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Identification of proteins associated with reducing regrowth and with the longevity of cane fields

Rone Charles Maranho[1], Mariana Mancini Benez[2], Gustavo Barizon Maranho[2], Adriana Gonela[3], Adeline Neiverth[4], Marise Fonseca dos Santos[4], Ana Lúcia O. Carvalho[5], Claudete Aparecida Mangolin[6], Maria de Fátima P. S. Machado[6*]

[1]Postdoctoral in Genetics and Breeding, State University of Maringá, Maringá, PR 87020-900, Brazil
[2]Postgraduate in Agronomy, State University of Maringá, Maringá, PR 87020-900, Brazil
[3]Department of Agronomy, State University of Maringá, Maringá, PR 87020-900, Brazil
[4]Department of Biosciences, Federal University of Paraná, Palotina, PR 86950-000, Brazil
[5]Proteomic Mass Spectrometry Unit, Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-901, Brazil
[6]Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá, PR 87020-900, Brazil

***Corresponding author: mfpsmachado@uem.br**

Abstract

Budding axillary buds are crucial for the establishment and longevity of commercial and industrial sugarcane varieties (*Saccharum* spp.). Increasing the longevity of sugarcane varieties beyond the fifth or sixth cutting stage without decreasing agricultural productivity is a promising economic outlook for producers. Identifying the factors that limit the longevity of cane fields can be an alternative to bypass the regrowth limits of axillary buds. This study aimed to evaluate the bud sprouting rates of axillary buds of the variety RB867515 from the first to the fifth cut and to identify the prevalent proteins in the axillary buds of sugarcanes of the first, third, and fifth cutting stages. The ultra-performance liquid chromatography in tandem with quadrupole-time-of-flight electrospray ionization from one-dimensional gel electrophoresis (1DE-UPLC-ESI-QTOF) mass spectrometry approach was used to obtain the proteome of the axillary buds in the first, third, and fifth cuts. The sprouting rate was evaluated using a caliper and considered positive when the budding of the axillary buds increased by at least 6 mm. Proteins were extracted in biological triplicate for each cutting stage, and the identification of proteins, based on acquired MS/MS spectra, was performed using the Mascot tool from Matrix Science against Viridiplantae within the SwissProt/UniProtKB and databases UP7305_Z_mays in Uniprot. The budding rate in the axillary buds of the first cut was 69.8%. In contrast, the germination rate of the axillary buds of the third cutting was 36.4%. Exclusive proteins were identified in the first, third, and fifth cutting stages, and a 49.3% reduction in the number of proteins was observed in the third cut compared to the first one. Absence of essential proteins/enzymes involved in *i*) carbohydrate metabolism, *ii*) folding and degradation of proteins (proteolytic functions), *iii*) 40S and 60S ribosomal subunits, and *iv*) proteins involved in biotic and abiotic stresses, are factors that may limit the longevity of cane fields from the third cut before the fifth or sixth cutting stage, when productivity in the field became economically unfeasible.

Keywords: *Saccharum* spp.; cutting stage; sugarcane productivity, sprouting rate, shotgun proteomics.
Abbreviations: 1D SDS-PAGE_one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis; LC-MS/MS_liquid chromatography coupled with sequential mass spectrometry; PPI_protein-protein interaction network; UPLC-ESI-TOF-MS_ultra performance liquid chromatography-electrospray ionization-time of flight-mass spectrometer.

Introduction

Sugarcane (*Saccharum* spp.) is a monocot with a high sucrose content and lignocellulosic biomass (cellulose, hemicellulose, and lignin) production per cultivated area. Sucrose is used to produce sugar and ethanol, whereas the lignocellulosic fibers are used for fertilization, animal feed, energy cogeneration, second-generation ethanol production (E2G) (Tyagi et al., 2019; Huang et al., 2020), among other applications (Miyahara et al., 2018). Sugarcane-derived ethanol is a renewable biofuel that is a viable alternative for reducing carbon dioxide (CO_2) emissions in the long term (Yuan et al., 2008). Sugarcane ethanol use in the Brazilian global transport sector can offset approximately 86% of CO_2 emissions compared with petroleum use (Jaiswal et al., 2017).

Commercial and industrial cultivation of sugarcane is characterized by the planting of stem segments containing axillary buds (stalk or seed pieces) or pre-sprouted seedlings (MPB) (Landell et al., 2012) that correspond to the plant-

cane stage. After the first harvest (first cut), the axillary buds remain in the soil sprout, forming a new aerial part harvested after maturation (second cut), corresponding to the ratoon cane stage. This regrowth process is repeated five to six times until its maintenance becomes economically unfeasible. Thus, the budding potential of axillary buds is a deciding factor for the establishment and longevity (number of cutting stages) of commercial and industrial sugarcane varieties (Ehsanullah et al., 2011).

