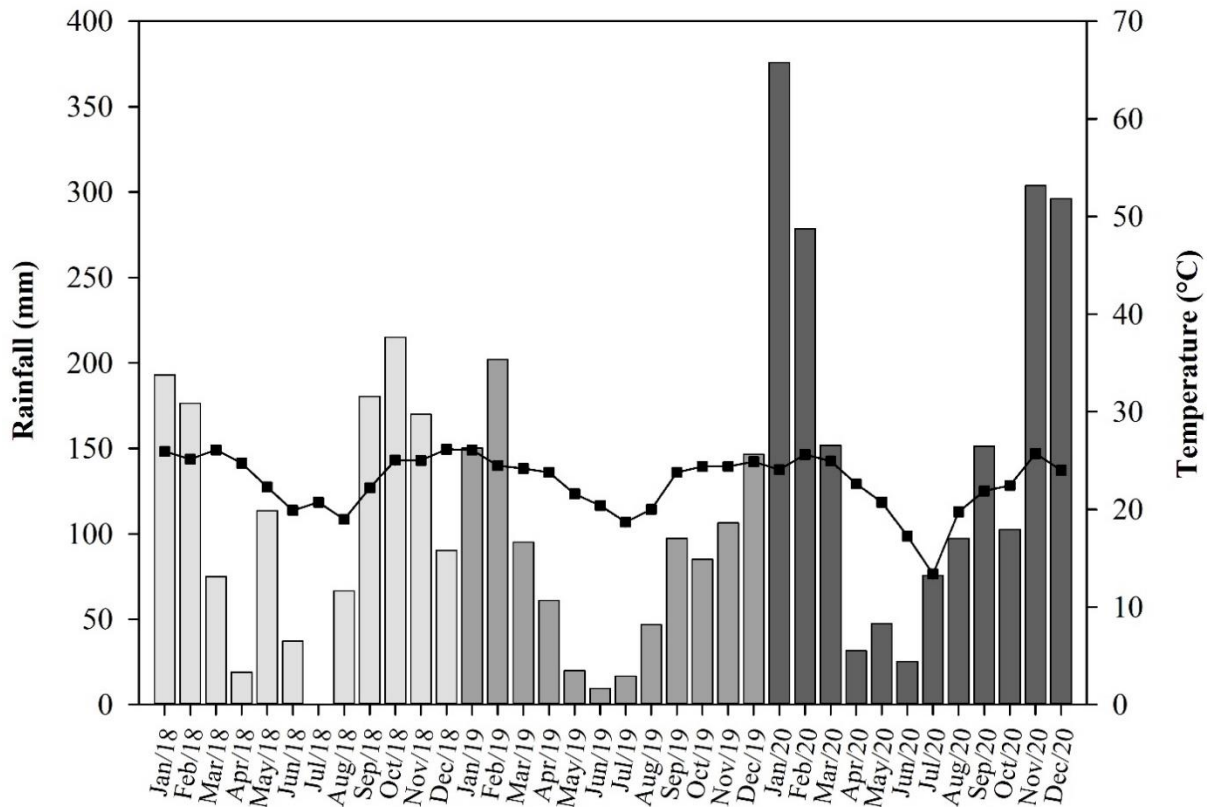
The field experiments of sugarcane (*Saccharum* spp. hybrids) were carried out under drought conditions in three different harvest seasons: site 1 (2018), site 2 (2019), and site 3 (2020) in 4th, 5th, and 3rd sugarcane ratoons, respectively. Experiments were conducted over drought season in South-Central region of Brazil from June to September, in: (*site 1*) Bungue mill's area in Dourados – MS (22°.13'.18" S, 54°.48'.23" W); (*site 2*) São Martinho mill's areas in Pradópolis – SP (21°.21 '.34" S, 48°.03'.56" W); and (*site 3*) São Martinho mill's areas in Motuca – SP, Brazil (21°.30 '.30" S, 48°.09'.12" W). Average elevations of areas are (*site1*) 448, (*site 2*) 538 and (*site 3*) 615 m asl. The soil is classified according to (STAFF, 2014), and soil chemical characteristics were determined before field experiments installation. Therefore, ten soil subsamples were collected from the experimental area between the ratoon rows and combined into one composite soil sample of each site. Soil chemical analysis from experimental area are presents in Table 1.

Table 1 - Soil classification and chemical characteristics of experiments at each site

Site	Experiment	Soil classification	Depth	pH	M.O.	P _(resin)	S	Al ⁺³	H+Al ⁺³	K	Ca	Mg	SB	CTC	V%
				CaCl ₂	g dm ⁻³	mg dm ⁻³				----- mmol _c dm ⁻³ -----					%
Site 1 Dourados (MS)	1	Rhodic Eutrudaf	0.00-0.20	5.2	29	32	37	0	35	2.2	66	18	86	121	71
			0.20-0.40	5.1	39	23	55	0	39	2.4	54	16	73	111	65
Site 2 Pradópolis (SP)	2	Rhodic Eutradox	0.00-0.20	5.1	26	19	9	0	40	2.6	42	15	60	100	60
			0.20-0.40	5.1	23	16	29	0	42	1.7	33	15	49	91	53
Site 3 Motuca (SP)	3	Rhodic Hapludox	0.00-0.20	5.3	17	55	27	0	21	2.6	27	11	41	61	67
			0.20-0.40	5	14	34	33	1	25	0.8	17	6	24	49	49

Site 1 has a humid subtropical climate (Cfa), and sites 2 and 3 have a tropical savannah climate (Aw), according to the Koppen-Geiger climatic classification system (ALVARES et al., 2013). The annual average temperatures are 21.3, 23.4, and 21.6 °C and the average precipitations are 1700, 1419, and 1344 mm, in sites 1, 2, and 3, respectively (CEPAGRI, 2021) (Fig. 1).

Figure 1 - Monthly rainfall and mean temperatures



Monthly rainfall (bars) and mean temperatures (line/squares) during experimental period of 2018, 2019 and 2020 in sites 1, 2 and 3, respectively. Different colors of gray mean different years of experiment conduction.

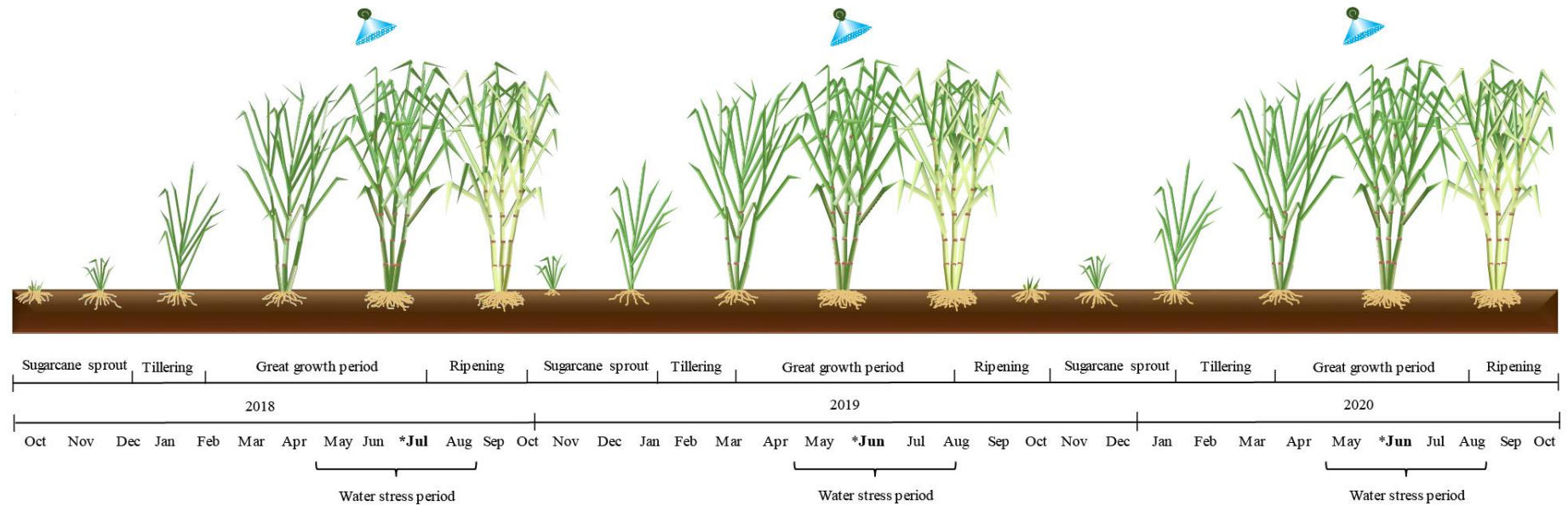
The used varieties were RB855536 (site 1) and SP803280 (sites 2 and 3). RB855536 has a high agricultural and industrial productivity in favorable environmental conditions. Excellent ratoon regrowth, high tillering, high sucrose content, medium to late ripening. SP803280 is characterized as high sucrose content and ratoon yield, moderate tillering, great ratoon regrowth, and medium to late ripening.

3.2 Experimental design and treatments description

The experimental design was carried out in randomized blocks (RBC), plots consisted of eight rows of 10 m in length and spacing between the planting rows of 1.5 m with two treatments: (1) Control, no Seaweed extract (SWE) application; and (2)

SWE application, presence of algae extract biostimulant based on *Ascophyllum nodosum*, with twelve replications. The SWE applications were performed in July in site 1 and in June in sites 2 and 3. Harvest for all sites was performed in October from the respective experimental years (Fig. 2).

Figure 2 - Schematic experimental timeline with description of experiment conduction and biostimulant seaweed extract application in each year (*)



The dose applied of biostimulant SWE was 500 ml i.a ha⁻¹, with a recommended dose per hectare of 0.5 L - 1 L ha⁻¹ for a 100 L ha⁻¹ water volume. Product warranties are given in (g/l): Organic carbon = 78.0, N = 13.0, S = 40.3, B = 1.17, Co = 0.78, Fe = 16.9, Cu = 13.0, Mn = 14.3, Mo = 0.52, Zn = 29.9. The algae extract is classified as a class A organomineral fertilizer, and has technology based on *Ascophyllum nodosum* in its composition, with a density of 1.30 g/ml at 20°C.

All applications were carried out when environmental conditions were suitable using pressurized costal equipment (CO₂), with a single application tip 1/4KLC-9 brass fieldjet, coupled to a rod of 2.6 m long, enabling simultaneous and homogeneous application in four plant rows, reaching an application range of 7.5 m. The working pressure used was 344 kPa for a water volume of 100 L ha⁻¹. The application and dosage of the products follow the specifications recommended by the manufacturers.

The experimental areas did not present problems with pests, weeds, or diseases. Thus, the crop management were carried out according to the recommendation of each site, following the mill's calendar for cultivation practices.

3.3 Enzymatic sampling for laboratory procedures

The leaves TVD (Top Visible Dewlap) or leaf +1 were collected according to Kuijper (VAN DLLLEWIJN, 1952), discarding the tip and base of the leaf, using only the middle third for metabolic, to posteriorly evaluate the contents of hydrogen peroxide (H₂O₂), malondialdehyde (MDA), trolox equivalent antioxidant capacity (TEAC) and enzymatic analysis superoxide dismutase, catalase, peroxidase and polyphenoloxidase. The samplings were performed within 9:00 and 10:00 am at 90 days after SWE applications, prior to harvest.

For enzymatic evaluation, the sampled tissue was quickly frozen in liquid N, and stored at - 80°C in falcon tubes.

