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RESEARCH ARTICLE

Impact of *Leifsonia xyli* subsp. *xyli* titer on nutritional status, and metabolism of sugar cane

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Abstract

Aims Sugarcane plants infected with *Leifsonia xyli* subsp. *xyli* (*Lxx*) have their primary metabolism affected with decreased levels of sugars and amino acids. Cysteine and methionine are sulfur-containing essential amino acids used for bacterial growth and the *Lxx* titer in sugar cane leaves could affect the animo acid concentrations. The goal of this study was to evaluate how the increase in *Lxx* titer affects the nutritional status and sulfur metabolism in sugar cane leaves.

Methods Susceptible sugar cane (*Saccharum officinarum*) genotype: CB49260 was used in this study with low (256 cells) and high (2090 cells) *Lxx* titers and macronutrients and primary metabolites assessed from leaves and culms.

Results Plants with high *Lxx* titers accumulated more biomass in the main culm, leaves, and shoots than plants with low *Lxx* titers. Additionally, plants with high *Lxx* titers had 26% more sulfur content in leaves than plants with low *Lxx* titers. Higher levels of sulfate, sucrose, maltose, raffinose, shikimic acid, malate, putrescine, glycerol, and, erythritol were also present in plants with high *Lxx* titers; but decreased

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levels of methionine and glutathione in leaves. In the culm, plants with high *Lxx* titers also had increased levels of maltose; but decreased levels of threonine, ornithine, phenylalanine and *myo*-inositol when compared with plants with low *Lxx* titers.

Conclusions This study demonstrated that high bacterial titers increase sulfur demand in sugar cane; however, the increased S content in the leaf did not result in higher sulfur assimilation, verified by increased sulfate but decreased methionine and glutathione levels. Therefore, our study showed that lower methionine availability, and methionine catabolism to putrescine in the leaves may fail to meet the increased sulfur organic compound demand of *Lxx*. The decrease in glutathione biosynthesis may reflect impaired biosynthesis or a drain on this antioxidant resulting from oxidative stresss by pathogenesis of *Lxx*.

Keywords Ratoon stunting disease · Plant pathogenic bacteria · Plant-microbe interaction · Sulfur metabolism · Metabolomics · Glutathione · Polyamines

Abbreviations

APS	Adenosine phosphosulfate
DAP	Days after planting
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic acid
GR	Glutathione Reductase
GSH	Reduced glutathione
GSSH	Oxidized glutathione
GST	Total glutathione
Lxx	*Leifsonia xyli* subsp. *xyli*
MDA	Malonoaldeyde
NADPH	Nicotinamide adenine dinucleotide phosphate
PVPP	Polyvinylpolypyrrolidone
RSD	Ratoon stunting disease
SAM	S-adenosylmethionine
TCA	Tricarboxylic acid

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Introduction

Ratoon stunting disease (RSD) caused by the bacterium *Leifsonia xyli* subsp. *xyli* (*Lxx*) is the main disease in sugar cane (Chakraborty et al. 2023). RSD drastically reduces sugar cane productivity by reducing plant growth and the number of culms, especially in ratoon sugar cane (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. 2021a). It is possible that many sugar cane plants contain low bacterial titers that are not detected due to the plants being asymptomatic. Urashima et al. (2017) reported a wide range sugar cane genotypes on fields in the center-south region of Brazil were infected by *Lxx*, with 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 stealthily 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. 2021b; Kashyap et al. 2021; Urashima et al. 2017). Heat treatment (50°C for 2 h) is one of the disease control methods used 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 bacterium that contains only a 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 have insect vectors to introduce them into xylem vessels (Panta et al. 2022). The bacterium is introduced into healthy plants during harvest by contaminated knives after cutting infected material (Young 2018). *Lxx* colonizes and multiplies in the xylem vessels from which it systematically spreads to other plant organs such as leaf mesophyll and phloem (Guo et al. 2019; Marques et al. 2022; Quecine et al. 2016).

RSD causes changes in sugar cane growth that are associated with transcriptional, proteomic, and metabolic changes due to growth of the bacterium in the plant (Cia et al. 2018; Castro-Moretti et al. 2021). The changes in sugar cane growth include a reduced number and size of culms, as well as red coloring of the vascular bundles (Garcia et al. 2021b; Kazeem and Ikotun, 2019; Young, 2018). Decreased sugar cane growth is not associated with water deficit conditions caused by *Lxx* colonization (Garcia et al. 2021b; Zhang et al. 2016a). Rather,

this defective plant growth is associated with decreased levels of gibberellin, auxin, and ethylene hormones and increases in abscisic acid (Garcia et al. 2021b; Zhang et al. 2016a). The reduction in sugar cane growth is also associated with decreases in photosynthetic rates and sugar partitioning to the organs (Garcia et al. 2021b; Zhang et al. 2016b). Sugar cane with high bacterial titers had decreased sugar partitioning to meristem regions and tillers (Garcia et al. 2021a; Marques et al. 2022). Additionally, 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. 2016a; Guo et al. 2019).

Although it has not yet been investigated whether or not *Lxx* colonization affects the nutritional status of sugar cane, information on changes in nutritional status of RSD infected plants is essential to understand progress of the disease in the field. The establishment of *Lxx* in plant organs involves the expression of genes of the antioxidant system and the acquisition of the amino acids cysteine and methionine by the apoplast (Faria et al. 2020; Monteiro-vitorello et al. 2004). Since the survival of bacterium in the plant requires the consumption of methionine and cysteine, it is likely that growth of the bacterium will increase the demand for sulfur in the plant. Sulfur is a constituent of organic molecules that perform critical physiological functions, such as synthesis of the amino acids cysteine and methionine; coenzymes acetyl-CoA, thiamine and biotin, secondary metabolites, glucosinolates, and phytoalexins (Bloem et al. 2007; Koprivova and Kopriva 2016). Some pathogens alter the nutritional status of the plant to cause physiological damage that leads to host susceptibility (Fatima and Senthil-Kumar 2015; Yang et al. 2022). For example, increased sulfur 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). Moreoever, higher sulfur content in the plant's tissues has been be related to a defense response. For instance, Castro-Moretti et al. (2021) reported that 120 days after the inoculation of *Lxx* in sugar cane plants, there was an increase in leaf cysteine levels, which is a important amino acid associated with plant immunity (Álvarez et al. 2012). The aims of our study were to: (i) evaluate changes in the nutritional status of plants with high and low *Lxx* titers and (ii) assess how the increase in bacterial titers affects sulfur metabolism in the plant.

Materials and methods

Plant material

This study was carried out on RSD asymptomatic and symptomatic sugar cane (*Saccharum* spp.) using the genotype CB 49,260. 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. (2021b). The symptomatic and asymptomatic culms were cut into mini culms containing one bud each, using a disinfected cane knife that had been immersed in a bactericide solution (12.5% Benzalkonium chloride, 1 mL liter^{-1} of water) after each cut. 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) 22 days after planting (DAP). Pots were watered daily to field capacity throughout the experiment. The soil water content (% vol/vol) was controlled using the gravimetric method until the soil reach 80% of field capacity, for adequate water availability. Plant water transpiration (kg H_2O pot^{-1} day^{-1}) was assessed by placing plants on a precision balance and recording daily changes in pot weight, with three plants being harvested and weighted every 30 days to maintain the pot weight accuracy. A control pot without plants was used to measure soil evaporation (Santos et al. 2021). At 60 DAP, bacterial titer was quantified from a leaf sheath according to Young et al. (2017). Bacterial titer 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. 2021b). At 127 DAP, leaf and culm samples of the main shoot were put in liquid nitrogen and stored in a freezer at 80 °C.

