Received: 16 February 2024 | Accepted: 26 June 2024

DOI: 10.1111/ppl.14430

ORIGINAL RESEARCH

Physiologia Plantarum

Salt stress alters the cell wall components and structure in *Miscanthus sinensis* stems

Kasper van der Cruijsen[1] | Mohamad Al Hassan[1] | Gijs van Erven[2,3] | Nicole Kollerie[1] | Bas van Lent[1] | Annemarie Dechesne[1] | Oene Dolstra[1] | Maria-João Paulo[4] | Luisa M. Trindade[1]

[1]Laboratory of Plant Breeding, Wageningen University & Research, Wageningen, The Netherlands

[2]Wageningen Food and Biobased Research, Wageningen University & Research, Wageningen, The Netherlands

[3]Laboratory of Food Chemistry, Wageningen University & Research, Wageningen, The Netherlands

[4]Biometris, Wageningen University & Research, Wageningen, The Netherlands

Correspondence
Luisa M. Trindade,
Email: luisa.trindade@wur.nl

Funding information
HORIZON EUROPE Innovative Europe, Grant/Award Number: 745012

Edited by R. Le Hir

Abstract

Miscanthus is a perennial grass suitable for the production of lignocellulosic biomass on marginal lands. The effects of salt stress on Miscanthus cell wall composition and its consequences on biomass quality have nonetheless received relatively little attention. In this study, we investigated how exposure to moderate (100 mM NaCl) or severe (200 mM NaCl) saline growing conditions altered the composition of both primary and secondary cell wall components in the stems of 15 *Miscanthus sinensis* genotypes. The exposure to stress drastically impacted biomass yield and cell wall composition in terms of content and structural features. In general, the observed compositional changes were more pronounced under severe stress conditions and were more apparent in genotypes with a higher sensitivity towards stress. Besides a severely reduced cellulose content, salt stress led to increased pectin content, presumably in the form of highly branched rhamnogalacturonan type I. Although salt stress had a limited effect on the total lignin content, the acid-soluble lignin content was strongly increased in the most sensitive genotypes. This effect was also reflected in substantially altered lignin structures and led to a markedly reduced incorporation of syringyl subunits and p-coumaric acid moieties. Interestingly, plants that were allowed a recovery period after stress ultimately had a reduced lignin content compared to those continuously grown under control conditions. In addition, the salt stress-induced cell wall alterations contributed to an improved enzymatic saccharification efficiency.

1 | INTRODUCTION

Over the last decades, the number of soils contaminated with high contents of salt has increased globally due to natural processes, such as weathering, and due to human-driven processes, like improper irrigation practices (Daliakopoulos et al., 2016; Ivushkin et al., 2019). This development is problematic as plant growth is hampered due to the influx of excessive levels of sodium and chloride ions, which induces osmotic stress in a matter of days, followed by ion toxicity and oxidative stress upon prolonged exposure (Isayenkov and Maathuis, 2019; Zhao et al., 2020). Plants are not necessarily exposed to constant stress because salinity levels tend to fluctuate throughout the growing season. These fluctuations are influenced by precipitation and evapotranspiration and also depend on the occurrence and intensity of saltwater intrusion events (Rengasamy, 2010; DiCara and Gedan, 2023). Nevertheless, lowered productivity due to salinity leads to considerable economic losses and renders substantial amounts of land to be considered degraded and marginal

(Zörb, Geilfus and Dietz, 2019; Hayat et al., 2020; Pancaldi and Trindade, 2020). Therefore, there is an increasing interest in developing crops that are better adapted to salinity and suitable for cultivation in such environments.

Miscanthus is a perennial grass that received widespread interest for its high yields in combination with the favourable characteristics of its lignocellulosic biomass. It has been shown that Miscanthus species possess genetic variation regarding their resilience to saline-rich environments (Chen et al., 2017; Zheng, Xiao, et al., 2022). Nevertheless, salt stress affects many critical physiological and biochemical plant growth-related processes. In this regard, changes within the cell wall biosynthesis are required to adjust the structural integrity and extensibility of the cell wall (Le Gall et al., 2015; Landi et al., 2017; Arif et al., 2020; Rui and Dinneny, 2020; Zhao et al., 2020). Several studies have suggested that these adjustments are crucial for plant stress adaptation and could be related to salt tolerance (Uddin et al., 2013; An et al., 2014; Corrêa-Ferreira et al., 2019; Tang et al., 2022). However, the inconsistent observations between species suggest that multiple response mechanisms exist, complicating the assignment of their biological relevance to tolerance (Le Gall et al., 2015; Corrêa-Ferreira et al., 2019). Additionally, since the majority of lignocellulosic biomass consists of cell walls, it is expected that structural modifications could have a considerable impact on feedstock quality as the composition affects further downstream processing (van der Cruijsen et al., 2021).

Miscanthus has typical grass cell walls consisting of the polysaccharides cellulose, hemicellulose and pectin that, together with lignin, form a complex network and provide rigidity and structure to the plant (van der Cruijsen et al., 2021). The major component of these cell walls is cellulose (Schäfer et al., 2019), composed of linearly linked glucose molecules that are present in crystalline form (Somerville, 2006). The hemicellulose in Miscanthus is composed of a xylan backbone that is substituted with arabinosyl units (Schäfer et al., 2019). These arabinosyl units can be extensively esterified with (di)ferulic acid (FA) and, to a lesser extent, with *p*-coumaric acid (*p*CA) (Pauly et al., 2013; Hatfield, Rancour and Marita, 2017). Secondary cell walls are deposited during plant maturation, and here, cellulose and hemicellulose are crosslinked together with lignin. Lignin is an aromatic polymer that in grasses consists of p-hydroxyphenyl (H), syringyl (S) and guaiacyl (G) subunits, with *p*CA, FA, acetic acid and the flavonoid tricin also incorporated (Ralph, Lapierre and Boerjan, 2019; van Erven et al., 2023). Hemicellulose plays a central role in connecting the different cell wall biopolymers, as it is able to adhere to cellulose but also forms crosslinks with lignin through their mutual linkage with FA (Gao et al., 2020; Kirui et al., 2022; Tryfona et al., 2023). Pectin is a relatively minor component, which in the cell walls of Miscanthus plants mainly consists of homogalacturonan and rhamnogalacturonan-I (RG-I) (De Souza et al., 2015). Homogalacturonan is formed by linear chains of galacturonic acid (GalA), while RG-I has a backbone of alternating galacturonyl and rhamnosyl units, in which the latter can be further substituted with arabinan, galactan and arabinogalactan branches (Carpita, 1996; Mohnen, 2008).

In several grass species, salt stress has been reported to distinctively affect the major cell wall components that are known to impact the enzymatic saccharification efficiency in the aboveground biomass (Kumar et al., 2018; Tiwari et al., 2018; Oliveira et al., 2020; Zheng, Xiao, et al., 2022). Saccharification is a critical post-harvest step to break down cellulose into glucose molecules that can, in turn, be fermented into ethanol (Saini, Saini and Tewari, 2015). Multiple studies have pointed out a drop in cellulose content when plants experience salt stress (Oliveira et al., 2020; Zheng, Xiao, et al., 2022), meaning less substrate would be available for saccharification and fermentation. Lignin is well known to be the main limitation for the (enzymatic) conversion of biomass into biofuels, as it acts as a barrier that prevents contact between cellulase and cellulose (Huang et al., 2022). However, studies on the impact of salt stress on lignin have yielded contrasting results; while some have observed an increase in lignin as a response to salt stress, others have reported a decrease or no significant change (Kumar et al., 2018; Tiwari et al., 2018; Stavridou, Webster and Robson, 2019; Oliveira et al., 2020). Most studies on grasses have left pectin uncharacterized, likely because of its relatively low content in the grass cell walls. However, in maize and several non-grass species, increased pectin levels and changes in its composition have been mentioned as a response to salinity (De Lima et al., 2014; Corrêa-Ferreira et al., 2019; Oliveira et al., 2020). Despite its low content pectin has been implied to contribute to differences in saccharification efficiency in Miscanthus (De Souza et al., 2015; Da Costa et al., 2019).