3.3.1 Sugarcane leaf metabolites evaluation

Twenty leaves were collected and, after drying, were placed in an oven with forced air circulation at 65°C, for 72 hours, then the material was milled in a Willey mill, in a sieve with mesh of 1 mm in diameter. From samples was measured total sugars,

and soluble sugars, starch and sucrose content, and the results are expressed in (%) for each 100 g sample (NELSON, 1944; SOMOGYI, 1945).

3.3.2 Oxidative stress and antioxidant enzymes

The malondialdehyde (MDA) contents were evaluated, as well as the activities of superoxide dismutase (SOD; EC: 1.15.1.1), catalase (CAT; EC: 1.11.1.6), peroxidase (POD; EC: 1.11.1.7), polyphenoloxidase (PPO; EC: 1.10.3.1) and Trolox effective antioxidant capacity (TEAC - Trolox equivalent antioxidant capacity).

Lipid peroxidation was evaluated according to the method of Heath; Packer (1968) to calculate the MDA content. A molar extraction coefficient of 155 mM L^{-1} was used, and the results were expressed in nanomoles of MDA per gram of fresh weight. Extractions for enzymatic analysis were performed according to the methodology described by (BRADFORD, 1976) to determine the total protein content. The H_2O_2 content was determined (Alexieva et al., 2001), and the content was calculated based on a calibration curve and expressed in $\mu\text{mol g}^{-1}$ of fresh weight (FW).

SOD activity was evaluated as described by Giannopolitis; Ries (1977), and the results were expressed in units (U) of SOD per gram of protein. CAT activity was evaluated as described by Havir; mchale (1987), and the results were expressed in nanomoles per minute per milligram of protein. POD activity was evaluated according to Allain et al (1974), and the results were expressed in micromoles of H_2O_2 per minute per gram of fresh weight. Finally, the PPO enzyme was evaluated according to Kar; Mishra (1976), and the results were expressed in $\mu\text{mol transformed catechol.min}^{-1}.\text{g}^{-1}$ fresh weight.

Relative antioxidant capacity occurred according to the Mustafa Ozgen et al (2006), in terms of Trolox-equivalent antioxidant capacity (TEAC) in 2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The antioxidant capacity equivalent to Trolox in DPPH (TEAC-DPPH) was expressed in mg trolox fresh weight 100 g^{-1} ; and Trolox in ABTS (TEAC-ABTS) was expressed in mM trolox fresh weight kg^{-1} .

3.4 Sugarcane biometric evaluations

All evaluations were carried out at ripening phenological stage prior to harvest. Biometry parameters were performed to evaluate the sugarcane development, using 20 random stalks collected per plot. The parameters measured were: (1) plant height (m), measured from the distance from the ground to the auricular region of the leaf +1, and (2) stalk diameter (using a digital caliper) (MARAFON, 2012).

3.5 Sugarcane Technological evaluations

At the same time of biometric parameter evaluation, ten stalks were cleared at the height of the apical bud, defoliated and forwarded to the mill's PCTS laboratory to analyze the following characteristics: Sucrose (%) (sucrose concentration in the fresh weight of stalk), Fiber % (dry water-insoluble matter in the sugarcane), Purity % (sucrose present in the total solids content in cane juice), Total Reducing Sugar (TRS) (all forms of sugars in sugarcane in the form of reducing or inverted sugars), Reducing Sugars (RS %) (reducing substances in cane and sugar products calculated as invert sugar - predominantly hexoses), according to the methodology defined in the Sucrose Content-Based Sugarcane Payment System, in accordance with Consecana's semiannual updates for the technological evaluations described by (FERNANDES, 2011).

3.6 Stalk and sugar yields

Two planting lines were defined as useful area in each plot, where two doubled meters totaling 4 m linear were used for the evaluations of stalk mass at ripening phenological stage prior to harvest. The stalks inserted in the 4 m line were weighed and extrapolated to obtain stalk yield in Mg ha^{-1} . Then, sugar yield (Mg ha^{-1}) was carried out by multiplying the values of stalk yield (Mg ha^{-1}) by the TRS, and divided by 1000.

3.7 Biomass and energy production

Fiber and stalk yield were used for calculation of bagasse at 50% moisture, and trash yield was calculated considering 140 kg of trash per Mg of stalk and considering 60% collection from the soil surface (HASSUANI, 2005). Energy production was calculated considering that 1 Mg of trash has 4.96 MWh of primary energy and 1 Mg of bagasse has 4.94 MWh of primary energy (1 MWh = 3,600.00 MJ) (HASSUANI, 2005).

3.8 Statistical analysis

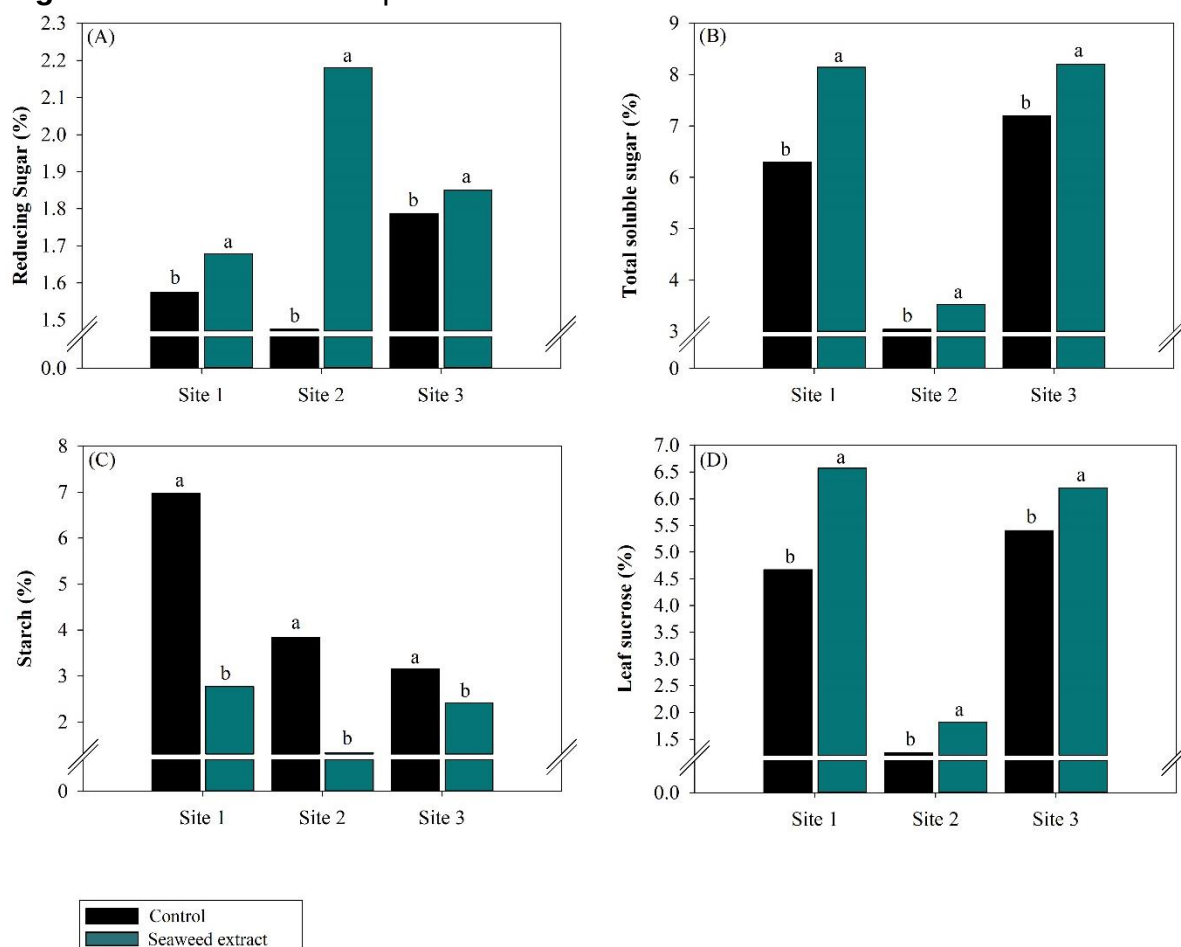
The results were submitted to the Shapiro-Wilk normality test. Then, results were submitted to analysis of variance by the f test at $p < 0.10$.

4 RESULTS

Leaf metabolites were carried out in all sites and were influenced by biostimulant application (Fig. 3). Sugarcane receiving SWE increased ($p < 0.10$) the reducing sugar (average by 35%) and rates of total soluble sugar (average by 21%) in all sites (Fig. 3A and 3B). Increases also occurred for leaf sucrose that gains were higher in SWE by 40.6, 45.6, and 13.1% in sites 1, 2, and 3, respectively, in relation to the control (4.67, 1.25, and 5.48% in sites 1, 2, and 3, respectively) (Fig. 3D).

Contrarily, rates of starch were decreased in the leaf from control to treatment receiving SWE. SWE reduced to 2.77, 1.50, and 2.50% at sites 1, 2, and 3, and control reaching 6.98, 3.85, and 3.14% at sites 1, 2, and 3, respectively (Fig. 3C).

Figure 3 – Leaf metabolic parameters



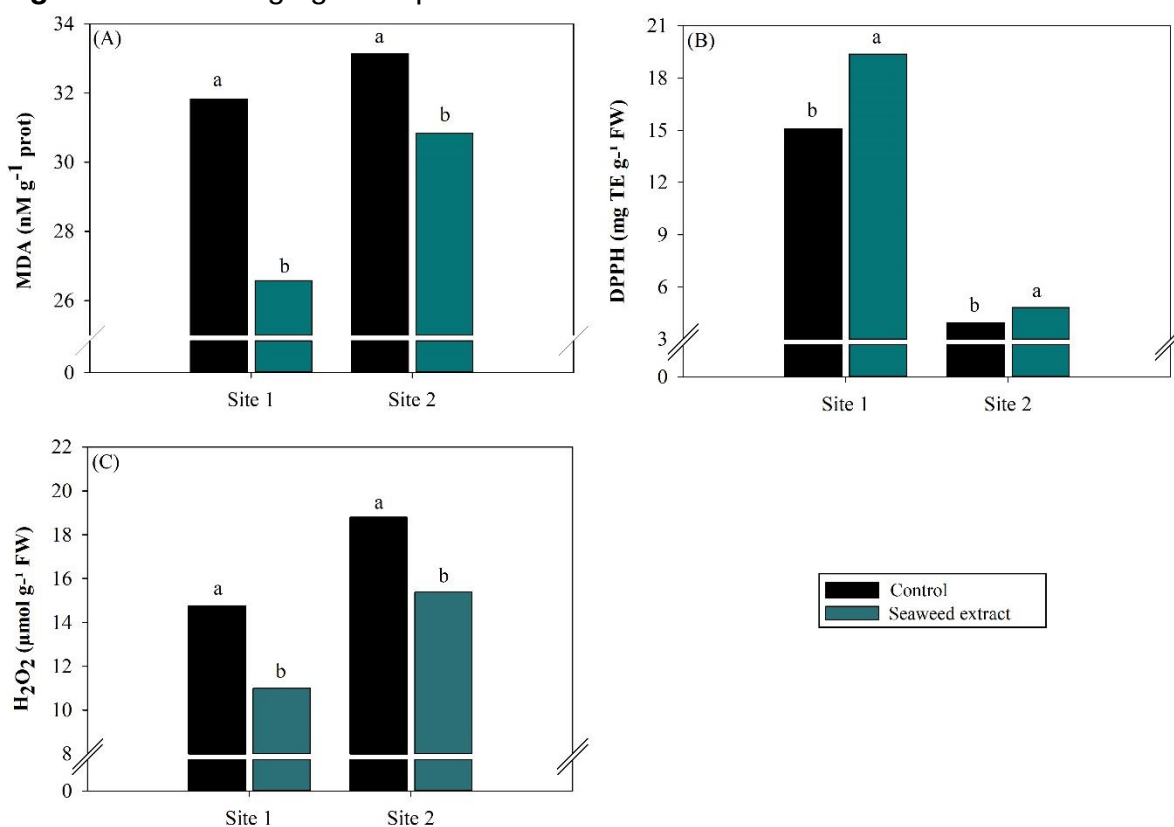
Sugarcane leaf metabolic parameters: Reducing sugars (%); Total soluble sugars (%); Starch (%); Leaf sucrose (%), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

The organic compound marker for oxidative stress MDA showed a decrease in its reactive potential for the SWE treatment at the sites where the enzyme evaluations were carried out (site 1 and 2), decreasing from 31.82 nM g⁻¹ prot and 33.15 nM g⁻¹ prot to 26.57 nM g⁻¹ prot and 30.83 nM g⁻¹ prot, respectively (Fig. 4A).

