# Sulfur metabolism in sugarcane is affected by high titers of Leifsonia xyli subsp. xyli

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# Abstract

**Aims** *Leifsonia xyli* subsp. *xyli* (*Lxx*) is the most common sugarcane bacterial pathogen that affects plant development and primary metabolism. For example, cysteine and methionine are sulfur-containing essential amino acids used for bacterial growth and the title of *Lxx* in sugarcane plants might affect sulfur metabolism. The goal of this study were to evaluate how the increase in bacterial titers affects nutritional status and sulfur metabolism in sugarcane.

**Methods:** The study was carried out with a susceptible sugarcane (*Saccharum officinarum*) genotype CB49260, with low and high *Lxx* titers, evaluating the mineral status and levels of primary metabolites.

**Results:** Plants with high *Lxx* titers increased leaf sulfur content (S) compared to plants with low *Lxx* titers where plants with high *Lxx* titers displayed increased levels of sulfate, sucrose, maltose, raffinose, shikimic acid, malate, putrescine, glycerol, and, erythritol but decreased levels of methionine and glutathione in leaves. In the culm, plants with high *Lxx* titers displayed increased contents of maltose but decreased levels of threonine, ornithine, phenylalanine and *myo*-inositol when compared with plants with low *Lxx* titers.

**Conclusions:** This study thus demonstrated that high bacterial titers increase sulfur demand in sugarcane. However, the increase in S content in the leaf did not result in higher sulfur assimilation, which was verified by increases sulfate level and decreases in methionine and glutathione levels. Therefore, our study showed that plant metabolism fails to meet the increased sulfur organic compound demand due to lower methionine and glutathione biosynthesis and methionine catabolism to putrescine biosynthesis in the leaves.


# Introduction

Ratoon stunting disease (RSD) is the main disease in sugarcane caused by the bacterium *Leifsonia xyli* subsp. *xyli* (*Lxx*). RSD drastically reduces sugarcane productivity by reducing plant growth and the number of culms, mostly in ratoon crops (Bailey and Bechet 1997). Disease symptoms are noticeable in ratoon crops when the *Lxx* increases in titer in the cane plant (Davis et al., 1988; Garcia et al., 2021). It is possible that many sugarcane fields present low bacterial titers that are not detected due to the plants being asymptomatic. Urashima and Silva (2017) found that in the center-south region of Brazil 67% of the sugarcane fields were infected by *Lxx*, and the sugarcane genotypes presented low bacterial titers. The absence of specific symptoms of RSD and the lack of symptoms in plants with low bacterial titers make diagnosing the disease difficult. Therefore, RSD spreads silently in the fields during harvesting due to cutting both infected cane and healthy plants (Young, 2018). If infected culms with high bacterial titers are planted, RSD severity can be greater (Garcia et al., 2021; Kashyap et al., 2021; Urashima, 2017). Heat treatment (50°C for 2 h) is one of the control methods that contributes by reducing the bacterial titers in plant material before planting (Andreato et al. 2022; Carvalho et al., 2016; Dias et al., 2019).

*Lxx* is a gram positive fastidious bacteria that contains few pathogenicity genes in its genome (Davis et al., 1984; Evtushenko et al., 2000; Monteiro-vitorello et al., 2004). *Lxx* cannot penetrate through natural openings and does not contain insect vectors to introduce them into xylem vessels (Panta et al. 2022). The bacteria is introduced into healthy plants during harvest by cutting infected material (Young 2018). *Lxx* colonizes and multiplies in the xylem vessel, systematically spreading to other plant organs, and can be found in the leaf mesophyll and phloem (Guo et al., 2019; Marques et al. 2022; Quecine et al., 2016).

RSD causes changes in the sugarcane growth, which are associated with transcriptional, proteomic, and metabolic changes due to the growth of bacterial titers in the plant (Cia et al. 2018; Castro-Moretti et al. 2021). The changes in sugarcane growth include reduced number and sizes of culm, as well as red coloring of the vascular bundles (Garcia et al., 2021; Kazeem and Ikotun, 2019; Young, 2018). The decreases in sugarcane growth are not associated with water deficit conditions caused by *Lxx* colonization (Garcia et al., 2021; Zhang et al., 2016). Rather, this defective plant growth is associated with changes in the hormone levels, such as decreases in gibberellin, auxin, and ethylene and increases in abscisic acid levels (Garcia et al., 2021; Zhang et al., 2016). The reduction in sugarcane growth is also associated with decreases in photosynthetic rates and sugar partitioning in the organs (Garcia et al. 2021; Zhang et al. 2016b). Sugarcane with high bacterial titers displayed a decrease in sugar partitioning from meristem regions and tillers (Garcia et al. 2021a; Marques et al. 2022). Adittionally, in severe RSD cases, photosynthetic rates are reduced due to the decrease in chlorophyll content and activities of phospho*enol*pyruvate carboxylase, NADP-malic enzyme, and pyruvate phosphate dikinase (Zhang, *et al.* 2016; Guo et al. 2019).

Sulfur is a constituent of organic molecules that perform several critical physiological functions, such as amino acids cysteine and methionine and coenzymes Acetyl-CoA, thiamine and biotin, and secondary metabolites glucosinolates and phytoalexins (Bloem et al. 2007; Koprivova and Kopriva 2016). Some pathogens alter the nutritional status of the plant, bringing physiological damage that leads to host susceptibility (Fatima and Senthil-Kumar 2015, Yang et al. 2022). For example, increased S assimilation makes the plant more resistant against vascular pathogens such as *Pseudomonas syringae* pv. *actinidiae*, *Verticillium dahliae*, *Fusarium oxysporum* and *Ralstonia solanacearum* (Williams and Cooper 2003; Bloem et al. 2005; Zhang et al. 2021). Sulfur content, for instance, is altered due to the colonization of vascular pathogens, and the increase in its assimilation may be related to a defense response (Williams and Cooper 2003).

The establishment of the *Lxx* in plant organs involves the expression of the genes of the antioxidant system and the acquisition of the amino acids cysteine and methionine by the plant (Faria et al., 2020; Monteiro-Vitorello et al., 2004). Since the survival of the bacteria in the plant requires the consumption of methionine and cysteine, it is likely that the growth of the bacterial titer will increase the demand for S in the plant. Castro-Moretti et al. (2021) showed that at 120 days after the inoculation of *Lxx* in sugarcane plants, there was an increase in leaf cysteine levels. In this study we hypothesized that the growth of the bacterial titer increases the sulfur demand in the plant and that plants with high bacterial titers would display a greater accumulation of sulfur compounds. However, it has not yet been investigated whether

*Lxx* colonization affects the nutritional status of sugarcane. Information on the changes nutritional status in sugarcane caused by RSD is essential to understand the progress of the disease in the field. The aims of our study were to: (i) evaluate changes in the nutritional status among plants with high and low *Lxx* titers and (ii) verify how the increase in bacterial titers affects sulfur metabolism in the plant.