Macronutrient quantification in sugarcane leaves

The quantification of macronutrients was performed on the third fully expanded leaves (leaf + 3) of the main shoot (Malavolta et al. 1997) using 0.5 g of dry matter (DM) placed in test tubes containing 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 (Malavolta et al. 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 by inductive coupling plasma analysis (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 to 98%.

Quantification of sulfate in the leaf

The sulfate content was quantified spectrophotometrically (Malavolta et al. 1997) by mixing 10 mL of the extract with 1 mL of an 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 min at room temperature. Samples were read in a spectrophotometer at 420 nm. Sulfate content was determined from an S standard curve (0–50 mg L^{-1}).

Quantification of reduced and oxidized glutathione

Quantification of reduced and oxidized glutathione was obtained spectrophotometrically (Lima et al. 2018) by homogenizing 0.8 g of fresh tissue from the first fully expanded leaf (leaf + 1) of the main shoot with 5% sulfosalicylic acid (1 mL) and centrifuging at 10,000 g at 4°C for 20 minutes. Two hundred uL (200 µL) of the supernatant was transferred into a new eppendorf tube 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 reading in a spectrophotometer at 412 nm. Then, 100 µL of 0.4 mM 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 from a GSH standard curve (0–1 mM).

Metabolomics

The first fully expanded leaf (leaf + 1) and culm of the main shoot were harvested, stored in liquid nitrogen, transported to the lab and macerated in liquid nitrogen. Four biological replicates per treatment were used in the metabolomic analyses (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 for derivation, and analysis of metabolites in the sample was performed exactly as described by Lisec et al. (2006).

Quantification of malonoaldeyde content (MDA)

The evaluation of lipid peroxidation in the first fully expanded leaf (leaf + 1) and culm of the main shoot was carried out according to Heath and Packer (1968) by macerating, 0.2 g (200 mg) of fresh plant material (shoot or stem) in 2 mL of 0.1% TCA containing PVPP. The macerated tissue was transferred to an eppendorf tube and centrifuged at 13,000 g for 10 min 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 min. Each sample was cooled on ice and then left in the dark for 15 min at room temperature. Readings were performed in a spectrophotometer at 532 and 600 nm. The concentration of MDA was calculated using the equation: ABS (532–600) /ε155 = X (in mM L^{-1}.); X/1000 = X in mM mL^{-1} where ε = 155 mM^{-1} cm^{-1}.

Growth measurements

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

Statistical analysis

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

Results

Growth measurements

Plants with high *Lxx* titers accumulated more biomass in the main culm, leaves, and shoots than plants with low *Lxx* titers (Table 1). In contrast, plants with high *Lxx* titers had lower tiller biomass than plants with low *Lxx* titer. Root biomass and total biomass were similar regardless of bacterial titer.

High bacterial titers change nutrient concentrations in sugar cane

The concentration (titer) of bacteria affected the nutritional status of the plants (Fig. 1A-F). Although all of the macronutrients were within reported nutrient sufficiency ranges for sugar cane (Bryson, et. al., 2014), leaves with high *Lxx* titers had 26% more S, 13% more K, and 22% more Mg compared to plants with low *Lxx* titers (Fig. 1A, B, and C respectively). Although, levels of N ($p = 0.12$; Fig. 1D), Ca ($p = 0.056$; Fig. 1E), and P ($p = 0.55$; Fig. 1F) on plants leaf tissue were not changed.

Table 1 Biomass in main culm, leaves on main culm, main shoot, tillers, roots, and total biomass (g dry weight) in sugarcane genotype CB 49,260 with low and high *Lxx* titers

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

Statistical difference was represented by * $p < 0.05$ ($n = 6 \pm$ SE)

Amino acids, gluthathione, sulfate and mda contents in the leaf

High bacterial titers reduced the levels of amino acids 21%, and total glutathione 38% in leaves compared to plants with low *Lxx* titers ($p < 0.05$; Fig. 2A-F). Oxidezed glutathione and reduced glutathione contents were similar regardless of bacterial titer in the leaves. On the other hand, the sulfate content was 210% higher in plants with high *Lxx* titers to suggest that high bacterial titers reduced sulfur assimilation in sugar cane leaves. In adittion to increasing sulfate, high bacterial titers also increased oxidative stress in leaves as evidenced by higher MDA content than sugar cane leaves with low *Lxx* titers.

Amino acids, gluthathione, and MDA content in the culm

Bacterial titers also affected the levels of glutathione, amino acids, and MDA in the culm ($p < 0.05$; Fig. 3A-E) where plants with high *Lxx* titers had 49% less amino acid content compared to plants with low *Lxx* titers. Oxidized glutathione, reduced glutathione and total glutathione contents were similar regardless of bacterial titer in the culm. High bacterial titers in sugar cane increased MDA 28% when compared to plants with low *Lxx* titers.

Changes in metabolite levels in sugar cane relative to bacterial titer

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 sugar cane. From these 43 compounds, 19 metabolites were altered by bacterial titer levels in the leaf and culm (Fig. 4). The sugar cane with high *Lxx* titers had lower concentrations of glutamine, homoserine, methionine, and valine amino acids in leaves (Fig. 4). The sugar content in leaves was higher in plants with high *Lxx* titers than low *Lxx* titers. Plants with high *Lxx* titers had more sucrose, maltose and raffinose than those with low *Lxx* titers (Fig. 5); however, trehalose was decreased in plants with high *Lxx* titers. 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 (Fig. 5).

