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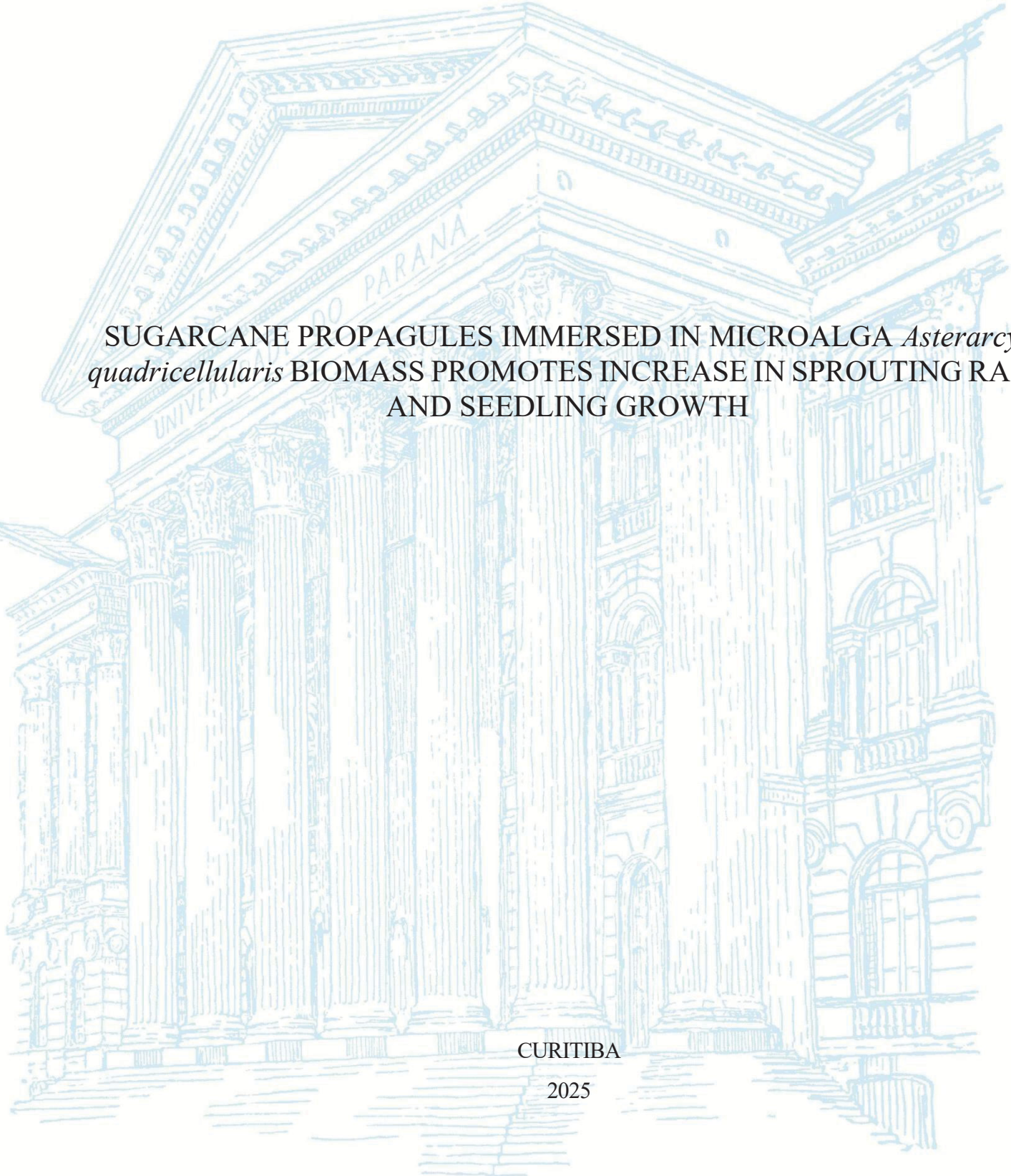

SUGARCANE PROPAGULES IMMERSED IN MICROALGA *Asterarcys quadricellularis* BIOMASS PROMOTES INCREASE IN SPROUTING RATE AND SEEDLING GROWTH

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, área de concentração em Produção Vegetal, Departamento de Fitotecnia e Proteção Vegetal, do Setor de Ciências Agrárias da Universidade Federal do Paraná, como parte dos requisitos para obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. João Carlos Bespalhok Filho
Coorientador: Prof. Dr. Átila Francisco Mógor
Coorientador: Prof. Dr. Ricardo Augusto de Oliveira

CURITIBA

2025

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Lara, Gabriel Bocchetti de
Sugarcane propagules immersed in microalga *Asterarcys quadricellularis* biomass promotes increase in sprouting rate and seedling growth / Gabriel Bocchetti de Lara. – Curitiba, 2025.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Agronomia (Produção Vegetal).
Orientador: Prof. Dr. João Carlos Bespalhok Filho
Coorientador: Prof. Dr. Átila Francisco Mógor
Coorientador: Prof. Dr. Ricardo Augusto de Oliveira

1. Bulbilho. 2. Cana de Açúcar. 3. Microalga. 4. Insumo agrícola. 5. Crescimento. I. Bespalhok Filho, João Carlos. II. Mogor, Átila Francisco. III. Oliveira, Ricardo Augusto de. IV. Universidade Federal do Paraná. Programa de Pós-Graduação em Agronomia (Produção Vegetal). V. Título.

Bibliotecária: Talita Nunes Silva Gonçalves CRB-9/2244

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **GABRIEL BOCCHETTI DE LARA**, intitulada: **SUGARCANE PROPAGULES IMMERSED IN MICROALGA *Asterarcys quadricellularis* BIOMASS PROMOTES INCREASE IN SPROUTING RATE AND SEEDLING GROWTH**, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 04 de Agosto de 2025.

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JOÃO CARLOS BESPALHOK FILHO
Presidente da Banca Examinadora

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05/08/2025 10:47:38.0
GILDA MÓGOR
Avaliador Externo (AUTONOMA)

Dedico a toda a minha família, especialmente a minha esposa e companheira de vida Kauana, por acreditar em mim e me fazer acreditar que podemos alcançar sonhos como este.

AGRADECIMENTOS

Agradeço primeiramente a Deus, por me dar saúde, proteção e sabedoria para aproveitar as oportunidades da vida.

Ao professor orientador Dr° João Carlos Bespalhok Filho, pelos ensinamentos, acompanhamento contínuo e auxílio durante o Doutorado e escrita desta Tese.

Ao professor coorientador Dr° Ricardo Augusto de Oliveira, pela assistência e oportunidade de aprofundar meus conhecimentos nessa linha de pesquisa.

Ao professor coorientador Dr° Átila Francisco Mógor, pela amizade, profissionalismo e ensinamentos que hoje compõem minha bagagem acadêmica e profissional.

A professora e companheira de trabalho Drª Gilda, pela constante ajuda, voz de apoio e pelas oportunidades que tornaram minha jornada acadêmica mais leve e proveitosa.

A amiga e companheira de trabalho Drª Juliana, por ter me ajudado desde o primeiro dia que entrei como estagiário, dando suporte sempre quando precisei, e pela parceria mesmo nos momentos mais difíceis.

A minha família que sempre me apoiou e me deu suporte para que eu conseguisse alcançar o meu melhor. Sem vocês nada disso seria possível.

E por fim, a todos que de alguma forma participaram desta longa caminhada, dando conselhos, acolhendo quando os desafios apareciam e comemorando junto nossas conquistas. A todos vocês, meu muito obrigado!

RESUMO

O uso de propágulos de uma gema (OBP) como método de plantio de cana-de-açúcar é amplamente difundido devido aos seus benefícios logísticos. Propágulos de diferentes posições do colmo apresentam idades fisiológicas distintas, bem como diferentes taxas de brotação e crescimento inicial, o que pode afetar a uniformidade do estande. No entanto, seu crescimento inicial pode ser melhorado com a aplicação de insumos sustentáveis. O potencial biotecnológico das microalgas tem ganhado interesse devido à sua bioatividade para diversos usos. Na cana-de-açúcar, fertilizantes biológicos têm sido relatados como capazes de aumentar a eficiência de absorção de nutrientes e melhorar o crescimento e a produtividade. Portanto, este estudo teve como objetivo, primeiramente, determinar a concentração de máxima eficiência (MEC) do extrato da microalga *Asterarcys quadricellularis* (AQ) na taxa de brotação e nas alterações biométricas do OBP da cana-de-açúcar (cultivar RB036152); e, em segundo momento, examinar sua bioatividade na promoção de alterações bioquímicas no OBP. Todos os resultados biométricos mostraram um padrão quadrático positivo semelhante, correspondendo a concentrações crescentes de AQ até rendimentos biométricos decrescentes. A MEC resultou em um aumento de 23,1% na taxa de brotação em comparação ao controle. O desenvolvimento das plantas foi estimulado durante os 10 dias iniciais de crescimento da cana-de-açúcar em OBP na câmara B.O.D. O crescimento também foi promovido após o transplante para vasos, favorecendo o desenvolvimento da parte aérea e das raízes. A MEC média da AQ de todos os dados biométricos foi de 2,38 g L^{-1}. Para o segundo experimento, foi utilizada uma concentração de 2,5 g L^{-1}. As análises bioquímicas indicaram alterações nos níveis de açúcar, especialmente com aminas bioativas (poliaminas e indolaminas), mostrando níveis reduzidos de putrescina e aumento do teor de triptofano no OBP. Esses resultados podem, pelo menos em parte, estar relacionados ao teor de L- aminoácidos livres na biomassa da microalga, que ativa aminas bioativas como metabólitos-chave envolvidos nos efeitos promotores do crescimento vegetal. Este estudo também oferece insights valiosos para o desenvolvimento de técnicas de campo ecologicamente corretas para aprimorar e padronizar a brotação e o crescimento inicial da cana-de-açúcar.

Palavras-chave: Bioinsumos; Clorófitas; Extrato; Microalga; Poliaminas; *Saccharum* spp.

ABSTRACT

The use of one-bud propagules (OBP) as a sugarcane planting method is widespread due to logistical benefits. Propagules from different stem positions have varying physiological ages and exhibit different sprouting and early growth rates, which can affect stand uniformity. However, their initial growth can be improved with the application of sustainable inputs. The biotechnological potential of microalgae has gained interest because of their bioactivity for many uses. In sugarcane, biological fertilizers have been reported to increase nutrient uptake efficiency and enhance growth and yield. Therefore, this study aimed first to determine the maximum efficiency concentration (MEC) of the microalga *Asterarcys quadricellularis* biomass extract (AQ) on the sprouting rate and biometric changes of sugarcane OBP (cultivar RB036152); and second, to examine its bioactivity in promoting biochemical changes in OBP. All the biometric results showed a similar positive quadratic pattern corresponding to increasing AQ concentrations until biometric diminishing returns. The MEC resulted in a 23.1% increase in sprouting rate compared to the control. Plant development was stimulated during the initial 10 days of sugarcane OBP growth in the B.O.D. chamber. Growth was also promoted after transplanting into pots, enhancing shoot and root development. The average MEC of AQ derived from all biometric data was 2.38 g L^{-1}. For the second experiment, a concentration of 2.5 g L^{-1} was used. Biochemical analysis indicated changes in sugar levels, and especially with bioactive amines (polyamines and indoleamines), showing reduced putrescine levels and increased tryptophan content in OBP. These results may, at least partly, be related to the L-free amino acid content in the microalga biomass, which triggers bioactive amines as key metabolites involved in the microalga's growth-promoting effects. This study also offers valuable insights for developing environmentally friendly field techniques to enhance and standardize sugarcane sprouting and early growth.

Keywords: Bioinputs; Chlorophyta; Extract; Microalga; Polyamines; *Saccharum* spp.

SUMMARY

1 INTRODUCTION

Brazil is the world's largest producer of sugarcane and the second largest in terms of sugar production. Several factors contribute to the high yield of the crop, such as the correct choice of cultivars and technologies, which have a positive impact on crop yields. In this sense, the economic importance of research aimed at providing sustainable solutions to this problem is remarkable when combined with renewable sources, such as bioinputs that can promote plant growth.

In Normative Instruction No. 61, of July 8, 2020, art. 2, XXIII, biofertilizers are defined as a product that contains an active principle or organic agent, free of pesticides, capable of acting, directly or indirectly, on all or part of cultivated plants, increasing yield, without considering their hormonal or stimulating value (BRAZIL, 2020). Recently, the Brazilian National Bioinputs Law (Law No. 15.070/2024), issued on December 23, 2024, established a comprehensive regulatory framework for biological inputs. This law defines bioinputs to include products of plant, animal, or microbial origin, as well as those derived from biotechnological processes, encompassing biofertilizers, soil conditioners, biostimulants, semiochemicals, metabolites, macromolecules, and biological control agents (BRAZIL, 2024).

One main classification of bioinputs involves their ability to promote plant growth and development. These substances and/or microorganisms' primary function, when applied to the plant, is related to triggering or enhancing endogenous physiological processes (Barbosa et al., 2025). Among these plant physiological changes are increased tolerance to abiotic stresses, improved nutrient use efficiency, and enhanced crop quality, independently of directly providing nutrients (Santos et al. 2024).

The action of bioinputs obtained from microalgae biomass is attributed to the presence of bioactive compounds (Gitau et al., 2022; González-Pérez et al., 2022), such as polysaccharides, polyamines (PAs) (Braun and Colla, 2023; Mógor et al., 2017), as well as free L-amino acids (L-AAs) (Renuka et al., 2018; Cordeiro et al., 2022a). Research on sugarcane applied with microalgae is scarce, but it has been reported that immersion in microalgae biomass stimulated bud sprouting and initial growth (Mógor et al., 2022).

Among these sources, the biochemical composition of microalgae stands out as a raw material for developing new products to improve plant growth (Garcia-Gonzalez and Sommerfeld, 2016; El Arroussi et al., 2018; Mógor et al., 2018), offering a viable alternative to reduce dependence on chemical fertilizers. The potential of these substances has been explored and is considered a

renewable, ecological, and economically feasible source, capable of increasing plant yield in a sustainable way (Mahajan et al., 2003).

In this scenario, some microalgae biomass rich in amino acids, such as the Chlorophyta *Asterarcys quadricellularis* (AQ), are being studied for their ability to serve as a source of these biomolecules for bioinputs (Ghosh et al., 2017). However, the mechanisms induced by microalgae in plants are complex and not yet fully understood. Their action involves the interaction of several molecules (Barone et al., 2018), linked to an intricate signaling network (Mógor et al., 2018), which activate genes linked to the biosynthesis of amino acids and bioactive amines, which will trigger metabolic responses.

Thus, given the efficiency of microalgae and the possible benefits they can bring to various crops, including sugarcane, this research aimed to study the effect of immersion in AQ biomass, and its concentration of maximum efficiency (MEC), on the production of sugarcane one-bud propagules (OBP), involving sprouting rate, and biometrical and biochemical changes.

1.1 HYPOTHESIS

The production of sugarcane OBP can be enhanced with the use of bioinputs composed of the biomass extract of the microalga *A. quadricellularis*. The bioactive compounds in this biomass can act as metabolic signaling agents, activating metabolic pathways that trigger physiological changes, such as promoting plant growth. Likewise, it can alter the biochemical levels of bioactive amines and polyamines. This effect could provide benefits from the time the OBP are planted in growth trays, promoting a higher sprouting rate and better initial development, and after transplanting into pots, enhancing the vegetative growth of sugarcane.

1.2 OBJECTIVES

1.2.1 General objective

Evaluate the sprouting rate of sugarcane OBP after immersion in *A. quadricellularis* as well as their capacity to promote plant growth and biochemical changes.

1.2.2 Specific objective

Determine the concentration of maximum efficiency (MEC) of the microalga *A. quadricellularis* biomass extract on sugarcane OBP.

2. BIBLIOGRAPHICAL REVIEW

2.1 SUGARCANE

2.1.1 History and importance

The main origin center of *Saccharum officinarum* (Linnaeus, 1753) was in New Guinea, located in Southwest Asia (James, 2004).

The introduction of sugarcane in Brazil took place around 1530, at the beginning of the colonial period, by Martim Affonso de Souza, who created the first sugar mill and sugar activities (Nocelli et al., 2017). These activities expanded 40 years later, mainly in the Northeast and Center South regions, acquiring a high economic importance that to this day remains one of the pillars of the Brazilian economy (Rodrigues, 2020).

The *S. officinarum* species has adapted well to the region's soil, cultivated mainly in the country's coastal areas. During colonization, Bahia and Pernambuco were the places that developed the most from the resources of these sugar activities (Oliver, 2014). Later, at the end of the 19th century, this crop expanded to other states, such as São Paulo, which at the time was experiencing a crisis in coffee production (Araújo and Santos, 2013).

In the beginning, the sugar mills were only focused on refining sugar, but in 1975, with the creation of the National Alcohol Production Plan (Proálcool), which aimed to, in part, replace fossil fuels with alcohol, there was a great expansion of the sugar-alcohol sector, especially in the interior of São Paulo, placing Brazil as a leading country in the production of renewable energy (Mozambani et al., 2006).

Currently, the geographical distribution of this crop has grown and developed in several locations with varying climates in the country, in the main sugarcane-producing states, according to the National Supply Company (CONAB, 2025).

Even with the market changing and expanding to other forms of economic development, Brazil remains the main country in the large-scale production of this crop, and by exporting its sugar worldwide, it has gained global prominence in the sugar-energy sector (Unica, 2024). It can be seen then how sugarcane, being a high-value-added crop, has gained great relevance in the Brazilian agribusiness sector (Matoso et al., 2020).

Its by-products are widely used in agricultural areas for soil correction, fertilizers, organic fertilizers, and as a nutritional additive in the diet of different animals (Dos Santos, 2022). Its main product is sucrose for sugar production, followed by sugarcane bagasse, which is a by-product used as

an alternative fuel (Palacios-Bereche et al., 2022), as well as the fiber itself, which can be used by the mills to produce energy (Rodrigues, 1995).

In this scenario, where the search for renewable energy sources is being promoted, sugarcane is gaining focus as it is considered a clean matrix (Cortez, 2012) and one of the best sources of renewable energy, with extremely favorable prospects (Nocelli et al., 2017). This has encouraged the production of flex-fuel vehicles, which can run on both gasoline and ethanol (Barbosa et al., 2020).

Other uses of sugarcane include its "*in natura*" form as fodder in animal diets, as well as its great value in the food industry to produce *rapadura*, molasses, *cachaça*, among other products (Galo, 2013).

2.1.2 General and morphological aspects of sugarcane

Sugarcane (*Saccharum officinarum* L.) is a plant species classified as perennial grass belonging to the division Magnoliophyta, subdivision Angiospermae, kingdom Plantae, class Liliopsida, order Poales, family Poaceae, genus *Saccharum,* and species *Saccharum officinarum* (Nascimento et al., 2015).