The Trolox-equivalent antioxidant capacity test, which measures the antioxidant capacity of a given plant tissue sample, increased as a consequence of a lower MDA measurement. Accordingly, SWE increased ($p < 0.10$) the Trolox-equivalent antioxidant (DPPH) by 28.3 and 21.7% in both sites 1 and 2, respectively, in relation to the control (15.1 mg TE g⁻¹ and 3.95 mg TE g⁻¹ in sites 1 and 2, respectively) (Fig. 4B).

The level of H₂O₂ in plants treated with SWE decreased considerably ($p < 0.10$) in the evaluated sites. Site 1 measured a drop of 25.5%, while site 2 dropped 18.2%, both related to the control: 14.8 µmol g⁻¹ FW and 18.8 µmol g⁻¹ FW, respectively (Fig. 4C).

Figure 4 – Scavenging ROS parameters



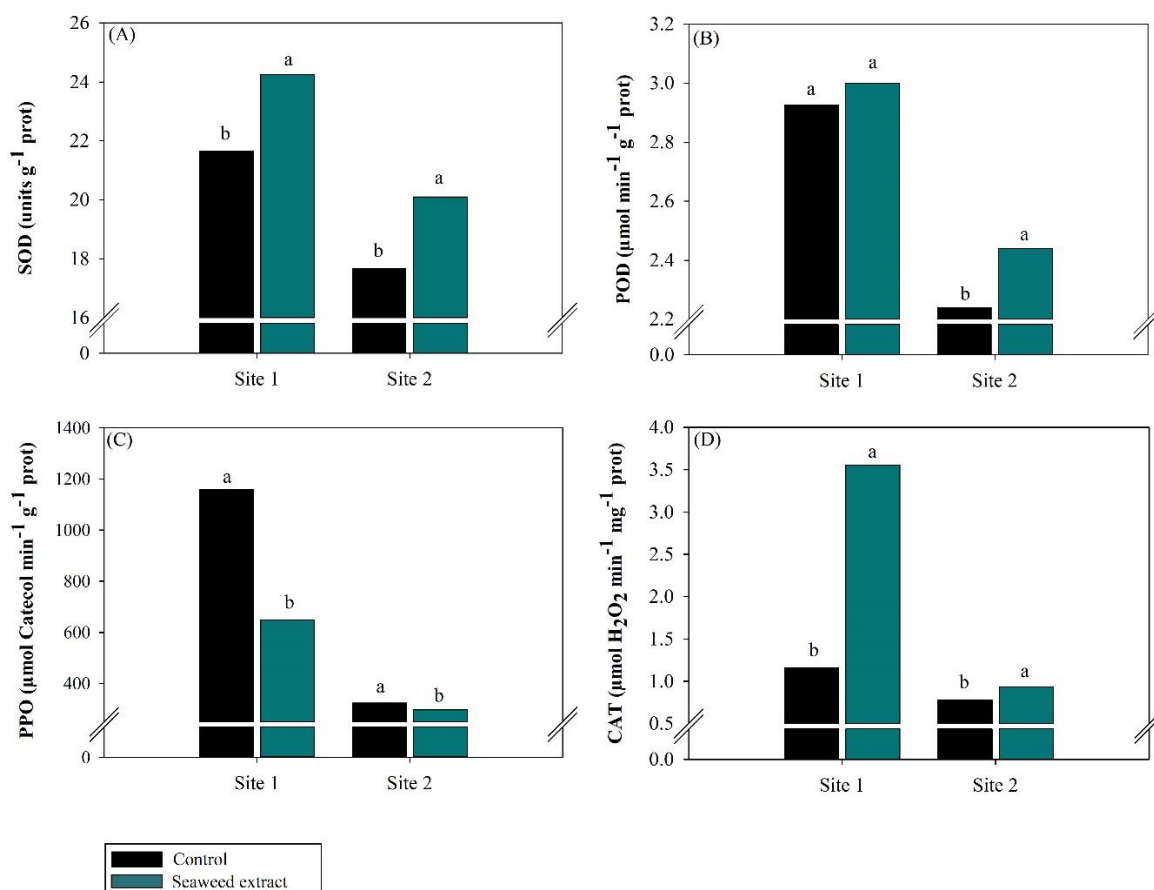
Sugarcane ROS scavenging parameters: MDA (nM g⁻¹ prot); DPPH (mg trolox/100g FW) and H₂O₂ (µmol g⁻¹ FW), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*,

applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

The increase ($p < 0.10$) in the activity of the antioxidant system enzymes SOD, CAT and POD was also observed for both sites (Fig. 5). For sugarcane at site 1 the application of SWE increased by 11.9% for SOD and 2.3% for POD compared with control 21.7 units g^{-1} prot and 2.93 $\mu\text{mol min}^{-1} \text{g}^{-1}$ prot, respectively. Sugarcane at site 2 had increases by 13.8% for SOD and 8.9% for POD relative to the control 17.66 units g^{-1} prot and 2.24 $\mu\text{mol min}^{-1} \text{g}^{-1}$ prot, respectively (Fig. 5A – B). CAT had increases from 1.2 (site 1) and 0.78 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$ prot (site 2) to 3.6 and 0.93 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$ prot at sites 1 and 2, respectively (Fig. 5D).

However, polyphenoloxidase (PPO), an enzyme that has an oxidizing characteristic and causes the sample to darken in the presence of hydrogen peroxide (H_2O_2) had decreases when SWE was applied at both sites, its values were lower by 43.9 at site 1 and 8.2% at site 2 compared to the control 1158 and 323.16 $\mu\text{mol Catecol min}^{-1} \text{g}^{-1}$ prot, respectively at the same sites (Fig. 5C).

Figure 5 – Enzimatic Parameters



Sugarcane enzymatic parameters: SOD (units g^{-1} prot); POD ($\mu\text{mol min}^{-1} \text{g}^{-1}$ prot); PPO ($\mu\text{mol Catecol min}^{-1} \text{g}^{-1}$ prot); CAT ($\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$ prot), at harvest affected by SWE application strategy.

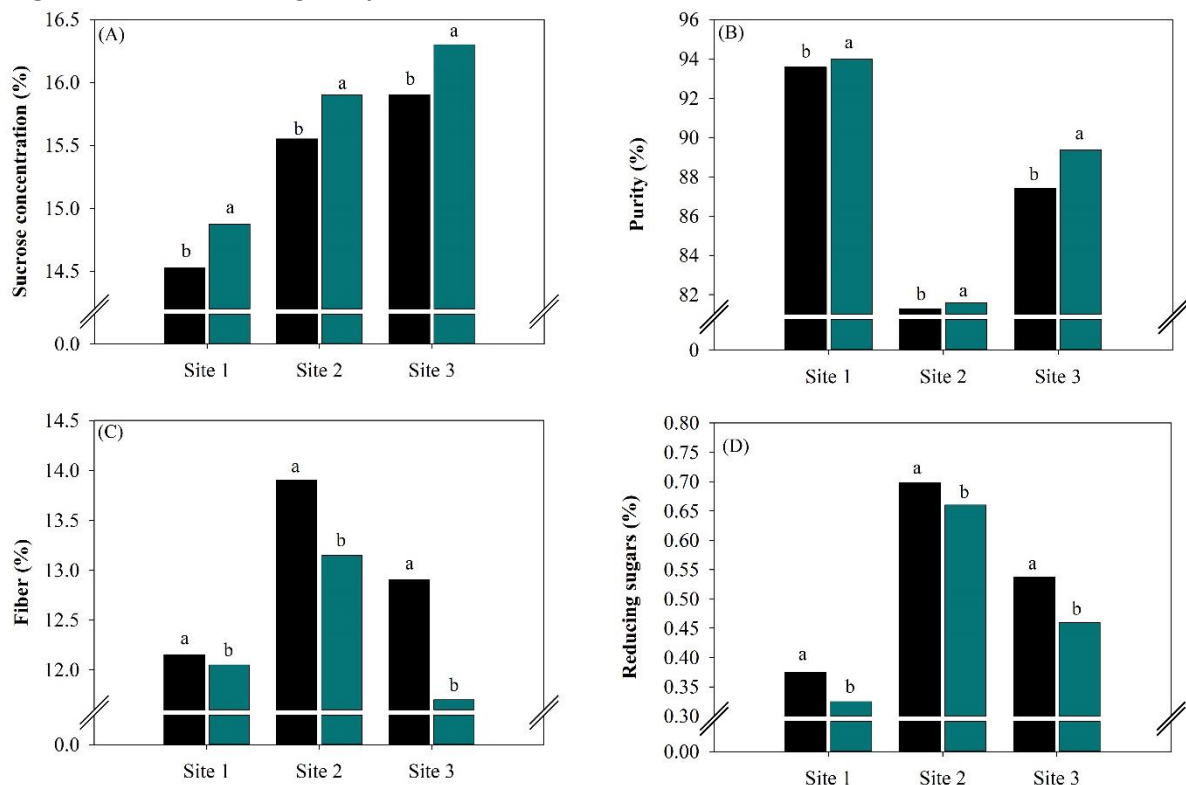
Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

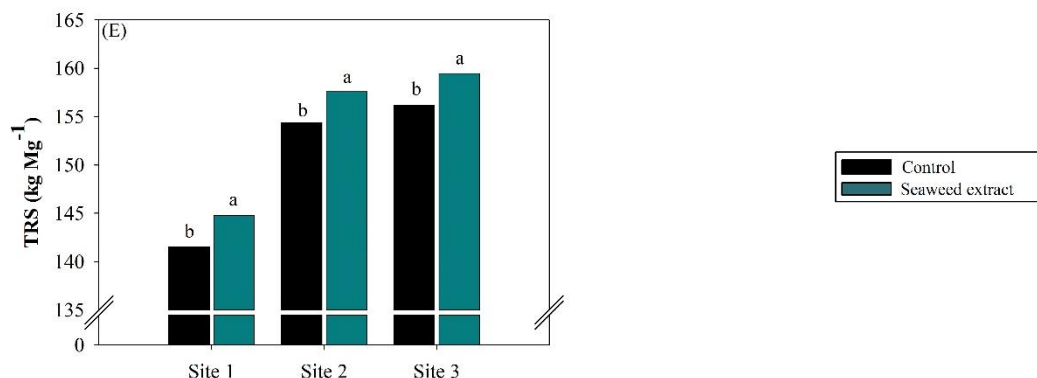
The sucrose concentration (%) was influenced ($p < 0.10$) by the action of the SWE biostimulant, which raised up to: 2.7%, 2.6% and 2.6%, compared to control: 14.52, 15.55, and 15.90% at sites 1, 2 and 3, respectively (Fig. 6A). Therefore, the levels of reducing sugars also changed and followed the pattern of that of sucrose concentration, decreasing in all sites due to a higher sucrose amount (Fig. 6D).