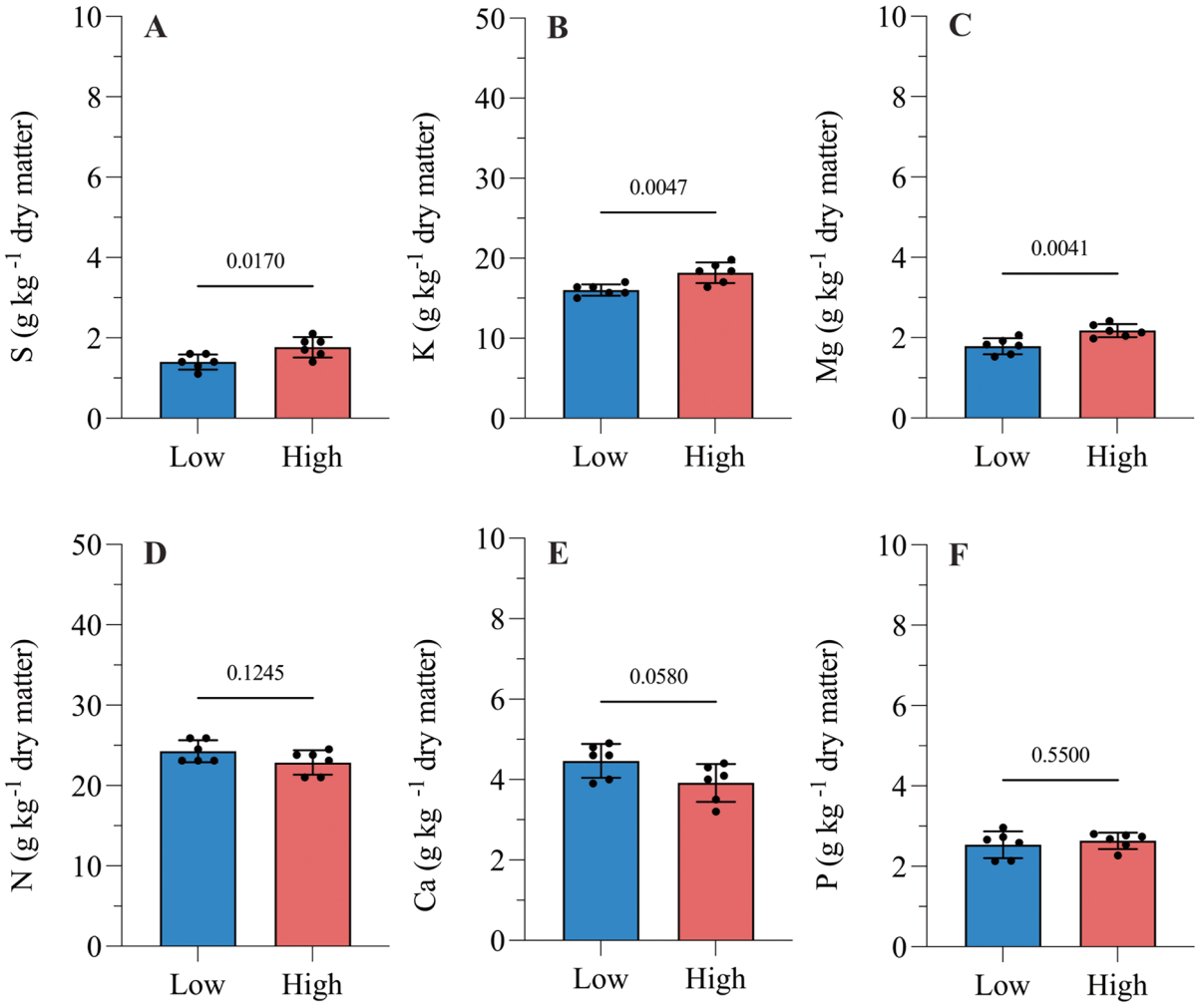

Fig. 1 Sugarcane genotype CB 49,260 with low and high titers of *Leifsonia xyli* subsp. *xyli* (*Lxx*). Leaf concentration of **A** S, **B** K, **C** Mg, **D** N, **E** Ca, **F** P. Mean values ($n = 6 \pm$ SE) differentiated by the t test at $p < 0.05$

Culms with high *Lxx* titers had more maltose and threonine, ornithine, phenylalanine and myo-inositol than culms with low *Lxx* titers (Fig. 6).

Discussion

Ratoon Stunting Disease (RDS) is one of the most important diseases that sugar cane growers face (Chakraborty et al. 2023). This disease reduces sugar cane 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. 2021b) and, sometimes, RSD symptoms are confused with nutritional deficiency (Young 2016). In this study, we demonstrated that high *Lxx* titers affect the nutritional status and sulfur metabolism of the plant since sulfur accumulated in the leaves but sulfur assimilation was reduced.

The higher level of sulfur, especially as sulfate, in the leaves and culm with the high bacterial titer in sugar cane, could indicate an increased sulfur demand by the plant and be correlated with the decreased content of methionine and glutathione (Figs. 1, 2 and 5). That suggests that there is an increased demand for organic sulfur compounds in leaves caused by higher acquisition of cysteine and methionine by *Lxx*, as the bacterium does not synthasize cysteine or methionine

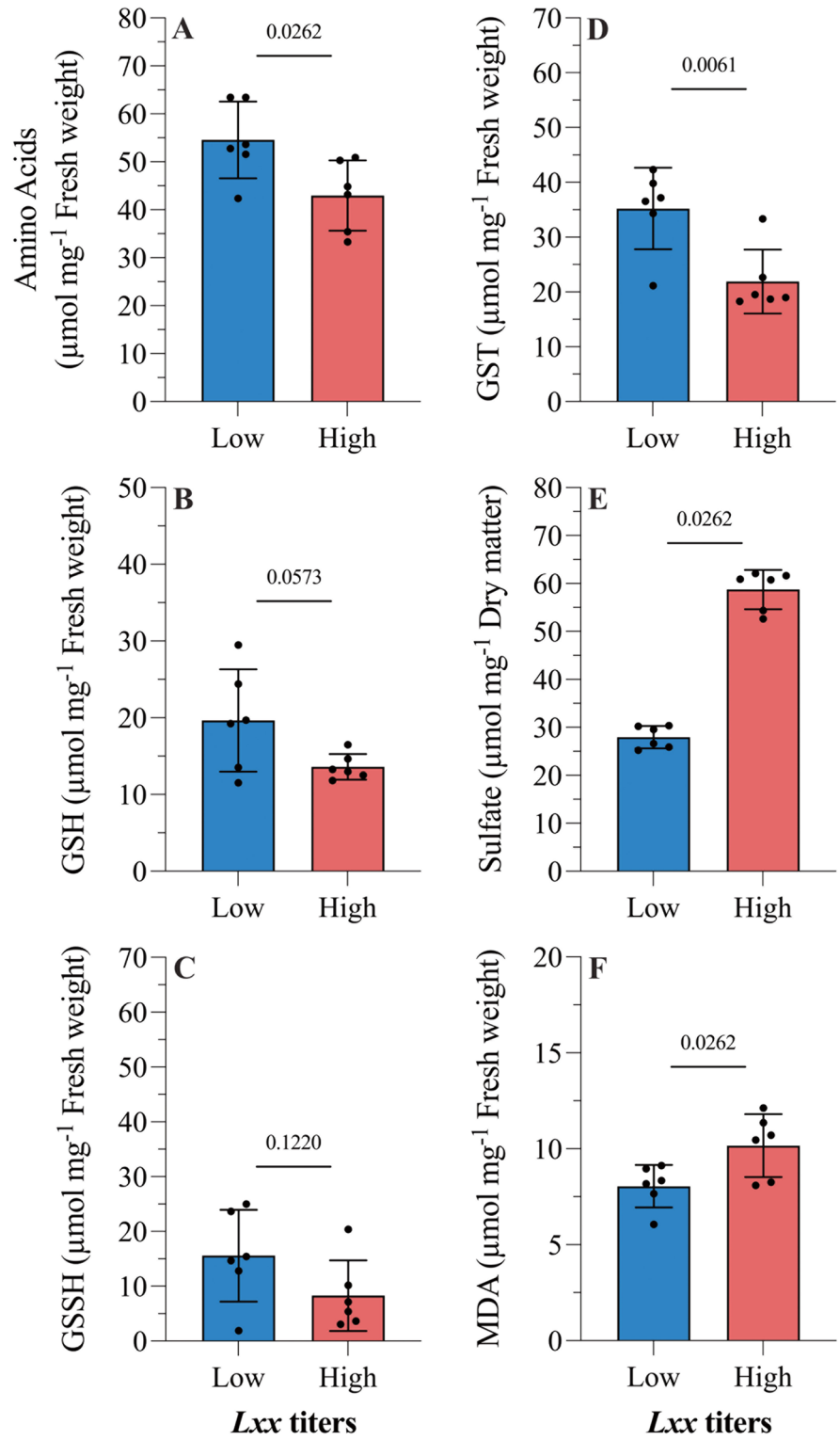

Fig. 2 Physiological analysis of sugarcane genotype CB 49,260 leaves with low and high *Leifsonia xyli* subsp. *xyli* (*Lxx*). **A** Amino acids, **B** GSH (reduced glutathione), **C** GSSG (oxidized glutathione), **D** GST (total glutathione), **E** Sulfate **F** MDA (Malonoaldeyde). Mean values ($n = 6 \pm$ SE) differentiated by the t test at $p < 0.05$