## Material And Methods

# PLANT MATERIAL

The study was carried out on RSD asymptomatic and symptomatic sugarcane (*Saccharum* spp.), using the genotype CB 49260. Plants were maintained in greenhouse conditions (30°C and 70% relative humidity) at the Department of Plant Pathology and Nematology, “Luiz de Queiroz” College of Agriculture (ESALQ-USP) in Piracicaba, Brazil (22°42'32"S, 47°37'45" W, elevation 546 m). The asymptomatic and symptomatic plants were obtained from culms of 586 days-old plants as described by Garcia et al. (2021). The symptomatic and asymptomatic culms were cut into mini culms containing one bud each, using a disinfected cane knife, and immersed in a bactericide solution (12.5% Benzalkonium chloride, 1 mL liter$^{-1}$ of water). Culms were planted in a commercial substrate (Basaplant) in a 32-cell tray. The plants were transplanted to 6 L pots containing the same substrate amended with fertilizer (7.5 g of 10-10-10 NPK and 7.89 g of ammonium sulfate) at 22 days after planting (DAP). Pots were watered daily throughout the experiment to the field capacity. At 60 DAP bacterial titers were quantified from a leaf sheath according to Young et al. (2017). Bacterial titers in the plant was performed by qPCR according to Carvalho et al. (2016). By qPCR analysis a 13-fold difference was found between plants with high (2090 cells) and low (262 cells) *Lxx* titers, respectively, for plants with and without RSD symptoms (Garcia et al. 2021). At 127 DAP, leaf and culm samples of the main shoot were put in liquid nitrogen and stored in a refrigerator at 80°C.

# MACRONUTRIENT QUANTIFICATION IN SUGARCANE LEAVES

The quantification of macronutrients was performed in the third leaves totally expanded (leaf + 3) of the main shoot (Malavolta, 1997), using 0.5g of dry matter (DM) placed in test tubes with the addition of nitro perchloric acid (1/2 v/v) and incubated overnight. Leaf samples were digested by incubating them in heating blocks, where the temperature was slowly increased in the block until reaching 210°C for 120 min spectrophotometry (Malavolta, 1997). After digestion, the samples were resuspended in 50 mL of distilled water in a falcon tube, and 5 mL of each sample was used for quantification of macronutrients and micronutrients in inductive coupling plasma (ICP-OES, Thermo Scientific, model iCAP 6200). The accuracy and precision of the analytical method were assured by the use of standard reference materials – SRM (NIST 1515 – apple leaves, and NIST 1573a – tomato leaves). Elemental recovery from SRM ranged from 95–98%.

# QUANTIFICATION OF SULFATE CONTENT ON THE LEAF

The sulfate content was quantified by spectrophotometry (Malavolta, 1997). Briefly, 10 mL of the leaf digestion extract was mixed with 1 mL of HCL solution containing 20 mg $L^{-1}$ of S. Then 500 mg of $BaCl_2.H_2O$ crystals were added to the solution and incubated for 1 minute at room temperature. Samples were read in a spectrophotometer at 420 nm. Sulfate content was determined by an S standard curve (0–50 mg $L^{-1}$).

# QUANTIFICATION OF REDUCED AND OXIDIZED GLUTATHIONE

Quantification of reduced and oxidized glutathione was obtained by spectrophotometry (Lima et al. 2018). 0.8 g of fresh tissue of first leaves totally expanded (leaf + 1) of the main shoot, was homogenized with 5% sulfosalicylic acid (1 mL) and centrifuged at 10,000 g at 4°C for 20 minutes. 200 µL of the supernatant was transferred into a new eppendorf, and 1.8 mL of 100 mM potassium phosphate, 0.5 mM EDTA (pH 7) and 100 µL of 3 mM Ellman's reagent (DTNB) were added. The samples were left in the dark for 5 minutes before the readings in a spectrophotometer at 412 nm. Then, 100 µL of 0.4 mM of NADPH and 2 µL of GR (205 U $mg^{-1}$) were added to the sample and kept in the dark for 20 minutes before the second reading. Glutathione quantification was determined by GSH standard curve (0–1 mM).

# METABOLOMICS

The first leaves totally expanded (leaf + 1) and culm of the main shoot were harvested and stored in liquid nitrogen, transported to lab and macerated in liquid nitrogen. Four biological replicates per treatment were used in the metabolomic analyses, according to Caldana et al.(2013). To 50 mg of frozen plant material, 700 µL of methanol was added before incubating the material for 1 h under agitation at 70°C. Then the samples were centrifuged at 12,000 x g, and the supernatant was removed. Chloroform (300 µL) and water (300 µL) were added to the collected supernatant to separate the polar and apolar phases. Then 1 mL of the polar phase was taken and dried. Ribotol was added as an internal standard and derivation, and analysis of metabolites in the samples was performed exactly as described by Lisec et al. (2006).

# QUANTIFICATION OF MALONOALDEYDE CONTENT (MDA)

The evaluation of lipid peroxidation in the The first leaves totally expanded (leaf + 1) and culm of the main shoot was carried out according to Heath and Packer (1968). Briefly, 0.2 g (200 mg) of fresh plant material (shoot and stem) was macerated in 2 mL of 0.1% TCA, containing PVPP. The macerated tissue was transferred to an eppendorf and centrifuged at 13,000 g for 10 minutes at 4ºC. Then, 1 mL of TCA (20%, containing 0.5% thiobarbituric acid) was added to 0.250 mL (250 uL) of the supernatant and incubated at 95°C for 30 minutes. Each sample was cooled on ice and then left in the dark for 15 minutes at room temperature. Readings were performed in a spectrophotometer at 532 and 600 nm. Knowing that ε = 155 $mM^{-1}$ $cm^{-1}$, determinations of MDA concentrations were performed using the equation: ABS (532–600) /ε155 = X (in mM $L^{-1}$.); X/1000 = X in mM $mL^{-1}$.

# GROWTH MEASUREMENTS

The plant material was harvested and separated into main culm, leaves, tillers, and roots and kept in oven at 60°C for 3 days. Then, dry biomass of the main culm, leaves, shoots (main culm + leaves), tillers, roots, and total plant biomass (shoot + tillers + roots).