The species belonging to the *Saccharum* genus number more than thirty, with six species currently recognized: *S. barberi*, *S. edule*, *S. officinarum*, *S. robustum*, *S. sinese,* and *S. spontaneum* (Castro, 2015). The sugarcane cultivars used commercially in Brazil are interspecific clonal hybrids, resulting from crossbreeding and the selection of the robustness characteristic of the *Saccharum spontaneum* species and the accumulation of a large amount of sugar in *S. officinarum* (Pedrozo et al., 2008).

Sugarcane is an allogamous species; its inflorescence is of the panicle type (Rodrigues, 1995), and it can reproduce in two ways: sexually, used mainly for genetic improvement, and asexually, commonly used in commercial plantations (CONAB, 2025). Vegetative propagation provides greater speed and uniformity in the production of seedlings (Rodrigues, 1995).

It is an erect, perennial, rhizomatous plant. The stalks are cylindrical, hairless, and trichome-free of variable color and, internally, with entirely primary and widely dispersed vascular bundles. The nodes are protuberant or contrite. Sugarcane leaves are simple, alternate, and lanceolate (EMBRAPA, 2022).

It has a C4 photosynthetic metabolism, since it has capable of forming organic compounds with four carbons in their chain, a high capacity for storing sucrose in the tissues of the stalks, and good fixation of CO_2 from the atmosphere (Hitchcock, 1923).

Sugarcane produces a high level of photosynthesis in environments with higher temperatures (30 to 40°C), regions with more light, and rainfall rates of 1,500 to 2,500 mm, making it a crop that thrives in tropical and subtropical climates (Marafon, 2012). This temperature range is ideal for promoting sprouting, but for there to be good propagules sprouting, the crop requires moisture in the soil to promote swelling of the buds and root primordia located in the node region (Ripoli et al., 2006).

Sugarcane develops in the form of clumps, divided into two parts: its aerial part, located above the ground, formed by stalks, consisting of nodes and internodes, responsible for supporting the leaves, conducting water and nutrients from the soil to the plant, as well as storing sugar, and its underground part, which is made up of roots and rhizomes (Valsechi, 2008). Sugarcane has morphological differences, such as the shape of the clump, the type of culm, the inflorescence, the rhizome, and the fasciculate root (Mozambani et al., 2006).

The leaves of this plant are very characteristic, greenish in color, sessile, lance-shaped, linear, broad, and acute, with a distribution along its culm fixed at the nodes, interspersed in opposite and alternating rows (Sobrinho et al., 2019). Its leaves are elongated and flat, with a length of between 0.5 and 1.5 m and a width of between 2.5 and 10 cm, consisting of a sheath, a collar, and a leaf blade with silica on its edges (Scarpari and Beauclair, 2008).

The anatomy of its stalks is specially developed for the accumulation of sucrose. Its root system is fasciculate, located predominantly in its first 50 centimeters (Ripoli et al., 2006). The tillers are shoots formed after sprouting, occur in the underground part, and, in the case of sugarcane, are limited, except in some varieties of the *S. spontaneum* species, whose tillering is unlimited (Vasconcelos and Casagrande, 2008).

In sugarcane cultivation, the number of tillers provides the number of productive stalks at the end of the crop cycle (Segato et al., 2006), which is why knowledge of the factors that affect tillering dynamics in the field is necessary for proper sugarcane crop management.

The duration of each phase varies depending on the variety and climatic conditions. The vegetative development of sugar cane can be divided into three phases: initial (sprouting, emergence, and tillering), middle (stem elongation, growth), and final (maturation) (Smit and Singels, 2006).

This crop has different names according to when it is harvested, being called plant cane until its first harvest, while its second name is determined according to its growth period, which can vary between 12 and 18 months (Tavares, 2009). Therefore, sugarcane harvested at around 12 months is called year cane, while sugar cane harvested at 18 months is called year and a half cane (Vitti et al., 2008).

The production cycle of this species is divided into four phases: the first consists of the emergence of primary shoots, the second is tillering and crop establishment, which covers the period from the emergence of shoots to the end of tillering, the third is intensive growth from the end of tillering to the start of sucrose accumulation, and the fourth is maturation, when sucrose accumulation in the stalks becomes intense (Segato et al., 2006).

The sprouting of the ratoon occurs 20 - 30 days after planting. Then, the sprout breaks off the bud and is called the primary stalk, which grows towards the surface of the soil, and below it, the roots of the stalk emerge (Sobrinho et al., 2019). This is followed by leaf formation and the emergence of secondary shoots called tillers above the ground, which grow using the reserves present in the culm and the nutrients supplied by the first roots (Manhães et al., 2015).

The peak of tillering accompanies younger leaves, reaching pre-maturation with a higher concentration of sugar below the top, and finally, the cane reaches maturity with a similar distribution of sugar between the stalks (Manhães et al., 2015).

Proper management and effective treatment are essential for the plant to develop well throughout its crop cycle. Even though this plant organism has a high-water tolerance, it is important to provide water at the necessary times, as well as balanced fertilization (Adorna, 2011). This care is indispensable for farmers who aim for a good economic return in sugar and alcohol production (De Oliveira et al., 2019).

2.1.3 Genetic improvement

The genetic improvement of sugarcane has made it possible to obtain highly yielding cultivars that are adapted to different environments, which has made it possible to expand this crop in various regions of the country. The chromosomal difference between the species used to obtain the new clones allows for a high level of ploidy and aneuploidy among the cultivars (Blackburn, 1984; Janoo et al., 1999).

Currently, efforts to expand the genetic base of sugarcane have been added to the hybridization process, especially in pre-improvement work, since genetic gains are decreasing over time (Natarajan et al., 2019). The entire breeding process is slow and takes around 15 years, i.e., from the crossing stage to the release of a new cultivar (Costa et al., 2011; Natarajan et al., 2019).

Sugarcane improvement can be classified, historically, into five phases, in which it is observed that today commercial varieties are hybrids resulting from these crosses: 1st = Crossing and selection of noble sugarcane clones to obtain other noble cultivars; 2nd = Interspecific hybridization between *S.*

officinarum and *S. spontaneum*, followed by successive backcrosses with *S. officinarum* (nobilization); 3rd = Crossing of nobilized canes to obtain improved hybrids; 4th = Crossing between hybrids from the previous phase and those from the nobilization phase; 5th = Recovery of variability through the use of germplasm banks, especially using wild accessions (Lopes, 2011).

In Brazil, the genetic improvement of sugarcane is strongly focused on industrial production, with the selection of genotypes with high sugar yield per area (Barbosa et al., 2015; Carneiro et al., 2015). Furthermore, breeding programs have developed varieties adapted to environments with specific soil and climate conditions, harvest times, greater tolerance to water stress, greater resistance to pests and diseases, and better adaptation to mechanized harvesting, among others (Morais et al., 2011).

The Sugarcane Breeding Program at the Federal University of Paraná (UFPR) is part of the Inter-University Network for the Development of the Sugar-Energy Sector (RIDESA), which is composed of 10 Brazilian Federal Universities that manage the flowering and crossing stations (Brasileiro et al., 2024). At present, RB cultivars are planted on 60% of the total sugarcane area in Brazil, including six of the 10 most widely planted varieties (Oliveira et al. 2021).

One of the major challenges of the program is to breed varieties that perform well in restrictive environments (Brasileiro et al., 2024). Therefore, the main objective of RIDESA is the development of sugarcane cultivars with different maturation cycles, resistance to major pests and diseases, while obtaining high yields under different growing and management conditions (Carneiro et al. 2015, Daros et al. 2017, Diniz et al. 2019, Berton et al. 2020).

2.1.4 Cultivar (cv.) RB036152

RB036152 is a new variety that was launched by RIDESA (Interuniversity Network for the Development of the Sugar-Energy Sector), developed at the Federal University of Paraná, with superior results to the standard variety RB867515. It is a cross between SP83-5073 x RB867515 varieties (RIDESA, 2015).

It has fast initial growth, good inter-row closure, and a tall stature. It has a high yield potential and broad adaptability, and stability of agricultural production. Tolerant of the main sugarcane diseases. The RB036152 sugarcane cultivar is known for its high cane yield and high sucrose content. It has a medium to late ripening cycle, making it an ideal option for summer planting in the center-south region of Brazil (Brasileiro et al., 2024).

Sucrose accumulation begins in July, with increasing concentrations until November, a trend typical of medium-ripening cultivars in restrictive and favorable environments. This makes RB036152 an excellent option for guaranteeing satisfactory yields of stalks and sugar in restrictive environments.

This cultivar is particularly suited to mechanized planting and harvesting, demonstrating excellent performance in challenging environments over several growing seasons. RB036152 has consistently shown high yields, superior sucrose content, and excellent plant health (Brasileiro et al., 2024). Its performance, which surpassed that of RB867515 – the most widely planted variety in Brazil's restrictive environments – makes it an excellent option for medium to low fertility soils. RB036152 also exhibited the main target traits required for genotypes adapted to restrictive environments and approached the ideotype for low to medium fertility conditions (Brasileiro et al., 2024).

RB036152 demonstrated excellent plant health throughout the trials and can be classified as resistant to smut (*Ustilago scitaminea*), sugarcane leaf scald (*Xanthomonas albilineans*), sugarcane mosaic virus (SCMV), orange rust (*Puccinia kuehnii*), and brown rust (*Puccinia melanocephala*) (Brasileiro et al., 2024).

Overall, RB036152 is characterized by excellent adaptation to restrictive environments, rapid initial growth and canopy closure, high yield potential, and suitability for mechanized planting and harvesting.

2.1.5 Propagation, planting, and development

Propagation of sugarcane is commonly carried out through the distribution of stalk cuttings, which, after being planted, develop the first fixation roots, and the plant survives for approximately 30 days only on nutritional reserves, in addition to water and mineral salts absorbed by the roots of the primary tillers (Landell et al., 2012).

There are three types of planting most used in Brazil today: manual, semi-mechanized, and mechanized. In the semi-mechanized planting system, only furrowing is carried out mechanically; the other operations are carried out manually, such as dethatching and distribution of stalk cuttings (Landell et al., 2012).

In the manual planting system, 6 to 8 t ha^{-1} of propagation material is used (Xavier, 2014). In the mechanized planting system, all manual operations are eliminated; therefore, furrowing, fertilization, distribution of cuttings, and covering are mechanized. Consequently, there is a reduction in implementation costs, and it facilitates the management of the system (Pinto and Moraes, 1997).

In the mechanized planting system, 18 to 20 t ha^{-1} of propagation material is used (Xavier, 2014). Thus, the cost of propagation material is higher in this system compared to the manual one, benefiting only from the reduction of labor, generating a field efficiency of 75% (Pinto and Moraes, 1997).

Many plants are returning to the manual system of distribution of cuttings, due to the high expenditure and costs of propagation material in the mechanized system (May and Ramos, 2019). Thus, in deep planting furrows, the stalks are divided into 3-4 buds, or entire stalks are distributed (May and Ramos, 2019). About 3 months after planting, the crop depends solely on the roots of the tillers (Tavares, 2009).

The primary stalk then develops from each bud, forming clumps through the tillering of sugarcane (Tavares, 2009). Under the conditions found in Brazil, 6 to 12 buds are normally planted per linear meter of furrow, capable of producing approximately 15 tillers (Tavares, 2009). The first regrowth of sugarcane that occurs after the first harvest is also known as ratoon, and the others that occur annually until the crop is renewed are called second ratoon (Tavares, 2009).

Because most Cerrado soils are extremely poor in nutrients, including phosphorus and nitrogen, this crop requires a high demand for these nutrients in all its development phases, which are mostly compensated by using large quantities of chemical fertilizers (Silva et al., 2021). When nitrogen is lacking in the plant, it results in deficient growth and development throughout the crop cycle, presenting symptoms such as older, narrow, yellowish leaves and reduced tillering (Martins et al., 2016). Therefore, techniques that provide and enhance the absorption of nutrients for the plant, as well as promote plant growth, can contribute to the development of the crop and a sustainable increase in yield, since this crop can reach high levels of yield in aerated, deep soils, with moisture retention and high fertility (EMBRAPA, 2022).

Therefore, due to its development, it is necessary to have planning for the management of this crop, since environmental factors can influence its regrowth potential, such as the incidence of sunlight, temperature, humidity, and nutrients in the soil (Scapari and Beauclair, 2008). Among the environmental factors capable of influencing the yield potential of sugarcane, water resources are considered an essential factor, since this crop is formed by approximately 30% dry matter and 70% water, according to its phenological stage. Therefore, large amounts of water are needed to supply its production cycle (Mozambani et al., 2006).

2.1.6 Pre-sprouted seedling system – MPB

The pre-sprouted seedling system (or "mudas pré-brotadas" - MPB) is a multiplication method developed by the Sugarcane Program of the Agronomic Institute – IAC (Campinas-SP), which consists of using a cutting block to produce sugarcane seedlings in a controlled environment, involving early sprouting and establishment (Landell et al., 2012).

This system aims to reduce the amount of propagation material used for planting by up to 80% (Jain et al., 2010), as well as renew and expand sugarcane areas, in addition to yield and uniformity in planting across the planted area and phytosanitary control of seedlings in nurseries (Landell et al., 2012).

In commercial sugarcane plantations, when older planting techniques are practiced, it is common for the volume of the propagative material to be high, since failures are frequent and, to reduce possible losses, the use of seedlings has increased (Landell et al., 2012). With the development of the MPB technique, mini-stalks of approximately 3 cm with a single bud are used. Thus, the volume of propagation material can be reduced to 2 t ha^{-1}, reducing approximately 18 t ha^{-1}, using material that can be destined for the industry (Landell et al., 2012).

In the MPB system, the risk of pathogen infestation is reduced, since the buds are selected and treated before they reach the nurseries for the subsequent production of sugarcane seedlings (Landell et al., 2012). Healthy and vigorous seedlings reduce the chances of failures in the planting stand, optimizing and increasing the yield potential. The use of pathogen-free propagation material is one of the most important points for the initial development of the crop in the field. Thus, the buds must come from disease-free nurseries, aged six to ten months, and without varietal mixing. Such nurseries must be provided with heat treatment, accompanied by roguing procedures and samples for disease diagnosis, if necessary (Landell et al., 2012).

The use of MPBs enables economic gains in the implementation of nurseries, replanting of commercial areas, and renewal and expansion of sugarcane areas. The use of MPB can reduce the volume of seedlings used in planting by up to 90% (Landell et al., 2012).

In this way, from one ton of seedlings, it is possible to produce MPB to plant an area of up to 300 hectares in 17 months, while in the conventional system, the extension would not reach 30 hectares (Coplana Produtor, 2013).

One of the major challenges in implementing the MPB technique is water availability during the transplanting period, which is the most critical period for seedlings, as the plant enters the field with active leaf area and transpiration (Martins et al., 2015). Therefore, irrigation management would

be essential at this stage, but not all properties have water resources available for this type of management.

Low water availability causes changes in plant performance, and the ability to reverse such changes will vary depending on the genotype, duration, severity of stress, and phenological phase (Inman-Bamber and Smith, 2005; Smit and Singels, 2006).

2.2 STAGES OF PRODUCTION OF PRE-SPROUTED SEEDLINGS (MPB)

2.2.1 First stage - Preparation of mini-stalks (OBP)

To produce seedlings, stalks with a physiological age of six to ten months are required, coming from basic nurseries that undergo a rigorous quality and management protocol (Landell et al., 2012).

First, the straw is removed. This should be done outside the perimeter of the nursery to avoid the transport of phytopathogens. The process must be done manually to avoid damage. To remove the stalks, a cutting instrument is used, which must be disinfected with products based on quaternary ammonia (Landell et al., 2012).

To separate the stalks into mini-cutting, a guillotine with a disinfected double blade is used (Xavier et al., 2008). It is recommended that the distance between the blades for this process be 3 cm, since this step determines the size of the mini-stalks (Landell et al., 2012), which, when single-budded, are called one-bud propagules (OBP).

2.2.2 OBP treatment

According to Landell et al. (2012), treatment consists of immersing the OBP in an Azoxystrobin or Pyraclostrobin 0.1% solution for three minutes. Another alternative is heating treatment, with a temperature of 52 ºC applied for 30 minutes, or 50 ºC for two to three hours. At this stage, a visual selection of the OBP is also performed to verify that they are visually healthy, removing those that do not present homogeneous characteristics.

2.2.3 Sprouting

After treatment, the OBP are transferred to plastic boxes containing substrates, with a capacity for 80 units (Landell et al., 2012).

The OBPs must be completely covered by the substrate and stored in chambers, such as B.O.D. or greenhouses, subject to a controlled temperature of 32°C and sufficient irrigation to complete the pre-sprouting process (Landell et al., 2012).

The duration of this stage varies from seven to ten days, depending on the variety and physiological age of the bud (Landell et al., 2012).

2.2.4 Pricking out

Immediately after pre-sprouting, the sprouted buds are individualized into seedling tubes containing substrates and fertilizers to provide better rooting conditions (Landell et al., 2012).

2.2.5 First acclimatization phase

The seedlings remain in the greenhouse or chambers for 21 days. In this location, they are protected with shade cloth, which allows 50% of the shade, which will be gradually removed after the first week (Landell et al., 2012).

The pre-sprouted seedlings are subjected to high relative humidity to reduce the harmful effects imposed by high temperatures. They require irrigation shifts defined according to the development of the plants (Landell et al., 2012).

Before moving on to the second acclimatization phase, the plants receive leaf pruning to stimulate root development and minimize water loss (Landell et al., 2012).

2.2.6 Second acclimatization phase

The seedlings are exposed to full sunlight to simulate the reality of the field. In addition, they receive four irrigation shifts totaling 4 mm day^{-1} and intense leaf pruning, totaling three by the end of this stage, which is completed after 21 days (Landell et al., 2012). The seedlings are then removed from the tube, packaged, and sent for planting.

2.3 SUGARCANE ECONOMIC AND SOCIAL ASPECTS

Alternatives are being sought for generating electricity from sugarcane to reduce production costs, operate more efficiently, and contribute to increasing the sustainability of the activity (CONAB, 2025). Thus, the sugar and ethanol sectors make Brazil the world's largest producer of sugarcane and the only country in the world to implement an alternative fuel to petroleum on a large scale.

Brazil has stood out in carbon credit projects registered with the UN, where most of the projects are related to the generation of electricity from sugarcane bagasse (Junqueira, 2006). Currently, alcohol is recognized worldwide for its environmental, social, and economic advantages, and first-world countries are already interested in our technology.

In this scenario, Brazil stands out worldwide as the largest producer of sugarcane (*Saccharum* spp.), an input of fundamental importance for the sugar and alcohol industry (Silveira et al., 2015; CONAB, 2025). In Brazil, the estimate made in March/2025 for the sugarcane harvest production per year is 699.2 million tons, with an average yield of 75.4 t ha^{-1} (IBGE, 2025).