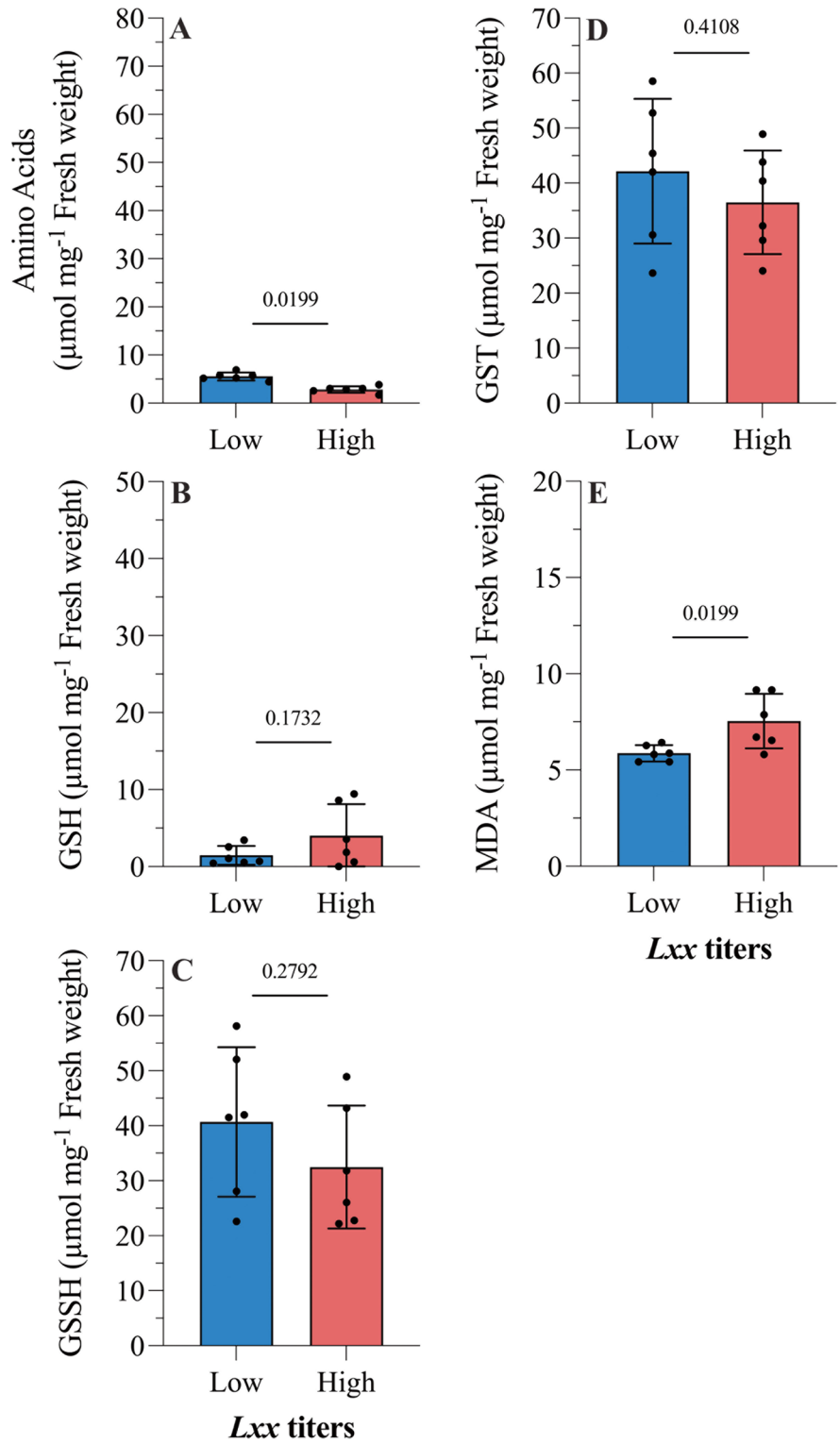

Fig. 3 Physiological analysis of culm tissues in sugarcane genotype CB 49,260 with low and high *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers. **A** Amino acids, **B** GSH (reduced glutathione), **C** GSSG (oxidized glutathione), **D** GST (total glutathione), **E** MDA (Malonoaldeyde). Mean values ($n = 6 \pm$ SE) differentiated by the t test at $p < 0.05$