# STASTISTICAL ANALYSIS

The experiments were organized in a completely randomized design, and data were submitted to analysis of variance (ANOVA) using the Software Sisvar (Ferreira 2011). The differences between the means were performed using the t test ($p < 0.05$).

## Results

# HIGH BACTERIAL TITERS CHANGES NUTRIENT LEVEL IN SUGARCANE

The level of bacterial titer affected the nutritional status of the plants (Fig. 1A-F). Plants with high *Lxx* titers presented an increase of 26% in S, 13% in K, and 22% in Mg compared to plants with low *Lxx* titers (Fig. 1A, B and C). On the other hand, plants did not show a difference in the levels of N ($p = 0.12$; Fig. 1D), Ca ($p = 0.056$; Fig. 1E) and P in the leaf ($p = 0.55$; Fig. 1F).

## Amino Acids, Gluthathione, Sulfate And Mda Contents In The Leaf

The high bacterial titers reduced the levels of the amino acids (-21%), and total glutathione (-38%) on the leaves compared to the plants with low *Lxx* titers ($p < 0.05$; Figs. 2A-F ). The oxidezed glutathione, and reduced glutathione contents in did not show a difference in function level bacterial titers in the leaves. On the other hand, the content of sulfate was 210% higher in the plants with high *Lxx* titers, suggesting that high bacterial titers reduced sulfur assimilation in sugarcane leaves. In adittion, the high bacterial titers increased in stress oxidative in leaf, evidencing by higher MDA contents when compared sugarcane with low *Lxx* titers.

## Amino Acids, Gluthathione, And Mda Contents In The Culm

The level of bacterial titers also affected the levels of the glutationa, amino acids, and MDA in the culm ($p < 0.05$; Figs. 3A-E ). In the culm, plants with high *Lxx* titers had 49% less amino acid content compared to plants with low *Lxx* titers. The oxidezed glutathione, reduced glutathione and total glutathione contents in did not show a difference in function level bacterial titers in the culm. Additionally, high bacterial titers in sugarcane increase 28% MDA contents when compared to plants with low *Lxx* titers.

## Changes In Metabolite Levels In Sugarcane With High Bacterial Titers

A total of 43 compounds: 17 amino acids, 14 organic acids, six sugars, four polyols and two miscellaneous compounds, were identified in the metabolite profiles of leaves and culms of sugarcane. From these 43 compounds, 19 metabolites were altered by bacterial titer levels in the leaf and culm (Fig. 4). The sugarcane with high *Lxx* titers displayed lower contents of amino acids in the leaf, which was especially prominent for glutamine, homoserine, methionine, and valine (Fig. 4). The sugar content in the leaf was higher in plants with high *Lxx* titers than low *Lxx* titers. Plants with high Lxx titers displayed higher sucrose, maltose and raffinose contents than those with low *Lxx* titers (Figs. 5). However, trehalose content was decreased in plants with high *Lxx* titers. The shikimic acid, malate, putrescine, glycerol, and erythritol levels increased in plants with high *Lxx* titers, whereas *myo*-inositol decreased when compared to plants with low *Lxx* titers (Figs. 5). In the culm, plants with high *Lxx* titers showed increased maltose content and decreased levels of threonine, ornithine, phenylalanine and myo-inositol when compared with plants with low *Lxx* titers (Figs. 6).

# GROWTH MEASUREMENTS

The plants with high *Lxx* titers showed higher biomass accumulation on main culm, leaves, and shoots, when compared to plants with low *Lxx* titers. Plants with high *Lxx* titers had a higher tillers biomass than plants with high *Lxx* titers. The root biomass and total biomass did not differ by bacterial titers (Table 1).

Table 1
Biomass in main culm, leaves on main culm, main shoot, tillers, roots, and total biomass in sugarcane genotype CB 49260 with low and high *Lxx* titers. Statistical difference was represented by * $p < 0.05$ (n = 6 ± SE)

| Organs | Low *Lxx* titers | High *Lxx* titers |
|---|---|---|
| Main culm (g) | 34.71 ± 5.67 | 60.42 ± 7.37 * |
| Leaves on main culm (g) | 20.73 ± 1.85 | 35.67 ± 2.94 * |
| Main shoot (g) | 55.44 ± 6.79 | 96.09 ± 9.02 * |
| Tillers (g) | 55.55 ± 4.27 | 38.85 ± 6.19 * |
| Roots (g) | 35.24 ± 3.17 | 34.35 ± 3.31 |
| Total Biomass (g) | 146.23 ± 6.74 | 169.29 ± 12.98 |

## Discussion

Ratoon Stunting Disease (RDS) is one of the most important diseases that sugarcane growers face, as the disease reduces sugarcane productivity by limiting both growth and the number of culms. RSD symptoms are hard to diagnose unless the plants have high bacterial titers (Garcia et al. 2021), and sometimes RSD symptoms are confused with nutritional deficiency (Young, 2016). In this study, we

demonstrated that sugarcane plants with high *Lxx* titers have their nutritional status and sulfur metabolism affected, showing increased sulfur content in the leaves but low sulfur content assimilation.

The increase in the levels of S in the leaves and culm was caused by enhanced bacterial titers in sugarcane. The increased sulfur demand in the plant may be correlated with the decreased content of free amino acids, methionine and glutathione (Figs. 2 and 5). That suggests that there is an increase in the S demand for organic sulfur compounds in leaf caused by higher acquisition of cysteine and methionine by bacteria, as *Lxx* do not synthesize these amino acids (Monteiro-Vitorello et al. 2004). Previous studies described that plants inoculated with *Lxx* have an increased content of free amino acids in their tissues (Zhu et al. 2017; Castro-Moretti et al. 2021). Castro-Moretti et al. (2021) observed that asymptomatic sugarcane plants CB 49260 (the same variety used in this study) displayed an increase in cysteine level after 120 dai with *Lxx*. In our study, plants with high *Lxx* titers (symptomatic plants) had a higher sulfur content than plants with low *Lxx* titers (asymptomatic plants). The growth of bacterial titers throughout sugarcane growth requires an increase in the biosynthesis of cysteine and methionine, which caused an increase in S absorption by the plants.