São Paulo is responsible for producing 351.8 million tons, which represents 50.3% of national production (IBGE, 2025). However, crops were harmed by adverse weather (hot and dry) throughout 2024 and by fires in August in the Center-South region of the country, especially in São Paulo, which may impact the 2025 harvest (IBGE, 2025). The Center-South region of the country is expected to have a higher agricultural yield in the next sugarcane cycle (2025/26), but with a smaller harvest and production area, also due to unfavorable weather conditions (SCA, 2025). Crop yield could reach between 78 and 82 tons per hectare in the Center-South in 2025/26, compared to 78 tons in 2024/25 (SCA, 2025).

As a result of the drought and fires last year, the renewal of sugarcane fields was affected, and the area to be harvested is expected to decrease by around 400 thousand hectares in 2025/26, to 7.4 million hectares (SCA, 2025). Thus, sugarcane yield will be between 592 million and 607 million tons in the next cycle in the Center-South, compared to around 620 million in 2024/25 (SCA, 2025). The share of sugar in the production mix of the mills is expected to increase from 48% to 51%, which should guarantee a supply of 40.7 million tons, compared to 40 million in this 2024/25 season (SCA, 2025).

According to the IBGE (2025), the drop in national yield has occurred due to the climate problems faced throughout the cycle of this crop, highlighting the importance of new techniques and management to promote its yield, since, for good sugarcane development, it is necessary that environmental factors, such as the presence of solar radiation, water availability and nutrients, are aligned.

Currently, sugarcane crops represent a source of great financial profitability for producing ethanol and sugar in Brazil. The production of this crop stands out in Brazil, as the sugar and alcohol industry represents approximately 8% of national exports (the fourth most representative sector in the country), in addition to generating 8% of agro-industrial jobs, thus contributing effectively to the growth of the national domestic market (MDIC, 2025).

In addition, approximately 2% of Brazil's Gross Domestic Product (GDP) is generated by the sugarcane agro-industrial sector, which is responsible for the generation of more than two million jobs for the Brazilian population (CNM, 2023; MDIC, 2025).

The crop stands out worldwide for being one of the best options among renewable energy sources and for having a debatable social role, since its cultivation and processing generate several direct and indirect jobs. In 2024, the bioenergy sector generated approximately 2.2 million direct and indirect jobs in the country (Unica, 2024).

With the lower volume of sugarcane harvested in 2025, there was a 3.4% drop in sugar yield in the country, estimated at 44.1 million tons (CONAB, 2025). The country recorded a 4.4% growth in total ethanol production, reaching 37.2 billion liters, despite a 1.1% drop in fuel produced from crushed sugarcane, with a total of 29.35 billion liters (CONAB, 2025).

Brazilian sugar exports remain at high levels, consolidating Brazil as the world's leading supplier of the product, with 35.1 million tons and revenue of US$16.7 billion (CONAB, 2025). On the other hand, Brazilian ethanol exports in the 2024/25 harvest closed with a shipped volume of 1.75 billion liters of ethanol, a 31% drop compared to the volume of the 2023/24 harvest (CONAB, 2025).

Therefore, new agronomic techniques to help improve sugarcane yield are being developed and applied in commercial plantations, such as the correct choice of soil and climate, pest and weed control, search for new fertilizers, and even bioinputs to enhance plant development and harvest yield (Adorna, 2011).

2.4 BIOFERTILIZERS (BIOINPUTS)

2.4.1 Historical and current importance

The growing demand for food resulting from population growth has been generating an excessive dependence on synthetic inputs (Mahanty et al., 2017). To achieve high yields, a large amount of pesticides and an excess of inorganic fertilizers are used, which can cause a series of damages to the soil and human and animal health, resulting in a major imbalance in the ecosystem (Garcia et al., 2012). Thus, the use of bioinputs has emerged as an alternative to the application of chemical products, amid environmental and food safety concerns.

In this scenario, the adoption of alternative technologies aimed at optimizing cultivation and making the process more sustainable is increasing. The use of bioinputs in agriculture is an important strategy, not only in organic production systems where natural inputs are essential. They are relevant in all systems that aim to increase yield, with a focus on sustainability, to meet the growing demand for

food and energy sources. The literature shows how this topic has been the subject of research over the last few decades (Mógor et al., 2008; Vasconcelos and Gonçalves, 2013).

Global sales of bioinputs and biological products were estimated to be between US$5 and US$8 billion annually by 2020, with the largest producers being the United States, Brazil, China, India, Malaysia, and South Africa (Araujo-Abad and Collahuazo-Reinoso, 2019). The bioinputs market in Brazil registered growth of nearly 15% in the 2023/24 harvest, generating sales of around R$5 billion, with emphasis on plant growth promoters (Santos et al., 2024).

2.4.2 Legislation and classes

Bioinputs can be classified into three main groups based on their functionality: (I) those that promote plant growth and development, (II) those that serve as biological pest control agents, and (III) those that contribute to soil conditioning or restoration. Each of these categories is further subdivided into specific subgroups according to their mode of action and biological mechanisms. Notably, one bioinput can often perform multiple functions simultaneously, for example, promoting plant growth while also protecting against pests and diseases (Barbosa et al., 2025).

Furthermore, substances and/or microorganisms whose primary function, when applied to the plant or its rhizosphere, is to trigger or enhance endogenous physiological processes are called biostimulants (Barbosa et al., 2025). These physiological changes include improved nutrient use efficiency, increased tolerance to abiotic stresses, and enhanced crop quality (Santos et al., 2024). Biostimulant formulations may be composed of microbial inoculants, algal extracts, humic and fulvic acids, protein hydrolysates, or chitosan, but their mode of action involves the stimulation of plant metabolic pathways rather than supplying nutrients themselves (Barbosa et al., 2025). By modulating plant metabolism, biostimulants can enhance nutrient use efficiency, optimizing root uptake and assimilation of available elements (Barbosa et al., 2025).

Some effects of bioinputs on plants relate to the improvement in biometric and biochemical parameters. Studies report that bioinputs are capable of increasing crop yield, increasing the content of proteins, essential amino acids, and vitamins. In addition to demonstrating positive effects on the bioremediation of metals and pesticides, reducing the population of plant-parasitic nematodes and boosting the photosynthetic activity of the plant, increasing the expression of antioxidant enzymes and chlorophyll content in the leaves (Mahanty et al., 2017).

They can be applied directly to the seeds, to the surfaces of the plants, or to the soil, and can colonize the rhizosphere or the interior of the plants (Beltrán-Pineda and Bernal-Figueroa, 2022). The composition of bioinputs is vast and can include algae biomass, humic substances, protein hydrolysates, and beneficial microorganisms, which, when applied to plants, can produce metabolic changes (Du Jardin, 2015), also improving the chemical and biological quality of the soil or stimulating plant growth (Abdel-Raouf et al., 2012).

Normative Instruction No. 61 (IN61), of July 8, 2020, establishes the rules on definitions, requirements, specifications, guarantees, tolerances, registration, packaging, and labeling of organic fertilizers and biofertilizers, intended for agriculture. Subdividing fertilizers into those of amino acids, humic substances, algae extracts or processed algae, plant extracts, compost, and others (BRAZIL, 2020). Recently, the Brazilian legislation classified these substances as bioinputs (BRAZIL, 2024).

Yakhin et al. (2017) add that even in small concentrations, they can provide great results, since they would improve the physiological and biochemical processes, seeking the maximum genetic and yield potential of plants. In this context, their form of action is related to the stimulation of physiological responses when applied to plants through signaling action (Mógor et al., 2017; Stadnik et al., 2017).

Research shows that its application can biometrically increase roots and leaves, help plant physiology, or even stimulate responses to biotic and abiotic stresses (Nardi et al., 2016; Stadnik et al., 2017). Other studies show that, due to the diversity of compositions, the use of these substances and organisms as bioinputs can bring several advantages to crops (Alvarez et al., 2021). Such as L-amino acids, protein hydrolysates, and microalgae, in promoting plant growth (Mógor et al., 2008); polysaccharides, stimulating growth, greater yield, nutrient absorption, and stress resistance (Tarraf et al., 2015; El Arroussi et al., 2016). Furthermore, the supply of amino acids in foliar solutions provides plants with the elements necessary for the development of structures, saving metabolic energy (Garcia et al., 2013; Plaza et al., 2018), and is related to a series of metabolic processes that will be addressed in a later topic.

Among these sources, emphasis is placed on the biochemical composition of microalgae as a raw material to develop new products to improve plant growth (Garcia-Gonzalez and Sommerfeld, 2016; El Arroussi et al., 2018; Mógor et al., 2018), offering a viable alternative to reduce dependence on chemical fertilizers. The potential of these substances has been explored and is considered a renewable, ecological, and economically feasible source, capable of increasing plant yield in a sustainable way (Mahajan et al., 2003).

In this scenario, it is observed that some microalgae biomass rich in amino acids are being studied for its ability to serve as a source of these biomolecules for bioinputs. Studies on the application of these substances in plants indicate results of agronomic importance, such as improvement of the total content of soluble proteins in plants, promotion of better nitrogen assimilation, and stimulation of amino acid metabolism (Nardi et al., 2016).

Other effects include: their performance as stress-reducing agents, source of amino acids, and hormone precursors (Zhao, 2010). Corroborating Nardi et al. (2016) and Zhao (2010), other authors report that the use of microalgae benefits plant development by producing growth-promoting molecules, amino acids, polypeptides, and polyamines that improve plant growth and yield (Safi et al., 2014). Thus, studying this Chlorophyta microalgae reveals great potential for scientific research into new sources of bioinputs with the potential to promote plant growth.

2.4.3 Potential of algae in agriculture

Algae constitute a large group of photosynthetic organisms, including eukaryotic microalgae, macroalgae, and prokaryotic cyanobacteria (Andersen et al., 2013), with increasing environmental and economic importance (Renuka et al., 2018). Under natural conditions, microalgae are preferably autotrophic and capable of using carbon dioxide and nutrients such as nitrogen, phosphorus, and potassium from aquatic environments for their metabolism (Brennan and Owende, 2010).

Algae (micro and macroalgae) are examples of raw materials for bioinputs, contributing to the synthesis of hormones, the provision of nutrients, and activation of enzymatic activities, promoting healthy plant growth (Trivedi, 2023). With the development of these products, specific regulations were implemented, encouraging their use in agriculture and increasing the demand for their production. Among the most researched, seaweed-based bioinputs stand out for improving plant growth, strengthening their defense, and optimizing nutrient use efficiency (Trivedi, 2023).

Most algae play an important role in carbon sequestration and are responsible for 50% of total photosynthesis on Earth (Moroney et al., 2009). The nature of these organisms reveals their extreme adaptability, with high growth rates and resistance to environmental stresses; their composition contains molecules that give them and their products these characteristics (Azaman et al., 2017).

In this context, historically, seaweed extracts are one of the oldest sources used in agriculture, although the effects of their application on plants have only recently been identified (Du Jardin, 2015). It is estimated that there are around 800 thousand species of microalgae in the world, of which approximately 6% are described (Suganya et al., 2016).

Regarding green microalgae and cyanobacteria, studies show their action in mineralization, incorporation, and mobilization of organic and inorganic nutrients, and in the production of many bioactive compounds (Prasanna et al., 2014).

Therefore, each microalgae's potential depends on the composition of its biomass. These diverse metabolisms produce a range of compounds of great interest, including nutraceuticals and bioactive compounds such as carotenoids (Borowitzka, 2013), polyunsaturated fatty acids (Ratledge, 2004), polysaccharides (Ishaq et al., 2016), polyamines (Mógor et al., 2022), carbohydrates (Khan et al., 2005), high-value proteins (Ishaq et al., 2016), and free amino acids (Renuka et al., 2018), such as L-amino acids (Mógor et al., 2018). Their rich compositions can induce metabolic changes when applied to plants. As examples, researchers cite the microalgae *Chlorella vulgaris* and *Spirulina platensis* as promoters of these changes, increasing the levels of total sugars, amino acids, and phenolic compounds in onion plants (Dineshkumar et al., 2020). In addition, other research cites applications with the microalgae *Dunaliella salina* as capable of attenuating the effects of oxidative stress in tomatoes under the effect of salinity (El Arroussi et al., 2018).

The performance in primary metabolism was studied through increased cell division and expansion in plants, as well as in secondary metabolism (El Naggar et al., 2020). These molecules can cause changes in the levels of reducing sugars, such as glucose and fructose, which are not very mobile in the phloem, in addition to non-reducing sugars, such as sucrose, which are more mobile (Taiz and Zeiger, 2017). Other authors also cite its effectiveness in the germination of sugarcane buds (Mógor et al., 2022).

Another form of interaction between the biomass of microalgae and plants is through a phytohormonal-like effect, since algae present these molecules in their composition (Amatussi et al., 2020). In this same study with a biofertilizer based on calcareous algae, rich in humic substances, an auxin-like effect was observed when observing the promotion of plant growth, improving the quality of crops (Amatussi et al., 2020).

Research also shows promising results in the metabolic modifications of plants, promoting yield gains concomitantly with the increase in the concentration of biomolecules, such as carbohydrates and proteins (Dineshkumar et al., 2018). Regarding changes in the root part, Barone et al. (2018) observed biometric increases in the roots and increased expression of genes related to the acquisition of nutrients by the roots of beet plants treated with microalgae of the genus *Scenedesmus* and *Chlorella*.

2.4.4 *Asterarcys quadricellularis*

The microalgae *A. quadricellularis* (AQ) belongs to the phylum Chlorophyta, which is composed of unicellular green microalgae found in fresh and marine water, ranging from 2 to 10 μm in diameter and dependent on light for their autotrophic growth (Hong et al., 2012).

To better understand this microorganism, it is worth observing other organisms of the Chlorophyta, since in the genus *Asterarcys,* there is only one species (Hegewald et al., 2010). Some of the Chlorophyta genera (e.g., *Scenedesmus* and *Chlorella*) have potential related to their bioactive molecules that can include amino acids and proteins, which are studied for their ability to promote plant growth. Some species of microalgae have 50-56% of their dry weight in proteins (Ishaq et al., 2016).

Under controlled cultivation conditions, this microalga can have a high content of proteins, amino acids, lipids, polysaccharides, and pigments (Varshney et al., 2018; Singh et al., 2019). Its protein content can vary between 39-45% of its biomass, which is rich in different amino acids (Ghosh et al., 2017; Xupeng et al., 2017), like aspartate and glutamate, which constitute a large proportion of its content (Tab. 1) (Cordeiro et al., 2022).

Table 1: Free L-amino acids composition of *A. quadricellularis* (CCAP 294/1) biomass

Amino acids	Percentage (%)	Amino acids	Percentage (%)
Glutamic acid	4.27	Glycine	1.54
Aspartic acid	3.32	Threonine	1.45
Alanine	2.41	Isoleucine	1.41
Leucine	2.36	Phenylalanine	1.37
Arginine	2.17	Tyrosine	0.95
Lysine	2.11	Histidine	0.71
Valine	1.81	Methionine	0.51
Serine	1.68	Tryptophan	0.37
Proline	1.60	Cysteine	0.29

Adapted from Cordeiro et al. (2022)

Both these amino acids play crucial roles in plant growth, development, and stress responses, as they serve as building blocks for proteins, precursors for other amino acids, and participate in nitrogen metabolism and signaling pathways (Kumar et al., 2017).

In addition, these molecules in the levogyre form would provide bioactivity, causing an effect on improving plant growth (Mógor et al., 2018). This potential has been recorded in a few recently published studies (Mógor et al., 2022; Lara et al., 2025).

As can be seen in this section, the agricultural potential of microalgae is related to their ability to provide a wide range of bioactive and effective molecules for biofertilization. Furthermore, it is concluded that the mechanisms induced by microalgae in plants are complex and have not yet been fully understood. Most likely, their action involves the interaction of several molecules (Barone et al., 2018), which are not necessarily linked to the supply of nutrients, but rather to an intricate network of signaling (Mógor et al., 2018), which activate genes linked to the biosynthesis of amino acids and polyamines, which will trigger metabolic responses.

In this sense, their ability to act on plant metabolism, causing greater yield through a sustainable way, is remarkable and requires further research to understand their full potential.

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3. ARTICLE 1 - MAXIMUM EFFICIENCY CONCENTRATION OF THE MICROALGA *Asterarcys quadricellularis* BIOMASS IN THE GROWTH OF SUGARCANE OBP

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ABSTRACT

The production of pre-sprouted seedlings is a technique that brings advantages to implementing the sugarcane crop in the field. Their initial growth can be enhanced with the application of sustainable input. The biotechnological potential of microalgae has grown in interest due to their bioactivity. In sugarcane, biological fertilizers have been reported to increase nutrient uptake efficiency, improve growth and yield. Thus, this study aimed to determine the maximum efficiency concentration of the microalga *Asterarcys quadricellularis* biomass in the sprouting and development of sugarcane one-bud propagules (OBP) (cultivar RB036152). Regression analysis of the biometric results of sugarcane OBP was used to determine the maximum efficiency concentration (MEC) of this microalga biomass used as an immersion solution. The biometric results showed a similar MEC pattern to the concentration increase of *A. quadricellularis* biomass. The MEC represented an increase of 23.1 in % sprouting rate of the propagules over the control. Plant development was stimulated in the initial growth (10 days) of sugarcane OBP in the B.O.D. chamber. Plant growth is also promoted after transplanting into pots, boosting the development of shoots and roots. The average MEC of *A. quadricellularis* biomass from all the biometric results was 2.38 g L^{-1}, promoting plant growth and a higher sugarcane sprouting rate.

Keywords: Bioinputs, MEC, Microalgae, Roots, *Saccharum* spp., Sprouting

INTRODUCTION

Brazil is the world's largest producer of sugarcane (*Saccharum* spp.) (CONAB, 2025). It leads in sugar production and ranks second in ethanol production, playing a significant role in the Brazilian economy by creating thousands of direct and indirect jobs (Silva et al., 2021). In this context, increased growth and development of sugarcane can lead to higher yields, benefiting various sectors of the economy and the environment when sustainable practices are adopted.

Sugarcane is a plant with a high capacity for photosynthesis, characteristic of plants with a C4 metabolism, that is, with a high ability to concentrate CO_2 due to reduced photorespiration, reaching high photosynthesis rates under intense solar radiation (Marafon, 2012). One crucial factor for successful sugarcane cultivation involves the health and production traits of the propagules and, consequently, the seedlings. The sugarcane sprout is a miniature culm that emerges above the soil surface. From this point, the vegetative apical bud takes over growth in height, resulting in a series of nodes and internodes that form the sugarcane stalk.

The conventional method of planting sugarcane involves using stalk cuttings with more than one bud; however, reserves differ based on physiological age, resulting in uneven or defective sprouting (Baracat et al., 2017). To reduce these losses, producing pre-sprouted sugarcane seedlings allows for the selection of individual propagules with buds of similar physiological ages (Singh et al., 2023), making it a practical and accessible approach (Mohanaselvan et al., 2024). Therefore, using propagules from a single sugarcane bud offers economic benefits, such as lowering costs related to harvesting and transporting stalks, as well as logistical benefits by decreasing machinery traffic in the sugarcane fields (Fluminhan and Fluminhan, 2020).