The structural modifications of the polysaccharides and lignin polymers due to salt stress have so far remained largely unknown in Miscanthus. Since Miscanthus cultivation is mainly restricted to marginal lands, it is important to identify additional factors that contribute to its tolerance against salinity. Also, being a lignocellulosic feedstock, it is important to evaluate the effect that potential changes in cell wall composition would have on biomass quality. The aim of this study was to evaluate cell wall remodelling in *Miscanthus sinensis* stems under moderate and severe saline conditions. Additionally, plants underwent a recovery period to assess the cell wall readjustments after temporary exposure to stress. In-depth biochemical analyses were performed on a set of 15 genotypes that previous studies had shown to represent a range of tolerance to both drought and salinity. This setup allowed us to infer any possible relationships between cell wall modulation and tolerance to salt.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental set-up

The research was performed using 15 *M. sinensis* genotypes (Table S1), which were selected from the collection at Wageningen University & Research for their varying degrees of salt tolerance (Chen et al., 2017). These genotypes were propagated through tissue culture, and young plantlets were grown for six weeks on a hydroponic system with half-strength Hoagland solution to provide the required nutrients. Greenhouse conditions were set at a day/night cycle of 16 h at 20°C and 8 h at 16°C. For each genotype, 12 plants were grown at three different concentrations of NaCl: 0 mM (control), 100 mM (moderate stress) or 200 mM (severe stress). The experiment was set up in a randomized

split-block design. Half of the plants (270 in total, 90 per treatment) were harvested after a 4-week stress period. For the remaining plants, salt was flushed out of the hydroponic system and replaced with water containing half-strength Hoagland solution. Subsequently, the plants were allowed a 4-week recovery period until harvest. After each harvest, the fresh and dry weight (three days at 60°C) of leaves and stems were recorded for six plants per genotype for each treatment. The reductions in plant yields between control and stress conditions were used to identify differences in salt tolerance among genotypes. Biochemical analyses were performed on pooled samples from the stems of two identically treated plants of the same genotype that were harvested at the same timepoint, yielding three biological replicates per analysis.

2.2 | Cell wall isolation and compositional analysis

The cell walls were extracted by using the alcohol-insoluble residue (AIR) protocol (Pettolino et al., 2012), followed by monosaccharide determination according to the NREL procedure (Sluiter et al., 2008), with the modifications described by Petit et al. (2019). In short, Miscanthus stems were milled on a cross beater mill equipped with a 1 mm sieve and subsequently ball milled for 2 min at 25 Hz (Retsch MM 200, Verder Scientific) in a 10 mL stainless steel grinding jar containing two 12 mm stainless steel grinding balls. Cell walls were extracted following the upscaled AIR protocol, where milled biomass (500 mg) was extracted with 15 mL 80% (vol/vol) ethanol on ice for 30 min. Three rounds of ethanol extraction were followed by acetone extraction (10 min, room temperature) and methanol extraction (10 min, room temperature). Samples were centrifuged 5 min at 10,000 *g* between each extraction and the supernatant was discarded. The extracted cell walls were dried in a RapidVap (Labconco) and treated with α-amylase (Megazyme). Samples were placed in a boiling water bath (5 min) and afterwards incubated with α-amylase solution (2 U mg^{-1} of carbohydrate in 10 mM Tris-maleate buffer) for 1 h at 40°C under constant shaking, followed by adding one-half the amount of α-amylase solution and incubating for 30 min at 40°C under constant shaking. Four volumes of cold ethanol were added to the samples for precipitation at −20°C for 1 h. Subsequently, the samples were centrifuged at 1,500 *g* (5 min, room temperature), after which the supernatant was discarded. Samples were washed an additional three times with cold absolute ethanol, being centrifuged (1,500 *g*, 5 min, room temperature) after each washing step, after which the supernatant was discarded. The remaining pellets were dried in a RapidVap and provided the starch free cell wall samples (CW) used for all further analyses.

For compositional analysis, the polysaccharides within the isolated cell walls (30 mg) were hydrolyzed with sulfuric acid (72% w/w, 30°C, 1 h) that was then diluted and heated (4% w/w, 120°C, 1 h). Hydrolysates were filtered using a 0.45 µm PTFE syringe filter and diluted 5 x and 50 x with MQ-filtered water. A set of calibration standards that included multiple monosaccharides (glucose, xylose, arabinose, galactose, mannose and rhamnose) and uronic acids (galacturonic acid and glucuronic acid) in concentrations ranging from 0.0005–0.1 mg ml^{-1}, were used to quantify the individual components in each sample. The monosaccharides and uronic acids in the diluted samples and calibration standards were separated at a flow rate of 0.25 mL min^{-1} at 30°C using HPAEC-PAD (Dionex ICS-5000, Thermo Scientific) equipped with a Dionex CarboPac PA-1 column (2 × 250 mm) and similar guard column. The elution of neutral sugars was initiated using an isocratic method with 20 mM sodium hydroxide for 25 min, followed by a linear gradient beginning with 60 mM sodium acetate in 100 mM NaOH for 15 min and concluding with 200 mM sodium acetate in 100 mM NaOH to elute uronic acids. Subsequently, the column was washed with 1 M sodium acetate in 100 mM NaOH for 5 minutes and re-equilibrated with 20 mM sodium hydroxide for 30 min. Values were corrected for degradation by using sugar recovery standards, and anhydro correction was also applied, as described by Sluiter et al. (2008). Each sample was analyzed in triplicate, and the averaged values are reported on a dry weight basis as grams per 100 g of cell wall material, expressed as percent cell wall (%CW). Mannose and glucuronic acid levels were too low to be accurately quantified and, hence are not further reported.

Residues after acid hydrolysis were used to gravimetrically determine the acid-insoluble lignin (AIL) content within the cell walls. The remaining biomass was recovered on glass fiber filters, flushed with ample water to remove remaining acid, and dried overnight at 103°C before the samples were weighed. Acid soluble lignin (ASL) was spectrophotometrically quantified in the hydrolysate at 205 nm (110 L g^{-1} cm^{-1} as the absorptivity constant) using the formula reported by van der Weijde et al. (2016). Also, here each sample was analyzed in triplicate, and the average values are reported on a dry weight basis as grams per 100 grams of cell wall material (%CW).

2.3 | Ferulic- and p-coumaric acid quantification

Esterified ferulic acid (FA) and *p*-coumaric acid (*p*CA) within cell walls (15 mg) were hydrolyzed by adding 300 µL of 4 M NaOH to the sample. Samples and recovery standards were heated (121°C, 4 h), and subsequently cooled to room temperature and diluted 4 times with purified water within the vial. Samples were further diluted an additional 4 times in 1.5 mL Eppendorf tubes and centrifuged for 10 min at 16,000 *g* before the supernatant was transferred into UPLC vials. Samples and (recovery) standards were analyzed on a UPLC system (Waters ACQUITY H-Class PLUS System, Waters Corporation) and separated by a C18 HyPURITY column (3 µm, 150 × 3 mm, Thermo Fisher Scientific). The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a flow rate of 1 mL min^{-1} (95% A, 5% B). UV absorbance at 280 nm was used for the quantification of both FA and *p*CA content. Samples were analyzed in triplicate and reported as previously described for monosaccharides and lignin.