The variety RB867515, distributed in Brazil in 1997 by the Ridesa breeding program (Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético), has overcome challenges since RB867515 has remained the most planted variety in the central and southern regions of Brazil for more than two decades. Despite the release of new varieties in the last two decades, the RB867515 variety still occupies 18% of the sugarcane planted area (http://www.CanaOnline.com.br/). RB867515 is tolerant to charcoal (*Sporisorium scitamineum*), scald (*Xanthomonas albilineans*), and mosaic and resistant to brown rust (*Puccinia melanocephala*). RB867515 responds well to mechanized harvesting, showing good regrowth of the axillary buds and robust agronomic characteristics (Daros et al., 2015), providing assurance and safety to producers.

Although the RB867515 variety shows good regrowth of axillary buds and has high agronomic potential, its longevity (productive cycle) is five or six cuts, similar to that of other sugarcane varieties. Increasing the longevity of sugarcane varieties without decreasing agricultural productivity is a promising economic outcome for producers. Identifying the factors limiting the longevity of cane fields can be an alternative to bypassing the regrowth limits of axillary buds. Quantitative and qualitative alterations in the proteome of axillary buds of RB867515 in the fifth cutting stage have been shown (Benez et al., 2019; Maranho et al., 2019) and in axillary buds and roots of other sugarcane varieties (Maranho et al., 2021). These changes were also observed at the end of the regrowth period. However, whether proteome changes in the axillary buds of RB867515 occur before the fifth cut and whether they are related to a gradual reduction in the budding potential of the axillary buds are still unknown. This study hypothesized that alterations in the proteome of axillary buds may occur before the fifth cutting. Identifying which proteins are altered can be useful for developing strategies to retard the decreasing agricultural productivity of the fifth cut. This study aimed to evaluate the bud sprouting rates of the axillary buds of sugarcanes from the first to the fifth cutting stage and to identify the prevalent proteins in the axillary buds from the first, third, and fifth cutting stages.

Results

Bud germination

The budding rates in the axillary buds of the first and second cuts were 69.8% and 67.2%, respectively. In contrast, the germination rate in the axillary buds of the third cut was 36.4%. From the fourth cut onwards, the sprouting rate remained low, at approximately 35–37% (Figure 1).

Protein profile in 1D SDS-PAGE of the axillary buds info in the three cutting stage

The protein mixture obtained by the trichloroacetic acid (TCA)/acetone method adapted to sugarcane axillary buds (Maranho et al., 2018) showed good separation by one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D SDS-PAGE) for the three cutting stage, presenting a protein profile varying from 120 to 10 kDa (Figure 2). Specific proteins were observed in the axillary buds of sugarcanes from the first cut (red arrows) and the third and fifth cuts (black arrows). Despite not showing apparent proteins ranging from 220 kDa to 121 kDa and below 10 kDa on the electrophoresis gel, proteins in these regions were identified by tryptic digestion and injection into the mass spectrometer.

Identification of proteins by mass spectrometry

The number of proteins identified in the axillary buds of RB867515 differed at each cutting stage (Figure 3). Exclusive proteins were identified in the first (77), third (39), and fifth (44) cutting stages. A proteome reduction of 49.3% and 42.8% was observed in the third and fifth cuts, respectively, compared to that in the first cut. The total and differential proteomes at each cutting stage (first, third, and fifth) are shown in Tables S1, S2, and S3, respectively. The upregulated and downregulated proteins at the three cutting stages are shown in Tables S4, S5, S6, S7, S8, and S9.

Biological processes and subcellular location

Differential proteins from each cutting stage and the proteome shared between the third and fifth cutting stages were analyzed based on their biological processes and subcellular locations (Tables S10, S11, S12, and S13). The most common biological processes were carbohydrate metabolism, biosynthesis, folding, catabolism, translation of proteins, amino acid biosynthesis, response to biotic and abiotic factors, and plant resistance. The expression of proteins involved in the biological responses to biotic and abiotic stresses was increased with the advancement of the cutting stages:7.8%, 15.4%, and 18.2% in the first, third, and fifth cuts, respectively (Figure 4).

Most proteins were identified in the cytoplasm and cytosol (Figure 4). The cell wall, extracellular, and secreted proteins were prominent among the identified proteins, mainly in the differential proteome of the fifth cut and in the shared proteome between the third and fifth cuts.

Protein-protein interaction (PPI)-Network

The analysis in STRING v.11.0 databases of the differential proteins of each cutting stage and the proteomes shared between each cutting stage generated protein-protein interaction networks to identify the biological relationship between proteins and peptides (Figure 5). The highest number of interactions was observed between the proteomic proteins shared between all cutting stages (Figure 5D) and in the differential proteome of the axillary buds of sugarcanes at the first cutting stage (Figure 5X).