Sugarcane yield depends on vegetative growth and the sugar reserve in the stalk (Nalawade et al., 2018; Singh et al., 2018). Additionally, root growth enhances the ability to exploit the soil, enabling greater water and nutrient absorption, and leading to better crop establishment (Azevedo et al., 2011). Propagules from the apical sections sprout earlier and exhibit higher sprouting percentages than those from the median and basal positions, due to their age and, consequently, greater metabolic activity (Ferreira et al., 2018; Figueiredo et al., 2020; Mógor et al., 2022). Therefore, developing an effective method to produce mini-stalks (i.e., one-bud propagules, OBP) helps achieve higher sprouting rates and fewer failures, resulting in improved crop establishment and potentially higher yields (Singh et al., 2023).

According to Brazilian Normative Instruction No. 61, dated July 8th, 2020, art. 2, XXIII, a biofertilizer is defined as a product containing an active principle or organic agent, free of pesticide substances, capable of acting either directly or indirectly on all or part of cultivated plants, enhancing their yield without considering their hormonal or stimulating value (BRAZIL, 2020). Additionally, Brazilian legislation classifies them as bioinputs (BRAZIL, 2024).

The effects of bioinputs derived from microalgae biomass are linked to the presence of bioactive compounds (Gitau et al., 2022; González-Pérez et al., 2022), such as polysaccharides, polyamines (PAs) (Braun and Colla, 2023; Mógor et al., 2017), and free L-amino acids (L-AAs)

(Renuka et al., 2018; Cordeiro et al., 2022a). Research on microalgae in sugarcane is limited, but it has been found that immersion in microalgae biomass promotes bud sprouting and initial growth (Mógor et al., 2022). Additionally, sugarcane is known to show biometric improvements in response to other bioinput applications in areas such as leaves (Liao et al., 2019), culm (Prihandarini et al., 2018), and roots, particularly in thinner roots (dos Santos et al., 2019).

The microalga *Asterarcys quadricellularis* (*AQ*) biomass has a high content of free L-amino acids (L-AA) (Cordeiro et al., 2022a), proteins, and polysaccharides (Ghosh et al., 2017). This green microalga is a Chlorophyta from the Scenedesmaceae family with notable plant growth-promoting activity (Cordeiro et al., 2022b; Lara et al., 2022; Marques et al., 2025) and remarkable results in sugarcane (Mógor et al., 2022).

We examined how different levels of *AQ* biomass concentrations and their MEC as a bioinput, applied through pre-planting immersion, affect the biometric changes of sugarcane OBP (RB036152), from sprouting to early development (30 days after transplanting).

MATERIAL AND METHODS

Plant material

Sugarcane (*Saccharum* spp.) OBP supplied by RIDESA Brazil of the cultivar RB036152 (Brasileiro et al., 2024) was produced from propagules from the apical third of stalks grown in Paranavaí at the Federal University of Paraná's Sugarcane Research Station.

OBP with 50 mm long sections, with an average diameter of 22 mm, containing only one bud and homogeneous characteristics, were selected, as the size of the bud will influence the reserve for sprouting (Mógor et al., 2022). Those with flawed, irregular buds or those that did not reach the appropriate BRIX value for evaluation were discarded.

The percentage of Brix (total soluble solids) was determined using a field refractometer to determine the stalk ripeness index. The index was determined to be 0.60, indicating that the OBP was in good condition to be planted (Silveira et al., 2015).

Microalga biomass

The biomass of the microalga *A. quadricellularis* (K. Behre) (*AQ*) was supplied by Alltech® Crop Sciences Brazil. This biomass was produced in mixotrophic cultivation and spray-dried to produce a fine, greenish powder. Its AAs concentration was 90.9 mg g^{-1}, corresponding to 9% free L-amino acids by weight, and was determined using 0.2 mg of dry biomass diluted in 1.7 mL of 80%

ethanol to prepare an extract from which 1.0 mL was diluted in distilled and deionized water to carry out the colorimetric reaction (Winters et al., 2002).

Immersion treatments

For the immersion solutions, the biomass was diluted in concentrations of 1.25, 2.50, 3.75, and 5.0 g L^{-1}, as well as a control (water only). The OBP was immersed for 30 minutes in the respective treatments. The OBP were then placed in a plastic tray, covered with vermiculite, and taken to a B.O.D. (biological oxygen demand) chamber at 32 °C for 10 days in the dark to stimulate sprouting. After this stage, the biometric data was collected, and the sprouted OBP were transplanted into 5 L pots with commercial substrate and placed in an agricultural greenhouse for 30 days with daily irrigation control.

Experimental area

The research was performed in the laboratory and agricultural greenhouse at the Organic Horticulture Research Area of the Federal University of Paraná, located in the municipality of Pinhais-PR, Brazil at 25° 23' 30'' S and 49° 07' 30'' W, at an average altitude of 920 m, with a Cfb type temperate climate according to the Köppen classification.

Biometric analyses in the B.O.D. chamber

After 10 days in the B.O.D. chamber, non-destructive biometric analyses were carried out on all the OBP in the vermiculite trays. The following were evaluated at this stage: sprouting (sprouts above 0.5 mm) (%), culm height (cm), and culm diameter (mm). The sprouting rate was calculated according to the equation below.

$$\text{Sprouting (\%)} = \frac{\text{Number of sprouted buds}}{\text{Total sugarcane mini setts}} * 100$$

Culm height was defined as the distance from the soil surface to the sprout top, using a measuring tape, while shoot diameter was defined as the width of the base of the sprout base, using a digital caliper.

Biometric analysis in greenhouse

The sprouted OBP were then transplanted into 5-liter pots with commercial substrate and placed in an agricultural greenhouse with irrigation control. After 30 days, non-destructive biometric evaluations were made of the number of leaves and tillers, plant height (culm height plus longest leaf blade) with a measuring tape (cm), average culm diameter with a digital caliper (mm); and destructive analyses of the fresh and dry mass of the roots and aerial part using a precision scale (g). For the dry masses, the plant materials were placed in an oven at 65 ºC for 3 days, until the mass became constant.

In addition, root biometric data were obtained through readings with an Epson Expression 836XL 3D scanner at a resolution of 150 dpi and data processing with the WinRhizo computer program, involving: length (cm), diameter (mm), volume (cm^3), area (cm^2), and stratification by diameter (mm).

Statistical analysis

The experimental design adopted was completely randomized. The treatments used involved the control (water only) and 1.25, 2.5, 3.75, and 5.0 g L^{-1} *AQ* concentrations, each with 4 replicates. The regression test was performed based on the biomass concentrations of *AQ* for each biometric variable analyzed. When a quadratic response was identified, the maximum efficiency concentration (MEC) of *AQ* was estimated by calculating the second-order derivative of the equation.

The statistical program Assistat 7.7 Beta (Silva and Azevedo, 2016) was used for data analysis. The data was tested for homogeneity of variances using Bartlett's test and then analyzed based on concentrations using the Regression analysis.

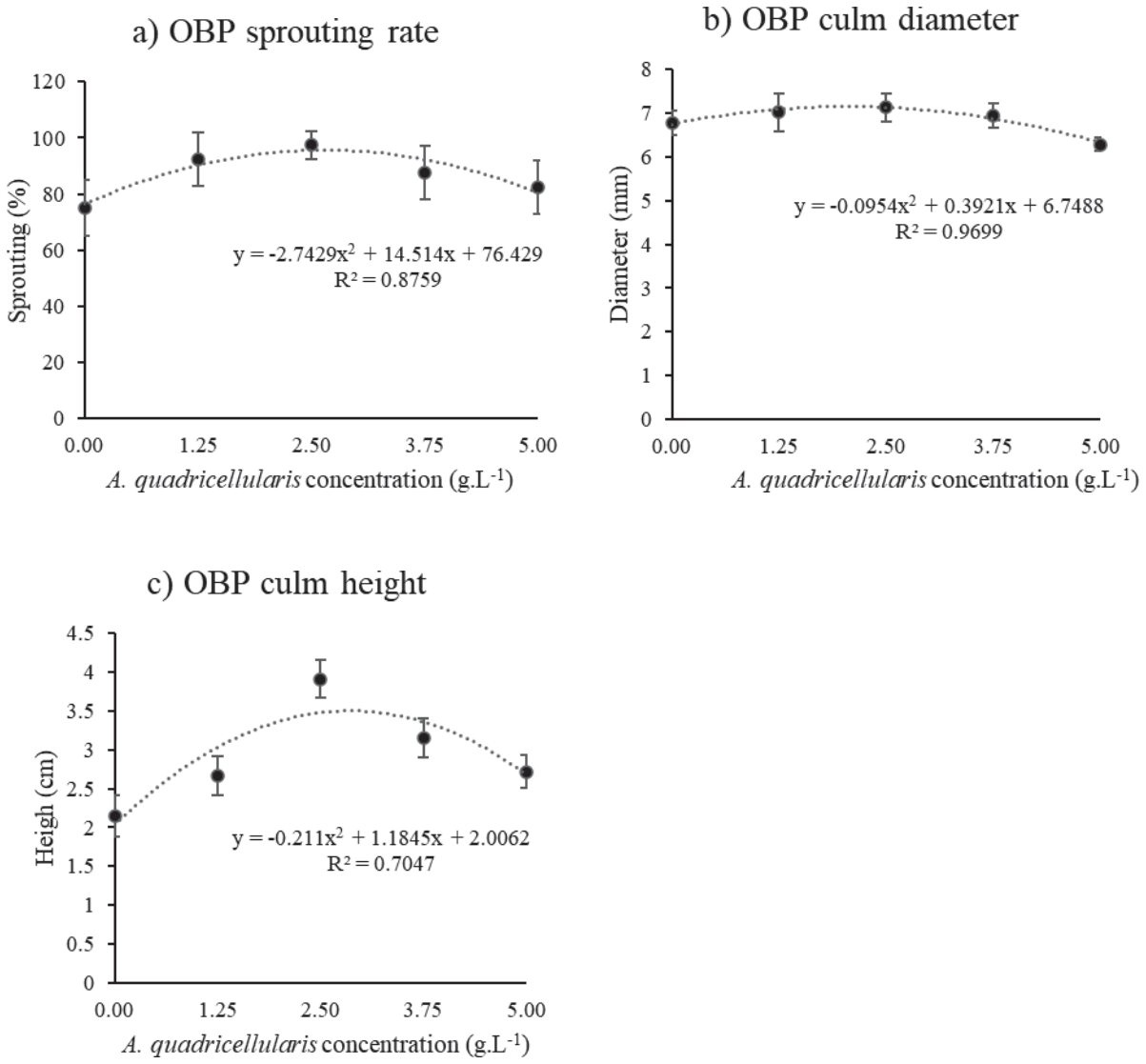
RESULTS

B.O.D. chamber tests

Increasing concentrations of *A. quadricellularis* (*AQ*) biomass did not influence the variables of the number of leaves and tillering of sugarcane (RB036152). The other variables showed progressive increases up to the MEC, with diminishing returns after that point.

The OBP sprouting rate (Fig. 1a), the sprout diameter (Fig. 1b), and the sprout height (Fig. 1c) were influenced by immersion in *AQ*, showing increases with a quadratic pattern. The MEC of these variables and the increase compared to the control were: 2.65 g L^{-1} (23.1%); 2.06 g L^{-1} (5.97%); and 2.86 g L^{-1} (71.99%), respectively.

Figure 1: Regression analysis of *A. quadricellularis* (*AQ*) concentrations applied via immersion in sugarcane (RB036152) in the B.O.D. chamber, of a) the sprouting rate, b) culm diameter, and c) culm height.



Biometrics in Greenhouse

The height of the aerial part in OBP (Fig. 2a), culm diameter (Fig. 2b), leaf fresh mass (Fig. 2c), leaf dry mass (Fig. 2d), root fresh mass (Fig. 3a), root dry mass (Fig. 3b), as well as the length (Fig. 3c), volume (Fig. 3d), and root area (Fig. 3e) were influenced by immersion in *AQ*, showing increases in a quadratic pattern.

The MEC of the shoots of the OBP grown in the greenhouse had a range of greater *AQ* efficiency, similar to the variables analyzed in the B.O.D. chamber experiment.

The MEC and the increase compared to the control were for aerial part height 2.36 g L^{-1} (27.6%), culm diameter 2.04 g L^{-1} (7.95%), leaf fresh mass 2.27 g L^{-1} (40.24%), and leaf dry mass 2.20 g L^{-1} (41.49%). The root variables also followed the same quadratic pattern. The MEC and the increase compared to the control were for root fresh mass 2.05 g L^{-1} (27.3%), root dry mass 2.46 g L^{-1} (84.5%), as well as length 2.57 g L^{-1} (28.9%), volume 2.53 g L^{-1} (104.3%), and root area 2.54 g L^{-1} (61.2%).

Figure 2: Regression analysis of *A. quadricellularis* (*AQ*) concentrations applied via immersion in sugarcane OBP (RB036152) in the pot experiment, of a) the aerial part height, b) culm diameter, c) leaf fresh mass, and d) leaf dry mass.

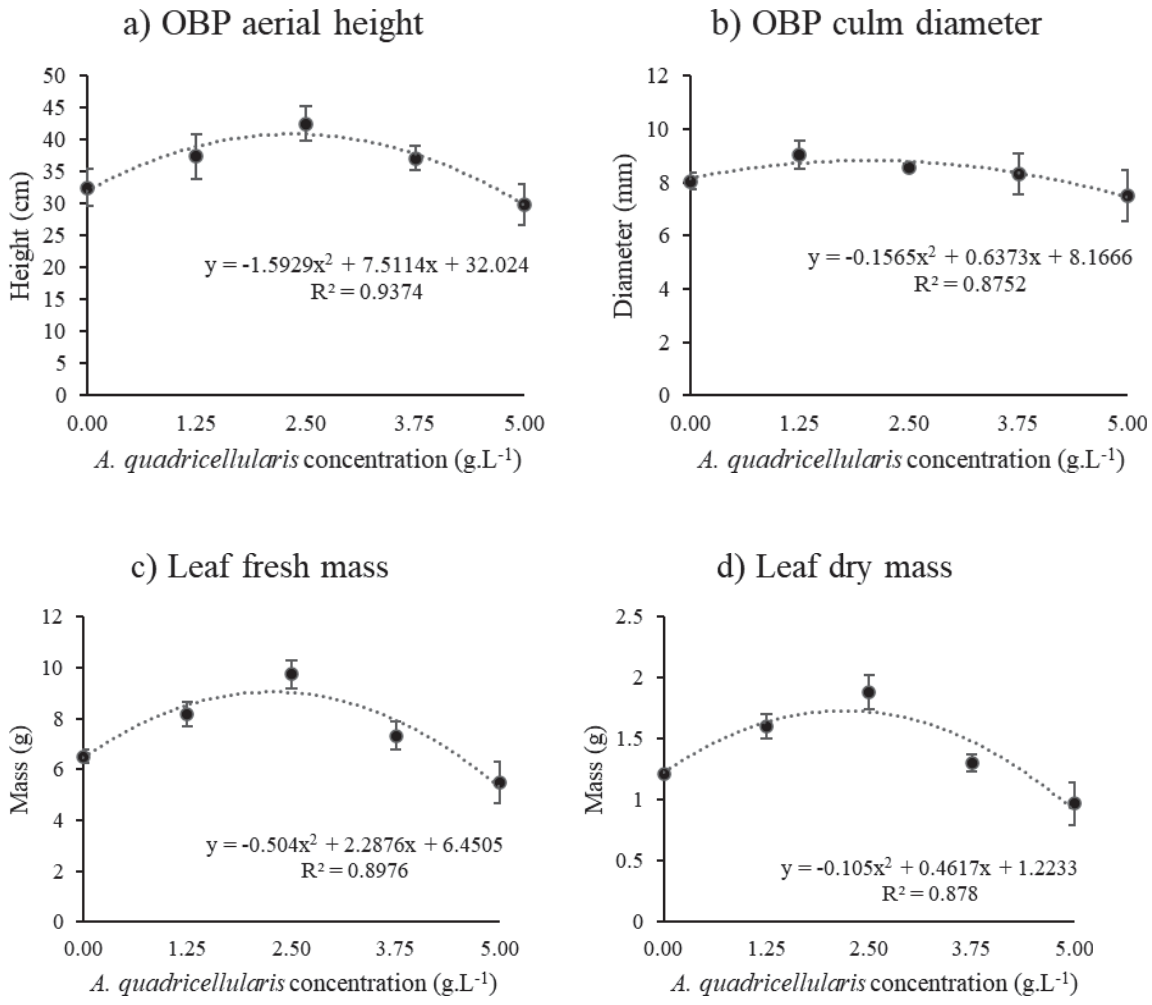
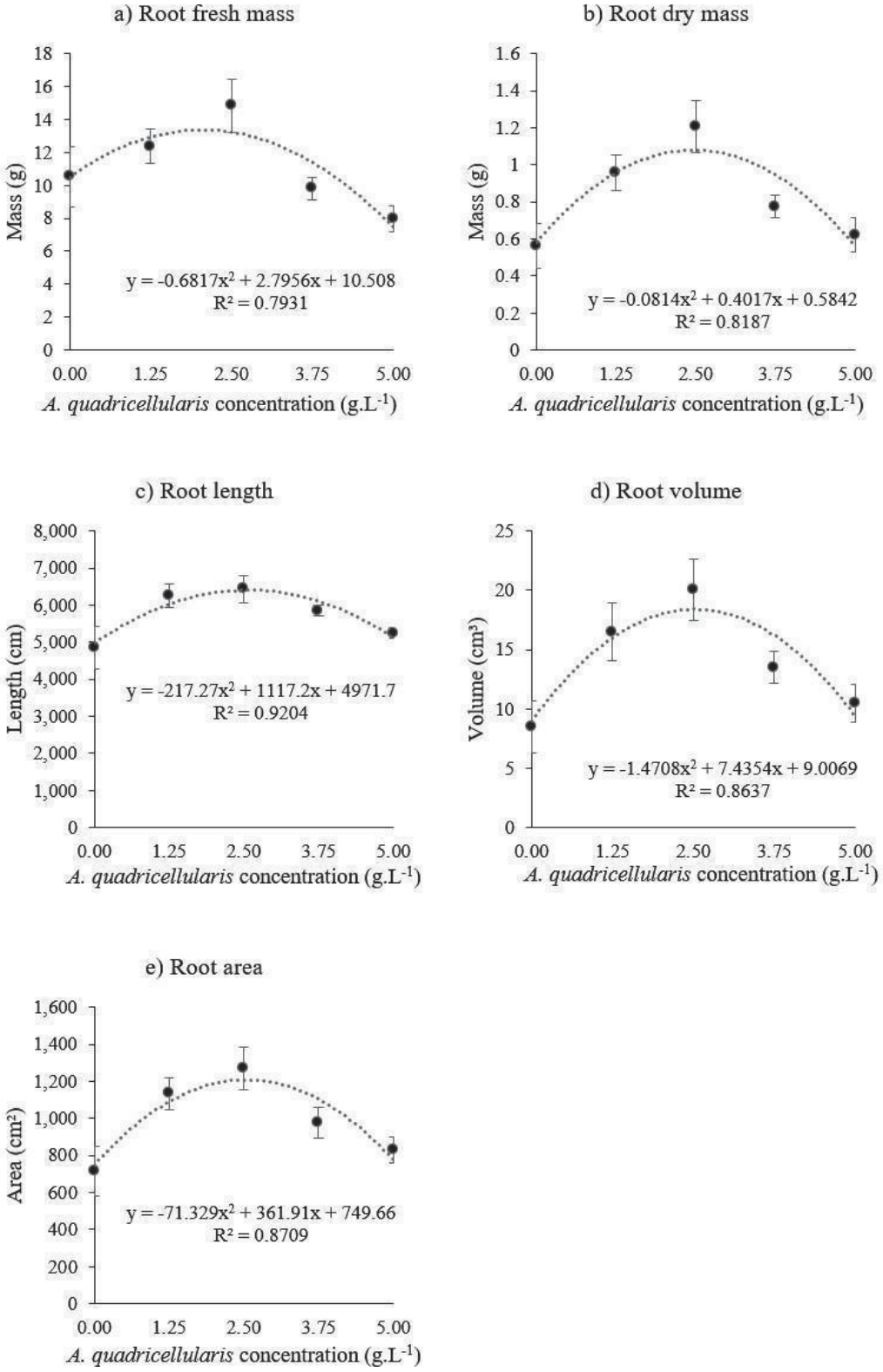


Figure 3: Regression analysis of *A. quadricellularis* (*AQ*) concentrations applied via immersion in sugarcane OBP (RB036152) in the pot experiment, of root a) fresh mass, b) dry mass, c) length, d) volume, and e) area.