Although the level of S was higher in plants with high *Lxx* titers than plants with low *Lxx* titers, there was a incrase sulfate level and a reduction in the levels of amino acids, methionine and glutathione in the leaf. The nutritional and physiological disorders in the plant were detected and associated with different plant pathogen infections, which affected the absorption or assimilation of nutrients (Fatima and Senthil-Kumar, 2015; Martins et al. 2015). In S assimilation, the sulfate absorbed by the root is transported to the leaf, where it is assimilated in bundle sheath cell through synthesis of the cysteine (Kopriva and Koprivova 2005; Weckopp and Kopriva 2014; Jobe et al. 2019). Cysteine is the first product of sulfur assimilated and is used as a substrate to synthesize methionine and glutathione (Kopriva and Koprivova 2005b; Jobe et al. 2019). The high sulfate level attached with low contents of methionine and glutathione suggested an decrease sulfur assimilation in the plant with high *Lxx* titers. The high S level in the leaf associated with low levels of sulfur organic compound also may be explained by higher synthesis of putrescine in the leaf (Fig. 7), since methionine is a precursor to putrecine biosynthesis (Heidari et al. 2020).). *Lxx* does not contain enzymes for encoding spermine, which is one of the essential polyamines for adaptation of microorganisms to the host (Cia et al. 2018). Putrescine is also a polyamine may be a metabolic signal related to the increase in the bacterial titer *Lxx* in sugarcane, being a metabolite that favors colonization in leaf tissues. Studies showed that putrescine is to virulence of bacteria in plants and for the development of the morphological structures of fungus (Vilas et al. 2018; Sánchez-Elordi et al. 2019), as it is also associated with a defense response against pathogenic microorganisms. Putrescine is an essential metabolite for disease severity, such as bacterial wilt in tomato caused *by Ralstonia solanacearum* (Lowe-Power et al. 2018). In *Pseudomonas syringae*, the absence of putrescine inhibits the growth of the bacteria in the plant, while the accumulation of putrescine in the apoplast favors colonization in the leaf (Vilas et al. 2018).

**Can the reduction in sulfur content in sugarcane be linked to the susceptibility of sugarcane to disease?**
Sulfur is an important nutrient involved in plant defense against vascular pathogens (Willians and

Copper, 2003). Increases in sulfate and S assimilation have been shown to increase plant resistance against pathogens, causing physiological changes and increasing enzyme activities related to the antioxidant system and secondary metabolism (Chen et al. 2007; Gu et al. 2021; Yang et al. 2022). Gu et al. (2021) showed that sulfur induces resistance against cankers by increasing phenolic compounds caused by higher activities of phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase. The upregulated expression of high affinity sulfate transporters associated with higher synthesis sulfur organic compounds increases tomato resistance against *Verticillium dahliae* (Fu et al. 2016). In our study, an increase in sulfur in the leaves did not result in an increase in sulfur organic compound. By contrast, there was a decrease in the methionine and glutathione levels. The decrease in glutathione level may be a signal of the susceptibility of the sugarcane to *Lxx* colonization. Glutathione is essential to maintain cellular redox homeostasis, mostly when plants are subject to biotic and abiotic stresses (Lu et al. 2022; Madhu et al. 2022). The reduction in glutathione levels in the plants with high *Lxx* titers caused an increase in oxidative stress, indicated by higher MDA levels in both leaves and culms. Studies have shown that susceptible plant genotypes displayed decreased glutathione content after infection with bacteria, fungi and virus (Zechmann 2020). Low glutathione and cysteine contents are associated with the susceptibility of tomato to colonization by *Verticillium dahliae* (Willians and Copper, 2003). High glutathione levels improve the defense system through upregulation of the key genes *PR-1a* and *PR5* (Chen et al. 2007).

**Sulfur metabolism may be affected by changes sugar metabolism in sugarcane with high** Lxx **titers**

Plants with high *Lxx* titers showed the highest sugar contents in the leaf and culm when compared with plants with low *Lxx* titers. Plants with high *Lxx* titers also showed the highest sucrose, maltose in the leaf and maltose in the culm, which occurred by reduced sugar partitioning to tillers production (Garcia et al., 2021). Symptomatic plants presented higher biomass in main culm, which caused higher sugars storage in parenchyma of the culm due to the lower tillers biomass (Garcia et al., 2021; Saez et al., 2019). However, the plants with high *Lxx* titers showed a decrease in trehalose content on the leaf when compared to low *Lxx* titers. Castro-Moretti et al. (2021) reported that susceptible plants displayed 10 times higher trehalose content than resistant genotypes. The decrease threalose contents is caused by increase bacterial titers in the plant. Trehalose may be the mostly sugars acquired in plants by bacterium though ABC transporters (Monteiro-Vitorello 2004).

The decreases in sulfur assimilation may also be caused by decreases in carbon skeletons and reduced power produced in photosynthesis and respiration. The increase in respiration rate in plants with high *Lxx* titers may be caused by the decrease in free amino acid levels (Garcia et al. 2021). Possibly, nutritional disorders have been more evident in plants with high *Lxx* titers in winter conditions and water deficit conditions due to lower sulfur assimilation, caused by decreases in photosynthesis (Takahashi et al. 2011; De Souza et al. 2018; Jobe et al. 2019). Therefore, plant with high *Lxx* titers might have affected S status by increasing the demand for products of sulfur assimilation or by reducing sulfur assimilation in plants due to the photosynthesis limitation.

# Conclusion

Our study showed the high bacterial titers in sugarcane invoked nutritional and metabolic changes in the plants. Surprisingly, we observed that plant metabolism fails to meet the increased sulfur organic compound demand caused by the growth of bacterial titers. Plants with high bacterial titers decreased sulfur organic compound due to low S assimilation and high methioninte catabolism to putrecine biotynthesis. This observation is an important tool to understand the mechanism by which the disease affects plant nutritional status. Further studies will be needed to investigate whether changes in nutritional status may be associated with plant disease resistance.

# Declarations

### AUTHOR CONTRIBUITIONS

FHSG designed the experiments, FHSG, APDJ, MN, SP, and JF performed the experiments, FHSG and SJM wrote the manuscript, FHSG and SP analyzed the data, FHSG and SP created and edited figures, FHSG, APDJ, MN, SP, JL, SJM, ARF, and RAK revised this draft by rewriting, discussing and commenting. All authors read and approved the manuscript.