2.4 | Saccharification efficiency

Saccharification efficiency was determined as described by Resch et al. (2015) with modifications. Extracted cell walls (10 mg) were dispersed in 1 mL 50 mM sodium citrate buffer (pH 5.0) containing cellulase (CTec2, concentration of 30% g enzyme / g cellulose) and 0.02%

(v/v) Proclin to prevent microbial growth (He et al., 2019). All samples, including enzyme and substrate blanks, were incubated at 50°C for 48 h in a Thermomixer at 750 rpm. After the reaction was terminated by incubating at 99°C for 10 min, samples were centrifuged (16,000 *g*), diluted (200 x) and analyzed in triplicate for glucose release as previously described for monosaccharides. Glucose release was used to calculate the glucose conversion levels, as it takes into account the differences in cellulose content between samples:

$$Glucose\ conversion\ (\%) = \frac{Glucose\ release\ (mg)}{Glucose\ content\ (mg)} \times 100$$

2.5 | Structural characterization of lignin by pyrolysis-GC–MS

The structural characterization of lignin was performed by analyzing the alcohol-insoluble samples by pyrolysis gas chromatography mass spectrometry (pyrolysis-GC–MS) using an Agilent VF-1701 ms column (30 m x 0.25 i.d. 0.25 μm film) for chromatographic separation, as previously described (van Erven et al., 2019). Uniformly ^{13}C-labeled lignin, isolated from ^{13}C wheat straw (97.7 atom% ^{13}C) (IsoLife BV) was used as an internal standard (^{13}C-IS) (van Erven et al., 2017). To each accurately weighed sample (80 μg), 10 μL of a ^{13}C-IS solution (1 mg ml^{-1} ethanol/chloroform 50:50 v/v) was added. All samples were dried prior to analysis and analyzed in triplicate. Lignin-derived pyrolysis products were monitored in full MS mode on the most abundant fragment per compound (both non-labelled and uniformly ^{13}C labelled). Pyrograms were processed by the Thermo Scientific TraceFinder 5.1 software. Relative abundances of lignin-derived pyrolysis products were calculated and classified as described previously (Table S2) (van Erven et al., 2019).

2.6 | Statistical analysis

All statistical analyses were performed in R (R-4.1.0, R Core team, 2021). A linear mixed model was specified using lme4 (Bates et al., 2015) to determine if the aerial biomass, cell wall content and compositional traits were significantly affected by genotype and treatment at the different harvest timepoints. The trial had a split-plot design, wherein each treatment consisted of a plot that could be divided into three separate blocks. Each block contained two plants of the same genotype, which were pooled together for biochemical analyses. In the mixed-effects model, genotypes and treatments were specified as fixed factors, while blocks were included as a random factor:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \eta_{k(i)} + \varepsilon_{ijk}$$

Where Y_{ijk} represents the response variable, μ is the grand mean, α_i is the fixed effect of treatment, β_j is the fixed effect of genotype, $\alpha\beta_{ij}$ is the interaction term between treatment and genotype, $\eta_{k(i)}$ is the block effect, and ε_{ijk} is the residual error. Wald tests were conducted to determine significant differences among the fixed effects in the model using the car package (Fox and Weisberg, 2018).

The two harvest timepoints represented separate experiments and were thus analyzed independently. A Tukey HSD post-hoc test was performed to identify significant differences in aerial biomass between treatments for each genotype. Additionally, Tukey HSD tests were applied across all genotypes to assess the differences in cell wall content and compositional traits between treatments, focusing on the effects of stress intensity. All Tukey HSD tests were conducted using the emmeans package (Lenth, 2023). The reported means and standard errors were also derived from this package. Pearson correlation coefficients were calculated on individual data points within the observed data to assess the linear relationships between traits. Figures were created using the ggplot2 package (Wickham, 2016).

3 | RESULTS

Plants grown under saline conditions showed significantly impaired growth ($p < 0.001$), as exposure to 100 mM NaCl and 200 mM NaCl, respectively reduced their dry weight on average by 24.8 and 44.2% compared to plants grown under control conditions (Figure 1A). The significant interaction ($p < 0.05$) between genotypes and treatments indicated that the effect salt stress had on the aerial biomass differed between genotypes. Especially exposure to 200 mM of NaCl revealed large differences in susceptibility to salt across the tested *M. sinensis* genotypes, as biomass was reduced by roughly 20% in the least affected genotypes (2, 6 and 8) up to nearly 70% in the most affected ones (1, 4, 5, 7, 11 and 14). Results after the recovery period confirmed genotypes 6 and 8 as the least affected genotypes and genotypes 1, 7 and 14 as the most affected genotypes. However, for some genotypes, the results after the recovery period differed from those after the initial stress period (Figure 1B). For instance, for genotype 4 the difference between plants that remained at control conditions and plants that had previously received the severe stress treatments was no longer significant, while for genotype 2 the opposite occurred. Nevertheless, together, these data show significant genotypic differences in their ability to maintain plant growth under salt stress conditions.

The amount of cell wall that could be retrieved from the stem material after the 4-week stress period was 82.6% for plants grown under control conditions, while it was 81.9% for plants exposed to 100 mM salt and 74.2% for plants exposed to 200 mM salt (Figure S1). The evidently lower amount of cell wall retrieved after severe stress conditions indicates a significant reduction ($p < 0.001$) in cell wall content that was not seen after the moderate stress treatment. Although the reduction was observed in nearly all genotypes, it was much more apparent in some, such as 1 and 7, which led to a significant interaction between treatments and genotypes ($p < 0.001$).

3.1 | Salinity modifies the composition of cell wall polysaccharides

The content and composition of the cell wall polysaccharides were significantly altered as a consequence of salt stress. The extent of these