Discussion

The reduction in the sprouting rate of axillary buds indicated that the most marked reduction was observed from the first to the third cut, which may be related to the reduction in commercial productivity often reported for sugarcane. The survey of the Brazilian sugarcane crop in 2021/2022 (CONAB, 2021) indicated an average agricultural productivity of 99.90, 74.80, and 62.29 kg/ha for the first, third, and fifth cuts, respectively. Sugarcane productivity in Brazilian sugarcane fields during the 2021–22 harvest period showed a 25.12, 37.65, and 16.72% reduction between the first, third, and

fifth cuts, respectively. The sprouting rate of the axillary buds of ratoons is one of the most important factors for the agricultural productivity of a sugarcane field (Crusciol et al., 2010). Other factors, such as tillering, fertilization, and root systems (Matsuoka and Stolf, 2012) can also directly or indirectly determine agricultural productivity.

Under the experimental conditions of the present study, the sprouting rate markedly reduced from the first to the third cuts and from the third cut onward. Moreover, the sprouting rate remained relatively stable until the fifth cut. Therefore, from the third cut onward, other *in situ* factors, including the number of stalks and germinal buds remaining in the soil, tillering, stem thickness, and leaf area, are more determinants for the successive decrease in productivity from the third cut *in situ* under field conditions. These results showed that the metabolic/molecular alterations to which third-cut axillary bud-donor plants were subjected to the field persisted during bud sprouting under the experimental conditions in the absence of probable stresses that may be induced by conditions in the field.

The number of proteins identified at each cutting stage was reduced alongside a sprouting rate reduction from the first to the third cut. The highest number of specific proteins was detected in the axillary buds of first-cut sugarcane (77 proteins, 20.1% of the total identified proteome). In the axillary buds of the third and fifth cut sugarcanes (showing a reduction in sprouting rate), 10.2, and 11.5%, respectively, of the total proteome were detected. A reduction in the number of proteins in the axillary buds (Maranho et al., 2019; Benez et al., 2019) and set roots (Maranho et al., 2021) of fifth-cut canes have also been reported.

The PPI network of the axillary buds showed a greater number of interactions in the first cut sugarcane (Figure 5X) than in the third and fifth cuts (Figures 5Y and 5Z), suggesting that alterations in the protein constitution may be related to a reduction in the meristematic capacity of the axillary buds in successive cutting stages. Alterations in gene expression regulatory pathways by miRNAs, which act in the signaling of phytohormones in axillary buds, have been reported (Ortiz-Morea et al., 2013). However, changes in various biological processes of the differentially expressed proteins of the first cut sugarcane may harm the sprouting of axillary buds in subsequent cutting stages during the industrial cultivation of sugarcane.

The repression of some proteins involved in carbohydrate metabolism in the third and fifth cuts can negatively influence the sprouting of axillary buds during the sugarcane-cutting stages. The main proteins involved in carbohydrate metabolism exclusively detected in the first cutting stage were: α-1,4 glucan phosphorylase (EC:2.4.1.1); exhydrolase II; glucan endo-1,3-β-D-glucosidase (EC:3.2.1.39); chloroplastic β-glucosidase (EC:3.2.1.182); probable fructokinase-7 (EC:2.7.1.4), and L-lactate dehydrogenase A (EC:1.1.1.27).

Proteins involved in protein regulation, folding, and degradation, ribosomal protein components, and translational elongation factors were also observed in the proteome of the axillary buds of first-cut plants and were absent in the common proteome of the third and fifth cut sugarcanes. The T-complex protein 1, also known as chaperonin, contains T-complex polypeptide-1 (CCT) or T-complex polypeptide-1 ring complex (TRiC) is a eukaryotic cytosolic group II chaperonin (Ahn et al., 2019). T-complex protein 1 subunits gamma, epsilon, and zeta were detected only in the first cut axillary buds. The absence of these CCT subunits result in incorrect folding of proteins, including microtubules, drastically altering the growth and development of axillary buds and plants originating from budding. A decrease in stalk size, a reduction in leaf area, and cracks in stalks are phenotypic alterations often found in older sugarcane fields.

The 26S proteasome complex proteins were exclusively identified in the axillary buds of first cut plants and were observed in the group of proteins with the highest number of interactions with each other and with other proteins (Figure 5X; PPI network). The proteasome-ubiquitin system (UPS) provides an efficient and rapid strategy to control many different cellular processes by selectively removing regulatory proteins and thus plays a critical role in regulating various cytological and physiological processes (Xu and Xue, 2019). The removal of non-functional proteins from cells is crucial for maintaining homeostasis and physiological metabolic activities. Therefore, the functions of proteases are particularly important under stress conditions that induce protein damage or impairment (Ali and Baek, 2020). The absence of UPS proteins can decrease axillary bud sprouting from the third cut through the loss of protein turnover capacity, which consequently causes physiological and structural changes and an inefficient response to environmental factors of the axillary buds at the beginning of budding.