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### CONFLICT OF INTEREST

The manuscript does not present any kind of conflict of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

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# Figures

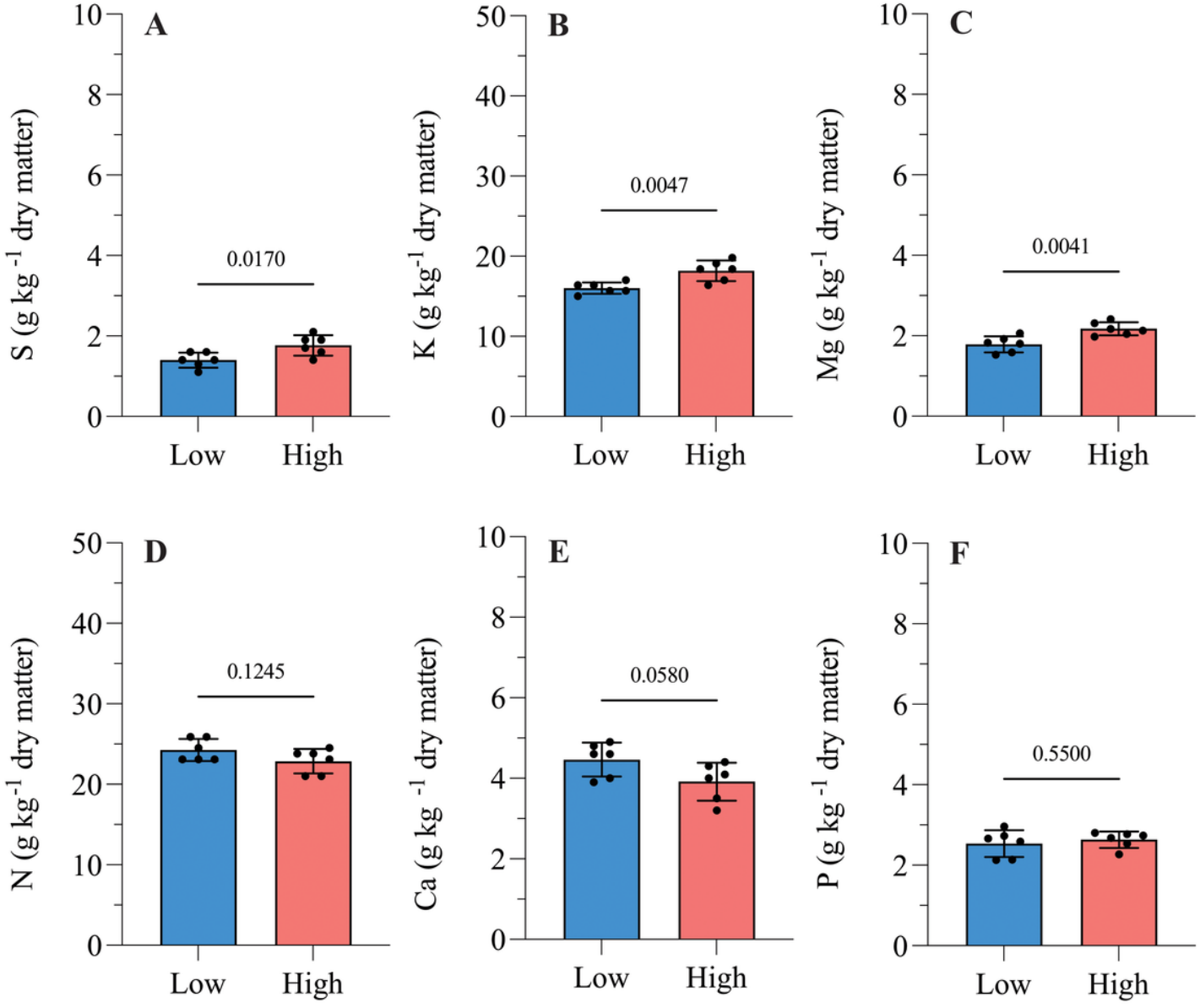
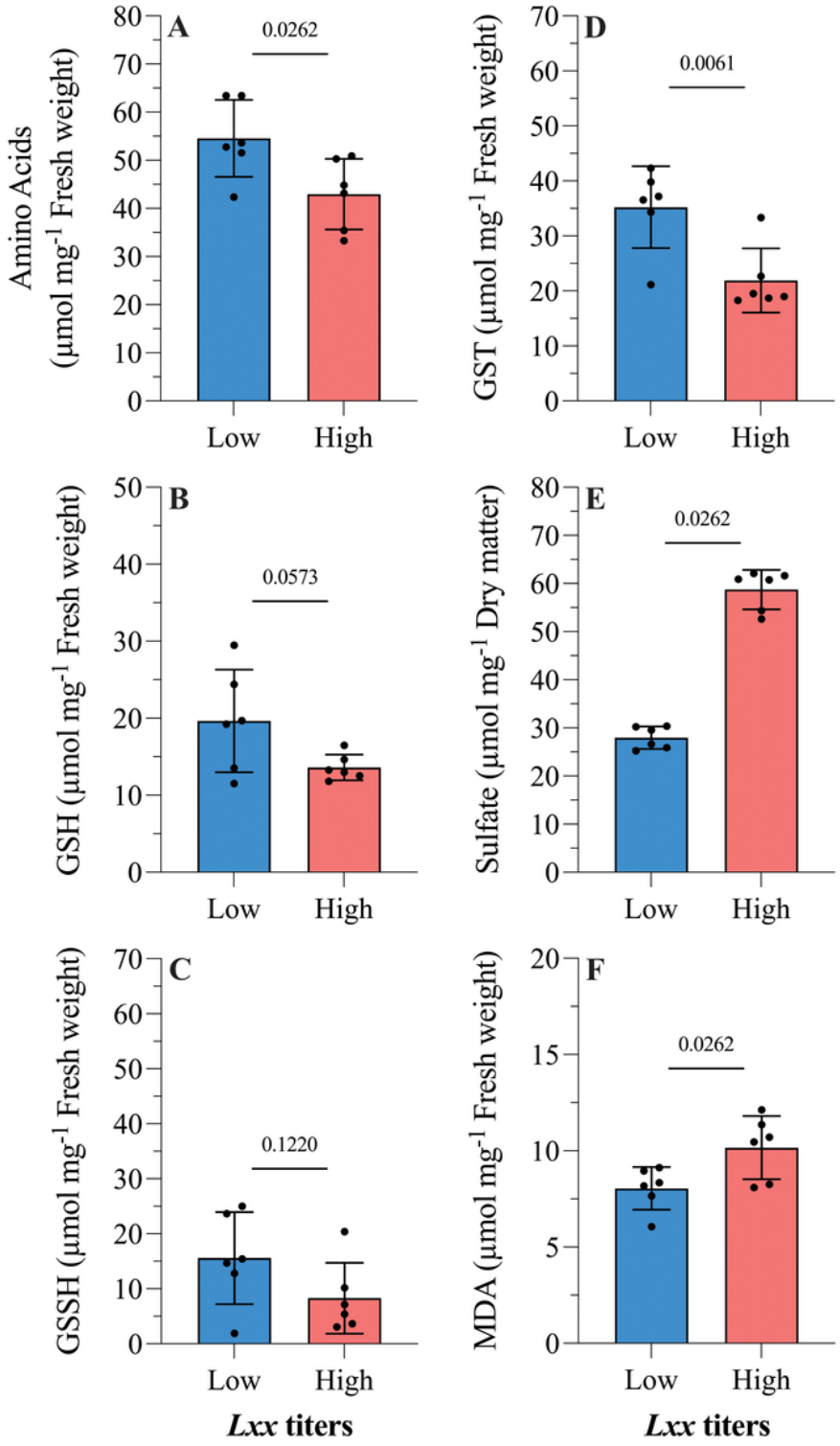