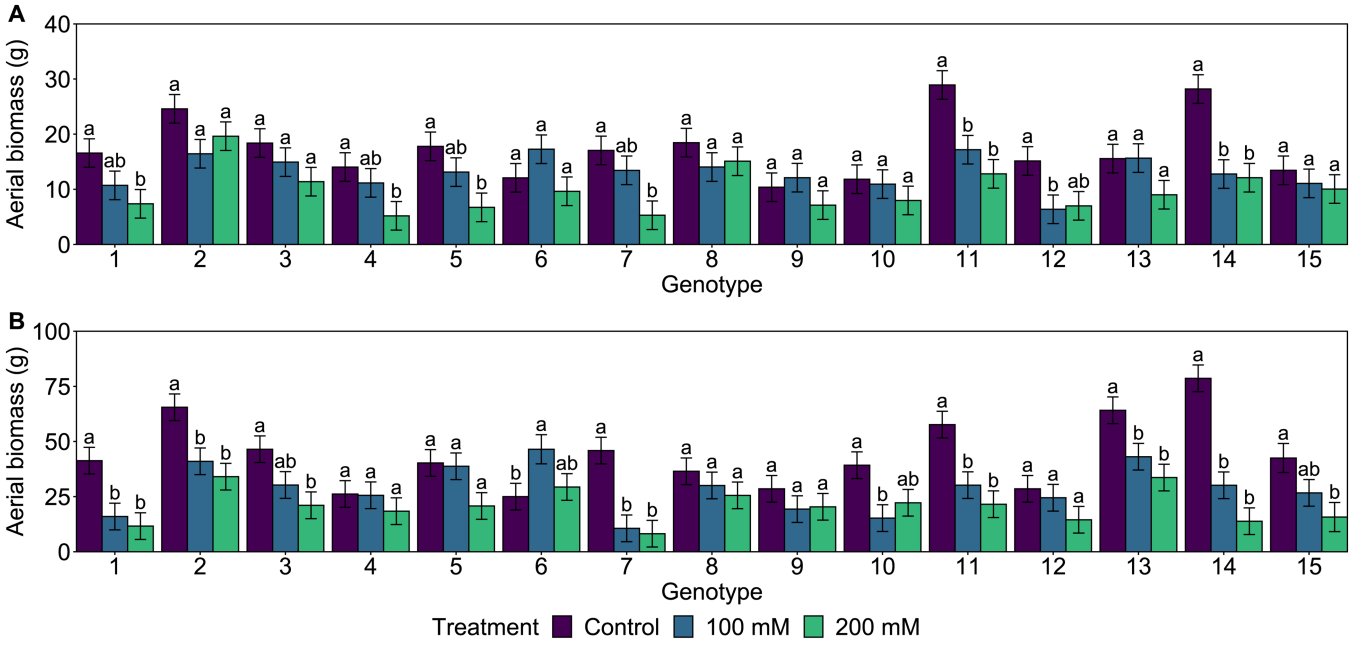

FIGURE 1 Aerial biomass weight (g) of 15 M. sinensis genotypes after a stress period (A) and a subsequent recovery period (B), grown under three treatments: Control (0 mM NaCl), 100 mM NaCl, or 200 mM NaCl. Different letters above the bars indicate significant differences ($p < 0.05$) between treatments within each genotype, as determined by the Tukey HSD test. Each bar represents the mean of $n = 6$ biological replicates; error bars indicate ± SE.

alterations was dependent on the genotype, as evidenced by significant interaction between genotypes and treatments for all analyzed monosaccharides ($p < 0.001$). Cellulose levels of the genotypes ranged between 41.5 and 46.7% in control plants at the end of the initial 4-week growth period. The amount of glucose was significantly lower in plants under stress ($p < 0.001$), with average glucose levels being reduced by 7.2% (100 mM NaCl) and 17.5% (200 mM NaCl) compared to control conditions. Each genotype showed an intermediate reduction in glucose levels at moderate stress levels compared to those at severe stress levels, indicating a stress severity-dependent effect (Figure 2A). Additionally, an overall significant difference ($p < 0.001$) between moderate and severe stress treatments further reflected this severity-dependent effect. The glucose reduction was also more pronounced in genotypes that displayed a higher sensitivity towards stress. On the contrary, most genotypes showed only a slight increase in xylose content upon stress, indicating a limited yet significant ($p < 0.05$) impact on hemicellulose content at both stress levels (Figure 2B). The most notable exception was genotype 7, which, at 200 mM NaCl, displayed a severe drop (39.9%) in xylose content. The average arabinose content in the control treatment was 2.12%. The presence of salt significantly increased ($p < 0.001$) these levels by 13.2% (100 mM) and by 42.9% (200 mM). The larger increase under severe stress conditions also led to significant differences between the two stress treatments ($p < 0.001$). The observed response at severe stress conditions also revealed that the increase largely followed the sensitivity gradient across genotypes, as the increase was over 80% in the ones most sensitive towards salt (1, 4, and 7). In contrast, the increase at severe stress conditions remained between 5 and 20% for the least affected genotypes (2, 6 and 8), with the remaining genotypes falling somewhere in between these extremities (Figure 2C). The variation for arabinose content between genotypes ranged from 2.13 to 4.80% in severely stressed conditions, which was much larger than the observed range of 1.78 to 2.60% in control conditions. Since arabinose is present as side-chains that are either attached to the xylan backbone or, to a lesser extent, rhamnose as a part of RG-I it contributes to more extensively branched polysaccharides within the cell wall.

The average galacturonic acid content in plants grown under control conditions was 1.08%, with genotypes ranging between 0.94 and 1.30% (Figure 2D). Under these conditions, galacturonic acid, along with rhamnose, which averaged 0.09% (range 0.07 to 0.12%) and galactose, which averaged 0.42% (range 0.26 to 0.64%), constituted the majority of pectin in the cell wall (Figure 2E-F). Adding these three components together, the estimated genotypic variation for the total pectin levels ranged between 1.27% and 2.04% in plants grown under control conditions. The stress induced an increase in galacturonic acid, rhamnose and galactose, all of which followed similar patterns, and their contents were highly correlated with each other ($r > 0.9$) as well as with arabinose (Figure S2). On average, the galacturonic acid levels increased by 10.2% in moderately stressed plants, while the increase was 38.9% for severely stressed plants. Rhamnose levels increased, on average, 17.8% for moderately stressed plants and 64.0% for severely stressed plants. The largest increase was observed in galactose content, which increased 16.7% in moderately stressed plants and 92.9% in severely stressed plants. On average, both stress treatments significantly increased these three components compared to control conditions ($p < 0.01$), while values in severely stressed plants