Six proteins of the 40S ribosomal subunit were also exclusively detected in the axillary buds of the first cutting stage and were downregulated from the third cutting stage onwards:40S ribosomal protein S3-1, 40S ribosomal protein S2-1, 40S ribosomal protein S3a, 40S ribosomal protein S5-2, 40S ribosomal protein S6-1, and 40S ribosomal protein S8. These ribosomal accessory proteins (RPs) are crucial for the formation of the 40S ribosomal subunit, which decodes mRNA during protein biosynthesis (Wang et al., 2019; Martinez-Seidel et al., 2020). In addition to their role in the maturation of ribosomal subunits, RPs are involved in plant development and responses to environmental changes (Williams et al., 2003). The 60S ribosomal protein L10 (RPL10), detected only in the axillary buds of first cut plants, is an essential component of the formation of the larger ribosome subunit (Ramos et al., 2020). A deficit in cytosolic ribosome assembly can lead to alterations in the regulation of post-transcriptional gene expression and protein synthesis during the early stages of sprouting after harvesting.

The proportion of proteins involved in the response to biotic and abiotic factors was higher in the axillary buds of sugarcane in the third (15.4%), fifth (18.2%), and both third and fifth (22.7%) cuts than in the axillary buds of the first cut plants (7.8%). Extreme variations in temperature and humidity, the incidence of agricultural pests and phytopathological microorganisms, and modern mechanized harvesting with soil compaction are factors that should stimulate the increase in stress protein synthesis over the years of vegetative propagation of sugarcane cultivated in ethanol and sugar mills. The upregulated proteins involved in the response to biotic and abiotic factors in the axillary buds of plants from the third cutting onwards can compose cellular mechanisms that ensure the survival of the plant in response to different stress factors. In contrast, some proteins involved in the response to biotic and abiotic factors were exclusively observed in the axillary buds of plants in the first cutting stage (upregulated in the first cutting stage). Aspartic proteases (APs; EC 3.4.2.3) are

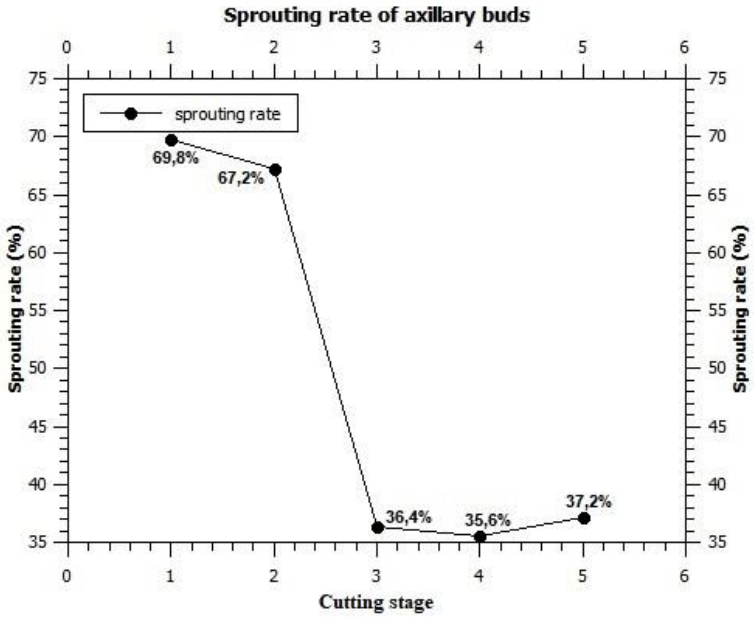

Fig 1. Sprouting rates of the axillary buds of cv. RB867515 at the first, second, third, fourth, and fifth cutting stages.

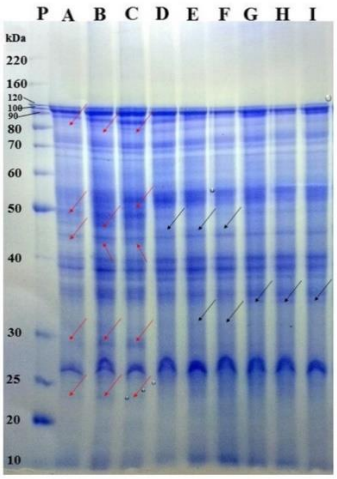

Fig 2. Total protein profile obtained by 1D SDS-PAGE. Red arrows indicate the protein fractions detected in the first cut sugarcanes. Black arrows indicate the protein fractions detected in the third and fifth cuts. P: molecular weight marker; A, B, and C: first cut; D, E, and F: fifth cut; G, H, and I: third cut. 1D SDS-PAGE, one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis.