The factor analysis investigating the effect of different concentrations of *AQ* on promoting root growth in different stratifications (Tab. 1) showed that for thinner roots (0 - 0.5 mm), the treatment with the best results was 2.5 g L^{-1}. Among the concentrations (g L^{-1}) used, the sequence of greatest influence in this stratification was 2.50>1.25=3.75>5.00>Control, while for the second stratification (0.5 - 1.0 mm) the effect was in the pattern 1.25=2.50=3.75>2.50=Control, and for the other stratifications no statistical difference was observed.

Table 1: Factor analysis of length by stratification of root diameter in sugarcane OBP (RB036152) after 40 days of immersion in different concentrations of *A. quadricellularis* biomass (*AQ*).

Sugarcane root length (cm) by stratification and treatment					
Stratification (mm)	*A. quadricellularis* concentrations (g L^{-1})				
Diameters	0	1.25	2.5	3.75	5.0
0.0-0.5	3,408 ± 325.4 aD	4,149 ± 234.1 aB	4,281 ± 253.2 aA	4,032 ± 162.2 aB	3,672 ± 121.2 aC
0.5-1.0	943.2 ± 112.8 bB	1,170 ± 78.2 bA	1,314 ± 62.9 bA	1,165 ± 51.7 bA	1,043 ± 43.2 bB
1.0-1.5	268.4 ± 69.2 cA	404.1 ± 26.1 cA	468.1 ± 39.8 cA	360.8 ± 52.6 cA	307.1 ± 57.2 cA
1.5-2.0	87.40 ± 14.6 dA	173.8 ± 8.6 dA	230.9 ± 14.8 dA	164.1 ± 11.0 dA	119.6 ± 11.0 dA

All data expresses the average of four replicates ± standard deviation. Lowercase letters = stratifications (columns) and uppercase letters = treatments (rows). Averages followed by the same letter do not differ statistically by Scott-Knott's test at a 5% probability level.

DISCUSSION

The production of sugarcane OBP can also benefit from the application of biostimulants, increasing the rate of sprouting and plant growth (Mógor et al., 2018), corroborating the data obtained in this study. These changes are induced by alterations in plant metabolism triggered by signaling processes based on the composition of the biomass applied (Yakhin et al., 2017). The biomass of microalgae, such as *AQ*, has many bioactive compounds, with L-AA standing out due to its high concentration and bioactivity (Mógor et al., 2018). L-AAs can participate in many metabolic pathways as a precursor to other AAs that are important for plant growth and development. Thus, relating, at least in part, to the promotional effects of *AQ* on sugarcane growth.

AQ has already demonstrated its efficiency as a biofertilizer for various crops (Cordeiro et al., 2022a; Cordeiro et al., 2022b; Lara et al., 2022; Palma et al., 2022; Marques et al., 2025), as well as sugarcane (Mógor et al., 2022). Microalgae offer advantages for the economy and environmental sustainability of agricultural production, providing various bioinputs (Gemin et al., 2019; Kapoore et al., 2021; Parmar and Srivatsan, 2023).

For sustainable agriculture, the biotechnological potential of microalgae has grown in interest with the identification of various substances in their biomass (Singh et al., 2016), including PAs (Incharoensakdi et al., 2010; Mógor et al., 2017) and AAs (Mógor et al., 2018).

Homogeneous and vigorous initial growth is desirable for sugarcane since biometric gains at this point can have repercussions on better crop development and yield per hectare (Otto et al., 2022). It should be noted that initial sprouting is one of the most important characteristics for the proper cultivation of sugarcane (Nalawade et al., 2018).

In this scenario, the use of bioinputs is remarkable as they have been described as capable of improving the initial performance of sugarcane (Gazola et al., 2017; Oliveira et al., 2018; Mógor et al., 2022; Almeida et al., 2024).

The sprouting rate result (Fig. 1a) indicated the MEC of 2.65 g L^{-1} in the B.O.D. chamber test, 23.1% higher than the control. This is similar to the result obtained with the same alga in OBP of different physiological ages (Mógor et al., 2022). This highlights the product influence on the sprouting rate, which is of fundamental importance for the establishment and development of sugarcane (Nalawade et al., 2018; Oñal Jr. et al., 2024). A better sprouting rate reduces losses due to failed buds, resulting in a greater number of plants in the stand, as well as a higher yield (Singh et al., 2018).

The variables that were not influenced by *AQ* are related to the characteristics of this new cultivar (Brasileiro et al., 2024) in the initial growth phase, since the number of leaves and tillering was expected not to increase significantly.

Other biometric gains were also recorded at an early stage in the B.O.D. chamber, such as in culm diameter (Fig. 1b), which indicated the MEC of 2.06 g L^{-1}, 5.97% higher than the control; and in the result for culm height (Fig. 1c), indicating the MEC of 2.86 g L^{-1}, 71.99% higher than the control. Gains were also found with other bioinputs for the initial growth of this crop (Mógor et al., 2022) and in the field (Prihandarini et al., 2018).

Sugarcane yield depends on vegetative growth from the moment it is established (Nalawade et al., 2018). Gains at this stage can lead to better performance from pre-sprouted seedlings when they are transplanted.

The results obtained in pots, on the biometric development of the culm, leaves, and roots, followed the behavior of gains in a quadratic pattern observed in the initial growth in the B.O.D. chamber. The height of the aerial part (Fig. 2a) indicated the MEC of 2.36 g L^{-1}, 27.6% higher than the control, while the culm diameter (Fig. 2b) indicated the MEC of 2.04 g L^{-1} in the pot experiment, 7.95% higher than the control. These results, together with the mass values (Figs. 2c-2d; 3a-3b), which are analogous to research with the same algae (Mógor et al., 2022), corroborate the hypothesis that the product's effectiveness has an ideal range, ascending to close to the MEC of 2.38 g L^{-1} and that after this, diminishing returns are observed. Bioinputs applied to sugarcane are described as effective products in promoting gains in leaf variables (Liao et al., 2019).

For roots in the tinner stratifications (Tab. 1), the results were in line with those for total root growth (Fig. 3c) (29% higher in the MEC compared to the control), indicating the influence of the product on this variable, which is of remarkable importance for sugarcane performance. This was also seen in other research with bioinputs (dos Santos et al., 2019), emphasizing how bioinputs can improve their root profile, especially the final ones, which are related to the potential for absorbing water and nutrients. The results were similar for root volume (Fig. 3d) and root area (Fig. 3e), with remarkable increases compared to the control at around 2.5 g L^{-1} (104.3% and 61.2%, respectively). This result, also recorded for other bioinputs in the root variables (dos Santos et al., 2019), corroborates the hypothesis that *AQ* applications can lead to the crop's greater capacity to exploit the soil.

The yield of sugarcane is intricately linked to its root development and biomass accumulation, making these two aspects crucial factors for achieving high yields (Azevedo et al., 2011; Oñal Jr. et al., 2024). The reserve in the propagules is also fundamental for the survival of the new plant in the first 60 days, progressively decreasing as the aerial part and roots develop (Landell et al., 2012; Baracat et al., 2017). Thus, products that promote an increase in these variables can help in the OBP development (Mógor et al., 2022).

Plant growth promotion with a quadratic pattern was frequent for the variables analyzed, in the culm, leaf, and root tissues. This suggests that sugarcane cell multiplication may be enhanced in the tissues of propagules immersed in *AQ*, due to its high L-AAs content (Cordeiro et al., 2022a). In addition, *AQ* biomass promotes the regulation of carbon and nitrogen metabolism, resulting in biometric changes in sugarcane (Mógor et al., 2022). Examples of this behavior can be seen in the biometric results, which followed the same quadratic pattern, indicating an optimum range around the MEC of 2.38 g L^{-1} for promoting plant growth while using biomass efficiently.

In this sense, future work should assess whether the effect of the stimuli on the buds immersed in *AQ* will continue after the initial growth, bringing long-term benefits that will reflect higher yields. It is therefore suggested that this microalga be used, based on its MEC, as a biostimulant for sugarcane OBP.

CONCLUSIONS

Immersing the OBP in increasing concentrations of *AQ* biomass before planting regulated the sprouting rate and promoted plant growth in culms, leaves, and roots, resulting in biometric increases in a quadratic pattern. The average MEC of *A. quadricellularis* biomass from all the biometric results was 2.38 g L^{-1}. This verifies the bioactivity of the *AQ* biomass and its ideal concentration range for promoting the sprouting and initial growth of sugarcane.

ACKNOLEDGEMENTS, FINANCIAL SUPPORT, AND FULL DISCLOSURE

To FUNPAR for providing the Doctoral Scholarship to the first author. To the Sugarcane Improvement Program at UFPR for supplying the plant material.

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Journal of Applied Phycology
https://doi.org/10.1007/s10811-025-03498-w

RESEARCH

Immersion of sugarcane propagules in *Asterarcys quadricellularis* biomass extract triggers bioactive amine production and promotes sprouting rate and early seedling growth

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Received: 2 May 2024 / Revised: 13 March 2025 / Accepted: 14 March 2025

Abstract

The use of one-bud propagules (OBP) as a sugarcane planting technique is widespread because of logistic advantages. Propagules from different culm positions have different physiological ages, and present different sprouting and early growth rate s, which is an issue for the uniformity of the stand. Therefore, this study aimed to investigate the effect of OBP immersion in a microalga biomass extract rich in L-free amino acids, looking for a natural alternative to hazardous chemical treatments to stimulate sugarcane sprouting and plant growth. Immersion of OBP for 30 min in a 2.5 g L^{-1} solution of the chlorophyte microalga *Asterarcys quadricellularis* (AQ) improved the sprouting rate of propagules from all culm positions, the effect being most evident in the medial position. Biochemical changes involving sugar levels, and especially the bioactive amines (polyamines and indoleamines), reduced putrescine content, and improved tryptophan content of OBP. Immersion in AQ also enhanced the early growth of sugarcane, with 50-day-old plants presenting gains in culm length and diameter, increments in leaf area, and fresh mass, variably depending on the OBP position. The biometrical gains also followed biochemical changes, increasing the serotonin content of leaves. The results could be related, at least in part, to the L-free amino acid content in microalga biomass, triggering the bioactive amines as key metabolites related to the microalga growth-promoting effect. This study also provides relevant information for developing a field technique to improve and unify sugarcane sprouting and initial growth in an eco-friendly way.

Keywords Biostimulant· Polyamines · Tryptophan· Tryptamine · Serotonin· Chlorophyceae ·*Saccharum* spp

Introduction

Brazil is the world's largest producer of sugarcane (*Saccharum* spp.), with sustainable leading results in sugar, ethanol, and energy production. Homogeneous and vigorous early growth is desirable to maximize sugarcane development and yield (Otto et al. 2022). Using sugarcane one-bud propagules offers advantages over the conventional method of planting, which uses longer parts of the culms (Baracat et al. 2017). These advantages involve economic issues related to reducing the costs associated with harvesting and transporting culms, and logistical problems by reducing machine traffic in the sugarcane fields (Fluminhan and Fluminhan 2020).

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Published online: 11 April 2025

The reserves in sugarcane propagules are fundamental for the initiation of growth and survival of the emerging plant (Baracat et al. 2017). The composition of reserves depends on the maturity of the propagule, which is related to the developmental stage of the culm. Sugarcane grows from the base to the top, so the physiological age of each bud that will form the propagule is older at the base of the culm than at the apex (Figueiredo et al. 2020). The sprouting of sugarcane buds is an energy-consuming process that relies upon the degradation of the propagule reserves such as carbohydrates, lipids, and proteins (Manhães et al. 2015). Thus, the reserves in the propagules are fundamental for sprouting and growth of the emerging plant in the early stage. These reserves progressively decrease as the aerial part and roots develop (Landell et al. 2012; Baracat et al. 2017). Bioactive amines (polyamines and indoleamines) participate in the transfer of reserves from the propagules to the buds (Ferreira et al. 2018; Mógor et al. 2022), and this is accomplished via the activation of enzymes and synthesis of hormones necessary for the promotion of cell division and elongation (May and Ramos 2019). The development of the emerging seedling is also related to the maturity (physiological age) of the propagules used for planting (Nalawade et al. 2018). Apical propagules have a higher sprouting rate than medial or basal ones due to their young physiological age and greater metabolic activity (Figueiredo et al. 2020). Therefore, propagules from different culm positions, with different physiological ages, present differences in sprouting and early growth rate, which could be an issue for the uniformity of the stand (Mógor et al. 2022).

The microalga *Asterarcys quadricellularis*,[1] a chlorophyte of the family Scenedesmaceae, is characterized by high protein, free L-amino acid (L-AA) (Cordeiro et al. 2022a), and polysaccharide (Ghosh et al. 2017) contents, with high potentiality of plant growth promotion activity (Cordeiro et al. 2022b; Lara et al. 2022; Marques et al. 2023, 2025). Microalgae transform solar energy and carbon dioxide into high-value products in their biomass (Baudelet et al. 2017; Machado et al. 2017) and can be cultivated on a large scale (Ghosh et al. 2022). Microalgae afford advantages to the economy and environmental sustainability of agricultural production by providing various inputs, such as biofertilizers and biostimulants (Gemin et al. 2019; Kapoore et al. 2021; Parmar and Srivatsan 2023). The biostimulant effect of microalgal biomass is attributed to the presence of a wide variety of bioactive compounds (Gitau et al. 2022; González-Pérez et al. 2022), such as polysaccharides and polyamines (Mógor et al. 2017; Braun and Colla 2023), as well as L-AA (Mógor et al. 2018, 2022; Renuka et al. 2018; Cordeiro et al. 2022a). Changes in plant metabolism can be initiated by signaling processes triggered by the application of beneficial bioactive compounds (Yakhin et al. 2017). Microalgae biomasses have many bioactive compounds, but the metabolic relations between these compounds and the physiology of plant tissues have complex patterns. Thus, some key metabolites, such as amino acids, sugars, and bioactive amines (polyamines and indoleamines) could be related to sugarcane sprouting and growth changes (Mógor et al. 2022).

Polyamines (PA) are aliphatic amines common in plants and participate in signaling processes. Their biosynthesis occurs through decarboxylation of L-arginine and L-ornithine, with specific distribution in tissues and organs and different localization patterns within cells related to their functions (Vera-Sirera et al. 2010). Putrescine (Put) is the first metabolite in the biosynthesis pathway of PA, which is converted to spermidine and spermine, whose activity and distribution are regulated depending on the type of tissue and stage of development (Chen et al. 2011; Fazilati and Forghani 2015). Stimulation of PA biosynthesis promotes plant metabolism and growth (Rakesh et al. 2021). Besides PA, the indoleamines: tryptophan, tryptamine and serotonin are bioactive amines closely related to plant growth (Negri et al. 2021).

Tryptophan (Try) is an aromatic amino acid produced from chorismate via the shikimate pathway and provides the structural backbone to auxin (indole-3-acetic acid; IAA) and the indoleamines—tryptamine (Tre) and 5-hydroxytryptamine (serotonin, Ser) (Erland and Saxena 2019), which share roles with auxin in plant growth and development (Arnao and Hernández-Ruiz 2018). Try, Ser and Tre are interrelated since Try is an aminoacid precursor of Ser, and Tre is a metabolite derived from Try, synthesized by tryptophan decarboxylase (Kang et al. 2008). These bioactive amines regulate carbon and nitrogen flow from the Try reservoir to the indoleamine pathway, thus inducing metabolic changes (Negri et al. 2021). This work investigates the effect of brief immersion of one-bud sugarcane propagules, differing in age, in extract of the microalga *A. quadricellularis* on propagules sprouting and biochemistry. Our findings will aid in developing an eco-friendly technique to improve sugarcane budding and early growth.

Materials and methods

Plant material

Sugarcane one-bud propagules (OBPs), obtained from the variety RB036152 *Saccharum* sp. (Brasileiro et al. 2024), were cultivated in Paranavaí municipality (Paraná state, Brazil) at the Research Station of the Sugarcane Genetic Development Program of the Federal University of Paraná (PMGCA/UFPR).

The OBPs were selected from basal, medial, and apical positions, corresponding to the 5th–7th, 10th–12th, and 15th–17th culm positions, from the base to the apex,

[1] In previous papers the alga is called *Asterarcys quadricellulare*.

respectively. Using a refractometer (RHBO-90), the Brix° percentage was determined at the basal, medial, and apical positions of culms to define their ripening status in terms of the maturation index (MI = Apical Brix° / Basal Brix°). The index of 0.60 indicates that the segments are suitable for planting (Silveira et al. 2015). OBPs measuring 50 mm in length and 22 mm in diameter, with homogeneous characteristics, were selected.

Microalga material

The biomass of the microalga *Asterarcys quadricellularis* used in the present work was supplied by Alltech® Crop Sciences, Brazil as *Asterarcys quadricellulare* (CCAP 294/1). This biomass was produced in a mixotrophic culture and spray-dried to make a fine, greenish powder.

Determination of the amino acid profile and protein content of *A. quadricellularis* followed the methodologies of Lucas and Sotelo (1980), White et al. (1986) and Hagen et al. (1989). The contents of amino acids were determined using an SPC1000 amino acid analyzer adapted to the pre-column derivatization method with phenyl isothiocyanate (PITC) and quantified by reverse-phase high-performance liquid chromatography (HPLC) using UV detection at 254 nm. The set consisted of a degasser, a quaternary pump module, a Rheodyne injection valve, an oven module, and a UV detection module, equipped with a Phenomenex LUNA C18 100 Å 5 µm column, 250 × 4.6 mm. The amino acid score was calculated as the ratio between the values of essential amino acids in the samples (mg g^{-1}) and the standard values (FAO/WHO 1991).