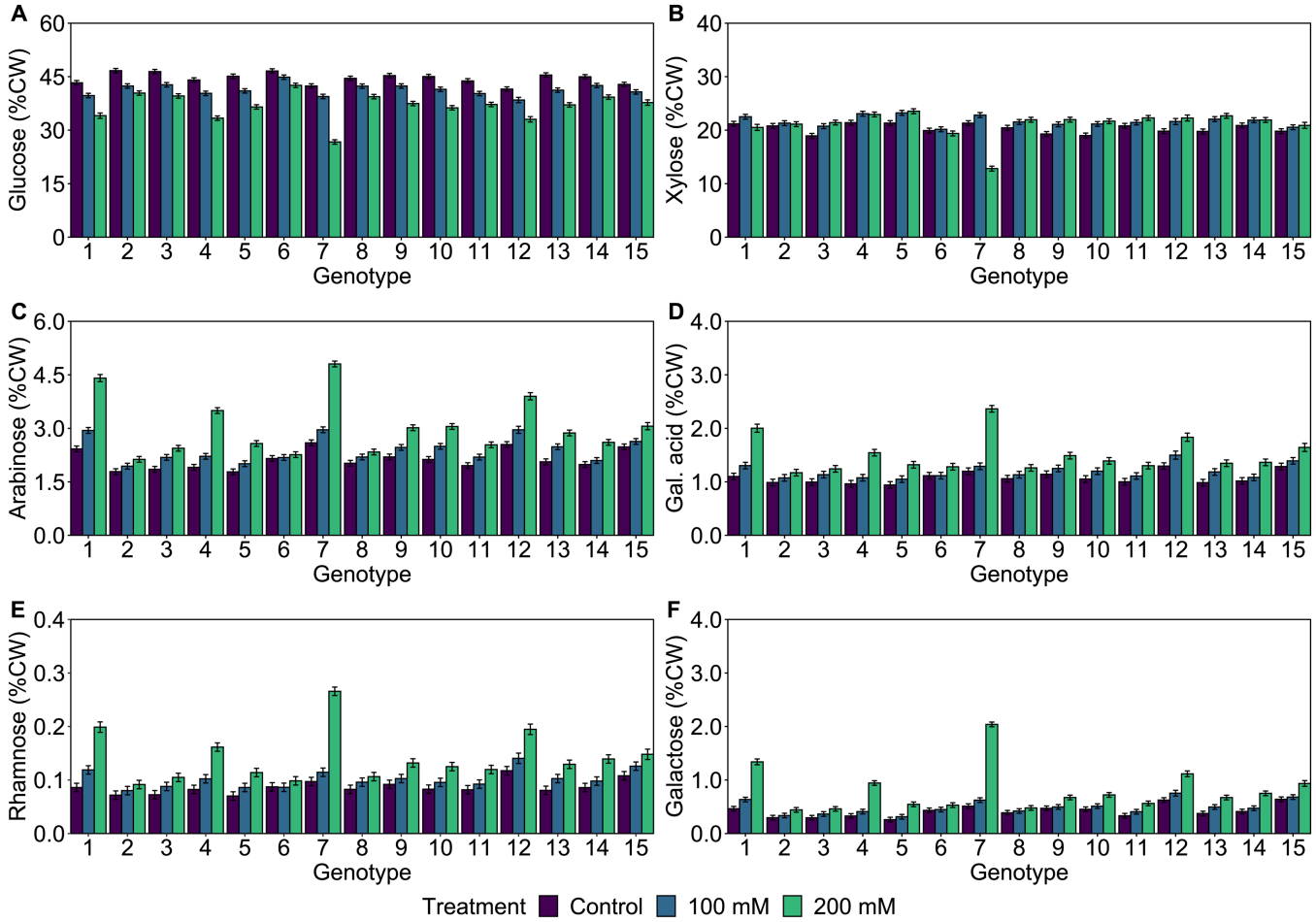

FIGURE 2 Analysis of monosaccharide content in cell walls (%CW) from stems of 15 M. sinensis genotypes grown for 4 weeks under control (0 mM NaCl), moderate salt stress (100 mM NaCl), or severe salt stress (200 mM NaCl) conditions. Each bar represents the mean of n = 3 biological replicates; error bars indicate ± SE.

were always higher than those in moderately stressed plants ($p < 0.001$). In stressed plants, there was also an increased variation in total pectin levels between genotypes, as these ranged from 1.45 to 2.39% for moderately stressed plants and from 1.70 to 4.67% for severely stressed plants. For all genotypes, the relative increase between control conditions and severe stress conditions was more substantial for galactose than for galacturonic acid and rhamnose. This was best illustrated in genotype 7, where the increase in galactose was 4-fold compared to 2.7-fold for rhamnose and 2-fold for galacturonic acid.

3.2 | Salt stress impacts lignin structural features and hydroxycinnamic acid content

The total lignin content (AIL + ASL) was, on average, 16.4% for plants that were grown under control conditions. The salt treatments had only a modest effect on the total lignin content for most genotypes after the 4-week stress period, being slightly reduced, increased or unchanged (Figure 3A). Although the observed differences were relatively small and appeared rather inconsistent, it was found that over all genotypes, the total lignin content was slightly, yet significantly higher after severe salt stress ($p < 0.05$). An interesting observation was that genotypes 1, 4, 7 and to a lesser extent, 14 showed a clear increase in ASL content at the expense of AIL content (Figure 3 A-D), leading to significant genotype and treatment interactions for both traits ($p < 0.001$). In these genotypes, the consequences of severe stress most likely caused a higher solubilization of the existing lignin polymer during the acid hydrolysis process, as also suggested by the strong negative correlation between AIL and ASL (Figure 3D). Contrasting effects were observed for the hydroxycinnamic acids. Ferulic acid content was, on average, 0.64% at control conditions and slightly increased ($p < 0.01$) by 4.7% for moderately stressed- and 6.3% for severely stressed plants (Figure 3E). The *p*-coumaric acid content was 2.30% at control conditions, and clear reductions ($p < 0.001$) of 12.6% for moderately stressed plants and 22.2% for severely stressed plants were observed (Figure 3F).

The lignin composition was analyzed between control and severely stressed plants of genotypes 1, 4, 7 and 14 to investigate if the increased ASL levels would be related to its structural features. Indeed, treatment with 200 mM NaCl substantially changed the

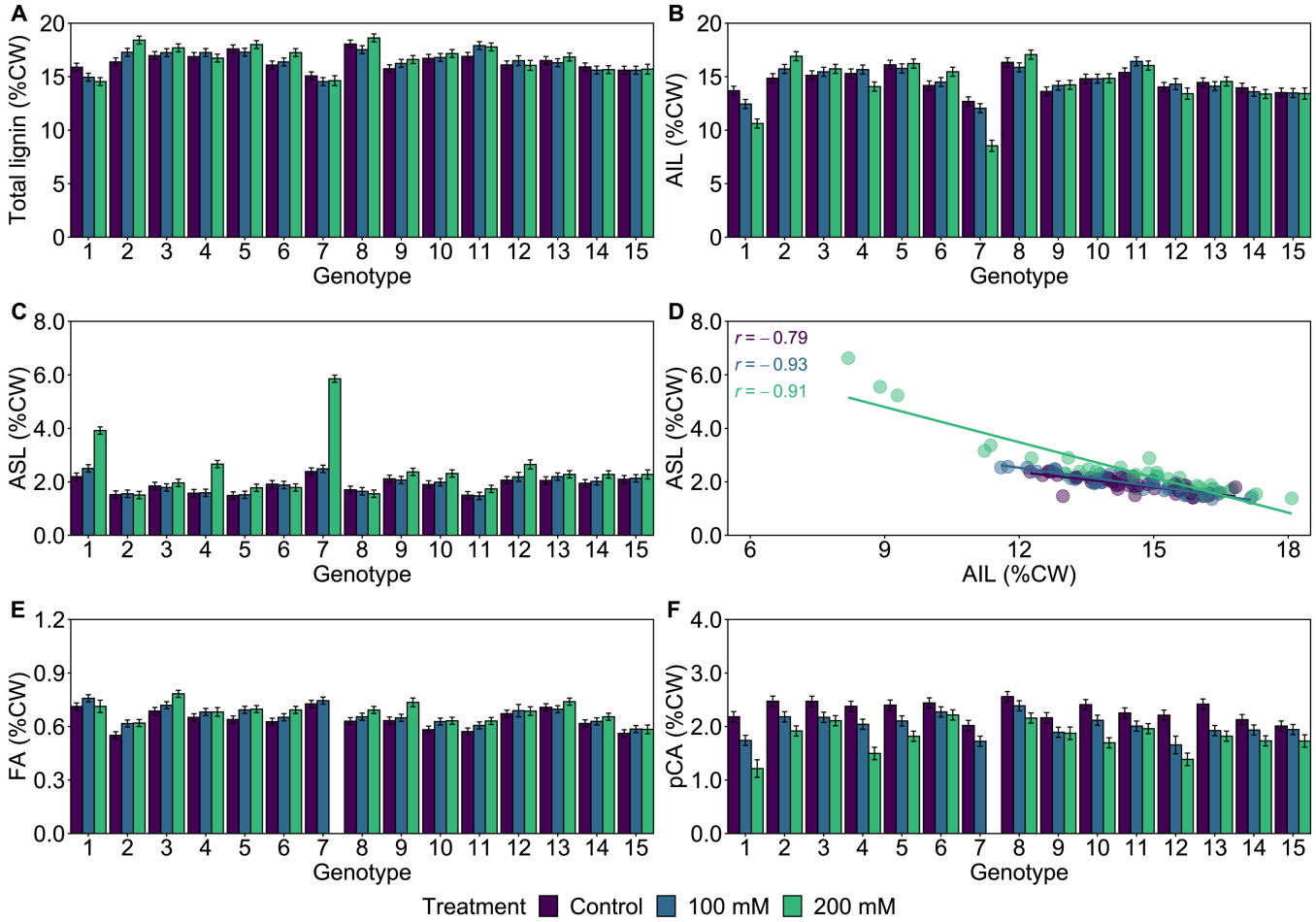

FIGURE 3 Total lignin (AIL + ASL), Acid Insoluble Lignin (AIL), Acid Soluble Lignin (ASL), Ferulic Acid (FA) and p-Coumaric Acid (pCA) content in cell walls (%CW) from stems of 15 M. sinensis genotypes grown for 4 weeks under control (0 mM NaCl), moderate salt stress (100 mM NaCl), or severe salt stress (200 mM NaCl) conditions. Each bar represents the mean of n = 3 biological replicates; error bars indicate ± SE. FA and pCA data are missing for Genotype 7 at 200 mM NaCl due to insufficient biomass to perform the analysis, for Genotype 1 only one biological replicate was available.