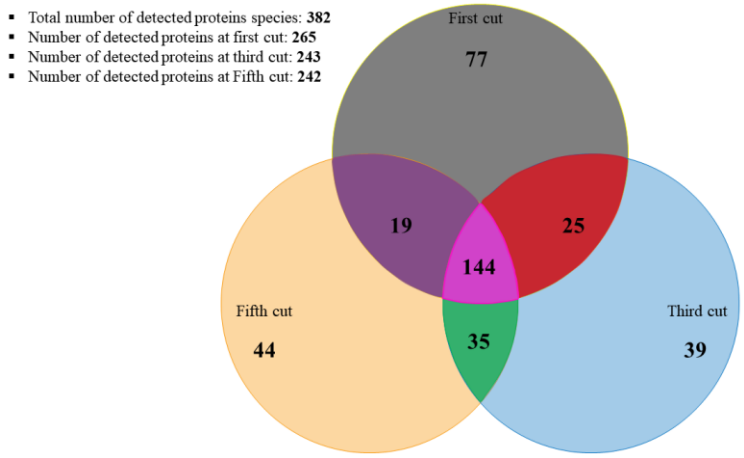

Fig 3. Venn diagram showing proteins identified by mass spectrometry in the axillary buds of cultivar RB867515 at the first, third, and fifth cutting stages. Exclusive proteins were identified in the first (77), third (39), and fifth (44) cutting stages, and 144 proteins in the three cutting stages. Twenty-five and nineteen proteins were identified in the first and third and first and fifth cutting stages, respectively, and thirty-five proteins were identified in the third and fifth cutting stages.

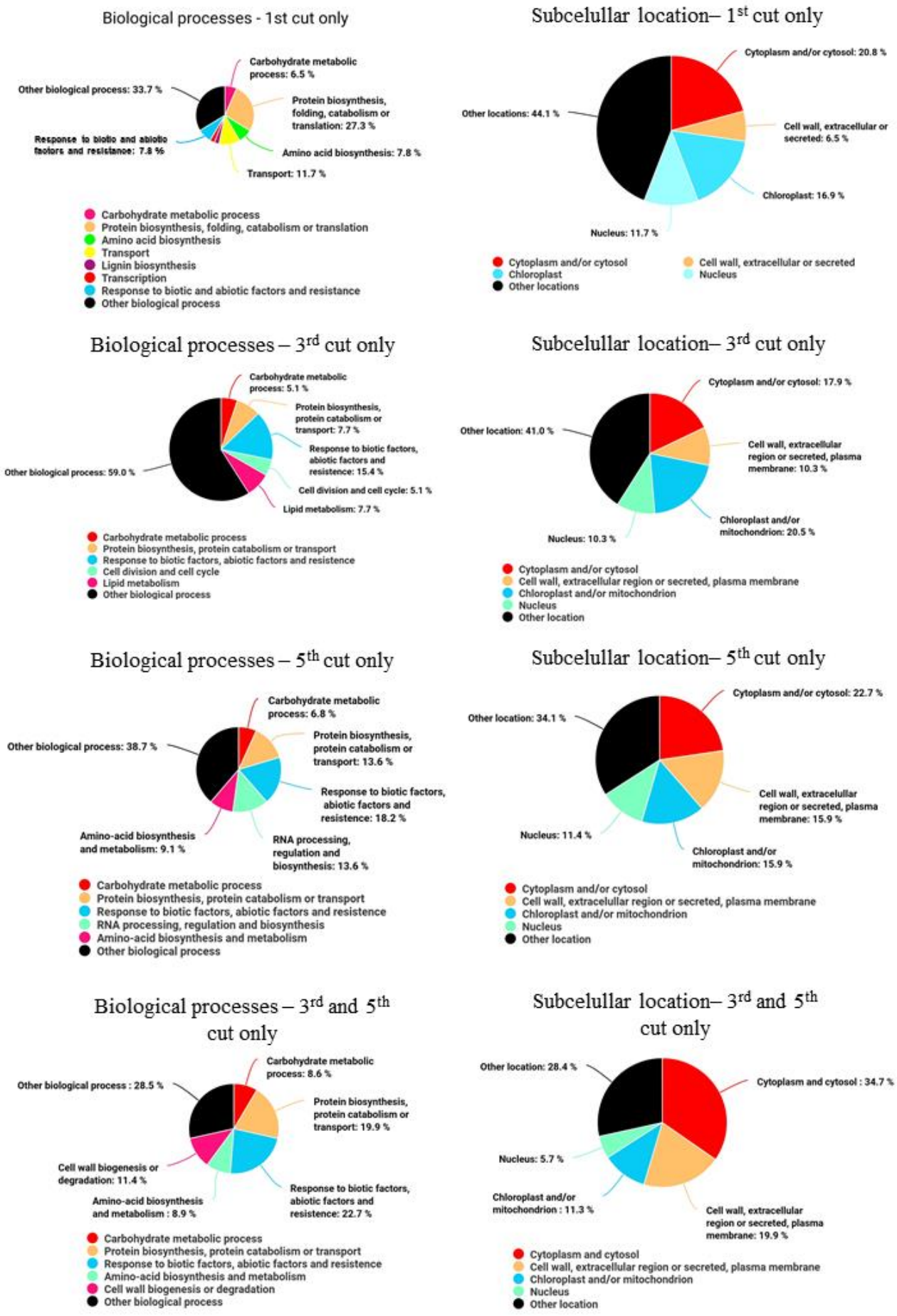

Fig 4. Biological processes and subcellular localization of proteins differentially expressed at each cutting stage (first, third, and fifth) and the differential proteome shared between the fifth and third cuttings.