The juice purity (%) was enhanced in SWE treatment (Fig. 6B). Thus, SWE improved the raw material quality for industrial use, reaching values of juice purity up to 80% at all sites. Contrarily to sucrose concentration and juice purity, fiber (%) was decreased by SWE application (Fig. 6C). Sugarcane when SWE applied at site 3 had the lowest value of fiber, which decreased by 9.3% in relation to the control (12.69%).

To calculate the total reducing sugars (TRS), sucrose concentration is used, as well as the levels of reducing sugars (Fig. 6E). Since there was influence ($p < 0.10$) of SWE treatment on sucrose concentration, TRS was increased in all sites by: 3.2 kg Mg^{-1} at sites 1 and 2, and 3.3 kg Mg^{-1} at site 3; while it was decreased to 141.6 , 154.4, and 156.2 kg Mg^{-1} at sites 1, 2, and 3, respectively, in control.

Figure 6 – Technological parameters

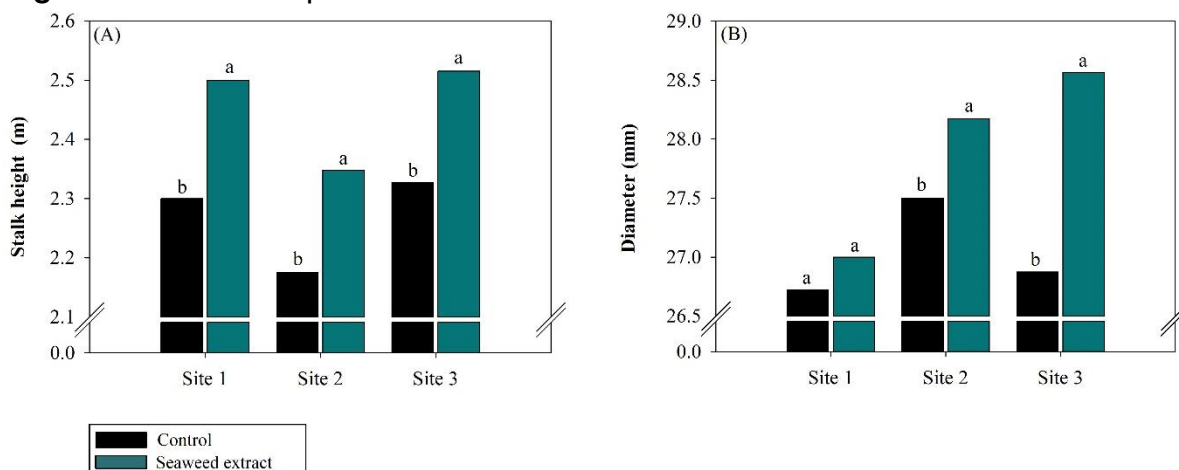




Sugarcane technological parameters: Sucrose concentration (%); Purity (%); Fiber (%); Reducing sugars (%) and Total reducing sugars (Kg Mg⁻¹), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

For biometric parameters the algae extract positively affected ($p < 0.10$) stalk height (Fig. 7A) in all sites, with average gain of 0.2 m at sites 1 and 3, and 0.18 m at site 2, in relation to control (2.30, 2.33, and 2.18 m, at sites 1, 2, and 3, respectively). Furthermore, the stalks diameter had influence of biostimulant's application, with larger diameters in sugarcane SWE treatment, except at site 1 that had no significant difference between treatments (Fig. 7B).

Figure 7 – Biometric parameters

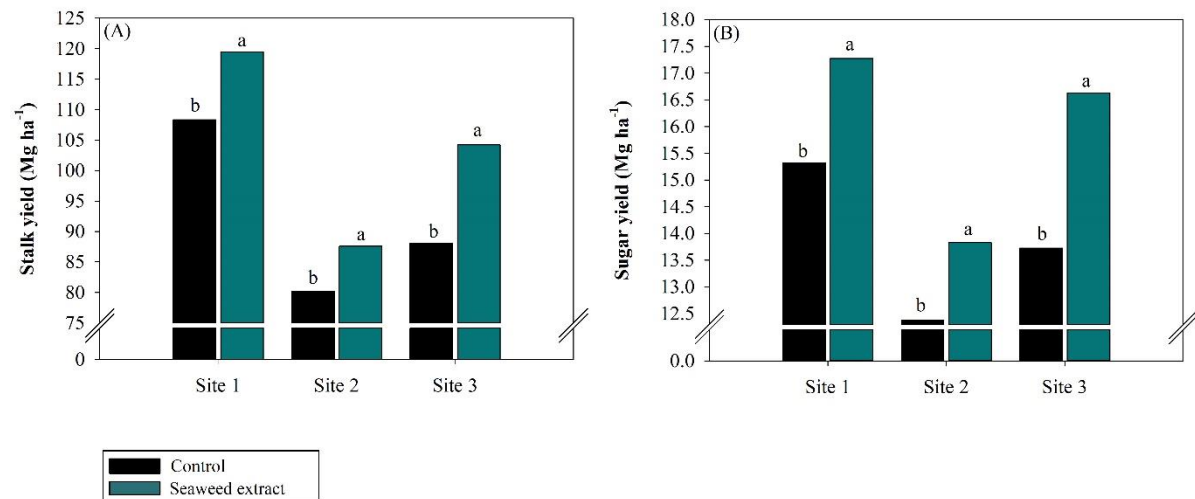


Sugarcane biometric parameters: Stalk height (m); Diameter (mm), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

The stalk yield was significantly increased when the biostimulant applied, which had average gains of site 1 - 10.2% (+11.1 Mg ha⁻¹), site 2 - 9.2% (+7.4 Mg ha⁻¹) and site 3 - 18.3% (+16.1 Mg ha⁻¹), related to the control (108.4, 80.2, and 88.07 Mg ha⁻¹, respectively) (Fig. 8A).

Considering that the calculation of sugar yield is based on the product between TRS and stalk yield, its increase is directly linked to the gains of stalk yield. Sugar yield was higher by 13.1% (+2 Mg ha⁻¹) in site 1, 11.3% (+1.4 Mg ha⁻¹) in site 2, and 20.9% (+2.9 Mg ha⁻¹) in site 3, related to the control (15.3, 11.3, and 20.9 Mg ha⁻¹, respectively) (Fig. 8B).

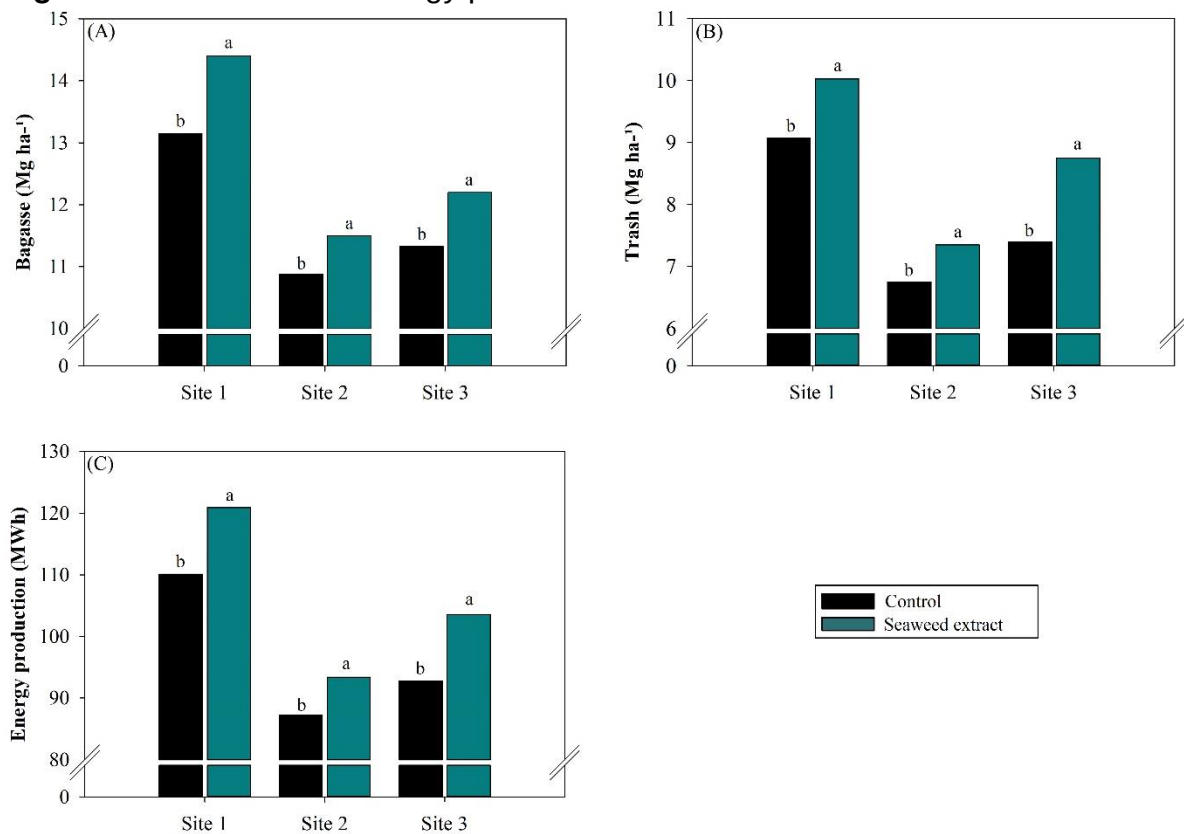
Figure 8 – Yield parameters



Sugarcane yield parameters: Stalk yield (Mg ha⁻¹); Sugar Yield (Mg ha⁻¹), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

In general, the application of SWE increased biomass production (bagasse and trash) in all experiments (Fig. 9). The higher bagasse was in sugarcane receiving SWE at site 1, which increased 1.2 Mg ha⁻¹ in relation to the control (13.2 Mg ha⁻¹) (Fig. 9A). Sugarcane had the highest trash production at site 3, with gain of 1.35 Mg ha⁻¹ compared to the control (7.4 Mg ha⁻¹) (Fig. 9B).

Both biomass and energy production were influenced by treatment application (Fig. 9). Energy production was increased in all experiments, which was higher by on average site 1 (9.8%), site 2 (7.1%) and site 3 (11.6%), in relation to the control (110.1, 87.2, and 92.7 MWh at sites 1, 2, and 3, respectively) (Fig. 9C).

Figure 9 – Biomass and Energy parameters

Sugarcane Biomass and Energy parameters: Bagasse (Mg ha⁻¹); Trash (Mg ha⁻¹) and Energy production (MWh), at harvest affected by SWE application strategy. Treatments are as follow: Control: no SWE application; Seaweed extract: application of SWE biostimulant based on *Ascophyllum nodosum*, applied in the drought season's beginning site 1 (July) and sites 2 – 3 (June). Averages followed by the same letters do not differ by the LSD test ($p < 0.10$).