structural composition (Figure 4), and samples most affected in terms of ASL content were also the most structurally changed. The analysis indicates reduced incorporation of syringyl subunits in the most affected plants (genotypes 1, 4, 7), both when the subunit composition was expressed in terms of all pyrolysis products (Figure 4A, Table S3) and the specific ratio of trans-sinapylalcohol to trans-coniferylalcohol (Figure 4B, Table S3). The latter parameter is especially relevant for pyrolysis-GC–MS lignin analysis of grasses like Miscanthus because it excludes interference from ferulic acid (van Erven et al., 2023). Upon pyrolysis, ferulic acid is converted into 4-vinylguaiacol, a product that is also formed from lignin, and its origin can thus not be distinguished. The difference observed for both ratios for genotype 14 might be due to a slightly changed ferulic acid content (Figure 3E). Analogously to ferulic acid, *p*-coumaric acid is converted into 4-vinylphenol and therefore, this pyrolysis product reflects the abundance of these moieties. In line with the analyzed pCA content (Figure 3F), pyrolysis-GC–MS analysis confirmed the reduction of *p*-coumaric acid upon salt stress (Figure 4C).

3.3 | Recovery period

Plants grown at control conditions got essentially an extended 4 weeks of growth with unchanged conditions during the recovery period. After the recovery period, control plants had an average cell wall content of 87.8%, indicating an increase between the harvest timepoints (Table 1). Although the cell wall content also increased for plants previously exposed to 100 mM salt (85.2%) or 200 mM salt (82.7%), the interaction between treatments and genotypes remained significant ($p < 0.01$). On average, the control plants were found to have glucose levels of 44.8% and xylose levels of 20.5%, which were similar to the values observed at the end of the initial period (Table 1). The average glucose levels were 43.1% for plants that were previously exposed to 100 mM salt and 42.4% for those previously exposed to 200 mM salt. Although glucose levels were still significantly lower ($p < 0.05$) in plants previously exposed to 200 mM salt, their values were much higher than those after the initial stress period. In general, the small increase in xylose levels that was observed in plants exposed

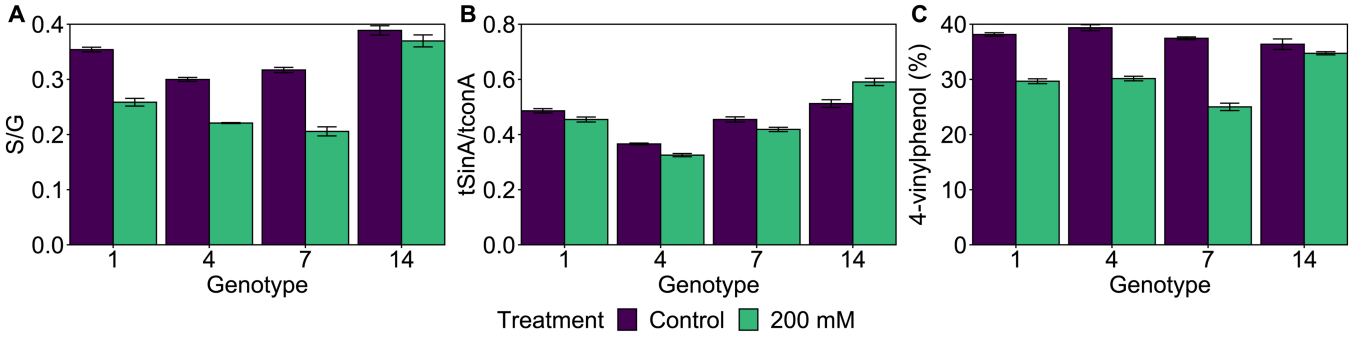

FIGURE 4 Pyrolysis-GC–MS analysis of lignin structural features. Ratios of Syringyl/Guaiacyl (A) and trans-sinapylalcohol/trans-coniferyl alcohol (B) and 4-vinylphenol abundance relative to all lignin-derived pyrolysis products (C) in 4 genotypes after control (0 mM NaCl) or severe salt (200 mM) stress treatment. Each bar represents the mean of n = 3 technical replicates; error bars indicate ± SD.

TABLE 1 Mean values (± SE) of the cell wall (% dry weight) and its components (% cell wall) from stems of the 15 M. sinensis genotypes grown at Control (0 mM NaCl), 100 mM NaCl or 200 mM NaCl conditions, harvested at the 4-week (stress) and 8-week (recovery) timepoints, with 45 observations per treatment per timepoint. The '%' shows the relative change from the 4-week timepoint to the 8-week timepoint.

	Control			100 mM			200 mM		
Trait	**4-week**	**8-week**	**%**	**4-week**	**8-week**	**%**	**4-week**	**8-week**	**%**
Cell wall (%DW)	82.6 ± 0.49	87.8 ± 0.74	6.3	81.9 ± 0.49	85.2 ± 0.74	4.03	74.2 ± 0.50	82.7 ± 0.74	11.5
Glucose (%CW)	44.5 ± 0.26	44.8 ± 0.51	0.7	41.3 ± 0.26	43.1 ± 0.51	4.4	36.7 ± 0.26	42.4 ± 0.51	15.5
Xylose (%CW)	20.3 ± 0.16	20.5 ± 0.23	1.0	21.7 ± 0.17	21.1 ± 0.23	−2.8	21.2 ± 0.17	21.2 ± 0.23	0.0
Arabinose (%CW)	2.12 ± 0.02	1.81 ± 0.02	−14.6	2.40 ± 0.02	2.16 ± 0.02	−10.0	3.03 ± 0.02	2.33 ± 0.02	−23.1
Galacturonic acid (%CW)	1.08 ± 0.02	0.88 ± 0.02	−18.5	1.19 ± 0.02	1.01 ± 0.02	−15.1	1.50 ± 0.02	1.08 ± 0.02	−28.0
Rhamnose (%CW)	0.09 ± 0.002	0.08 ± 0.004	−11.1	0.10 ± 0.002	0.10 ± 0.004	0.0	0.14 ± 0.002	0.11 ± 0.004	−21.4
Galactose (%CW)	0.42 ± 0.01	0.28 ± 0.02	−33.3	0.49 ± 0.01	0.38 ± 0.02	−22.4	0.81 ± 0.01	0.44 ± 0.02	−45.7
Acid insoluble lignin (%CW)	14.5 ± 0.11	17.7 ± 0.28	22.1	14.6 ± 0.11	16.6 ± 0.28	13.7	14.3 ± 0.11	15.4 ± 0.28	7.7
Acid soluble lignin (%CW)	1.89 ± 0.04	1.35 ± 0.04	−28.6	1.94 ± 0.04	1.59 ± 0.04	−18.0	2.46 ± 0.04	1.80 ± 0.04	−26.8
Total lignin (%CW)	16.4 ± 0.10	19.1 ± 0.24	16.5	16.5 ± 0.10	18.1 ± 0.24	9.7	16.8 ± 0.10	17.2 ± 0.24	2.4
Ferulic acid (%CW)	0.64 ± 0.007	0.77 ± 0.007	20.3	0.67 ± 0.007	0.84 ± 0.007	25.4	0.68 ± 0.007	0.86 ± 0.007	26.5
p-Coumaric acid (%CW)	2.30 ± 0.03	2.61 ± 0.04	13.5	2.01 ± 0.03	2.57 ± 0.04	27.9	1.79 ± 0.04	2.41 ± 0.04	34.6
Glucose conversion (%)	25.5 ± 0.51	14.3 ± 0.65	−43.9	25.6 ± 0.52	17.2 ± 0.65	−32.8	30.0 ± 0.57	21.8 ± 0.65	−27.3