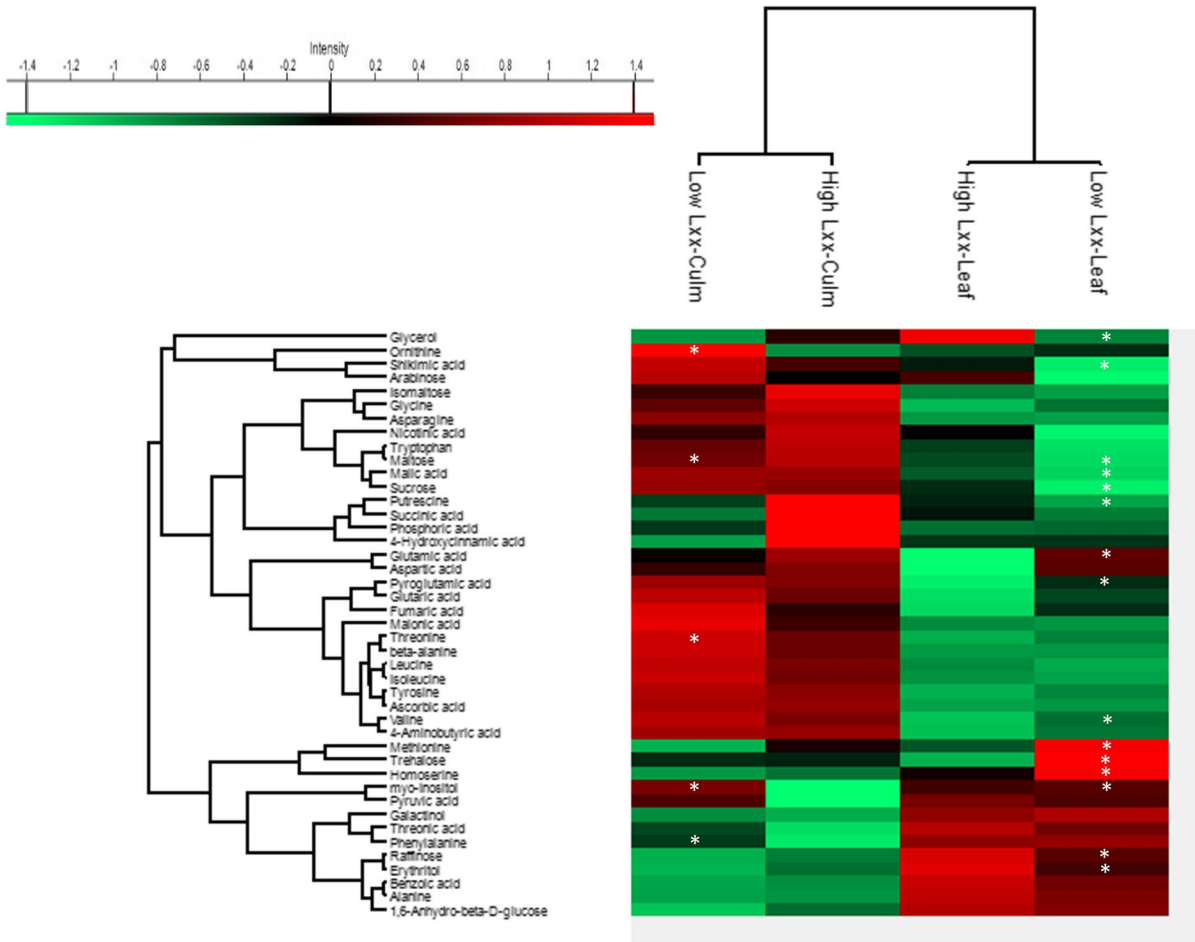

Fig. 4 The heat map showing the metabolite contents in the leaf and culm of plants with high and low titers of *Leifsonia xyli* subsp. *xyli* (*Lxx*) that was built using a Log2 Fold Change of relativized medians using perseus. Red indicates higher relative values, whereas green indicates lower relative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

(Monteiro-vitorello et al. 2004). Previous studies have reported 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 sugar cane CB 49,260 (the same variety used in this study) had more cysteine level after 120 days after inoculation 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 bacteria throughout sugar cane growth requires an increase in the biosynthesis of cysteine and methionine which caused an increase in sulfur absorption by the plants. Although the level of sulfur was higher in plants with high *Lxx* titers than plants with low *Lxx* titers, there was more sulfate and less of the sulfur amino acids, methionine and glutathione, in the leaf.

Various nutritional and physiological disorders in plants have been reported that are associated with different plant pathogen infections which affect the absorption or assimilation of nutrients (Fatima and Senthil-Kumar 2015; Martins et al. 2015). In sulfur assimilation, the sulfate absorbed by the root is transported to the leaf where it is assimilated in bundle sheath cells through synthesis of cysteine (Kopriva and Koprivova 2005; Weckopp and Kopriva 2014; Jobe et al. 2019). Cysteine is the first metabolite of assimilated sulfur and is used as a substrate to synthesize methionine and glutathione (Kopriva and

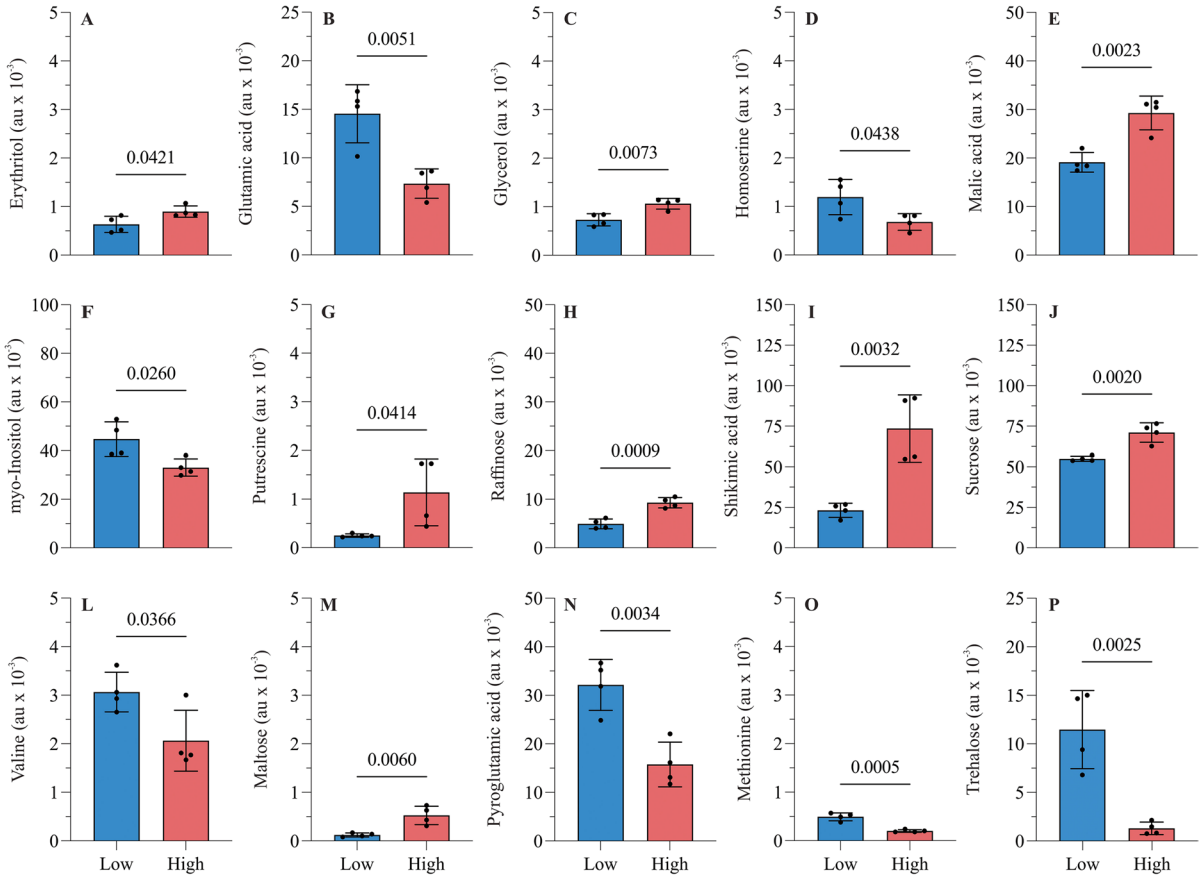

Fig. 5 Difference in metabolite levels in the leaf of plants with high and low *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers. Mean values ($n = 4 \pm$ SE) differentiated by the t test at $p < 0.05$

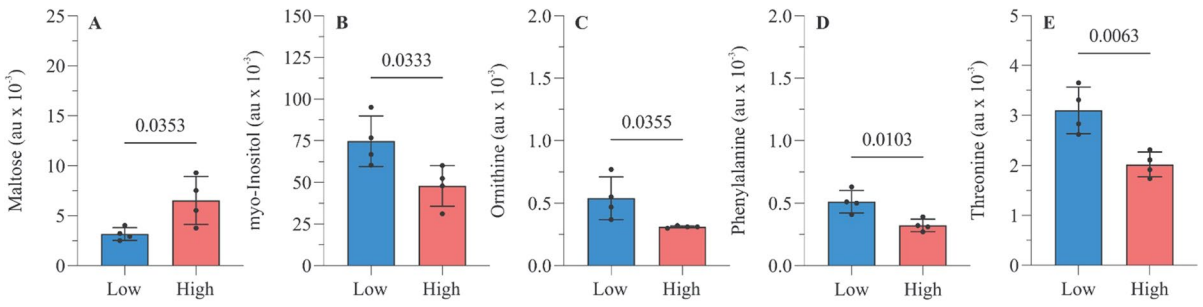

Fig. 6 Difference between metabolite levels in the culm of plants with high and low *Lxx* titers. Mean values ($n = 4 \pm$ SE) differentiated by the t test at $p < 0.05$

Koprivova 2005; Jobe et al. 2019). The high sulfate level and low content of methionine and glutathione suggested that there was a decrease in sulfur metabolism in the high *Lxx* plant. The high sulfur level in the leaf associated with low levels of sulfur organic compounds also may be explained by the higher synthesis of putrescine in the leaf (Fig. 7) since methionine is a precursor to putrescine biosynthesis (Heidari et al.