5 DISCUSSION

Strategies such as increased water uptake from the soil; stomatal closure; osmotic adjustment of the plant's tissue; in addition to hormonal signaling of abiotic stress, production of antioxidant species and production of metabolites (GUPTA; RICO-MEDINA; CAÑO-DELGADO, 2020), are adopted by plants to maintain the water balance of their physiological system. However, the plant's efficient response to the stress factor is inextricably linked to patterns of severity, duration, number of exposures, combinations of stresses and even the plant's genetic material. Thus, excessive environmental changes patterns may lead plants to irreversible stress conditions, preventing crops from reacting in a natural way, affecting its phenological plasticity and yield (HAUVERMALE; SANAD, 2019). Here we evaluated the strategy to mitigate the drought stress on sugarcane plants using a seaweed extract-based biostimulant. Significant increases in crop growth, better yields and greater resistance to abiotic stresses are described by the use of SWE (KHAN et al., 2009b).

Initially, in plants under drought stress conditions the conversion of sugars into starch is desirable to avoid a high sugar concentration that inhibits photosynthesis and generates a senescence response (PAUL; FOYER, 2001; ROSA et al., 2009), as a consequence of deficient solute transport in the phloem, reducing its strength, enabling the source (leaves) loading to sink (stalks) (GONG et al., 2015; LEMOINE et al., 2013; YAMADA et al., 2010).

The greater accumulation of starch in the leaves in the control plants (Fig. 1C), induces a lower sugar synthesis rate in the medium. With less solutes in the medium, the guard cells from the stomata increase their water potential due to a greater free water energy, then, cell loses its turgor and closes the stomata. Thus, it reduces excessive water loss to the atmosphere and responds better to drought stress (PRASCH et al., 2015; VALERIO et al., 2011), however, stomata closure directly affects plant's yield.

On the other hand, starch has a plasticity characteristic in plant metabolism and can respond to the stress factor in different and even controversial ways, thus, it cannot only be considered a reserve carbohydrate. It responds depending on the vegetal tissue type and its function in the plant, besides of its source-sink relation (DONG; BECKLES, 2019; THALMANN; SANTELIA, 2017).

Our results represent a punctual plant response to drought, which physiological starch mechanism was evidenced by the biostimulant action of the SWE application. The SWE application can provide a greater water use efficiency, as well as optimize the energy and carbon use, even in limiting conditions for photosynthesis. Thus, by mobilizing this carbohydrate reserve into total derived sugars, the plant increases its tolerance to drought and favorable conditions to its growth (THALMANN; SANTELIA, 2017), as sugars are considered substrates for protective proteins and other compounds synthesis, essentials for cellular membranes protection (TAIZ; ZEIGER, 2017).

Probably, the attenuation of abiotic stress caused by the application of SWE leads plants to consume more of its reserve carbohydrate, degrading starch present in the leaves. This process release, among other components, glucose, raising in source tissues reducing sugar index (ZEEMAN; KOSSMANN; SMITH, 2010).

The released monosaccharide play an important role in the leaf cytosol, where through the action of SPS enzyme will lead to the synthesis of sucrose (DONG; BECKLES, 2019), enhancing total soluble sugar. In addition, the metabolites generated by starch degradation (Fig. 1A and D) are essential for maintaining the osmoprotective balance of plant cells, maintaining cell volume, which prevent plants from losing water to the extracellular medium (THALMANN; SANTELIA, 2017).

Following physiological responses, the enzymic activity was also affected by application of SWE (Fig. 3B). In plants treated with SWE the activity of PPO enzymes decreased. This enzyme is concentrated in plastids, where has its participation in the control of oxygen levels in the region of photosystems I and II, collaborating with the ROS balance for a healthy cell functioning condition, i.e., contributing to the functioning of photosynthesis in condition of stress (BOECKX et al., 2015; THIPYAPONG et al., 2004).

In contrast, other studies clearly demonstrated that these enzymes are involved in the browning of sugarcane juice, an unwanted characteristic in the sugar industry (NAIKOO et al., 2019; VICKERS et al., 2005). The browning is caused by the reaction between the enzyme and phenolic compounds (flavonoids, tannins, hydroxycinnamate esters, and lignin), secondary metabolites synthesized in the cell cytosol and involved in plant defense mechanisms against stress (NAIKOO et al., 2019; VAUGHN; DUKE, 1984; VICKERS et al., 2005). Probably, by relieving the stress caused by drought, the

SWE contributed to the maintenance of enzyme levels, decreasing its oxidative potential against phenolic compounds.

Other examples in plants treated with SWE that showed positive response in the cellular redox balance, allowing the plant to reduce the deleterious effects caused by ROS, showing attenuation of drought effects in several compared crops were: bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*), soybean (*Glycine max*), sweet orange (*Citrus sinensis*), spinach (*Spinacea oleracea*) (CARVALHO et al., 2018; SHUKLA et al., 2018; SPANN; LITTLE, 2011; XU; LESKOVAR, 2015).

In a study carried out with non-stressed plants, GOÑI et al (2016) showed that there was an improvement in the expression of genes that encode the antioxidant system of plants, in the presence of *Ascophyllum nodosum* extract. In our findings, the presence of SWE increased antioxidant capacity measured in DPPH, enhancing radical scavenging activity in the leaves and contributes to raising cellular phenolic content levels, as also described in previous studies (ELANSARY; SKALICKA-WOŹNIAK; KING, 2016; FAN et al., 2011; LOLA-LUZ; HENNEQUART; GAFFNEY, 2014; SPANN; LITTLE, 2011). Therefore, the activity of antioxidant enzymes (SOD, CAT and POD) as well as non-enzymatic substances (α-tocopherol, ascorbate and β-carotene) (ALLEN et al., 2001; SHI et al., 2018; SHUKLA et al., 2019; YILDIZTEKIN; TUNA; KAYA, 2018), can be enhanced by exogenous applications of SWE.

The higher concentration of SOD in the treated plants (Fig. 4A) are related to the beneficial effect of SWE at eliminating ROS. This enzyme is a pioneer in catalyzing the dismutation of two O_2^- radicals, which is reduced to H_2O_2 and the other is oxidized to O_2 . Thus, by removing O_2^- this enzyme reduces the risk of originating cellular OH^- (BHATTACHARJEE, 2010; DINAKAR; DJILIANOV; BARTELS, 2012; DUBEY; PANDEY, 2011).

In addition to SOD, the activity of the enzymes CAT and POD were also enhanced (Fig. D and B). They are crucial for defense against oxidative stress as they use H_2O_2 as an oxidant, converting this molecule into H_2O , eliminating the excess generated by the enzymatic action of SOD (BALLESTEROS; WUNDERLIN; BISTONI, 2009; CHUIAN-FU KEN et al., 2005; NASCIMENTO; BARRIGOSSI, 2014). Similar results are reported by literature evidencing the SWE application on plants at eliminating cellular H_2O_2 , and consequently contributed for a stable redox state maintenance, alleviating drought stress (CAVERZAN; CASASSOLA; PATUSSI BRAMMER, 2016; DO ROSÁRIO ROSA et al., 2021).

Plants treated with SWE also show reduced levels of 3-carbon dialdehyde MDA (Fig. 3A). This metabolite is a product of the action of ROS on cell membranes that oxidize polyunsaturated fatty acids, causing irreversible cell damage (GOÑI; QUILLE; O'CONNELL, 2018a; SHUKLA et al., 2019). MDA acts intensely on organelles such as chloroplasts and mitochondria that have intense oxidative metabolism and high concentration of polyunsaturated fatty acids (MANO, 2012). We observed that a lower MDA level in plant cells when SWE was applied in sugarcane under drought stress. Probably, it relieves the oxidizing action of ROS in chloroplasts and mitochondria that, combined, they are responsible to convert the atmospheric carbon into substrate and metabolic energy through the photosynthesis and cell respiration.

Another important fact is that due to plant hormones in its composition, the SWE can also be classified as growth regulator, acting directly or indirectly in the metabolism of carbohydrates (ALI et al., 2016; CRAIGIE, 2011), affecting the raw material quality. The raw material quality is an important factor on sugarcane crop system because is direct related to the accumulation of sucrose in the stalks. Therefore, even being a biostimulant substance, SWE may also help sugarcane to reach sucrose content levels favorable to the industrialization in plants under water stress (Fig. 5A), in which sucrose level must be equal or above 13% (DEUBER, 1988).

Sucrose synthesis involves several enzymes, such as the SS (sucrose synthase), SPS (sucrose phosphate synthase), NI (neutral invertase) and SAI (acid invertase) (STURM, 1999; WINTER; HUBER, 2000). Previous studies suggests that great results of SPS determines the sucrose synthesis, and that of SAI enzymes are involved in the sucrose degradation, in addition increases of SPS activity in the leaves (source) and stalks (sink) samples, as well as a decrease of SAI enzyme activity, are related to SWE was applied (CHEN et al., 2021). In summary, sugarcane plants more tolerant to water stress work better on the sucrose metabolism, probably increasing the activity of precursor enzymes due to higher levels of substrate present in the cytosol cells.

As illustrated (Fig. 5), higher sucrose concentration leads to an increased evaluation in the technological parameters. Therefore, a higher purity was obtained in this study. The reducing rate of glucose, fructose and fiber resulted in a higher total recoverable sugar (TRS). Other studies also relate a strong influence of polysaccharides working on vegetative parameters, abundant in various algae extracts, including those based on *Ascophyllum nodosum* (BALTAZAR et al., 2021;

CRAIGIE, 2011; OMIDBAKHSHFARD et al., 2020; RASUL et al., 2021). These non-growth hormones compounds act in a way to modulate metabolic, lipid and transcription processes promoting phenotypic changes such as the resistance of plants to stress, reducing the presence of reactive oxygen species, loss of electrolytes due to cell damage and, by consequently, they enable the growth of plants (BALTAZAR et al., 2021; CRAIGIE, 2011; OMIDBAKHSHFARD et al., 2020; RASUL et al., 2021). It can be attributed to the SWE phytotonic characteristic derived from a complex metabolic composition rich in macro and micronutrients, carbohydrates, amino acids and plant hormones, such as auxins and cytokinin (DE ABREU; TALAMINI; STADNIK, 2008; TAN et al., 2021).

These plant growth-promoting complex can induce cell division and expansion, enhance the photosynthetic activity, contribute to the partition of metabolic energy and synthesis of biochemical components, and directly affect plant height, diameter and stalk yields (DESHMUKH; PHONDE, 2013; DIWEN et al., 2021; SHUKLA et al., 2019; SINGH et al., 2018; TAN et al., 2021). Furthermore, it is possible to state that mainly due to hormonal concentrations the SWE influences root growth (DIWEN et al., 2021; RATHORE et al., 2009; WOZNIAK et al., 2020), and provide conditions for the expression of genes linked to the transport of nutrients (KROUK et al., 2010; RATHORE et al., 2009), which allows greater soil exploration and contributes to improve nutrient uptake by plants (OMIDBAKHSHFARD et al., 2020; RASUL et al., 2021).

Therefore, our results suggest that the SWE biostimulant characteristic brought significant increases in vegetative parameters, inducing positively the biometric measurements of height and diameter for sugarcane (Fig. 6A and B). Thus, by allowing plants to growth even under stress condition, probably due to a more efficient primary metabolism, there are directly positive responses in stalk yields (Fig. 7A).

TRS and stalk yield in this study, productivity measurement units, confirmed that the SWE molecule respond in a satisfactory way when focusing on plants under water stress condition. Raising the sugarcane productivity patterns, thus, increasing rates of sucrose (Fig. 7B).