to stress was slightly less pronounced and no longer significant after the recovery period. Remarkably, the large drop in xylose content that was observed in severely stressed plants of genotype 7 was also no longer found. The patterns for arabinose, galacturonic acid, rhamnose and galactose levels remained the same as was observed after 4-weeks (Figure S3). The proportion of all minor monosaccharides remained higher ($p < 0.01$) in plants previously subjected to stress, especially in those genotypes more sensitive to stress. This observation was further supported by the significant interactions ($p < 0.001$) between treatments and genotypes. However, plants subjected to severe stress showed a greater relative reduction in these monosaccharides (Table 1). Consequently, rhamnose, galactose and galacturonic

acid levels no longer differed significantly between plants that had recovered from moderate and severe stress treatments. This suggests that the persistently high content of monosaccharides in these plants, relative to controls, likely resulted from a higher initial content at the start of the recovery period.

The total lignin content of plants that were maintained at control conditions was, on average, 19.1%, which was significantly higher ($p < 0.01$) than the total lignin content of 17.2% for severely stressed plants that recovered after stress. The largest portion of total lignin content belonged to AIL, which accounted for 17.7% (control), 16.6% (100 mM) and 15.4% (200 mM) of the total cell wall. For control plants, the additional growth period increased AIL levels by 22.1% on average compared to the values that were observed after the 4-week timepoint. After the recovery period, the increase of AIL in formerly stressed plants was 13.7% for plants that received the moderate stress treatment, while it was only 7.7% for plants that had been severely stressed. The reduced lignification rate of plants that had been stressed led to significant differences ($p < 0.01$) in AIL levels between treatments, while such differences were absent after the initial stress period. ASL levels were 1.35% in control plants, 1.59% in plants that had received 100 mM salt and 1.80% in plants that had received 200 mM salt. Although ASL levels were reduced in all treatments, they remained significantly higher ($p < 0.05$) in previously stressed plants. Altogether, the observed lower lignin levels in previously stressed plants after the recovery period applied to all of the tested genotypes (Figure S4), which contrasted with the results after the first harvest timepoint.

3.4 | Structural alterations increase saccharification efficiency

Average glucose conversion levels were 25.5% (control), 25.6% (100 mM) and 30.0% (200 mM) after the 4-week growth period (Table 1). Glucose conversion ranged from 18.8% to 35.1% between genotypes grown under control conditions. As mentioned above, in general, the cell wall composition remained similar between plants at control conditions and those that received the 100 mM stress treatment. This lack of changes indeed translated into similar glucose conversion levels between these treatments. In contrast, the glucose conversion levels of plants subjected to 200 mM of salt were significantly higher ($p < 0.01$) and varied between 15.4% and 54.5%. Statistical analysis showed a significant interaction between genotypes and treatments ($p < 0.001$), which was reflected in the data as genotypes 1, 4, 12 and 14 had an increase in glucose conversion between 29.4% and 86.6%. For genotypes 9 and 10, the increase was 15.7% and 13.4%, while for other genotypes, glucose conversion levels remained similar (Figure 5A). It should be noted that genotype 7 grown under severe stress conditions did not yield enough biomass to perform the enzymatic saccharification experiment. However, based on the altered cell wall composition, it is expected that it would also have significantly improved glucose conversion rates under these conditions. The extended growth period strongly reduced glucose conversion levels across all treatments, with average reductions of 43.9% in control, 32.8% in 100 mM and 27.3% in 200 mM treatments. The larger reduction in glucose conversion for control plants led to significant differences among all treatments ($p < 0.05$). Furthermore, all genotypes previously exposed to severe stress exhibited a higher glucose conversion rate compared to their respective controls after the recovery period (Figure 5B). This increase was also observed in genotypes where no differences were found initially.

The variation that is present in glucose conversion rates between genotypes can be explained by the differences in the cell wall composition. The composition, in turn, depends on the combination of genotype, treatment and harvest timepoint. Acid insoluble lignin (AIL) negatively influenced glucose conversion rates in all treatments for 4-week-old and 8-week-old plants, with correlation coefficients ranging from −0.56 to −0.93 (Figure 6A). The increase in AIL content between 4-week-old and 8-week-old plants is considered the main reason for the reduced glucose conversion rates between the two timepoints. In contrast, ASL showed a positive association with glucose conversion for all treatments at both harvest timepoints, with correlation coefficients ranging from 0.66 to 0.92 (Figure 6B). Therefore, the aforementioned shift to a less rigid form of lignin that was

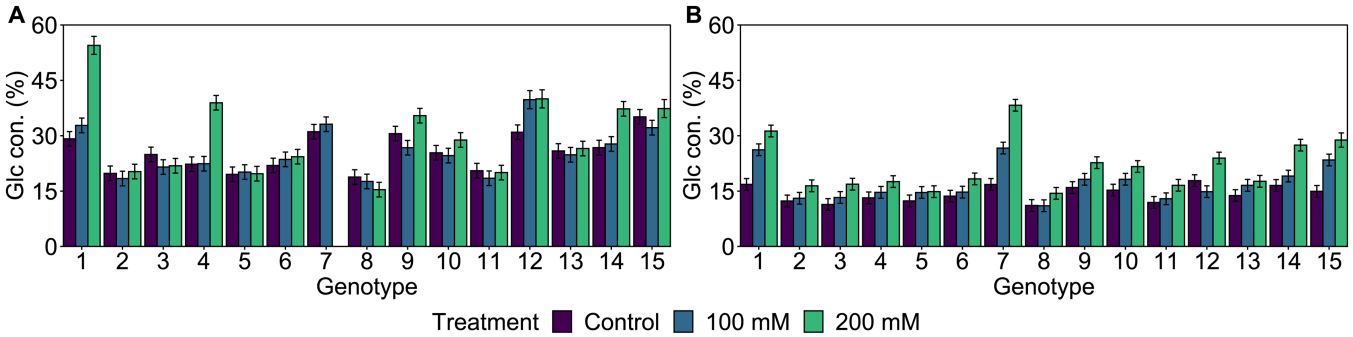

FIGURE 5 Glucose conversion (Glc con.) percentages from stems of 15 M. sinensis genotypes after growing 4 weeks (A) under control (0 mM NaCl), moderate stress (100 mM NaCl) or severe stress (200 mM NaCl) and after a subsequent recovery period (B). Each bar represents the mean of n = 3 biological replicates; error bars indicate ± SE. Glucose conversion levels for Genotype 7 at 200 mM NaCl are missing due to insufficient biomass to perform the analysis.