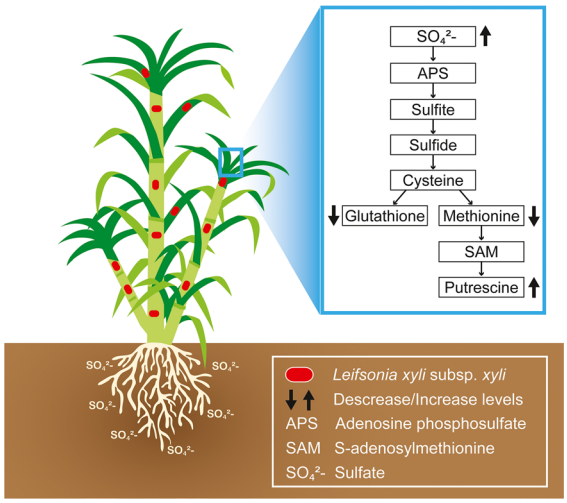

Fig. 7 Changes in sulfur metabolism caused by high *Leifsonia xyli* subsp. *xyli* (*Lxx*) titers in sugarcane plants

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 and may be a metabolic signal involved with the increased *Lxx* bacterial titer in sugar cane since it is a metabolite that favors colonization of leaf tissues. Studies have reported that putrescine is a virulence factor of bacteria in plants and necessary for the development of morphological structures of fungi (Vilas et al. 2018; Sánchez-Elordi et al. 2019). Putrescine is also associated with a defense response against pathogenic microorganisms. Putrescine is an essential metabolite for severe diseases such as bacterial wilt in tomato caused *by Ralstonia solanacearum* (Lowe-Power et al. 2018). In *Pseudomonas syringae*, the absence of putrescine inhibits the bacterial growth in plants but 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 (Williams and Copper 2003). Increases in sulfate and S assimilation have been shown to increase plant resistance against pathogens by 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 produced through higher activities of phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase. The upregulated expression of high affinity sulfate transporters associated with higher synthesis of 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 compounds. In contrast, there was a decrease in methionine and glutathione levels. The decreased glutathione level may be a signal for susceptibility of sugar cane to *Lxx* colonization. Glutathione is essential to maintain cellular redox homeostasis, especially when plants are subject to biotic and abiotic stresses (Lu et al. 2023; Madhu et al. 2022). The reduction in glutathione in the plants with high *Lxx* titers could increase oxidative stress as indicated by higher MDA levels in both leaves and culms. Studies have shown that susceptible plant genotypes have lower glutathione after infection with bacteria, fungi and virus (Zechmann 2020). Low glutathione and cysteine are associated with susceptibility of tomato to colonization by *Verticillium dahliae* (Williams and Copper 2003). High glutathione levels improve the defense system through upregulation of the key genes *PR-1a* and *PR5* (Chen et al. 2007). The shikimate pathway is another important metabolic pathway in disease resistance that may be compromised by high titers of *Lxx.* The high accumulation of shikimate and lower phenylalanine indicates that the metabolism for the production of aromatic amino acids, hormones, antibiotic products, etc. may be compromised.

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

Plants with high *Lxx* titers had higher sugar contents in the leaf and culm when compared with plants with low *Lxx* titers. Plants with high *Lxx* titers also had high sucrose and maltose concentrations in the leaf, and maltose in the culm as a result of reduced sugar partitioning for tiller production (Garcia et al. 2021b). Symptomatic plants had higher biomass in the main culm, which caused higher sugar storage in parenchyma of the culm due to lower tiller biomass (Garcia et al. 2021b; Saez et al. 2019). However, plants with high *Lxx* titers had

lower trehalose content in the leaf compared to low *Lxx* titer plants. Castro-Moretti et al. (2021) reported that susceptible plants had 10 times higher trehalose than resistant genotypes. The lower thehalose content was caused by high *Lxx* titer plants. Trehalose may be the most extensively acquired sugar in plants by bacterium through ABC transporters (Monteiro-vitorello 2004).

Decreased sulfur assimilation may also be caused by a shortage of carbon skeletons and reduced energy produced through photosynthesis and respiration. Increase respiration in plants with high *Lxx* titers may be caused by the decrease in free amino acids (Garcia et al. 2021b). Nutritional disorders have been more evident in plants with high *Lxx* titers under winter 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, plants with high *Lxx* titers might have altered sulfur status by increasing the demand for products of sulfur assimilation or by reducing sulfur metabolism in plants due to limited photosynthesis.

Conclusion

Our study showed that high bacterial titers of *Lxx* in sugar cane 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 bacterial growth. Plants with high bacterial titers had lower sulfur organic compound due to lower sulfur assimilation and higher methionine catabolism to putrescine. This observation is an important tool to understand the mechanism by which RSD affects plant nutritional status. Further studies will be needed to investigate whether changes in nutritional status may be associated with RSD resistance since the high shikimate with high infection indicates reduced function of this important pathway for disease resistance and stress response.

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Author contributions 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, SP, and SJM 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.

Data availability The data that support the findings of this study are available from the corresponding author with a reasonable request.

Declarations

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

References

Álvarez C, Ángeles Bermúdez M, Romero LC, Álvarez C, Ángeles Bermúdez M, Romero LC, Gotor C, García I (2012) Cysteine homeostasis plays an essential role in plant immunity. New Phytol 193:165–177. https://doi.org/10.1111/j.1469-8137.2011.03889.x

Andreato C, Gazaffi R, Oliveira MMA, Andreato C, Gazaffi R, Oliveira MMA, Camargo LEA, Urashima AS (2022) Effect of thermotherapy, Leifsonia xyli subsp. xyli titres, sugarcane genotype and diagnostic techniques on ratoon stunt control in Brazil. J Appl Microbiol 133:1676–1687. https://doi.org/10.1111/jam.15671

Bailey RA, Bechet GR (1997) Further evidence of the effects of ratoon stunting disease on production under irrigated and rainfed conditions. In Proc S Afr Sug Technol Ass 71:97–101

Bloem E, Haneklaus S, Schnug E (2005) Significance of sulfur compounds in the protection of plants against pests and diseases. J Plant Nutr 28:763–784. https://doi.org/10.1081/PLN-200055532

Bloem E, Haneklaus S, Salac I, Bloem E, Haneklaus S, Salac I, Wickenhäuser P, Schnug E (2007) Facts and fiction about sulfur metabolism in relation to plant-pathogen interactions. Plant Biol 9:596–607. https://doi.org/10.1055/s-2007-965420

Bryson GM, Mills HA, Sasseville DN, Jones JB Jr, Barker AB (2014) Plant analysis handbook III: a guide to sampling, preparation, analysis, interpretation and use of results of agronomic and horticultural crop plant tissue. Macro-Micro Publishing, Inc, Athens, Georgia