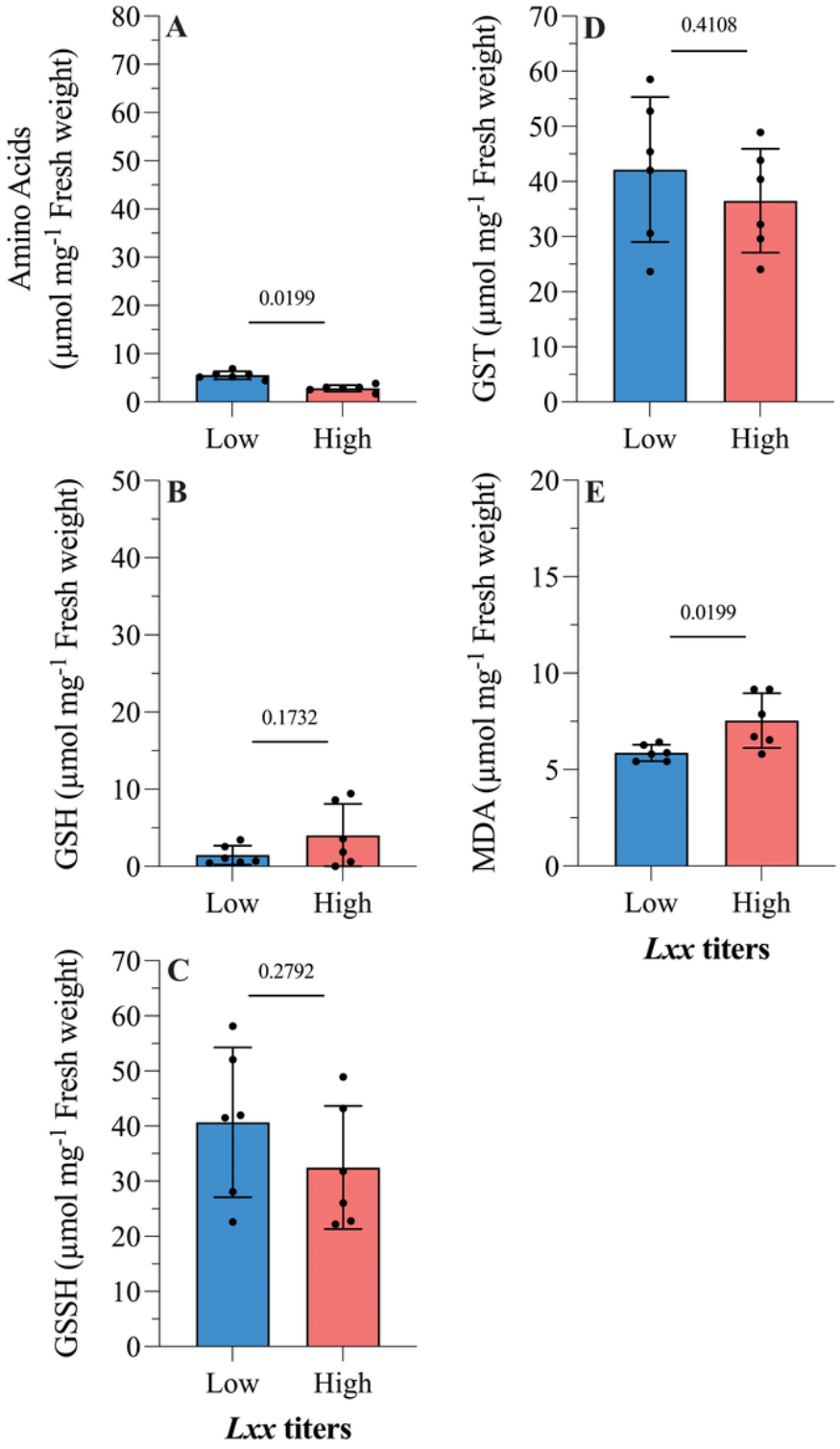

**Figure 1**

Sugarcane genotype CB 49260 with with low and high *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers. Leaf concentration of (A) S , (B) K, (C) Mg, (D) N, (E) Ca, (F) P. Mean values (n = 6 ± SE) differed for the t test at $p < 0.05$.