In turn, bagasse and straw are directly affected by vegetative variations in the plants, induced by the phytotonic SWE effect, especially direct products of stalk and fiber productivity. According to (Hassuani, Leal, and Macedo, 2005), at 50% humidity, stalk yield and fiber are used to calculate bagasse, while trash is calculated considering

140 kg of trash per Mg of stalk. Evidently, the biostimulant action of SWE in plants generated a vegetative potential that influenced these parameters for this study. Moreover, a greater productivity in biomass generates a direct response in the energy potential of plants treated with SWE biostimulant, due to the increase in vegetative parameters, even under drought conditions.

Thus, SWE use in sugarcane, as a management of water stress mitigation, proves to be an important tool in achieving greater yields and facing unfavorable environmental conditions to plants. Its substance action in plant physiological and enzymatic response allows the plant to protect metabolic processes, metabolizing carbohydrates essential for plant growth and raising biomass production per area.

6 CONCLUSION

Here we showed that a protective product based on algae extract applied in sugarcane is effective to quality and stalk yields under drought stress. The management of drought stress mitigation by SWE application improves sugarcane stalk yield and sugar production. Yet, SWE action represents an alternative to enhance the physiological and enzymatic sugarcane response of metabolic processes and stimulate the carbon assimilation and carbohydrates metabolization. The SWE positively acts on the plant antioxidant system and carbohydrates synthesis, which, in turn, reduce the concentration of reducing sugars and fiber, and increase purity of the juice. In addition, algae extract is a new option for managing sugarcane under stress by enhancing biometric parameters such as taller and thicker stalks, and, consequently, higher yields of stalk and sugar and a metabolically stronger plant. Relevant questions about how is the SWE effectiveness across a complete sugarcane cycle and in the long-term grown deserve further investigations.

REFERENCES

ALI, N. et al. The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions. **Journal of Applied Phycology**, v.28, p. 1353-1362, 2016.

ALI, N. et al. *Ascophyllum* extract application causes reduction of disease levels in field tomatoes grown in a tropical environment. **Crop Protection**, v. 83, p. 67–75, 1 maio 2016.

ALI, O.; RAMSUBHAG, A.; JAYARAMAN, J. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a tropical environment. **PLOS ONE**, v. 14, n. 5, p. e0216710, 1 maio 2019.

ALLAIN, C. C. et al. Enzymatic Determination of Total Serum Cholesterol. **Clinical Chemistry**, v. 20, n. 4, p. 470–475, 1 abr. 1974.

ALLEN, V. G. et al. Tasco: Influence of a brown seaweed on antioxidants in forages and livestock—A review. **Journal of Animal Science**, v. 79, n. suppl_E, p. E21–E31, 1 jan. 2001.

ALMESELMANI, M. et al. Protective role of antioxidant enzymes under high temperature stress. **Plant Science**, v. 171, n. 3, p. 382–388, 1 set. 2006.

ARIOLI, T.; MATTNER, S. W.; WINBERG, P. C. Applications of seaweed extracts in Australian agriculture: past, present and future. **Journal of Applied Phycology** **2015** **27:5**, v. 27, n. 5, p. 2007–2015, 14 abr. 2015.

ASADA, K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. **Plant Physiology**, v. 141, n. 2, p. 391–396, 1 jun. 2006.

AZEVEDO, R. A. et al. Sugarcane Under Pressure: An Overview of Biochemical and Physiological Studies of Abiotic Stress. **Tropical Plant Biology**, v. 4, n. 1, p. 42–51, 28 mar. 2011.

BALLESTEROS, M. L.; WUNDERLIN, D. A.; BISTONI, M. A. Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan. **Ecotoxicology and Environmental Safety**, v. 72, n. 1, p. 199–205, 1 jan. 2009.

BALTAZAR, M. et al. Recent Advances in the Molecular Effects of Biostimulants in Plants: An Overview. **Biomolecules** **2021**, Vol. 11, Page 1096, v. 11, n. 8, p. 1096, 25 jul. 2021.

BANERJEE, A.; ROYCHOUDHURY, A. Cold stress and photosynthesis. **Photosynthesis, Productivity, and Environmental Stress**, n. October, p. 27–37, 2019.

BARBOSA, M. R. et al. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciencia Rural**, v. 44, n. 3, p. 453–460, mar. 2014.

BARREIROS, A. L. B. S.; DAVID, J. M.; DAVID, J. P. Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. **Química Nova**, v. 29, n. 1, p. 113–123, 2006.

BATTIE LACLAU, P.; LACLAU, J. P. Growth of the whole root system for a plant crop of sugarcane under rainfed and irrigated environments in Brazil. **Field Crops Research**, v. 114, n. 3, p. 351–360, 12 dez. 2009.

BHATTACHARJEE, S. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. **Current Science**, v. 89, n. 7, p. 1113–1121, 2005.

BHATTACHARJEE, S. Sites of Generation and Physicochemical Basis of Formation of Reactive Oxygen Species in Plant Cell. In: **Reactive Oxygen Species and Antioxidants in Higher Plants**. 1. ed. [s.l.] Science Publishers, 2010. p. 1–30.

BLOKHINA, O.; VIROLAINEN, E.; FAGERSTEDT, K. V. Antioxidants, oxidative damage and oxygen deprivation stress: A review. **Annals of Botany**, v. 91, n. 2, p. 179–194, 1 jan. 2003.

BOECKX, T. et al. Polyphenol oxidase-mediated protection against oxidative stress is not associated with enhanced photosynthetic efficiency. **Annals of Botany**, v. 116, n. 4, p. 529–540, 1 set. 2015.

BOUKHARI, M. E. M. EL et al. Trends in Seaweed Extract Based Biostimulants: Manufacturing Process and Beneficial Effect on Soil-Plant Systems. **Plants 2020, Vol. 9, Page 359**, v. 9, n. 3, p. 359, 12 mar. 2020.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 7 maio 1976.

BRASIL, A. et al. Acta Scientiarum. Agronomy. **Acta Scientiarum. Agronomy**, v. 28, n. 2, p. 179–185, 2006.

BUCHELI, C. S.; ROBINSON, S. P. Food Chew. **Journal. Agriculture. Food Chem**, v. 42, p. 257–261, 1994.

CARMO-SILVA, A. E. et al. Rubisco activities, properties, and regulation in three different C4 grasses under drought. **Journal of Experimental Botany**, v. 61, n. 9, p. 2355–2366, 1 maio 2010.

CARR, M. K. V; KNOX, J. W. THE WATER RELATIONS AND IRRIGATION REQUIREMENTS OF SUGAR CANE (SACCHARUM OFFICINARUM): A REVIEW. **Experimental Agriculture**, v. 47, n. 1, p. 1–25, 2021.

CARVALHO, M. E. A. et al. Is seaweed extract an elicitor compound? Changing proline content in drought-stressed bean plants. **Comunicata Scientiae, ISSN-e 2177-5133, Vol. 9, Nº. 2, 2018, págs. 292-297**, v. 9, n. 2, p. 292–297, 2018.

CATHERINE J. HOWARTH. Genetic Improvements of Tolerance to High Temperature. In: M. ASHRAF P. I. C. HARRIS (Ed.). . **Abiotic Stresses**. 1. ed. [s.l.] CRC Press, 2005. p. 299–322.

CAVERZAN, A.; CASASSOLA, A.; PATUSSI BRAMMER, S. Reactive Oxygen Species and Antioxidant Enzymes Involved in Plant Tolerance to Stress. In: **Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives**. [s.l.] InTech, 2016. p. 463–480.

CHA-UM, S. et al. Evaluating sugarcane (Saccharum sp.) cultivars for water deficit tolerance using some key physiological markers. **Plant Biotechnology**, v. 29, n. 5, p. 431–439, 2012.

CHEN, D. et al. Effects of Seaweed Extracts on the Growth, Physiological Activity, Cane Yield and Sucrose Content of Sugarcane in China. **Frontiers in Plant Science**, v. 0, p. 865, 26 maio 2021.

CHUIAN-FU KEN, ‡ et al. Characterization of Fe/Mn–Superoxide Dismutase from Diatom *Thalassiosira weissflogii*: Cloning, Expression, and Property. **Journal of Agricultural and Food Chemistry**, v. 53, n. 5, p. 1470–1474, 9 mar. 2005.

CRAIGIE, J. S. Seaweed extract stimuli in plant science and agriculture. **Journal of Applied Phycology**, v. 23, n. 3, p. 371–393, 29 jun. 2011.

DAVAR, R.; DARVISHZADEH, R.; MAJD, A. Changes in antioxidant systems in sunflower partial resistant and susceptible lines as affected by *Sclerotinia sclerotiorum*. **Biologia (Poland)**, v. 68, n. 5, p. 821–829, 31 out. 2013.

DE ABREU, G. F.; TALAMINI, V.; STADNIK, M. J. Bioprospecção de macroalgas marinhas e plantas aquáticas para o controle da antracnose do feijoeiro. **Summa Phytopathologica**, v. 34, n. 1, p. 78–82, jan. 2008.

DE ALMEIDA SILVA, M. et al. BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY Photosynthetic Capacity and Water Use Efficiency in Sugarcane Genotypes Subject to Water Deficit During Early Growth Phase. **Arch. Biol. Technol.** v. 56, n. 5, p. 735–748, 2013.

DE AZEVEDO, A. C. B. et al. Enzymatic polyphenoloxidase inactivation with temperature and ozone in sugarcane variety RB 92579 to produce lower color sugar. **Brazilian Journal of Food Technology**, v. 22, 25 fev. 2019.

DESHMUKH, P. S.; PHONDE, D. B. Effect of seaweed extract on growth, yield and quality of sugarcane. **International Journal of Agricultural Sciences**, v. 9, p. 750–753, jun. 2013.

DEUBER, R. **Maturação da cana-de-açúcar na região sudeste do Brasil. In: Seminário de tecnologia agrônômica.** Piracicaba - SPCopersucar, 1988.

DI STASIO, E. et al. Ascophyllum nodosum-based algal extracts act as enhancers of growth, fruit quality, and adaptation to stress in salinized tomato plants. **Journal of Applied Phycology** 2018 30:4, v. 30, n. 4, p. 2675–2686, 28 mar. 2018.

DIETZ, K. J.; PFANNSCHMIDT, T. Novel regulators in photosynthetic redox control of plant metabolism and gene expression. **Plant Physiology**, v. 155, n. 4, p. 1477–1485, 1 abr. 2011.

DINAKAR, C.; DJILIANOV, D.; BARTELS, D. Photosynthesis in desiccation tolerant plants: Energy metabolism and antioxidative stress defense. **Plant Science**, v. 182, n. 1, p. 29–41, 1 jan. 2012.

DIWEN, C. et al. Alleviation Effect of Foliar Application with Seaweed Extract on Sugarcane under Drought Stress. **Chinese Journal of Tropical Crops**, v. 42, n. 5, p. 1348, 26 maio 2021.

DO ROSÁRIO ROSA, V. et al. Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and Ascophyllum nodosum (L.) seaweed extract. **Plant Physiology and Biochemistry**, v. 158, p. 228–243, 1 jan. 2021.

DONG, S.; BECKLES, D. M. Dynamic changes in the starch-sugar interconversion within plant source and sink tissues promote a better abiotic stress response. **Journal of Plant Physiology**, v. 234–235, p. 80–93, 1 mar. 2019.

DOS SANTOS, C. M. et al. Physiological Changes Associated with Antioxidant Enzymes in Response to Sugarcane Tolerance to Water Deficit and Rehydration. **Sugar Tech**, v. 17, n. 3, p. 291–304, 13 set. 2015.