Caldana C, Li Y, Leisse A, Caldana C, Li Y, Leisse A, Zhang Yi, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P (2013) Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in Arabidopsis thaliana. Plant J 73:897–909. https://doi.org/10.1111/tpj.12080

Carvalho G, Silva TGER, Munhoz AT, Carvalho G, da Silva TGER, Munhoz AT, Monteiro-Vitorello CB, Azevedo RA, Melotto M, Camargo LEA (2016) Development of a qPCR for *Leifsonia xyli* subsp. *xyli* and quantification of the effects

of heat treatment of sugarcane cuttings on *Lxx*. Crop Prot 80:51–55. https://doi.org/10.1016/j.cropro.2015.10.029

Castro-Moretti FR, Cocuron J-C, Cia MC, Castro-Moretti FR, Cocuron J-C, Cia MC, Cataldi TR, Labate CA, Alonso AP, Camargo LEA (2021) Targeted metabolic profiles of the Leaves and Xylem Sap of two sugarcane genotypes infected with the Vascular Bacterial Pathogen *Leifsonia xyli* subsp. *xyli*. Metabolites 11:234. https://doi.org/10.3390/metabo11040234

Chakraborty M, Ford R, Soda N et al (2023) Ratoon Stunting Disease (RSD) of Sugarcane : A Review Emphasizing Detection Strategies and Challenges. Phytopathology 1–46

Chen YP, Xing LP, Wu GJ, Chen Y-P, Xing L-P, Wu G-J, Wang H-Z, Wang X-E, Cao A-Z, Chen P-D (2007) Plastidial glutathione reductase from *Haynaldia villosa* is an enhancer of powdery mildew resistance in wheat (*Triticum aestivum*). Plant Cell Physiol 48:1702–1712. https://doi.org/10.1093/pcp/pcm142

Cia MC, de Carvalho G, Azevedo RA, Cia MC, de Carvalho G, Azevedo RA, Monteiro-Vitorello CB, Souza GM, Nishiyama-Junior MY, Lembke CG, Antunes de Faria RSdC, Marques JPR, Melotto M, Camargo LEA (2018) Novel insights into the early stages of Ratoon Stunting Disease of Sugarcane inferred from transcript and protein analysis. Phytopathology 108:1455–1466. https://doi.org/10.1094/PHYTO-04-18-0120-R

Davis MJ, Vidaver AM, Harris RW (1984) *Clavibacter*: a New Genus Containing Some Phytopathogenic Coryneform Bacteria, Including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and Pathogens That Cause Ratoon Stunting Disease of Sugarcane and Bermudagrass Stunting Disease *Clavibacter*. https://doi.org/10.1099/00207713-34-2-107

Davis MJ, Dean JL, Harrison NA (1988) Quantitative varability of *Clavibacter xyli* subsp. *xyli* population in sugarcane cultivars differing in resistance to ratoon stunting disease. Phytopathology 78:462–468

De Souza AP, Grandis A, Arenque-Musa BC, Buckeridge MS (2018) Diurnal variation in gas exchange and nonstructural carbohydrates throughout sugarcane development. Funct Plant Biol 45:865–876. https://doi.org/10.1071/FP17268

Dias VD, Filho RC, da Cunha MG (2019) Comparison of *Leifsonia xyli* subsp. *xyli* molecular detection in heat-treated sugarcane setts. Pesqui Agropecu Trop 49:1–7. https://doi.org/10.1590/1983-40632019v4955132

dos Santos Panta AM, Souza S, Gagliardi JL et al (2022) Investigation of *Leifsonia xyli* subsp. *xyli* in insects. Res Soc Dev 2022:1–9

Evtushenko LI, Dorofeeva LV, Subbotin SA et al (2000) Leifsonia poae gen. nov., sp. nov., isolated from nematode galls on Poa annua, and reclassification of ' Corynebacterium aquaticum ' Leifson 1962 as Leifsonia aquatica (ex Leifson 1962) gen. nov., nom. rev., comb. nov. and Clavibacter xyli Da. Int J Syst Evol Microbiol 50:371–380

Faria RSCA, Cia MC, Monteiro-Vitorello CB et al (2020) Characterization of genes responsive to osmotic and oxidative stresses of the sugarcane bacterial pathogen *Leifsonia xyli* subsp. *xyli*. Brazilian J Microbiol 51:77–86. https://doi.org/10.1007/s42770-019-00163-6

Fatima U, Senthil-Kumar M (2015) Plant and pathogen nutrient acquisition strategies. Front Plant Sci 6:1–12. https://doi.org/10.3389/fpls.2015.00750

Ferreira DF (2011) SISVAR: a computer analysis system. Cienc e Agrotecnologia 35:1039–1042

Fu X, Li C, Zhou X, Fu X, Li C, Zhou X, Liu S, Wu F (2016) Physiological response and sulfur metabolism of the *V. dahliae*-infected tomato plants in tomato/potato onion companion cropping. Sci Rep 6:1–11. https://doi.org/10.1038/srep36445

Garcia FHS, Daneluzzi GS, Mazzafera P et al. (2021a) Ratoon Stunting Disease (Leifsonia xyli subsp. xyli) affects source-sink relationship in sugarcane by decreasing sugar partitioning to tillers. Physiol Mol Plant Pathol 116. https://doi.org/10.1016/j.pmpp.2021.101723

Garcia FHS, Herculino T, Coelho RD et al (2021b) Impact of the colonization of *Leifsonia xyli* subsp. *xyli* in a susceptible sugarcane genotype on water status and physiological traits. Eur J Plant Plant Pathol 159:1–11. https://doi.org/10.1007/s10658-021-02209-8

Gu G, Yang S, Yin X et al (2021) Sulfur induces resistance against canker caused by *Pseudomonas syringae* pv. *Actinidae* via phenolic components increase and morphological structure modification in the kiwifruit stems. Int J Mol Sci 22:1–13. https://doi.org/10.3390/ijms222212185

Guo Y, Hu M, Liu LL et al. (2019) Activities of key enzymes in the C4 pathway and anatomy of sugarcane infected by Leifsonia xyli subsp. xyli. J Appl Microbiol 1–11. https://doi.org/10.1111/jam.14444

Heath R, Packer L (1968) Photoperoxidation in isolated chloroplasts of fatty acid peroxidation chlorophyll. Arch Biochem biophisics 126:189–198

Heidari P, Mazloomi F, Nussbaumer T, Barcaccia G (2020) Insights into the SAM synthetase Gene Family and its roles in Tomato Seedlings under Abiotic stresses and hormone treatments. Plants 9:586

Jobe TO, Zenzen I, Karvansara PR, Kopriva S (2019) Integration of sulfate assimilation with carbon and nitrogen metabolism in transition from C 3 to C 4 photosynthesis. 70:4211–4221. https://doi.org/10.1093/jxb/erz250

Kashyap A, Planas-Marquès M, Capellades M, Kashyap A, Planas-Marquès M, Capellades M, Valls M, Coll NS (2021) Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens. J Exp Bot 72:184–198. https://doi.org/10.1093/jxb/eraa444

Kazeem SA, Ikotun B (2019) Effect of Leifsonia xyli subsp. xyli on growth and yield of sugarcane in Nigeria. J Plant Pathol 101:587–595. https://doi.org/10.1007/s42161-019-00246-1