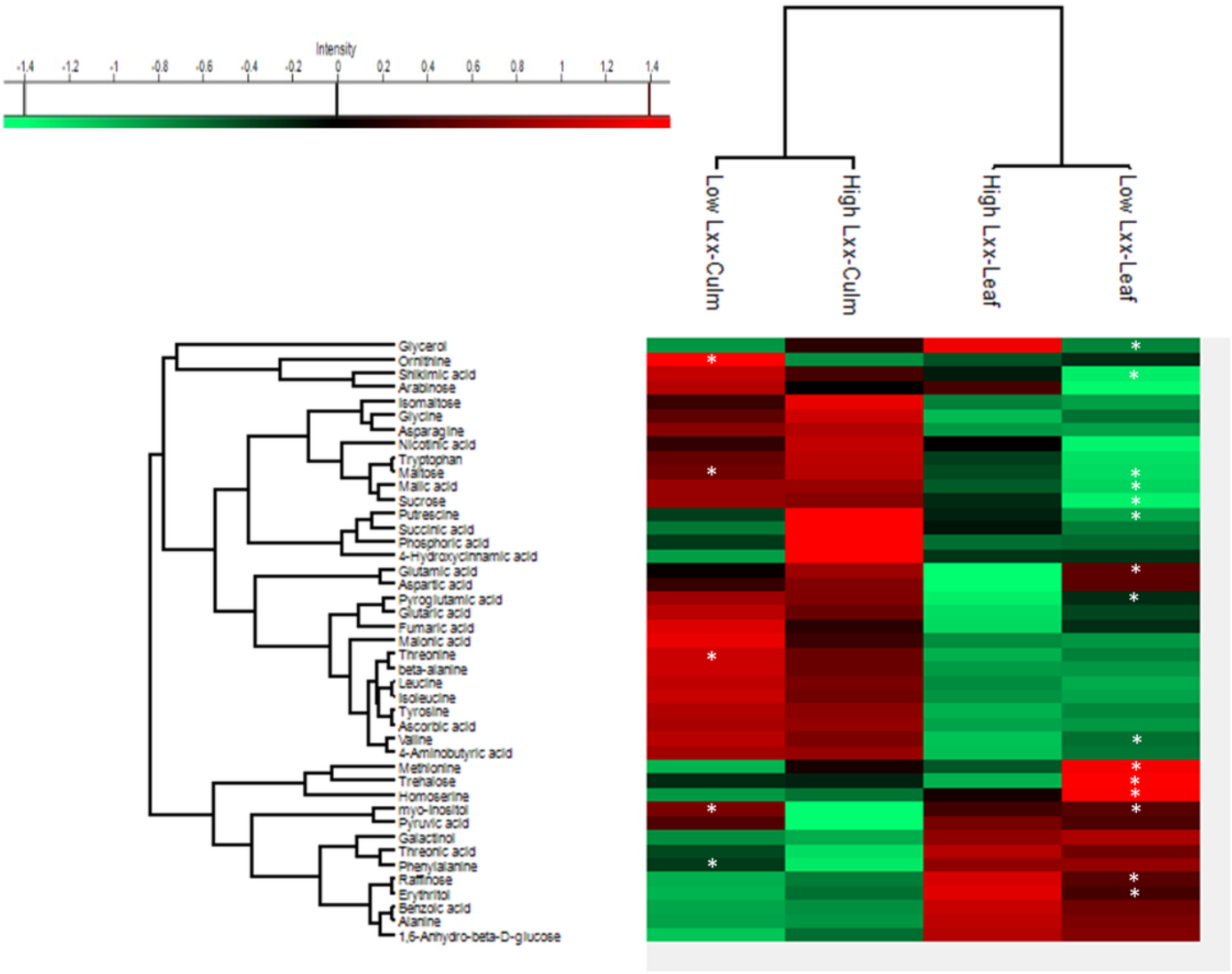

## Figure 2

Physiological anaylsis on sugarcane leaves genotype CB 49260 with low and high *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers. A) Amino acids, B) GSH (reduced glutathione), C) GSSG (oxidized glutathione), D) GST (total glutathione), E) Sulfate F) MDA (Malonoaldeyde). Mean values (n = 6 ± SE) differed for the t test at $p < 0.05$.



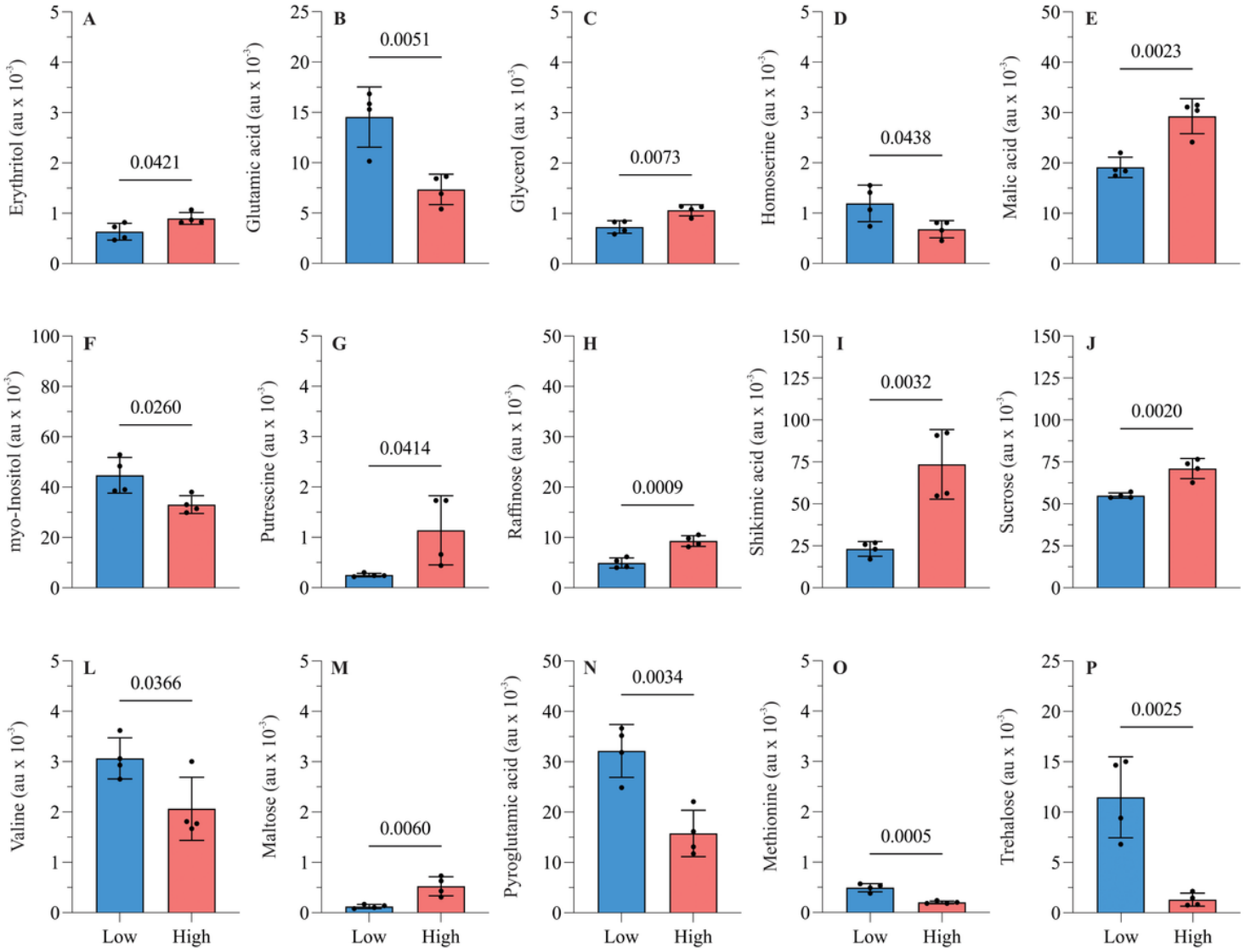

## Figure 3

Physiological anaylsis on culm in sugarcane genotype CB 49260 with low and high *Leifsonia xyli* subsp.*xyli* (*Lxx*) titers. A) Amino acids, B) GSH (reduced glutathione), C) GSSG (oxidized glutathione), D) GST (total glutathione), E) MDA (Malonoaldeyde). Mean values (n = 6 ± SE) differed for the t test at $p < 0.05$.