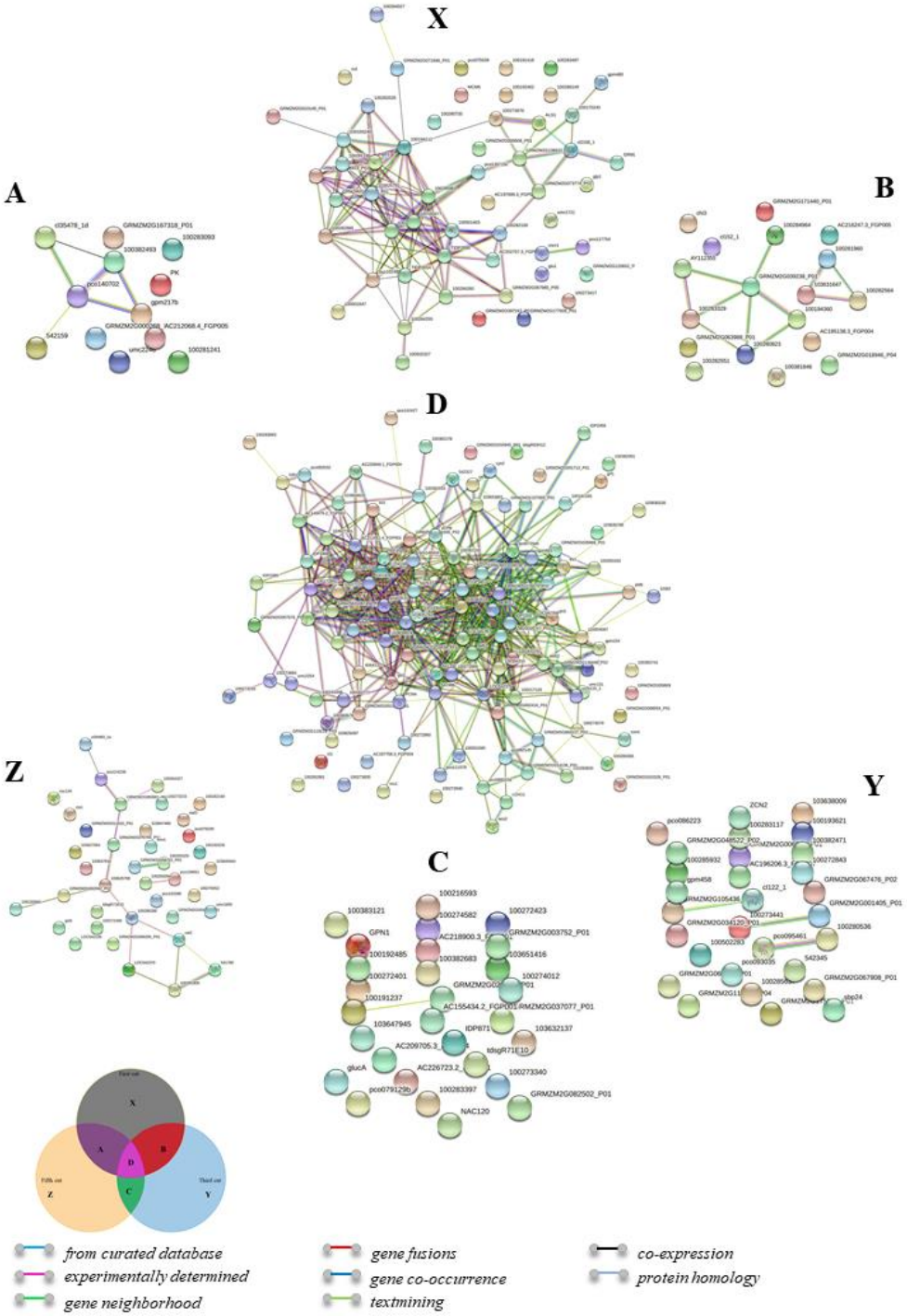

Fig 5. Protein-protein interaction network generated using STRING v.11.0 at the first (X), third (Y), and fifth (Z) cutting stages; exclusively at the first and fifth (A), the first and third (B), and the third and fifth (C) cutting stages; and in all cutting stages (D). PPI enrichment *p*-value: X= 3.2e-10; Y=0.788; Z=0.072; A=0.047; B=0.035; C=0.872; D= <1.0e-16, respectivelly. PPI, protein-protein interaction.