Kopriva S, Koprivova A (2005) Sulfate assimilation and glutathione synthesis in C4 plants. Photosynth Res 86:363–372. https://doi.org/10.1007/s11120-005-3482-z

Koprivova A, Kopriva S (2016) Hormonal control of sulfate uptake and assimilation. Plant Mol Biol 91:617–627. https://doi.org/10.1007/s11103-016-0438-y

Lima K, Borges R, Salvato F et al (2018) Temporal dynamic responses of roots in contrasting tomato genotypes to cadmium tolerance. 245–258. https://doi.org/10.1007/s10646-017-1889-x

Lisec J, Schauer N, Kopka J, Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc 1:387–396. https://doi.org/10.1038/nprot.2006.59

Lowe-Power TM, Hendrich CG, von Roepenack-Lahaye E et al (2018) Metabolomics of tomato xylem sap during

bacterial wilt reveals *Ralstonia solanacearum* produces abundant putrescine, a metabolite that accelerates wilt disease. Environ Microbiol 20:1330–1349. https://doi.org/10.1111/1462-2920.14020

Lu C, Jiang Y, Yue Y et al (2023) Glutathione and neodiosmin feedback sustain plant immunity. J Exp Bot 74:976–990. https://doi.org/10.1093/jxb/erac442

Madhu SA, Kaur A et al (2022) Glutathione peroxidases in plants: innumerable role in abiotic stress tolerance and plant development. J Plant Growth Regul. https://doi.org/10.1007/s00344-022-10601-9

Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do estado nutricional das plantas: Princípios e aplicações. Potafos, Piracicaba

Marques JPR, Cia MC, Batista de Andrade Granato A, Muniz LF, Appezzato-da-Glória B, Camargo LEA (2022) Histopathology of the Shoot Apex of Sugarcane Colonized by Leifsonia xyli subsp. xyli. Phytopathology® 112(10):2062–2071

Martins SJ, de Medeiros FHV, de Souza RM et al (2015) Common bean growth and health promoted by rhizobacteria and the contribution of magnesium to the observed responses. Appl Soil Ecol 87:49–55. https://doi.org/10.1016/j.apsoil.2014.11.005

Monteiro-vitorello CB, Camargo LEA, Van Sluys MA et al (2004) The genome sequence of the gram-positive sugarcane pathogen leifsonia xyli subsp. xyli. Mol Plant-Microbe Interact 17:827–836. https://doi.org/10.1094/MPMI.2004.17.8.827

Quecine MC, Silva TM, Carvalho G, Quecine MC, Silva TM, Carvalho G, Saito S, Mondin M, Teixeira-Silva NS, Camargo LEA, Monteiro-Vitorello CB (2016) A stable *Leifsonia xyli* subsp. *xyli* GFP-tagged strain reveals a new colonization niche in sugarcane tissues. Plant Pathol 65:154–162. https://doi.org/10.1111/ppa.12397

Saez JV, Mariotti JA, Vega CRC (2019) Source-sink relationships during early crop development influence earliness of sugar accumulation in sugarcane. J Exp Bot 70:5157–5171. https://doi.org/10.1093/jxb/erz251

Sánchez-Elordi E, de los Ríos LM, Vicente C, Legaz ME (2019) Polyamines levels increase in smut teliospores after contact with sugarcane glycoproteins as a plant defensive mechanism. J Plant Res 132:405–417. https://doi.org/10.1007/s10265-019-01098-7

Santos EF, Mateus NK, Rosario MO, Garcez TB, Mazzafera P, Lavres (2021) Enhancing potassium content in leaves and stems improves drought tolerance of eucalyptus clones. Physiol Plant 172:552–563. https://doi.org/10.1111/ppl.13228

Takahashi H, Kopriva S, Giordano M, Takahashi H, Kopriva S, Giordano M, Saito K, Hell R (2011) Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. Annu Rev Plant Biol 62:157–184. https://doi.org/10.1146/annurev-arplant-042110-103921

Urashima AS, Silva MF, Correa JJ, Moraes MC, Singh AV, Smith EC, Sainz MB (2017) Prevalence and severity of ratoon stunt in commercial Brazilian sugarcane fields. Plant Dis 101(5):815–821

Vilas JM, Romero FM, Rossi FR, Vilas JM, Romero FM, Rossi FR, Marina M, Maiale SJ, Calzadilla PI, Pieckenstain FL, Ruiz OA, Gárriz A (2018) Modulation of plant and bacterial polyamine metabolism during the compatible interaction between tomato and *Pseudomonas syringae*. J Plant Physiol 231:281–290. https://doi.org/10.1016/j.jplph.2018.09.014

Weckopp SC, Kopriva S (2014) Are changes in sulfate assimilation pathway needed for evolution of C4 photosynthesis? Front Plant Sci 5:773. https://doi.org/10.3389/fpls.2014.00773

Williams JS, Cooper RM (2003) Elemental sulphur is produced by diverse plant families as a component of defence against fungal and bacterial pathogens. Physiol Mol Plant Pathol 63:3–16. https://doi.org/10.1016/j.pmpp.2003.08.003

Yang Z, Hui S, Lv Y, Yang Z, Hui S, Lv Y, Zhang M, Chen D, Tian J, Zhang H, Liu H, Cao J, Xie W, Wu C, Wang S, Yuan M (2022) miR395-regulated sulfate metabolism exploits pathogen sensitivity to sulfate to boost immunity in rice. Mol Plant 15:671–688. https://doi.org/10.1016/j.molp.2021.12.013

Young AJ (2016) Possible origin of ratoon stunting disease following interspecific hybridization of *Saccharum species*. Plant Patholgoy 65:1403–1410. https://doi.org/10.1111/ppa.12545

Young A (2018) Turning a blind eye to ratoon stunting disease of sugarcane in Australia. Plant Dis 102:473–482. https://doi.org/10.1094/PDIS-06-17-0911-FE

Young AJ, Health C, Queensland S, Nock CJ (2017) Molecular detection of diverse *Leifsonia* strains Associated with Sugarcane. Plant Dis 101:1422–1431. https://doi.org/10.1094/PDIS-01-17-0016-RE

Zechmann B (2020) Subcellular roles of glutathione in mediating plant defense during biotic stress. Plants 9:1–21. https://doi.org/10.3390/plants9091067

Zhang X, Chen M, Liang Y et al (2016a) Morphological and physiological responses of sugarcane to *Leifsonia xyli* subsp. *xyli* infection. Plant Dis 100:1–8. https://doi.org/10.1094/PDIS-10-15-1134-RE

Zhang X, Liang YZ, Zhu K et al (2016b) Influence of Inoculation of *Leifsonia xyli* subsp. *xyli* on photosynthetic parameters and activities of defense enzymes in sugarcane. Sugar Tech 19:394–401. https://doi.org/10.1007/s12355-016-0479-1

Zhang Z, Long Y, Yin X, Yang S (2021) Sulfur-induced resistance against Pseudomonas syringae pv. Actinidiae via triggering salicylic acid signaling pathway in kiwifruit. Int J Mol Sci 22:12710. https://doi.org/10.3390/ijms222312710

Zhu K, Yuan D, Zhang X et al (2017) The physiological characteristics and associated gene expression of sugar cane inoculated with *Leifsonia xyli* subsp. *xyli*. J Phy 1–9. https://doi.org/10.1111/jph.12659