Thetotal free aminoacidcontent wasdetermined using the ninhydrin method according to Winters et al. (2002). An aliquot of 0.2 g dry algal biomass was extracted in 1.7 mL of 70% ethanol, of which 0.1 mL was diluted in 0.9 mL deionized water. Colorimetric measurements were made with a UV–VIS spectrophotometer (BEL 2000UV) at 570 nm.

The aminogram of the microalgal spray-dried biomass returned the contents (% DW) of L-AAs: aspartic acid 3.32, glutamic acid 4.27, serine 1.66, hlycine 1.54, histidine 0.71, arginine 2.17, threonine 1.45, alanine 2.41, proline 1.6, tyrosine 0.95, valine 1.81, methionine 0.51, cysteine 0. 29, isoleucine 1.41, leucine 2.36, phenylalanine 1.37, lysine 2.11, tryptophan 0.37; totaling to 9% of free L-AAs by weight; also, 37.94% protein was quantified in this biomass.

Plant growth and harvest

Before planting, the propagules were immersed for 30 min in 2.5 g L^{-1} of the *A. quadricellularis* extract (AQ). Water control was used for comparison. After that, propagules were planted in 3-L pots with a commercial substrate mixture of soil and pine bark, two propagules per pot. The substrate chemical analysis revealed the following composition: pH ($CaCl_2$) 6.6, pH (SMP buffer) 7.03, Al^{3+} not detected, $H^+ + Al^{3+}$ 2.32 cmol L^{-1}, Ca^{2+} 12.3 cmol L^{-1}, Mg^{2+} 3. 51 cmol L^{-1}, K^+ 1.94 cmol L^{-1}, P 193.82 mg L^{-1}; C 55.23 g L^{-1}; % Base Saturation 89.1, CEC 20.12 cmolc L^{-1}, Cu 1.81 mg L^{-1}, Mn 31.82 mg kg^{-1}, Fe 29.79 mg kg^{-1}, Zn 2.34 mg kg^{-1}, B 0.33 mg kg^{-1} and S 141.16 mg kg^{-1}. The pots were placed in a greenhouse under natural daylight (13 h photoperiod) at around 25 °C, and the soil moisture content was maintained at 80% of the water-holding capacity using an analogic tensiometer (SoilControl).

The sprouting percentage was determined 30 days after planting. After 50 days of growth, plants were harvested in the early morning for biometric determinations, and aliquots were frozen for biochemical analyses. Propagules were also collected and frozen for biochemical determinations.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design with a factorial scheme (2 × 3), with two factors and four replicates; the replicate was three pots, each containing two propagules. The first factor was the immersion treatment of OBP with two levels: (i) immersion in the microalga solution, and (ii) control with immersion in water. The second factor was the age or position of the OBP on the culm with three levels: (i) apical, (ii) medial, and (iii) basal.

The data homogeneity of variance was verified using Bartlett's test. Then, the data analysed by two-way ANOVA to reveal the effects of the main factors and their interactions on plant growth and biochemical composition. Tukey's test was used at 5% significance level. Data analysis was performed using the statistical program Assistat 7.7 Beta (Silva and Azevedo 2016).

Biometric determinations

Fresh mass (g) of the culms and leaves of 50-day-old plants were recorded using a precision scale. Culm diameter and culm length were measured using a digital caliper and a ruler, respectively. The leaf area (cm^2) was estimated using the WinRhizo program coupled to an LA1600 Scanner (Regent Instruments Inc., Canada).

Biochemical analyses

The apical, medial, and basal OBPs were harvested at the end of the experiment. Culms and leaves were analyzed for PAs (Put, Spd, and Spm), free amino acid (AAs), total soluble

sugars (TSS), reducing sugars (RS), non-reducing sugars (NRS); tryptophan (Try), serotonin (Ser) and tryptamine (Tre). Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were determined in the leaves.

Assay of amino acids, sugars, chlorophylls and carotenoids

Total free amino acids were extracted following the of Magné and Larher (1992). In test tubes citrate buffer pH 4.6 (0.2 M) and ninhydrin solution (1% ninhydrin and 0.03% ascorbic acid in methoxyethanol) were added to the frozen plant biomass. After vortexing, the test tubes were transferred to a water bath (approximately 100 °C) for 15 min. After cooling, 60% ethanol was added, followed by shaking. The total free amino acid content was quantified using amino acids standard curve of asparagine and glutamine (2 mM). Absorbance was read at 570 nm, against the reagent blank, and the results were expressed in μg AA g^{-1} dry weight.

The assay of total soluble and reducing sugars was performed as per Maldonade et al. (2013) using the DNS (dinitrosalicylic acid) test, based on the reaction between reducing sugars and 3,5-dinitrosalicylic acid as an oxidizing agent. The assay of total sugars was preceded by acid hydrolysis, to split the non-reducing sugars into reducing ones. The plant material was incubated with 2 N HCl in a water bath (approximately 100 °C) for 10 min, then cooled, and 2 N NaOH was added. The colorimetric reaction occurred in the dark, using the sample and the DNS reagent (3,5-dinitrosalicylic acid, NaOH, phenol, and sodium metabisulfite), first heating then cooling again and, finally a solution of sodium potassium tartrate (Rochelle salt) was added. The quantification of sugars was performed referring to a glucose standard curve (0–1 mg mL^{-1}). The results were expressed in mg glucose g^{-1} dry weight.

For the assay of chlorophylls and carotenoids, the pigments were extracted from the leaves using 80% acetone in dim light according to Lichtenthaler (1987). The slurry was centrifuged, and the absorbance of the supernatant was read at 663, 647, and 470 nm against 80% acetone. The estimated by applying the formulae of Lichtenthaler (1987) and Lichtenthaler and Buschmann (2001):

$$\text{Chlorophyll } \boldsymbol{a}(\text{Chl.a}) = 12.25\, A_{663} \;\text{—}\; 2.79\, A_{648}$$

$$\text{Chlorophyll } \boldsymbol{b}(\text{Chl.b}) = 21.50\, A_{646.8} - 5.10(A_{663})$$

$$\text{Total chlorophyll} = 7.15\, A_{663} + 18.71\, A_{646.8}$$

$$\text{Carotenoids} = \frac{1000\, A_{470} - 1.82(\text{Chl.a}) - 85.02(\text{Chl.b})}{198}$$

Assay of bioactive amines

The assay of tryptophan, serotonin, tryptamine and PAs was performed using high-performance liquid chromatography (HPLC). The HPLC analyses were conducted according to Diamante et al. (2019). The frozen plant samples were thawedand homogenized in 3 mL of 5% perchloric acid ($HClO_4$), kept in an ultrasonic bath for 30 min, and centrifuged at 6000 × *g* for 10 min (5 °C). To 200 μL of the supernatant, 400 μL dansyl chloride (2.5 mg mL^{-1} in acetone) and 200 μL saturated sodium carbonate solution were added. After stirring for one hour, the mixture stayed in the dark at 60 °C. Afterward, 200 μL proline (0.1 mg mL^{-1} in ultrapure water) was added. The mixture was kept at room temperature for 60 min and toluene (1000 μL) was used to extract the PAs. The samples were vortexed for 1 min and the supernatants were removed, dried in N_2, and suspended in 1 mL acetonitrile (HPLC, 99.9%). The mixture was then kept in an ultrasonic bath for 1 min and centrifuged for 5 min at 4000 × *g* (4 °C). The supernatant was filtered through 0.22 μm-mesh Millipore filters before injection into a UHPLC (ultra-high-performance liquid chromatograph). The chromatographic separation was conducted using a Thermo Scientific Dionex Ultimate 3000 system (Thermo Fisher Scientific, USA) coupled to a quaternary pump, an autosampler (model 3000RS) and a diode array detector (DAD-3000RS). Aliquots (20 μL) were injected and the chromatographic data were collected and processed with Chromeleon 7 software (Thermo Fisher Scientific, Germany) ata flow rate of 0.7 mL min^{-1}, using an Ace 5 C18 column (Advanced Chromatography Technologies, UK) (4.6 mm 9250 mm, particle size 5 μm) at 25 °C. Detection was set at 225 nm, and peak integration and calibration were conducted between 225 and 300 nm. The chromatography gradient was set to a solvent mixture of (A) 100% acetonitrile and (B) 50% acetonitrile, as follows: 0–2 min, 40% A + 60% B; 2–4 min, 60% A + 40% B; 4–8 min, 65% A + 35% B; 8–12 min, 85% A + 15% B; 12–15 min, 95% A + 5% B; 15–21 min, 85% A + 15% B; 21–22 min, 75% A + 25% B; 22–25 min, 40% A + 60% B. The identification and quantification of Put, Spd, Spm, Try, Ser, and Tre were based on the retention time of the standard.

Results

Sugarcane biometrics

Thesprouting percentage at 30 daysafter planting andthe biometric variables of 50-day-oldsugarcane plantsshowed significant differences associated with the propagule position

(Propg) and the *A quadricellularis* extract (AQ), with significant interaction between the two factors (Table 1).

Sprouting percentage (Fig. 1 A) and the measures of seedling growth (Fig. 1 B-F) were lowest in basal propagules but highest in top propagules. In addition, immersion in AQ enhanced propagule sprouting and seedling growth. However, the pattern of Propg-AQ interaction varied for the different growth measures.

The advantage of apical OBPs over the other culm positions was most evident in the control treatment for sprouting percentage and leaf area (Fig. 1 E), in the AQ treatment for culm fresh weight (Fig. 1 D) and leaf fresh weight (Fig. 1 F) but comparable in the two immersion treatments for culm length (Fig. 1 B) and culm diameter (Fig. 1 C). In turn, the beneficial effect of AQ immersion was most evident in the apical propagules for culm length, culm diameter, culm fresh weight and leaf fresh weight, in the medial propagules for sprouting percentage but in the basal propagules for leaf area.

Biochemicals of the one-bud propagules

The Propg-AQ interaction was observed for putrescine (Put). However, for Sspermidine (Spd), spermine (Spm), and amino acids (AA) only the average of each factor level differed from each other (Table 2).

Immersion in AQ altered the position pattern of Put. Whereas the highest Put content was found in the apical propagules of control treatment it was evident in the medial propagules of AQ treatment. In turn, propagule position modified the effect of AQ, which led to a Put content increase in medial and basal propagule while decreasing the content in apical propagules (Table 2).

Differences among OBP positions were observed for spermidine (Spd), free amino acids (AAs) and spermine (Spm) contents. The content of Spd was highest in the medial propagules but least in the apical propagules, irrespective of the immersion treatment. The content of AAs was comparable in the medial and basal propagules, being higher in both than the apical propagules (Table 2). AQ immersion increased spermidine (Spd) contents of all culm positions while lowering Spm content in all culm positions.

There was no interaction among factors on the contents of sugar fractions. Regarding OBP position, except for reducing sugars (RS) in the AQ treatment, the basal propagules exhibited the highest content of total soluble sugars (TSS) and non-reducing sugars (NRS). AQ immersion increased the RS content of all segment positions (Table 3). The highest content of Ser was found in the basal OBPs, of Tre content in the medial OBPs but Try exhibited almost comparable content in the three segment positions. AQ immersion increased the content of indoleamines (Try, Ser and Tre) similarly in all segment positions (Table 4).

Biochemicals of 50-day-old sugarcane culms

The content of Put was higher in culms emerging from apical and medial OBP than the basal OBP, irrespective of the immersion treatment (Table 5). While the Spd and Spm contents of control culms were higher in the apical OBP than the medial and basal OBPs, AQ immersion changed the pattern in favor of the culms from medial OBP against those from apical and basal OBPs. However, AQ immersion promoted no changes in AAs content in the culms and all segment positions were statistically equal (Table 5).

There was marked Propg-AQ interaction on the TSScontent of 50-day-old sugarcane culms. While in the control group, a non-significant difference in TSS content of culms was observed among the three Propg positions, AQ immersionrenderedthe culms from apical OBP withsignificantly higher TSS content than those from medial and basal OBP. AQ immersion significantly reduced TSS content of culms frommedial and basal OBPs, withalmost no effect in culms from apical OBPs. The effect of either factor on RS content of culms was non-significant, with non-significant interaction.

In the control group, culms from basal OBPs exhibited the highest NRScontent among the different positions, but AQ immersion changed the pattern in favor of those from apical OBPs. AQ immersion reduced NRS content in culms from all positions, particularly the medial ones (Table 6).

There was mild Propg-AQ interaction on Try content in culms of 50-day-old sugarcane plants. Whereas culms from basal OBP exhibited the lowest Try content in the control group, they had relatively high content in the AQ group. This was because of the differential effect of AQ treatment on Try content of culms from different positions, where AQ immersion led to a significant increase in Try content of culms from basal OBP but to non-significant effect on culms from medial and apical OBP. The effect of Propg position and AQ immersion and their interaction was non-significant on Ser, which averaged around 13.5 μg g^{-1} FW for all Propg-AQ combinations. Tre content was higher in culms emerging from apical and medial OBP than those from the basal OBP, irrespective of the immersion treatment which led to a comparable non-significant increase at all culm positions (Table 7).

Biochemicals of leaves of 50-day-old sugarcane plants

There was a marked Propg-AQ interaction on polyamine content of leaves (Table 8). The position pattern of Put and Spm contents of leaves was similar, with the highest contents of both metabolites found in the leaves from medial OBP of the control group, but in the leaves from basal OBP of the AQ group. This is because of the AQ-induced reduction in Put and Spm contents of leaves from medial OBPs versus an increase in the leaves from basal OBP. In the two immersion

Table 1 Two-way ANOVA showing the effect of the main factors: propagule position (Propg) and immersion in the microalga *Asterarcys quadricellularis* extract (AQ) and their interaction on growth and performance of sugarcane (*Saccharum* sp.), cv. RB036152. Total soluble sugars (TSS), reducing sugars (RS), non-reducing sugars (NRS), chlorophyll *a* (Chl. a), chlorophyll *b* (Chl. a), total chlorophyll (Chl. a + Chl. b) and carotenoids

Source of variation	df	Sprouting %		Culm length		Culm diameter		Culm fresh mass		Leaf area		Leaffresh mass	
		F	P	F	P	F	P	F	P	F	P	F	P
Propg	2	77.8	< 0.001	2.8	0.09	7.6	0.004	3.1	0.0707	41.9	< 0.001	17.0	< 0.001
AQ	1	52.5	< 0.001	18.7	< 0.001	16.2	0.0001	10.8	0.0041	560.4	< 0.001	89.8	< 0.001
Propg× AQ	2	127.3	< 0.001	22.0	< 0.001	25.0	< 0.001	24.9	< 0.001	252.1	< 0.001	55.5	< 0.001
Source of variation	df	Putrescine		Spermidine		Spermine		Amino acids		TSS		RS	
in one-budpropagules		F	P	F	P	F	P	F	P	F	P	F	P
Propg	2	6.7	0.0066	41.9	< 0.0001	2.0	0.1589	5.6	0.0127	36.0	< 0.0001	1.8	0.1886
AQ	1	7.3	0.0144	2.4	0.1369	23.1	< 0.0001	2.3	0.1488	14.9	0.0012	7.4	0.0139
Propg× AQ	2	14.6	0.0001	0.7	0.5022	0.2	0.7836	0.3	0.722	1.2	0.3368	1.0	0.4019
Source of variation	df	NRS		Tryptophan		Serotonin		Tryptamine					
in one-budpropagules		F	P	F	P	F	P	F	P				
Propg	2	31.0	< 0.0001	1.2	0.3158	24.5	< 0.0001	81.0	< 0.0001				
AQ	1	2.8	0.1086	62.3	< 0.0001	17.6	0.0005	142.9	< 0.0001				
Propg× AQ	2	0.2	0.8206	1.7	0.2043	3.8	0.0408	33.0	< 0.0001				
Source of variation of culms	df	Putrescine		Spermidine		Spermine		Amino acids		TSS		RS	
		F	P	F	P	F	P	F	P	F	P	F	P
Propg	2	8.7	0.0022	47.9	< 0.0001	14.2	0.0001	0.6	0.5704	1.3	0.2982	1.8	0.2022
AQ	1	0.2	0.6381	0.8	0.3956	3.3	0.0844	3.2	0.0889	11.8	0.0029	0.3	0.5838
Propg× AQ	2	2.1	0.1536	29.0	< 0.0001	4.6	0.0238	0.9	0.4123	4.3	0.03	1.4	0.2728
Source of variation of culms	df	NRS		Tryptophan		Serotonin		Tryptamine					
		F	P	F	P	F	P	F	P				
Propg	2	0.9	0.416	3.0	0.0748	1.7	0.2061	8.9	0.0019				
AQ	1	12.3	0.0025	2.1	0.0941	1.8	0.1889	3.5	0.0779				
Propg× AQ	2	1.9	0.1773	3.7	0.0456	2.0	0.1704	0.3	0.7189				
Source of variation of leaves	df	Putrescine		Spermidine		Spermine		Amino acids		TSS		RS	
		F	P	F	P	F	P	F	P	F	P	F	P
Propg	2	103.2	< 0.0001	22.3	< 0.0001	15.3	< 0.0001	2.7	0.0938	10.0	0.0011	10.6	0.0008
AQ	1	5.8	0.0273	44.7	< 0.0001	8.3	0.0099	0.1	0.7491	16.0	0.0008	6.9	0.0171
Propg× AQ	2	63.8	< 0.0001	25.6	< 0.0001	16.7	< 0.0001	0.01	0.9857	41.1	< 0.0001	39.4	< 0.0001
Source of variation of leaves	df	NRS		Tryptophan		Serotonin		Tryptamine					
		F	P	F	P	F	P	F	P				
Propg	2	3.9	0.0394	196.6	< 0.0001	2.9	0.0784	13.3	0.0002				
AQ	1	1.4	0.2583	0.7	0.4133	12.5	0.0023	13.7	0.0016				
Propg× AQ	2	4.4	0.027	10.5	0.0009	1.7	0.2039	18.7	< 0.0001				

Table 1 (continued)

Source of variation	df	Sprouting %		Culm length		Culm diameter		Culm fresh mass		Leaf area		Leaffresh mass	
		F	P	F	P	F	P	F	P	F	P	F	P
Source ofvariation in leaves	df	Chl. a		Chl. b		Chl. a+Chl. bB		Carotenoids					
		F	P	F	P	F	P	F	P				
Propg	2	2.0	0.1649	2.6	0.1003	1.7	0.207	1.9	0.1737				
AQ	1	3.7	0.0693	1.6	0.2217	0.9	0.3509	4.2	0.554				
Propg× AQ	2	0.8	0.4434	1.0	0.3916	0.2	0.8484	0.1	0.8896				

Not significant at $P>0.05$, significant at $P<0.05$, highly significant at $P<0.01$ and very highly significant at $P<0.001$, according to s

groups, the highest Spd content was detected in the leaves from basal OBP; meanwhile, the lowestcontent was foundeither in the leaves from apical OBPs in the control group or the leaves from medial OBPs in the AQ group. This is because AQ immersioninduceda markedreduction in Spd content of leaves from medial OBPs versus mild increases in the leaves fromapical and basal OBPs. The effects of OBP positionand immersion treatment on the amino acid content of leaves were non-significant, and the amino acid contentaveragedaround 1,5 μg g^{-1} leaf FW for all Prog-AQ combinations (Table 8).