Figure 6. Sugarcane stalks from cv. RB867515 with axillary buds of different cutting stages (A); seed pieces planted in vermiculite (B), with ungerminated axillary buds (negative sprouting) (C); and with germinated axillary buds (positive sprouting) (D).

proteolytic enzymes involved in protein processing and/or degradation in different plant organs, as well as in plant senescence, stress responses, programmed cell death, reproduction, and with plant-pathogen interactions (Figueiredo et al., 2021). The superfamily of thioredoxin (TRX; EC 1.8.1.9) oxidoreductases, which are upregulated in the axillary buds of plants at the first cut, are involved in different plant metabolic processes (Mata-Perez and Spoel, 2019). In higher plants, the TRX system is involved in controlling plant metabolic development (embryogenesis, mobilization of seed reserves, chloroplast development, and carbon metabolism), phytohormone pathways, and responses to abiotic and biotic constraints (Jedelská et al., 2020). Receptor-like kinases (RLKs), exclusively detected in the axillary buds of plants at the first cutting stage, are characterized by extracellular leucine-rich repeat (LRR) domains that interact with various ligands and regulate a wide range of processes (Kang and Hardtke, 2016). Leucine-rich repeat kinases (LRRKs) represent the largest subfamily of receptor-like kinases (RLK) and are involved in diverse functions in plants, such as growth, development, and survival, including organogenesis, morphogenesis, hormonal signaling, and abiotic and biotic stress (Dievart et al., 2020).

Thus, the absence of APs, TRX, and RLKs in the axillary buds at the third and fifth cutting stages may suppress an efficient response to various biotic and abiotic environmental stresses. In addition, some proteins, such as L-lactate dehydrogenase A (LDH-A; EC:1.1.1.27) and Cinnamoyl CoA reductase 1 (CCR1; EC:1.2.1.44), are involved in stress responses and important biological processes, such as cell proliferation, fermentation, and lignin biosynthesis (Ghosh et al., 2015; Eprintsev et al., 2018; Chakraborty et al., 2019). The absence of LDH-A and CCR1 may restrict axillary bud development. The proteomic approach and evaluation of axillary bud sprouting in three cutting stages applied to the study of sugarcane longevity in industrial cultivation were relevant to show that a marked reduction in the sprouting rate, as well as in the number of proteins, was observed in the axillary buds of plants at the third cutting stage, before the fifth or sixth cutting stage, when productivity in the field became irrelevant. Identifying the essential proteins observed in the axillary buds of plants at the first cutting stage, which are absent in the axillary buds of plants at the third cutting stage, may be useful for increasing the longevity of cane fields. The restoration of the transcriptionally active state of genes that encode these essential proteins/enzymes in the axillary buds of first cut plants that are absent in the axillary buds of third cut plants may be a useful strategy for increasing the longevity of cane fields. The identification of target genes encoding essential proteins/enzymes that may be related to greater molecular stability of the axillary buds of sugarcane plants from the third regrowth cycle may be useful for future studies using molecular tools, such as gene editing by Zinc Finger Nucleases, Tallens or CRISPR-Cas, to verify the effects of overexpression or repression of proteins that may increase the longevity of sugarcane fields.

Materials and methods

Sugarcane plants of cv. RB867515

Stalks from sugarcane at the first, second, third, fourth, and fifth cutting stages of approximately 10-month-old cv. RB867515 were collected from the Nova Aralco Industrial (20°53'29.82"S/50°26'55.25"W) farm in the state of São Paulo, Brazil (Figure 6A). Sugarcane plants were cultivated in dystrophic argisol-type soil with low water availability and medium cation exchange capacity. Only axillary buds from the fourth to the ninth nodes were used to avoid the influence of auxins.

Experimental design and traits evaluation

For budding induction and sprouting rate determination, 129 individual axillary buds from each cutting stage were planted in vermiculite labeled 10-L trays, with a space of 3 cm between samples (Figure 6B). The axillary buds were irrigated every two days. Sprouting occurred in a greenhouse at 22 °C after five days. The sprouting rate was evaluated using a caliper and considered positive when the budding of the axillary buds increased by at least 6 mm (Figure 6C). The sprouting rate (Rs) was calculated using the following equation: Rs: Bs/Bp × 100, where Bp is the total number of axillary buds planted and Bs is the total number of axillary buds sprouted. The Rs values are shown as percentages (%).