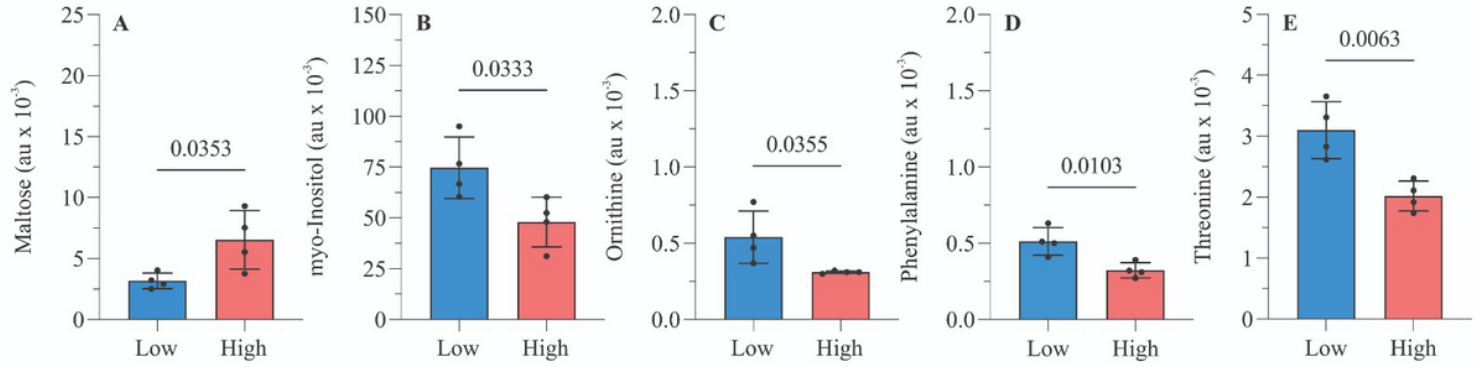

## Figure 4

The heat map showing the metabolite contents in the leaf and culm of plants with high and low *Leifsonia xyli* subsp. *xyli*(*Lxx*) titers was built using Log2 Fold Change of relativized medians using perseus. Red indicates higher relative values, whereas green indicates lower relative values. Statistical difference was represented by * $p < 0.05$ ($n = 4 \pm SE$).




## Figure 5

Difference between metabolite levels in the leaf between plants with high and low *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers. Mean values (n = 4 ± SE) differed for the t test at $p < 0.05$.




## Figure 6

Difference between metabolite levels in the culm between plants with high and low *Lxx* titers.Mean values (n = 4 ± SE) differed for the t test at $p < 0.05$.

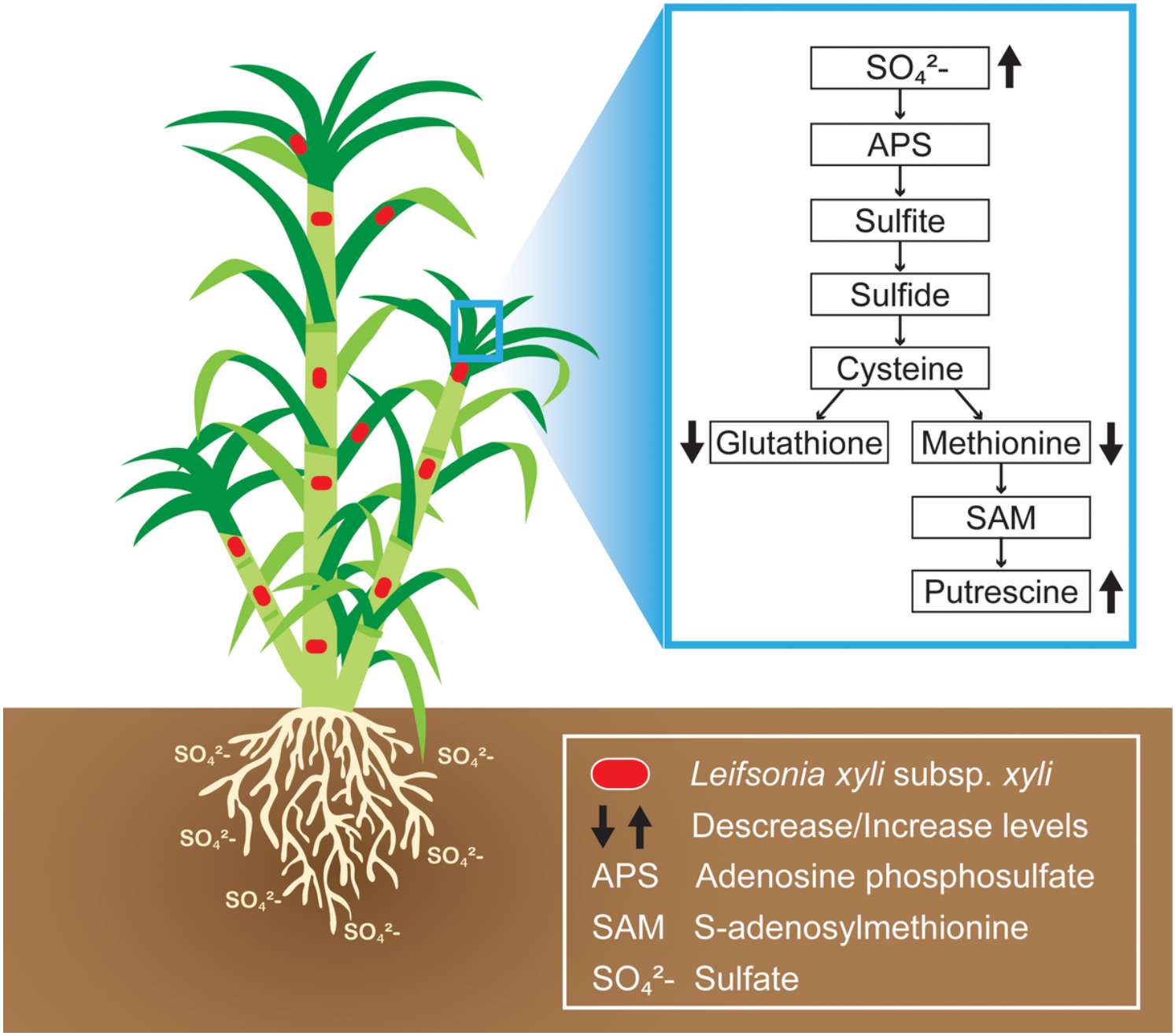



**Figure 7**

Changes in sulfur metabolism caused by high *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers in sugarcane plants