A Propg-AQ interaction was observed on all sugar fractions of leaves (Table 9); where AQ immersion changed the position pattern of sugar fractions of leaves. While the leaf contents of TSS and RS were higher in the medial OPBs than the other two positions in the non-treated group, they were highest in the basal OBPs but lowest in the medial OBPs of AQ group. This alteration in the position pattern of TSS and RS was because of the differential effect of AQ immersion in the three position groups. AQ immersion increased the content of TSS and RS in the leaves from apical and basal OBPs but reduced it in those from medial propagules. Similarly, while the NRS content of leaves was comparable in the three OBPs of the control group, it was relatively high in the leaves from medial but low in those from basal OBPs of the AQ group (Table 9).

Significant interactions among factors on Try and Tre contents of leaves (Table 10) were observed. Whereas Try content of control leaves was subtly higher in the basal and medial OBPs than the apical OBPs, the gradient was evident in the AQ group in favor of the leaves from basal OBP against those from apical OBP. This is because AQ immersion increased Try content of leaves from basal OBPs but decreased it in leaves from medial OBP with no effect on those from apical OBPs. The Tre content of control leaves was highest in the medial OBPs and least in the basal OBPs, but in the AQ group, it was higher in the apical than the basal and medial OBPs. This is because AQ immersion lowered Tre content of leaves from medial OBP without affecting the leaves from apical and basal OBPs. The content of Ser in the leaves was non significantly affected by the position of OBP. However, AQ immersion increased the Ser content of leaves from all segments (Table 10). The effects of the main factors (culm position and AQ immersion) and their interactions on the pigment content of leaves were non-significant. The contents of Chl *a*, Chl *b* and carotenoids in sugarcane leaves averaged around 0.21, 0.10 and 0.10 μg g^{-1} FW, respectively for all position-AQ combinations (Table 11).

Discussion

Using OBP as a sugarcane planting technique is widespread because of its logistic advantages. Expectedly, propagules of different physiological ages present differences in sprouting and early growth rates of seedlings. Younger propagules

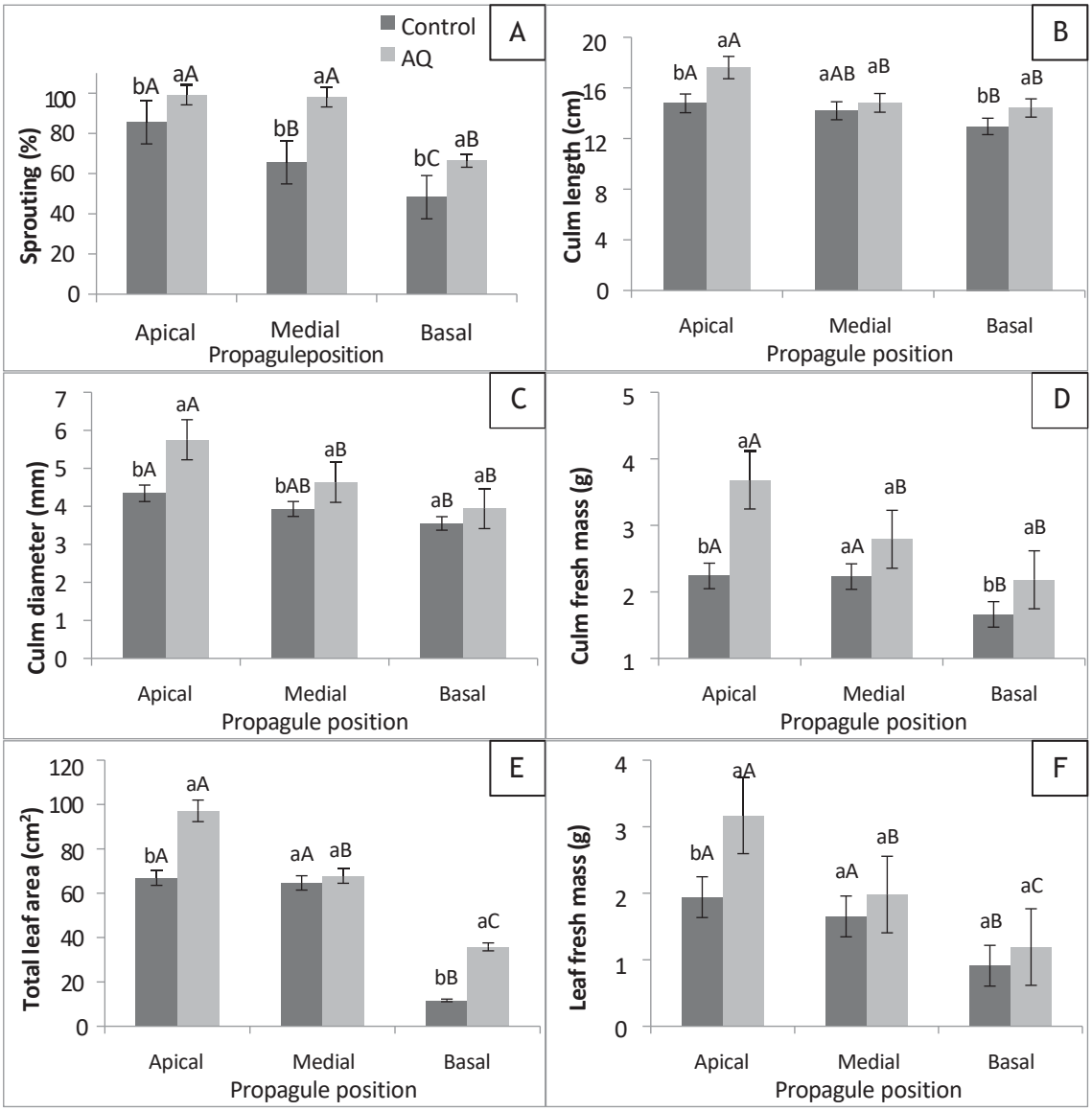

Fig. 1 (**A**) Percentage of sprouting (A), culm length (**B**), culm diameter (**C**), culm fresh mass (**D**), total leaf area (**E**) and leaf fresh mass (**F**) of 50-day-old sugarcane (*Saccharum* sp.) cv. RB036152 plants grown from one-bud propagules (apical, medial, and basal) and subjected to immersion in the *Asterarcys quadricellularis* extract (AQ). Bars indicate standard error ($n = 4$). Columns with the same letters do not differ statistically according to Tukey's test ($P \leq 0.05$), uppercase letters for propagule position and lowercase letters for immersion treatment.

have more accelerated metabolism, presenting the highest sprouting and initial growth capacity than propagules from the medial and basal culm positions (Baracat et al. 2017). The sprouting of sugarcane propagules can be enhanced by the application of organic amendments which induce changes in plant metabolism via initiating signaling processes (Yakhin et al. 2017). Microalgal biomass has many bioactive compounds, such as L-AA (Mógor et al. 2018) and polyamines (Incharoensakdi et al. 2010; Mógor et al. 2017), acting as plantgrowthpromoters. Amongstfree AA composition of microalgae, L-glutamic acid often constitutes the highest proportion (Lu et al. 2019). The AQ biomass, by their high content of L-AAs, can participate in many metabolic pathways as a precursor to other AAs important for plant growth and development through transamination (Forde and Lea 2007; Nunes-Nesi et al. 2010), thus triggering a series of metabolic changes (Lara et al. 2022; Marques et al. 2023). The L-AAs could act as bioactive compounds stimulating PA (Ronga et al. 2019) and indoleamine (Mógor et al. 2022) metabolism, which explains, at least in part, the promotive effect of AQ on sugarcane growth.

Table 2 Polyamines (putrescine, spermidine, and spermine) and total free amino acids contents (ng g^{-1} FW) in one-bud propagules of sugarcane (*Saccharum* sp.) cv. RB036152 from the apical, medial, and basalpositions on culmssubjected to immersion in the microalga *Asterarcys quadricellularis* extract (AQ) compared to the water control

Metaboliteand algal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Putrescine				
Control	146.0± 8.5^{aA}	115.3±19.9^{bB}	95.3 ± 5.4^{bB}	119.0±25.0^{b}
AQ	121.0±12.7^{bB}	146.8±14.0^{aA}	130.0± 8.6^{aAB}	133.0±16.0^{a}
$\overline{X}$	133.5± 17.0^{A}	131.0± 13.0^{A}	112.6± 5.0^{B}	
Spermidine				
Control	29.0 ± 2.2^{aA}	74.8 ± 13.9^{aA}	59.3 ± 2.1^{aA}	54.0 ± 21.0^{b}
AQ	41.0 ± 5.2^{aA}	77.3 ± 14.9^{aA}	62.0 ± 6.2^{aA}	60.0 ± 18.0^{a}
$\overline{X}$	35.0 ± 7.0^{C}	76.0 ± 13.0^{A}	60.5 ± 5.0^{B}	
Spermine				
Control	17.3 ± 5.4^{aA}	16.8 ± 2.8^{aA}	15.0 ± 4.8^{aA}	16.0 ± 4.0^{a}
AQ	12.0 ± 1.8^{aA}	9.8 ± 1.5^{aA}	7.5 ± 1.7^{aA}	10.0 ± 1.8^{b}
$\overline{X}$	14.6 ± 5.0^{A}	13.3 ± 4.0^{A}	11.2 ± 5.0^{A}	
Amino acids				
Control	187.6±24.1^{aA}	466.6± 222.6^{aA}	459.3± 140.7^{aA}	371.2± 193.5^{a}
AQ	347.7±24.0^{aA}	497.9± 123.8^{aA}	560.2± 253.8^{aA}	468.6± 174.9^{a}
$\overline{X}$	267.5±88.4^{B}	482.2± 167.6^{A}	509.7± 197.5^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters = immersion treatment.

Table 3 Total soluble sugars, reducing sugars, and non-reducing sugars contents (mg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 one-bud propagules from the apical, medial, and basal positions of culms subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water immersion control

Metabolite and ment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Total soluble sugars				
Control	17.7 ± 5.5^{aA}	19.5 ± 3.2^{aA}	38.9 ± 5.8^{aA}	25.4 ± 11.0^{b}
AQ	22.5 ± 4.3^{aA}	32.9 ± 4.5^{aA}	47.4 ± 8.8^{aA}	34.2 ± 12.1^{a}
$\overline{X}$	20.1± 5.3^{B}	26.2± 8.0^{B}	43.1 ± 8.2^{A}	
Reducing sugars				
Control	14.5 ± 4.7^{aA}	15.9 ± 3.0^{aA}	16.1 ± 3.6^{aA}	15.5 ± 3.6^{b}
AQ	17.3 ± 2.2^{aA}	24.1 ± 7.2^{aA}	19.4 ± 3.2^{aA}	20.3 ± 5.2^{a}
$\overline{X}$	15.9 ± 3.7^{A}	20.0 ± 6.7^{A}	17.8 ± 3.6^{A}	
Non-reducing sugars				
Control	3.2 ± 1.6^{aA}	3.6 ± 1.6^{aA}	22.7 ± 9.0^{aA}	9.9 ± 10.7^{a}
AQ	5.1± 4.0^{aA}	8.8 ± 6.3^{aA}	28.0 ± 8.4^{aA}	14.0 ± 12.0^{a}
$\overline{X}$	4.2± 3.0^{B}	6.2 ± 5.1^{B}	25.4 ± 8.5^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

Table 4 The indoleamines (tryptophan, serotonin and tryptamine) content (μg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 one bud propagules from apical, medial, and basal positions of culms subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water immersion control

Metabolite and treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Tryptophan				
Control	9.5± 0.4^{aA}	9.2 ± 0.3^{aA}		
AQ	11.5± 0.3^{aA}	11.1± 0.6^{aA}		
$\overline{X}$	10.5± 1.1^{A}	10.2 ± 1.1^{A}		
erotonin				
AQ	3.6 ± 0.3^{aB}	4.9 ± 0.6^{aA}	5.1 ± 0.2^{aA}	4.56±0.8^{a}
$\overline{X}$	3.5 ± 0.4^{C}	4.3 ± 0.8^{B}	4.9 ± 0.4^{A}	
Tryptamine				
Control	1.54 ± 0.06^{bB}	1.84 ± 0.04^{aA}	1.42 ± 0.05^{bC}	1.60 ± 0.19^{b}
AQ	1.71± 0.05^{aB}	1.91 ± 0.03^{aA}	1.84 ± 0.05^{aA}	1.82 ± 0.10^{a}
$\overline{X}$	1.63 ± 0.11^{B}	1.88± 0.05^{A}	1.63 ± 0.23^{B}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

Table5 Contentsofpolyamines (putrescine, spermidine, and spermine) (ng g^{-1} FW) and total free amino acids (μg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 culms of plants grown from one bud propagules (apical, medial, and basalculmpositions)subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to the watercontrol

Metaboliteand algal treatment	Propagule position			
	Apical	Medial	Basal	X
Putrescine				
Control	139.8±13.2^{aA}	129.3±12.3^{aA}	117.3±23.8^{aA}	128.8±18.0^{a}
AQ	132.3± 8.7^{aA}	143.0± 7.3^{aA}	102.5±15.6^{aA}	125.9±21.0^{a}
$\overline{X}$	136.1± 11.1^{A}	136.2± 11.9^{A}	110.0± 2.0^{B}	
Spermidine				
Control	25.5 ± 1.7^{aA}	13.5 ± 2.4^{bB}	12.0 ± 2.8^{aB}	17.0 ± 7.0^{a}
AQ	18.8 ± 2.1^{bB}	22.8 ± 1.0^{aA}	11.8 ± 2.2^{aC}	17.8 ± 5.0^{a}
$\overline{X}$	22.2 ± 4.0^{A}	18.2 ± 5.0^{B}	11.9 ± 2.0^{C}	
Spermine				
Control	8.0 ± 0.8^{aA}	6.8± 1.0^{bAB}	5.3 ± 1.0^{aB}	6.7 ± 0.1^{b}
AQ	7.5± 0.6^{aAB}	9.5 ± 1.7^{aA}	5.5 ± 1.3^{aB}	7.5 ± 0.2^{a}
$\overline{X}$	7.8 ± 0.1^{A}	8.2 ± 0.2^{A}	5.4 ± 0.1^{B}	
Amino acids				
Control	1,739±252^{aA}	1,698±121^{aA}	1,491±89^{aA}	1,642± 191^{a}
AQ	1,706±466^{aA}	2,380± 1,318^{aA}	2,160±338^{aA}	2,082± 806^{a}
$\overline{X}$	1,723±347^{A}	2,039±940^{A}	1,825±424^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters = immersion treatment.

Table 6 Contents of total soluble sugars, reducing sugars, and non-reducing sugars (mg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 culms of plants grown from one bud propagules (apical, medial, and basal culm positions) subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metabolite and algal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Totalsoluble sugars				
Control	31.1 ± 5.1^{aA}	33.8 ± 4.3^{aA}	35.8 ± 1.5^{aA}	33.6 ± 4.1^{a}
AQ	32.6 ± 6.8^{aA}	21.3 ± 4.5^{bB}	24.4 ± 7.4^{bAB}	26.1 ± 7.6^{b}
$\overline{X}$	31.8± 5.6^{A}	27.6± 7.8^{A}	30.1 ± 7.8^{A}	
Reducing sugars				
Control	18.2 ± 1.7^{aA}	19.1 ± 3.2^{aA}	16.7 ± 0.6^{aA}	18.0 ± 2.2^{a}
AQ	21.5 ± 3.7^{aA}	17.3 ± 4.0^{aA}	17.3 ± 3.5^{aA}	18.7 ± 4.0^{a}
$\overline{X}$	19.8± 3.2^{A}	18.2 ± 3.5^{A}	17.0 ± 2.4^{A}	
Non-reducing sugars				
Control	12.9 ± 5.4^{aA}	14.7 ± 7.4^{aA}	19.1 ± 1.0^{aA}	15.6 ± 5.5^{a}
AQ	11.1 ± 8.6^{aA}	4.0 ± 0.9^{aA}	7.2 ± 5.8^{aA}	7.4 ± 6.2^{b}
$\overline{X}$	12.0± 6.7^{A}	9.4 ± 7.5^{A}	13.1 ± 7.5^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

Table 7 The indolamines (tryptophan, serotonin, and tryptamine) content (μg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 culms of plant grown from one bud propagules (apical, medial, and basal culm positions) subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metabolite and algal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Tryptophan				
Control	14.7 ± 0.7^{aAB}	16.6 ± 0.9^{aA}	13.7 ± 2.6^{bB}	15.0 ± 2.0^{a}
AQ	15.0± 0.2^{aA}	16.2± 0.6^{aA}	16.7 ± 1.5^{aA}	16.0 ± 1.1^{a}
$\overline{X}$	14.8± 0.5^{A}	16.4± 0.7^{A}	15.1 ± 2.6^{A}	
Serotonin				
Control	14.2 ± 1.4^{aA}	12.9± 1.2^{aA}	12.2 ± 2.0^{aA}	13.2 ± 1.7^{a}
AQ	14.0± 0.4^{aA}	13.1± 0.8^{aA}	14.4 ± 1.1^{aA}	13.8 ± 0.9^{a}
$\overline{X}$	14.2± 0.9^{A}	13.0± 0.9^{A}	13.3 ± 1.9^{A}	
Tryptamine				
Control	0.62± 0.04^{aA}	0.60 ± 0.05^{aA}	0.51 ± 0.13^{aA}	0.58 ± 0.09^{a}
AQ	0.68 ± 0.03^{aA}	0.68 ± 0.06^{aA}	0.53± 0. 07^{aA}	0.63 ± 0.09^{a}
$\overline{X}$	0.65± 0.05^{A}	0.64± 0.06^{A}	0.52 ± 0.09^{B}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

Table8 Contentsofpolyamines (putrescine, spermidine, and spermine) (ng g^{-1} FW) and total free amino acids (µg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 leaves of plant grown from one-bud propagules (apical, medial, and basalculmpositions)subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metaboliteand algal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Putrescine				
Control	128.3± 4.3^{aB}	364.8±21.0^{aA}	156.3±27.6^{bB}	216.4± 111.7^{a}
AQ	115.3±13.5^{aC}	208.3±11.2^{bB}	258.3±40.3^{aA}	194.0±66.0^{b}
$\overline{X}$	121.8± 12.0^{C}	286.6± 85.1^{A}	207.3± 63.2^{B}	
Spermidine				
Control	109.0± 4.2^{aC}	162.5±20.9^{aB}	201.8±27.6^{aA}	157.8±43.7^{a}
AQ	130.0± 5.9^{aA}	90.5 ± 7.0^{bB}	130.0± 6.7^{bA}	116.8±20.3^{b}
$\overline{X}$	119.5±12.2^{B}	126.5±41.1^{B}	165.9±42.6^{A}	
Spermine				
Control	8.5 ± 1.7^{aC}	34.5 ± 12.1^{aA}	19.8 ± 3.7^{aB}	20.9 ± 12.9^{a}
AQ	9.5 ± 1.3^{aB}	10.0 ± 2.4^{bB}	24.0 ± 2.9^{aA}	14.5 ± 7.3^{b}
$\overline{X}$	9.0 ± 1.5^{B}	22.2 ± 15.4^{A}	21.9 ± 3.8^{A}	
Amino acids				
Control	1,657±368^{aA}	1,554±359^{aA}	1,255±77^{aA}	1,488±325^{a}
AQ	1,671±499^{aA}	1,601±245^{aA}	1,326± 315^{aA}	1,533±368^{a}
$\overline{X}$	1,664±406^{A}	1,578±286^{A}	1,290±216^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters = immersion treatment.