After determining the Rs, 200 mg of axillary buds from the first, third, and fifth cutting stages were isolated using a scalpel, immediately frozen in liquid nitrogen, and stored separately in an ultra-freezer at –80 °C until use. Proteins were extracted in biological triplicates for each cutting stage: first, third, and fifth. Each replicate (200 mg) was macerated with liquid nitrogen and used for protein extraction, as reported by Maranho et al. (2018). Proteins were pre-separated using a 1D SDS-PAGE, as reported by Maranho et al. (2019), to visualize the extraction quality. After electrophoresis, each lane of the gel was completely fragmented into 11 parts and used for tryptic digestion, as described by Shevchenko et al. (1996). Gel fragments from each lane were divided into protease-free microtubes and washed with methanol. Proteins were extracted from the 1D gel twice using a sonicator for 30 min. The extraction solution was removed, centrifuged in a Sigma-4-16K centrifuge at 2.935x *g,* and dried in a vacuum desiccator. The 1DE-UPLC-ESI-QTOF mass spectrometry approach was used to obtain the proteome of the axillary buds of sugarcanes from the first, third, and fifth cuts following the protocol of Maranho et al. (2019).

The protein identification (class, identity, and number of proteins) based on acquired MS/MS spectra was performed using the Mascot tool from Matrix Science (http://www.matrixscience.com/) against Viridiplantae within SwissProt/UniProtKB (https://www.uniprot.org/uniprot/) and databases UP7305_Z_mays in Uniprot (https://www.uniprot.org/).

Statistical parameters used in Mascot

Contaminant proteins were excluded using a specific database available on the Mascot and PRIDE_Contaminants database. Proteins with at least one matching peptide and a score above the limit indicated by Mascot were selected, with ions showing the identity or extensive homology (p<0.05). The principle of strict parsimony was used to avoid redundancy, and only one protein per identified peptide was considered, according to the highest Mascot score. A false discovery rate (FDR) filter was applied with a maximum of 1%. The peptide tolerance was 50 ppm, and the tandem mass spectrometry (MS/MS) tolerance was 0.3 Da.

Bioinformatics

Functional annotations of differentially regulated (expressed) proteins and their subcellular locations were

accessed using the SIB Bioinformatics Resource Portal (http://www.expasy.org/proteomics) with the UniProtKB complete proteome (https://www.uniprot.org/) annotation project database. The *Zea mays* database available in the STRING v.11.0 tool (Szklarczyk et al., 2019) was used to create the PPI network. The minimum required interaction core was set at 0.400 (medium confidence) for all STRING analyses. The PPI network was used to verify which differentially expressed proteins were key elements in the metabolic pathways involved in the budding of axillary buds at different cutting stages.

Conclusion

Among other physiological and agronomic factors limiting the longevity of sugarcane fields, our study showed that a reduction in the sprouting rate of axillary buds in the third cutting stage and the absence of essential proteins/enzymes involved in *i*) carbohydrate metabolism, *ii*) folding and degradation of proteins (proteolytic functions), *iii*) 40S and 60S ribosomal subunits, and *iv*) proteins involved in biotic and abiotic stresses, are factors that may limit the longevity of cane fields from the third cut reducing the frequently reported agricultural productivity in sugarcane fields.

Data Availability Statement

The data supporting this study are available from the **jPOST repository** https://repository.jpostdb.org/. **Accession numbers**: JPST000331 and JPST000335 and **jPOST repository** https://repository.jpostdb.org/. **Accession numbers**: JPST000331 and JPST000335.

Supplementary Material

Table S1. Total and differential proteome of the axillary buds of the RB867515 sugarcane at the first cutting stage. **Table S2**. Total and differential proteome of the axillary buds of the RB867515 sugarcane at the third cutting stage. **Table S3**. Total and differential proteome of the axillary buds of the RB867515 sugarcane at the fifth cutting stage. **Table S4**. Differentially expressed proteins (downregulated) in axillary buds of the RB867515 sugarcane at the first cut compared to the third cutting stage. **Table S5**. Differentially expressed proteins (upregulated) in axillary buds of the RB867515 sugarcane at the third cut compared to the first cutting stage. **Table S6**. Differentially expressed proteins (downregulated) in axillary buds of the RB867515 sugarcane at the first cut compared to the fifth cutting stage. **Table S7**. Differentially expressed proteins (upregulated) in axillary buds of the RB867515 sugarcane at the fifth cut compared to the first cutting stage. **Table S8**. Differentially expressed proteins (downregulated) in axillary buds of the RB867515 sugarcane at the third cut compared to the fifth cutting stage. **Table S9**. Differentially expressed protein (upregulated) in axillary buds of the RB867515 sugarcane at the fifth cut compared to the third cutting stage. **Table S10**. The 77 proteins detected in the axillary buds of the RB867515 sugarcane at the first cutting stage classified into functional categories based on their biological process and subcellular localization. **Table S11**. The 39 proteins detected in the axillary buds of the RB867515 sugarcane at the third cutting stage classified into functional categories based on their biological process and subcellular localization. **Table S12**. The 44 proteins detected in the axillary buds of the RB867515 sugarcane at the fifth cutting stage classified into functional categories based on their biological process and subcellular localization. **Table S13**. The 33 proteins detected in the axillary buds of the RB867515 sugarcane at the fifth and third cutting stages classified into functional categories based on their biological process and subcellular localization.

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