DU JARDIN, P. Plant biostimulants: Definition, concept, main categories and regulation. **Scientia Horticulturae**, v. 196, p. 3–14, 30 nov. 2015a.

DU JARDIN, P. Plant biostimulants: Definition, concept, main categories and regulation. **Scientia Horticulturae**, v. 196, p. 3–14, 30 nov. 2015b.

DUBEY, D.; PANDEY, A. Effect of Nickel (Ni) on chlorophyll, lipid peroxidation and antioxidant enzymes activities in black gram (*Vigna mungo*) leaves. **International Journal of Science and Nature**, v. 2, n. 2, p. 395–401, 2011.

ELANSARY, H. O.; SKALICKA-WOŹNIAK, K.; KING, I. W. Enhancing stress growth traits as well as phytochemical and antioxidant contents of *Spiraea* and *Pittosporum* under seaweed extract treatments. **Plant Physiology and Biochemistry**, v. 105, p. 310–320, 1 ago. 2016.

ENDRES, L. Photosynthesis and Water Relations in Brazilian Sugarcane. **The Open Agriculture Journal**, v. 4, n. 1, p. 31–37, 29 dez. 2010.

FAN, D. et al. Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress. **Food Chemistry**, v. 124, n. 1, p. 195–202, 1 jan. 2011.

FERNANDES, A. C. **Cálculos na agroindústria de cana-de-açúcar**. Piracicaba: STAB, 2011. 240 p.

GADJEV, I.; STONE, J. M.; GECHEV, T. S. Chapter 3: Programmed Cell Death in Plants. New Insights into Redox Regulation and the Role of Hydrogen Peroxide. **International Review of Cell and Molecular Biology**, v. 270, n. C, p. 87–144, 1 jan. 2008.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide Dismutases II. Purification and Quantitative Relationship with Water-soluble Protein in Seedlings. **Plant Physiology**, v. 59, n. 2, p. 315–318, 1 fev. 1977.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909–930, 1 dez. 2010.

GONG, X. et al. Arabidopsis AtSUC2 and AtSUC4, encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. **Physiologia Plantarum**, v. 153, n. 1, p. 119–136, 1 jan. 2015.

GOÑI, O. et al. Comparative Transcriptome Analysis of Two *Ascophyllum nodosum* Extract Biostimulants: Same Seaweed but Different. **Journal of Agricultural and Food Chemistry**, v. 64, n. 14, p. 2980–2989, 27 abr. 2016.

GOÑI, O.; QUILLE, P.; O'CONNELL, S. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. **Plant Physiology and Biochemistry**, v. 126, n. March, p. 63–73, 2018a.

GOÑI, O.; QUILLE, P.; O'CONNELL, S. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. **Plant Physiology and Biochemistry**, v. 126, p. 63–73, 1 maio 2018b.

GUPTA, A.; RICO-MEDINA, A.; CAÑO-DELGADO, A. I. The physiology of plant responses to drought. **Science**, v. 368, n. 6488, p. 266–269, 17 abr. 2020.

HASSUANI, S. **Biomass power generation : sugar cane bagasse and trash**. Piracicaba : CTC ed. Piracicaba ;Brasília: CTC ;;PNUD, 2005. v. 1

HATCH, M. D. C4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. **BBA Reviews On Bioenergetics**, v. 895, n. 2, p. 81–106, 1 jan. 1987.

HAUVERMALE, A. L.; SANAD, M. N. M. E. Phenological Plasticity of Wild and Cultivated Plants. **Plant Communities and Their Environment**, 8 maio 2019.

HAVIR, E. A.; MCHALE, N. A. Biochemical and Developmental Characterization of Multiple Forms of Catalase in Tobacco Leaves. **Plant Physiology**, v. 84, n. 2, p. 450–455, 1 jun. 1987.

HAYAT, S.; AHMAD, A. **Salicylic acid: A plant hormone**. Springer Science & Business Media, 2007.

HEATH, R. L.; PACKER, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics**, v. 125, n. 1, p. 189–198, 1 abr. 1968.

HELDT, H.-W. **Plant Biochemistry**. 3. ed. Oxford: Elsevier Inc., 2005.

JALEEL, C. A. et al. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. **Colloids and Surfaces B: Biointerfaces**, v. 59, n. 2, p. 150–157, 1 out. 2007.

JOSEMARA, R.; QUEIROZ, B. **RESPOSTA FISIOLÓGICA E MOLECULAR DE DOIS GENÓTIPOS DE MILHO À LIMITAÇÃO HÍDRICA**. [s.l.] Universidade Estadual Paulista (UNESP), 1 out. 2010.

KAR, M.; MISHRA, D. Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. **Plant Physiology**, v. 57, n. 2, p. 315–319, 1 fev. 1976.

KARTHIKEYAN, K.; SHANMUGAM, M. The effect of potassium-rich biostimulant from seaweed *Kappaphycus alvarezii* on yield and quality of cane and cane juice of sugarcane var. Co 86032 under plantation and ratoon crops. **Journal of Applied Phycology** 2017 **29:6**, v. 29, n. 6, p. 3245–3252, 15 jul. 2017.

KARUPPANAPANDIAN, T. et al. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. **Australian Journal of Crop Science**, v. 5, n. 6, p. 709–725, 2011.

KHAN, W. et al. Seaweed Extracts as Biostimulants of Plant Growth and Development. **Journal of Plant Growth Regulation** 2009 **28:4**, v. 28, n. 4, p. 386–399, 8 maio 2009a.

KHAN, W. et al. Seaweed Extracts as Biostimulants of Plant Growth and Development. **Journal of Plant Growth Regulation** 2009 **28:4**, v. 28, n. 4, p. 386–399, 8 maio 2009b.

KHOMPATARA, K. et al. Enhanced Resistance to Leaf Fall Disease Caused by *Phytophthora palmivora* in Rubber Tree Seedling by *Sargassum polycystum* Extract. **Plants** 2019, **Vol. 8, Page 168**, v. 8, n. 6, p. 168, 11 jun. 2019.

KRIEGER-LISZKAY, A.; FUFUZAN, C.; TREBST, A. Singlet oxygen production in photosystem II and related protection mechanism. **Photosynthesis Research**, v. 98, n. 1–3, p. 551–564, 9 set. 2008.

KROUK, G. et al. Nitrate-Regulated Auxin Transport by NRT1.1 Defines a Mechanism for Nutrient Sensing in Plants. **Developmental Cell**, v. 18, n. 6, p. 927–937, 15 jun. 2010.

LEMOINE, R. et al. Source-to-sink transport of sugar and regulation by environmental factors. **Frontiers in Plant Science**, v. 0, n. JUL, p. 272, 24 jul. 2013.

LOCATO, V. Reactive oxygen species and ascorbate glutathione interplay in signaling and stress responses. In: GUPTA, S. **Reactive Oxygen Species and Antioxidants in Higher Plants** . 1. ed. p. 45–64.

LOLA-LUZ, T.; HENNEQUART, F.; GAFFNEY, M. Effect on health promoting phytochemicals following seaweed application, in potato and onion crops grown under a low input agricultural system. **Scientia Horticulturae**, v. 170, p. 224–227, 7 maio 2014.

MACHADO, R. S. et al. Biometric and physiological responses to water deficit in sugarcane at different phenological stages. **Pesquisa Agropecuaria Brasileira**, v. 44, n. 12, p. 1575–1582, 2009.

MANO, J. Reactive carbonyl species: Their production from lipid peroxides, action in environmental stress, and the detoxification mechanism. **Plant Physiology and Biochemistry**, v. 59, p. 90–97, 1 out. 2012.

MARIANI, L.; FERRANTE, A. Agronomic Management for Enhancing Plant Tolerance to Abiotic Stresses—Drought, Salinity, Hypoxia, and Lodging. **Horticulturae** 2017, Vol. 3, Page 52, v. 3, n. 4, p. 52, 1 dez. 2017.

MEDEIROS, D. B. et al. Physiological limitations in two sugarcane varieties under water suppression and after recovering. **Theoretical and Experimental Plant Physiology**, v. 25, n. 3, p. 213–222, 2013.

MEYER, A. J.; HELL, R. Glutathione homeostasis and redox-regulation by sulfhydryl groups. **Photosynthesis Research**, v. 86, n. 3, p. 435–457, 11 dez. 2005.

MHAMDI, A.; NOCTOR, G.; BAKER, A. Plant catalases: Peroxisomal redox guardians. **Archives of Biochemistry and Biophysics**, v. 525, n. 2, p. 181–194, 15 set. 2012.

MICHALAK, I.; CHOJNACKA, K. Algal extracts: Technology and advances. **Engineering in Life Sciences**, v. 14, n. 6, p. 581–591, 1 nov. 2014.

MITTLER, R. Oxidative stress, antioxidants and stress tolerance. **Trends in Plant Science**, v. 7, n. 9, p. 405–410, 1 set. 2002.

MOREIRA, R. et al. Aqueous extracts of *Ascophyllum nodosum* obtained by ultrasound-assisted extraction: effects of drying temperature of seaweed on the properties of extracts. **Journal of Applied Phycology** 2017 29:6, v. 29, n. 6, p. 3191–3200, 21 maio 2017.

MUSTAFA OZGEN, † et al. Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Methods. **Journal of Agricultural and Food Chemistry**, v. 54, n. 4, p. 1151–1157, 22 fev. 2006.

NAIKOO, M. I. et al. Role and Regulation of Plants Phenolics in Abiotic Stress Tolerance: An Overview. **Plant Signaling Molecules: Role and Regulation under Stressful Environments**, p. 157–168, 1 jan. 2019.

NASCIMENTO, J. B.; BARRIGOSI, J. A. F. O papel de enzimas. **Agrarian Academy**, v. 1, n. 01, p. 234–250, 2014.

NELSON, N. A PHOTOMETRIC ADAPTATION OF THE SOMOGYI METHOD FOR THE DETERMINATION OF GLUCOSE. **Journal of Biological Chemistry**, v. 153, n. 2, p. 375–380, 1 maio 1944.

NEVES, M. F. Mais econômico e renovável, etanol conquista os consumidores brasileiros. In: **Caminhos da Cana**. [s.l.: s.n.]. p. 27–28.

NGOROYEMOTO, N. et al. Effect of organic biostimulants on the growth and biochemical composition of *Amaranthus hybridus* L. **South African Journal of Botany**, v. 124, p. 87–93, 1 ago. 2019.

OKOLIE, C. L.; MASON, B.; CRITCHLEY, A. T. **Seaweeds as a Source of Proteins for Use in Pharmaceuticals and High-Value Applications**. Novel Proteins for Food, Pharmaceuticals, and Agriculture: Sources, Applications, and Advances, v. 217, 2018.

OLIVEIRA, C. E. G. Acompanhamento de safra brasileira cana-de-açúcar - safra 2019/2020. **Companhia Nacional de Abastecimento**, v. 6, n. 4, p. 57, 2019.

OMIDBAKHSHFARD, M. A. et al. A biostimulant obtained from the seaweed *ascophyllum nodosum* protects *arabidopsis thaliana* from severe oxidative stress. **International Journal of Molecular Sciences**, v. 21, n. 2, p. 1–26, 2020.

PATADE, V. Y.; BHARGAVA, S.; SUPRASANNA, P. Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: Growth, osmolytes accumulation, and antioxidant defense. **Journal of Plant Interactions**, v. 6, n. 4, p. 275–282, dez. 2011.