The present findings reveal that brief immersion of sugarcane OBP in a solution of *A. quadricellularis* spray-dried biomass improved sprouting of all OBP positions, particularly the medial and basal OBPs (Fig. 1 A). Thus, the beneficial effect of AQ immersion was most evident in the aged OBPs of low metabolic activity relative to the apical OBP of high initial sprouting percentage before treatment. However, the AQ-propagule interaction on post-sprouting growth of seedlings exhibited different patterns than that of sprouting percentage, with minor variation among the different growth measures but with a common pattern of highest benefit from AQ immersion assigned to the apical OBP for all growth measures (Fig. 1 A-F).

Table 9 Contents of total soluble sugars, reducing sugars and non-reducing sugars (mg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 leaves of plants grown from one bud propagules (apical, medial and basal culm positions) subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metabolite and algaltreatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Total sugars				
Control	14.7± 1.0^{bB}	17.1 ± 0.6^{aA}	14.6 ± 1.1^{bB}	15.5 ± 1.5^{b}
AQ	16.8± 1.0^{aB}	13.9 ± 1.0^{bC}	20.7 ± 1.4^{aA}	17.1 ± 3.1^{a}
$\overline{X}$	15.8± 1.4^{B}	15.5± 1.9^{B}	17.6 ± 3.5^{A}	
Reducing sugars				
Control	11.6 ± 0.7^{aAB}	13.0 ± 0.9^{aA}	10.3 ±0.7^{bB}	11.6 ± 1.3^{b}
AQ	12.7± 1.4^{aB}	8.9 ± 1.8^{bC}	17.3 ± 1.5^{aA}	13.0 ± 3.9^{a}
$\overline{X}$	12.1± 1.2^{B}	10.9± 2.6^{B}	13.8 ± 3.9^{A}	
Non-reducing sugars				
Control	3.2± 0.3^{aA}	4.1 ± 0.5^{aA}	4.3 ± 0.6^{aA}	3.8 ± 0.7^{a}
AQ	4.1 ± 0.8^{aAB}	5.0 ± 1.0^{aA}	3.4 ± 0.7^{aB}	4.2 ± 1.1^{a}
~~X~~	3.6 ± 0.8^{B}	4.6 ± 0.9^{A}	3.8 ± 0.8^{AB}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

The metabolic changes in plant tissues in response to propagule position and AQ treatment exhibited different patterns in propagules, culms, and leaves, with some key metabolites, such as polyamines, affecting sprouting and growth changes (Ferreira et al. 2018). The metabolism of PAs in plants is closely connected to many other metabolic pathways (Chen et al. 2019). PAs can be directly or indirectly regulated through interaction with signaling metabolites (Kamiab et al. 2020) such as L-AA. Put is the first metabolite in the biosynthesis pathway of PAs through decarboxylation of L-arginine and L-ornithine (Vera-Sirera et al. 2010). Then Put is converted to Spd and Spm by the enzymes Spd and Spm synthases (Chen et al. 2019), respectively whose activity and distribution are regulated depending on the type of tissue and stage of development (Fazilati and Forghani 2015). Generally, the more theaminogroups in the PA molecule, the stronger will be its physiological activity (Chen et al. 2019). PAs participate in many metabolic processes, such as cell proliferation and differentiation,

Table 10 The indolamines (tryptophan, serotonin and tryptamine) content (μg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 leaves of plants grown from one bud propagules(apical, medialand basalculmpositions)subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metaboliteandalgal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Tryptophan				
Control	15.9 ± 0.6^{aB}	23.0 ± 0.5^{aA}	23.3 ± 1.1^{bA}	20.7 ± 3.7^{a}
AQ	15.6 ± 0.9^{aC}	21.6 ± 1.3^{bB}	26.0 ± 1.0^{aA}	21.1 ± 4.6^{a}
$\overline{X}$	15.7 ± 0.8^{C}	22.3 ± 1.2^{B}	24.7 ± 1.7^{A}	
Serotonin				
Control	23.7 ± 1.3^{aA}	24.5 ± 1.5^{aA}	24.7 ± 1.2^{aA}	24.3 ± 1.3^{b}
AQ	26.0 ± 0.9^{aA}	25.1 ± 1.5^{aA}	27.9 ± 1.8^{aA}	26.3 ± 1.8^{a}
$\overline{X}$	24.9 ± 1.6^{A}	24.8 ± 1.4^{A}	26.3 ± 2.2^{A}	
Tryptamine				
Control	0.88 ± 0.05^{aB}	1.04 ± 0.06^{aA}	0.77 ± 0.03^{aC}	0.89 ± 0.12^{a}
AQ	0.87 ± 0.04^{aA}	0.78 ± 0.06^{bB}	0.79 ± 0.06^{aAB}	0.82 ± 0.07^{b}
$\overline{X}$	0.88 ± 0.04^{A}	0.91 ± 0.15^{A}	0.78 ± 0.05^{B}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters = immersion treatment.

Table 11 The pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids) content (μg g^{-1} FW) in sugarcane (*Saccharum* sp.) cv. RB036152 leaves of plants grown from one bud propagules (apical, medial and basal culm positions) subjected to immersion in the *Asterarcys quadricellularis* extract (AQ) compared to water control

Metabolite and algal treatment	Propagule position			
	Apical	Medial	Basal	$\overline{X}$
Chlorophyll *a*				
Control	0.19 ± 0.01^{aA}	0.21 ± 0.01^{aA}	0.18 ± 0.02^{aA}	0.19 ± 0.02^{a}
AQ	0.21 ± 0.01^{aA}	0.21 ± 0.01^{aA}	0.21 ± 0.04^{aA}	0.21 ± 0.02^{a}
$\overline{X}$	0.20 ± 0.02^{A}	0.21 ± 0.00^{A}	0.19 ± 0.03^{A}	
Chlorophyll *b*				
Control	0.11 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{a}
AQ	0.10 ± 0.00^{aA}	0.10 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.10 ± 0.01^{a}
$\overline{X}$	0.11 ± 0.01^{A}	0.10 ± 0.00^{A}	0.10 ± 0.01^{A}	
Total Chlorophyll				
Control	0.30 ± 0.03^{aA}	0.31 ± 0.02^{aA}	0.28 ± 0.02^{aA}	0.30 ± 0.02^{a}
AQ	0.31 ± 0.01^{aA}	0.31 ± 0.01^{aA}	0.30 ± 0.05^{aA}	0.31 ± 0.03^{a}
$\overline{X}$	0.31 ± 0.02^{A}	0.31 ± 0.01^{A}	0.29 ± 0.04^{A}	
Carotenoids				
Control	0.09 ± 0.01^{aA}	0.10 ± 0.00^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{a}
AQ	0.10 ± 0.00^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.10 ± 0.01^{a}
$\overline{X}$	0.10 ± 0.01^{A}	0.10 ± 0.01^{A}	0.10 ± 0.01^{A}	

Each value is the mean of four replicates ± SE. Means followed by the same letter do not differ statistically according to Tukey's test ($P \leq 0.05$). Upper case letters = culms position. Lowercase letters =immersion treatment.

thus modulating plant growth and development (Srivastava et al. 2013; Mustafavi et al. 2018; Nandy et al. 2023). Spm and Spd are associated with plant ontogeny and growth promotion, while Put is associated with the early stages of PA metabolism or plant senescence (Anwar et al. 2015; Ahmed et al. 2017; Tyagi et al. 2023).

The PA profile of OBP was altered by AQ immersion, with Put contents being reduced in apical OBPs but increased in medial and basal ones, thus rendering Put content of treated medial OBPs the highest among all positions (Table 2). However, while AQ immersion increased Put content of leaves from basal OBPs, it led to a decrease in medial OBPs (Table 8). AQ immersion increased Spd content equally in propagules of different positions, with medial OBPs exhibiting the highest Spd content (Table 2). AQ immersion increased the Spd content of culms sprouted from medial OBPs, while decreasing it in culms sprouted from apical OBPs (Table 5). A similar decrease was stimulated by AQ immersion for Spd content in leaves from medial and basal OBPs (Table 8). The Spm content was decreased by AQ immersion in all OBPs (Table 2). However, while the culms sprouted from medial OBPs had Spm content increased by AQ (Table 5), the opposite was observed for leaves from medial OBPs (Table 8). The reduction in Put and increment in Spd contents (Tables 2, 5, 8) related to the enhancement of sprouting rates of apical propagules was previously reported by Mógor et al. (2022).

The AQ-induced increase in RS (e.g., glucose, fructose) of OBPs regardless of position (Table 3) indicates the effect of microalga biomass in changing sugar profile with accumulation of RS which are immobile in the phloem and with high participation in metabolism. TSS and NRS (e.g., sucrose, polyols) exhibited the highest content in basal OBP

among the different positions, suggestingalsoless mobilization to sprouts developing from basal OBPs relative to apical and medial OBPs (Table 3). Inculms sprouted from medial and basal OBPs AQ decreased TSS (Table 6). AQ immersionalso decreased NRS regardless of OBP position, while producing no change in RS levels in culms.(Table 6). AQ immersion increased Try content of OBPs (Table 4) with the same pattern of RS, regardless of OBP position. However, AQ immersion increased Try content of culms sprouted from basal OBPs, with no change in apical and medial OBPs (Table 7). Also, AQ immersion increased Ser and Tre contents of all OBPs, and the increment was particularly evident in the medial ones for Ser but in the basal andapical ones for Tre (Table 4). These AQ-induced metabolic changes in OBPs manifested as reduction of Spm content, alterations of Put, Ser, and Tre patterns, along with the highest contents of RS and Try, justify, at least in part, the AQ role in stimulation of sugarcane sprouting (Fig. 1 A) and are following the results obtained by Mógor et al. (2022),electing the bioactive amines as the compounds of nitrogen metabolism related to sugarcane sprouting. Previous works using *A. quadricellularis* biomass have already reported its plant growth promotioneffect in crops such as potatoes (Cordeiro et al. 2022a), onions (Cordeiro et al. 2022b), tomatoes (Lara et al. 2022), soybeans (Palma et al. 2022), beans (Marques et al. 2023) and red beets (Novaski et al. 2024).

Growth of the 50-day-old plants was affected by OBP immersion in AQ, with the culms of plants from apical OBPs exhibiting improvements in length, diameter, and fresh mass, while the culms from medial OBPs have been improved particularly in diameter and those from basal OBPs have been improved particularly in length and fresh mass (Fig. 1 B, C, D). Also, the beneficial effect of AQ immersion was particularly evident in leaf area of plants from apical and basal OBPs, and in leaf fresh mass of plants from apical OBPs (Fig. 1 E, F). The promotion of culm and leaf growth induced by AQ immersion was accompanied by biochemical alterations in the culms and leaves of 50-day-old plants. While AQ immersion induced a reduction in Spd content of culms from apical OBPs, there was an increase in the Spd and Spm contents of culms from medial OBPs, which can account for the increase in culm diameter, concomitant with the higher Put content (Table 5).

The PA content in theleaves of plants fromapical OBPs, which presented the highest leaf area and fresh mass (Fig. 1 E, F) did not change by AQ immersion (Table 8), indicating that the PA pattern of plants from the youngest propagules with more accelerated metabolism, are related to better growth. On the other hand, immersion in AQ reduced PA in leaves of plants from medial OBP, leading to non-significant area and fresh mass differences compared to the control. The improvement in leaf area of plants from basal OBP by AQ immersion was accompanied by increased Put content and reduced Spd content.

While the AA content has not been altered in OBPs by AQ immersion (Table 5, 8), for OBP position a higher AA content was observed for medial and basal propagules (Table 2). Similarly, AQ showed no influence on photosynthetic pigments (Table 11). However, changes in the leaf area of plants from basal and apical OBPs were observed in this important biometrics variable, for a better photosynthetic capacity (Fig. 1 E), and were accompanied by increased Put content and reduced Spd content (Table 8).

The PA and sugars cross-talk are related in part to changes that can occur in PA profile, with modifications in tissue sugar concentration and carbon–nitrogen signaling pathways (Anwar et al. 2015). PAs can improve the flow of sugars from the source to the sink (Luo et al. 2019), that is from the propagules to the emerging shoot (Mógor et al. 2022), which occurs through signaling processes stimulating cell division and elongation, thus improving seedling growth (May and Ramos 2019).

The growth-promoting effect of AQ immersion was well-characterized, but its role in the mobilization of sugars from OBPs to culms and leaves still needs thorough investigation. In general, growth promotion by AQ immersion implies consumption of sugar, thus reducing TSS content of culms from medial and basal OBP and NRS content in the culms regardless of OBP position (Table 6). Also, AQ immersion reduced the RS and TSS content of the leaves of plants that grew from medial OBPs (Table 9). It can be considered that the fast-growing plants could experience reduced sugar content in young tissues of high growth rates (Klopotek and Kläring 2014) as is the case in the early growth of 50-day-old sugarcane plants. The exception was the increase in TSS content of leaves of plants from apical and basal OBPs by AQ immersion (Table 9).

Furthermore, besides the changes in sugar and PA contents, there was a relation between the changes in the indoleamines' profile and the promotion of early growth of sugarcane seedlings by AQ. The AQ-induced increase in Try content of culms of plants emerging from basal OBPs (Table 7), those in Sercontent of leaves of plants emerging from all OBP regardless of their position (Table 10) justifies considering Tryand Seras the bioactivecompounds related to the metabolic flowin which Try is the AA precursor of the hormone auxin and the indoleamine Ser(Negri et al. 2021). The biosynthesis of serotonin is a multistep process coupled with two or more biocatalytic reactions; whereupon tryptophan is decarboxylated by tryptophan decarboxylase to generate the bioactive tryptamine, which is subsequently hydroxylated to form serotonin by tryptamine-5-hydroxylase. Auxin is a crucial regulator of plant growth and development and acts as a versatile coordinator of these processes. Both auxin and serotonin have functional similarities with

acommon biosynthetic pathway. Serotonin, likeauxin, has been implicated in plant growth (Mishra and Sarkar 2023). Microalgal biomass may promote plant growth through complex interactions between its components, resulting in synergistic or additive effects among various metabolites (Mazepa et al. 2021). Indoleamines and PAs can be regulated directly or indirectly through interaction with signaling metabolites, arising from nitrogen metabolism (amines and AAs) and carbon metabolism (sugars) (Kamiab et al. 2020; Nandy et al. 2023). This intricate regulatory feedback mechanism cancontrol the balance of bioactive amine levels, which has often beenseen in this work, associated with better sugarcane sprouting and early growth when OBPs were subjected to immersion in *A. quadricellularis* spray-dried biomass.

This work addresses a fundamental issue for sugarcane establishment and growth; it represents an attempt to obtain homogeneous and vigorous growth of sugarcane propagules to maximize sugarcane yield. The microalgal extract proved to level off the physiological differences between OBPs of different ages, by activating the aged basal propagules and bringing them to approach the behavior of the active apical ones. Expanding these biostimulant results to the field could increase the OBP sprout rate while enhancing and standardizing the plants for a better harvest.

Conclusion

Brief immersion of one-bud sugarcane propagules from apical, medial, and basal culm positions in the chlorophyte microalga *Asterarcys quadricellularis* extract (AQ) improved sprouting rate. This effect was accompanied by biochemical changes in the bioactive amines (polyamines and indoleamines) profile, manifested as reducing putrescine and improving tryptophan content in the apical OBPs. The immersion in AQ also enhanced the early growth of sugarcane, with increments in culm length and diameter, leaf area, and gain in leaf fresh mass, variably depending on the OBP position. These growth-promoting effects were also accompanied by biochemical changes in sugar content, and indoleamines profile, highlighting the role of enhanced serotonin content of leaves in plant growth. The results could be related to the L-free amino acid content of microalgal biomass triggering the bioactive amines as key metabolites. Application of the present findings on the field scale can aid in developing an eco-friendly and efficient technique to improve and unify sugarcane budding and early growth.

Acknowledgements The authors thank the Sugarcane Improvement Program at UFPR for supplying the plant material, and the first author thanks FUNPAR for his Doctoral scholarship.

Author contribution Conceptual Idea: Mógor, A.F.; Mógor, G.; Lima, G.P.P.; Oliveira, R.A.O.; Bespalhok Filho, J.C. Methodology design: Mógor, A.F.; Mógor, G.; Lima, G.P.P.; Oliveira, R.A.O.; Bespalhok Filho, J.C. Data collection: Lara, G.B.; Amatussi, J.O. Data analysis and interpretation: Lara, G.B.; Mógor, A.F.; Mógor, G.; Amatussi, J.O. Writing and editing: Lara, G.B.; Mógor, A.F.; Mógor, G.; Bespalhok Filho, J.C.

Funding The first author received a Doctoral Scholarship from FUNPAR.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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5. GENERAL CONCLUSION

The production of sugarcane OBP was enhanced with the use of the biomass extract of the microalga *Asterarcys quadricellularis*. Biochemical determinations reinforced the hypothesis that the bioactive compounds in the composition of this biomass could act as metabolic signaling agents. The activation of metabolic pathways of bioactive amines was evidenced in comparison to the control treatment. Morphological changes, involving plant growth promotion and physiological changes, such as an increase in the sprouting rate, were also observed. These changes, especially when using the maximum efficiency concentration of the microalga, resulted in a greater number of viable OBP for seedling production, as well as more vigorous seedlings after transplanting into pots.

6. FINAL CONSIDERATIONS

The vegetative growth was remarkable, as were the metabolic changes promoted at the maximum efficiency concentration of the *Asterarcys quadricellularis* biomass extract. The potential of renewable biomass and sustainable methods promotes gains in fundamental variables, such as sugar accumulation in the stem, for the sugar-alcohol industry, supporting sustainable development in a highly important sector for Brazil. Further studies could help identify natural products and their best concentration to increase sugarcane productivity. Therefore, the bioinputs application in the sugarcane OBP has yet to be evaluated under field conditions, considering whether the benefits found in growth chambers and agronomic greenhouses will be maintained until harvest time.

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