PAUL, M. J.; FOYER, C. H. Sink regulation of photosynthesis. **Journal of Experimental Botany**, v. 52, n. 360, p. 1383–1400, 1 jul. 2001.

PEREIRA, L. B. et al. Prospecting Plant Growth-Promoting Bacteria Isolated from the Rhizosphere of Sugarcane Under Drought Stress. **Current Microbiology** 2019 **76:11**, v. 76, n. 11, p. 1345–1354, 2 ago. 2019.

PHOTINI V. MYLONA; ALEXIOS N. POLIDOROS. ROS Regulation of Antioxidant Genes. In: S. DUTTA GUPTA. **Reactive Oxygen Species and Antioxidants in Higher Plants**. 1. ed. p. 102–117. 2010.

PRASCH, C. M. et al. β -amylase1 mutant Arabidopsis plants show improved drought tolerance due to reduced starch breakdown in guard cells. **Journal of Experimental Botany**, v. 66, n. 19, p. 6059–6067, 1 set. 2015.

PUTEH, A. B. et al. Chlorophyll fluorescence parameters of cultivated (*Oryza sativa* L. ssp. indica) and weedy rice (*Oryza sativa* L. var. nivara) genotypes under water stress. **Australian Journal of Crop Science**, v. 7, n. 9, p. 1277–1283, 2013.

QUAN, L. J. et al. Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. **Journal of Integrative Plant Biology**, v. 50, n. 1, p. 2–18, 1 jan. 2008.

RASUL, F. et al. Priming with a Seaweed Extract Strongly Improves Drought Tolerance in Arabidopsis. **International Journal of Molecular Sciences** 2021, Vol. **22**, Page **1469**, v. 22, n. 3, p. 1469, 2 fev. 2021.

RATHORE, S. S. et al. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. **South African Journal of Botany**, v. 75, n. 2, p. 351–355, 1 abr. 2009.

REIS, S. P. DOS; LIMA, A. M.; SOUZA, C. R. B. DE. Recent Molecular Advances on Downstream Plant Responses to Abiotic Stress. **International Journal of Molecular Sciences** 2012, Vol. **13**, Pages **8628-8647**, v. 13, n. 7, p. 8628–8647, 11 jul. 2012.

RIBEIRO, R. V. et al. Revealing drought-resistance and productive patterns in sugarcane genotypes by evaluating both physiological responses and stalk yield. **Experimental Agriculture**, v. 49, n. 2, p. 212–224, abr. 2013.

RODRIGUES GABRIEL SALES, C. et al. Trocas gasosas e balanço de carboidratos em plantas de cana-de-açúcar sob condições de estresses radiculares Cristina. **I**, v. 22, n. 3, p. 13084–200, 2012.

RONSEIN, G. E. et al. Oxidação de proteínas por oxigênio singlete: Mecanismos de dano, estratégias para detecção e implicações biológicas. **Química Nova**, v. 29, n.

3, p. 563–568, 2006.

ROSA, M. et al. Soluble sugars. <https://doi.org/10.4161/psb.4.5.8294>, v. 4, n. 5, p. 388–393, maio 2009.

SALAH, I. BEN et al. Seaweed polysaccharides as bio-elicitors of natural defenses in olive trees against verticillium wilt of olive. <http://mc.manuscriptcentral.com/tjpi>, v. 13, n. 1, p. 248–255, 1 jan. 2018.

SHARMA, I.; AHMAD, P. Catalase: A Versatile Antioxidant in Plants. In: **Oxidative Damage to Plants: Antioxidant Networks and Signaling**. Elsevier Inc., 2014. p. 131–148.

SHARMA, P. et al. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. **Journal of Botany**, v. 2012, 2012.

SHI, P. et al. Effects of *Ascophyllum nodosum* extract on growth and antioxidant defense systems of two freshwater microalgae. **Journal of Applied Phycology**, v. 30, n. 2, p. 851–859, 2018.

SHUKLA, P. S. et al. Seaweed extract improve drought tolerance of soybean by regulating stress-response genes. **AoB PLANTS**, v. 10, n. 1, 1 fev. 2018.

SHUKLA, P. S. et al. *Ascophyllum nodosum*-based biostimulants: Sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. **Frontiers in Plant Science**, v. 10, n. May, p. 1–22, 2019.

SILVA, M. A. DA et al. Biostimulant alleviates abiotic stress of mango grown in semiarid environment. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 24, n. 7, p. 457–464, 13 jul. 2020.

SILVA, M. DE A. et al. Pigmentos fotossintéticos e índice SPAD como Descritores de intensidade do estresse por deficiência hídrica em cana-de-açúcar. **Bioscience Journal**, v. 30, n. 1, p. 173–181, 2014.

SILVA, T. T. DE A. et al. Qualidade fisiológica de sementes de milho na presença de bioestimulantes. **Ciência e Agrotecnologia**, v. 32, n. 3, p. 840–844, 2008.

SINGH, I. et al. Can we not mitigate climate change using seaweed based biostimulant: A case study with sugarcane cultivation in India. **Journal of Cleaner Production**, v. 204, p. 992–1003, 10 dez. 2018.

SOMOGYI, M. A NEW REAGENT FOR THE DETERMINATION OF SUGARS. **Journal of Biological Chemistry**, v. 160, n. 1, p. 61–68, 1 set. 1945.

SPANN, T. M.; LITTLE, H. A. Applications of a Commercial Extract of the Brown Seaweed *Ascophyllum nodosum* Increases Drought Tolerance in Container-grown 'Hamlin' Sweet Orange Nursery Trees. **HortScience**, v. 46, n. 4, p. 577–582, 1 abr. 2011.

STURM, A. Invertases. Primary Structures, Functions, and Roles in Plant Development and Sucrose Partitioning. **Plant Physiology**, v. 121, n. 1, p. 1–8, 1 set. 1999.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. 6. ed. Porto Alegre: Artmed, 2017.

TAKAHASHI, S.; BADGER, M. R. Photoprotection in plants: A new light on photosystem II damage. **Trends in Plant Science**, v. 16, n. 1, p. 53–60, 1 jan. 2011.

TAN, C.-Y. et al. Regulation of algal and cyanobacterial auxin production, physiology, and application in agriculture: an overview. **Journal of Applied Phycology** 2021 **33:5**, v. 33, n. 5, p. 2995–3023, 19 maio 2021.

TEZARA, W. et al. Effects of water deficit and its interaction with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. **Journal of Experimental Botany**, v. 53, n. 375, p. 1781–1791, 1 ago. 2002.

THALMANN, M.; SANTELIA, D. Starch as a determinant of plant fitness under abiotic stress. **New Phytologist**, v. 214, n. 3, p. 943–951, 1 maio 2017.

THIPYAPONG, P. et al. Suppression of polyphenol oxidases increases stress tolerance in tomato. **Plant Science**, v. 167, n. 4, p. 693–703, 1 out. 2004.

TOMÁS-BARBERÁN, F. A.; ESPÍN, J. C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. **Journal of the Science of Food and Agriculture**, v. 81, n. 9, p. 853–876, 1 jul. 2001.

TRANTAPHYLIDÈS, C.; HAVAUX, M. Singlet oxygen in plants: production, detoxification and signaling. **Trends in Plant Science**, v. 14, n. 4, p. 219–228, 1 abr. 2009.

TRIPATHY, B. C.; OELMÜLLER, R. Reactive oxygen species generation and signaling in plants. **Plant Signaling and Behavior**, v. 7, n. 12, p. 167–173, 2012.

VALERIO, C. et al. Thioredoxin-regulated β -amylase (BAM1) triggers diurnal starch degradation in guard cells, and in mesophyll cells under osmotic stress. **Journal of Experimental Botany**, v. 62, n. 2, p. 545–555, 1 jan. 2011.

VAN HEERDEN, P. D. R. et al. Limitation of photosynthetic carbon metabolism by dark chilling in temperate and tropical soybean genotypes. **Plant Physiology and Biochemistry**, v. 42, n. 2, p. 117–124, 2004.

VAN OOSTEN, M. J. et al. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. **Chemical and Biological Technologies in Agriculture** **2017 4:1**, v. 4, n. 1, p. 1–12, 3 abr. 2017.

VAUGHN, K. C.; DUKE, S. O. Function of polyphenol oxidase in higher plants. **Physiologia Plantarum**, v. 60, n. 1, p. 106–112, 1 jan. 1984.

VICKERS, J. E. et al. Overexpression of Polyphenol Oxidase in Transgenic Sugarcane Results in Darker Juice and Raw Sugar. **Crop Science**, v. 45, n. 1, p. 354–362, 1 jan. 2005.

VRANOVA, E. Signal transduction during oxidative stress. **Journal of Experimental Botany**, v. 53, n. 372, p. 1227–1236, 15 maio 2002.

WAGNER, D. et al. The genetic basis of singlet oxygen-induced stress response of *Arabidopsis thaliana*. **Science**, v. 306, n. 5699, p. 1183–1185, 12 nov. 2004.

WATANABE, K. et al. Effects of Superabsorbent Polymer on Soil Water Content and Sugarcane Germination and Early Growth in Sandy Soil Conditions. **Sugar Tech** **2018 21:3**, v. 21, n. 3, p. 444–450, 28 set. 2018.

WINTER, H.; HUBER, S. C. **Regulation of sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes** **Critical Reviews in Plant Sciences** Taylor & Francis Group, , 2000. Disponível em: <<https://www.tandfonline.com/doi/abs/10.1080/07352680091139178>>. Acesso em: 10 set. 2021

WOZNIAK, E. et al. Biostimulant Mode of Action. **The Chemical Biology of Plant Biostimulants**, p. 205–227, 2 mar. 2020.

XU, C.; LESKOVAR, D. I. Effects of *A. nodosum* seaweed extracts on spinach growth, physiology and nutrition value under drought stress. **Scientia Horticulturae**, v. 183, p. 39–47, 12 fev. 2015.

YAKHIN, O. I. et al. Biostimulants in plant science: A global perspective. **Frontiers in Plant Science**, v. 7, p. 2049, 26 jan. 2017.

YAMADA, K. et al. Functional Analysis of an Arabidopsis thaliana Abiotic Stress-inducible Facilitated Diffusion Transporter for Monosaccharides *. **Journal of Biological Chemistry**, v. 285, n. 2, p. 1138–1146, 8 jan. 2010.

YILDIZTEKIN, M.; TUNA, A. L.; KAYA, C. Physiological Effects of the Brown Seaweed (*Ascophyllum nodosum*) and Humic Substances on Plant Growth, Enzyme Activities of Certain Pepper Plants Grown under Salt Stress. **Acta Biologica Hungarica** **2018** **69:3**, v. 69, n. 3, p. 325–335, 30 dez. 2018.

YUN-HEE KIM; SANG-SOO KWAK. The Role of Antioxidant Enzymes during Leaf Development. In: **Reactive Oxygen Species and Antioxidants in Higher Plants**. 1. ed. Boca Raton: CRC Press, 2010. p. 145–166.

ZEEMAN, S. C.; KOSSMANN, J.; SMITH, A. M. Starch: Its Metabolism, Evolution, and Biotechnological Modification in Plants. <http://dx.doi.org/10.1146/annurev-arplant-042809-112301>, v. 61, p. 209–234, 4 maio 2010.

ZHAO, D.; GLAZ, B.; COMSTOCK, J. C. Sugarcane leaf photosynthesis and growth characters during development of water-deficit stress. **Crop Science**, v. 53, n. 3, p. 1066–1075, 1 maio 2013.