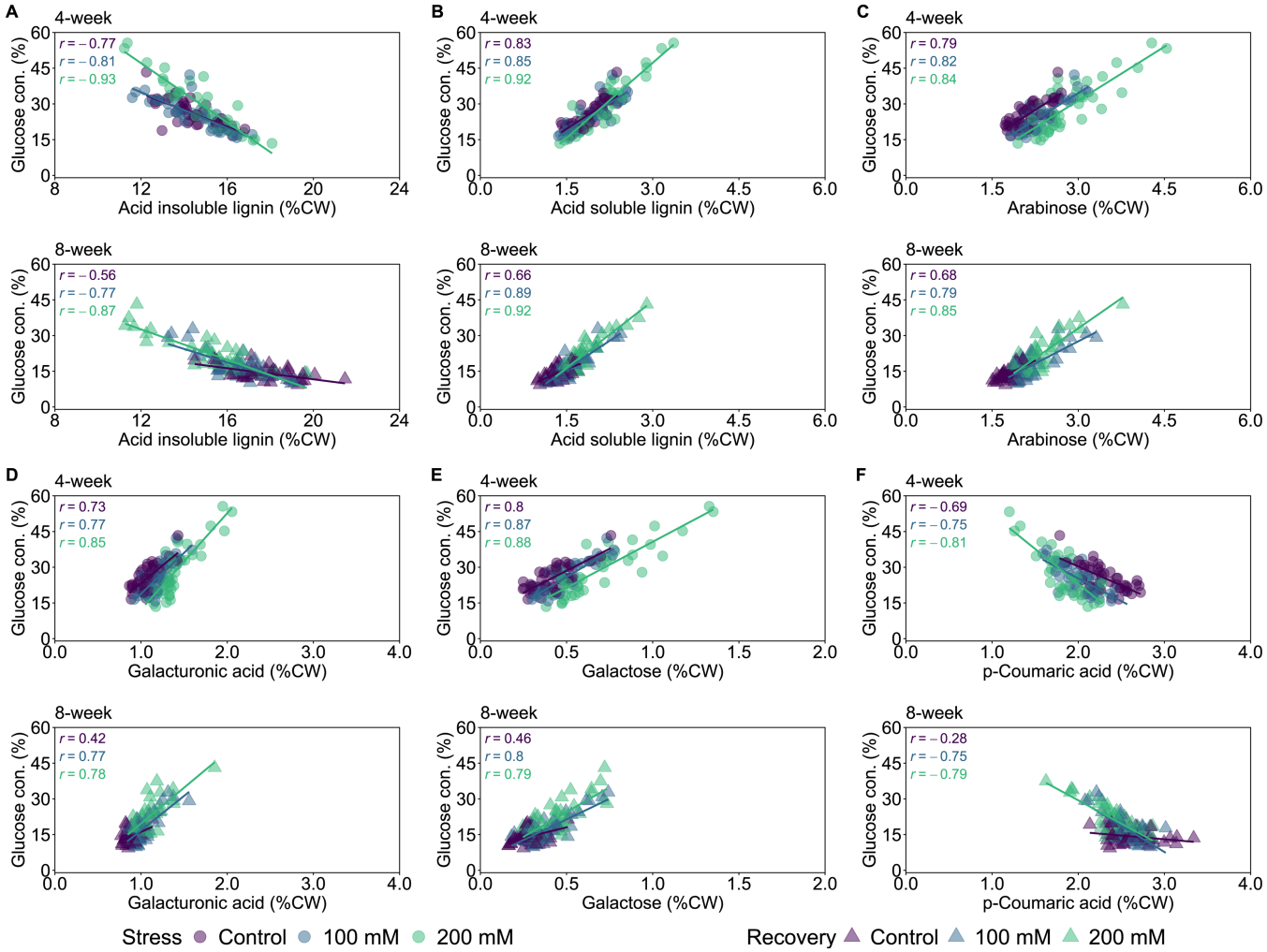

FIGURE 6 Pearson correlation coefficients between glucose conversion (%) and cell wall components in stems of M. sinensis plants after a stress period (4-week) and a subsequent recovery period (8-week), grown under three treatments: Control (0 mM NaCl), 100 mM NaCl or 200 mM NaCl. All correlations are significant ($p < 0.01$), except the correlation between Glucose conversion and p-Coumaric acid for the Control treatment at the recovery timepoint.

observed in some genotypes after severe stress seems to be an important factor that led to increased glucose levels. Like ASL, arabinose and the pectin components were also positively correlated with glucose conversion (Figure 6C-E) and negatively with lignin (Figure S2). It is likely that the increase in minor monosaccharides also contributed to glucose conversion, as the improved conversion rates of genotypes 9 and 10 after the 4-week stress period could not be explained by differences in lignin content. Interestingly, the data also showed that not only the content but also the genotypic variation of all favourable components declined as plants in the control environment matured (Figure 6).

4 | DISCUSSION

Exposure to salt stress alters the chemical composition of plant cell walls, and it has been hypothesized that these changes could be related to tolerance. In this study, we investigated the response of different genotypes that were previously identified to be contrasting in their response to salt and drought (Chen et al., 2017; van der Weijde, Huxley, et al., 2017). The variation that was observed for the relative reduction in biomass between control and salt-stressed plants confirmed that the selected genotypes indeed exhibited differences in tolerance (Figure 1). It was notable that plant yields for several genotypes, such as 6 and 8, were barely reduced, even under severe stress conditions. Although these genotypes did not produce the highest yields in the current experiments, they should be evaluated under field conditions, as yields in young plants are not necessarily indicative of yields in mature stands (Lewandowski et al., 2016). Regardless of their yields, these genotypes would be valuable resources for breeding programs, as they likely contain traits that could further enhance salt tolerance during early establishment in *M. sinensis*. The cell wall composition of *M. sinensis* stems contained, on average, 44.5% glucose, 22.4% arabinoxylan, 16.4% lignin and small amounts of pectin after

4 weeks of growth under control conditions (Table 1). These findings are consistent with those generally reported for Miscanthus, taking into consideration the young age of the plants (van der Cruijsen et al., 2021). The results show that the proportions of all these key components of the primary- and secondary cell walls were altered after exposure to saline conditions. The most pronounced alterations occurred after the severe stress treatment in genotypes that were most susceptible to salinity.

4.1 | Salinity affects the polysaccharide composition within the cell walls

Cellulose content decreased upon exposure to salt stress in all genotypes that were included in this study (Figure 2A). It was consistently observed that this reduction was higher under severe stress conditions (200 mM NaCl) compared to moderate stress conditions (100 mM NaCl). The decrease in cellulose levels as a response to salt stress has been reported previously for Miscanthus and other grass species (Uddin et al., 2013; Oliveira et al., 2020; Y. Yuan et al., 2021; Zheng, Yi, et al., 2022) as well as in Miscanthus exposed to drought (van der Weijde, Huxley, et al., 2017; Hoover et al., 2018). Osmotic and ionic stress affects the expression of multiple cellulose synthase (CesA) genes and non-CesA genes critical for regular cellulose biosynthesis (Wang, McFarlane and Persson, 2016). In addition, cellulose deposition is also prevented due to the disturbance of the microtubule arrangement required to guide cellulose synthase complexes to the plasma membrane (Wang, McFarlane and Persson, 2016). Thus, irregular cellulose biosynthesis as a consequence of salt stress is likely causing the reduction in cellulose content within the cell walls. In the formerly stressed plants, cellulose levels increased during the recovery period, ultimately being closer to the levels observed in control plants (Table 1). These values indicate that cellulose biosynthesis was largely restored within 4 weeks once the stressor was removed.

In contrast to the drop in cellulose, the amount of hemicellulose slightly increased upon exposure to salt (Figure 2B). A modest increase in xylose content implies expansion of the arabinoxylan backbone. The increase was found in all genotypes except genotype 7. Arabinose content increased to a much greater extent, indicating potentially a higher degree of substituted hemicellulose (Figure 2C). Although under normal conditions arabinose is more abundantly present as substitutions on the xylan backbone than on pectin (Schäfer et al., 2019), the method that we used for quantification of the monosaccharides cannot discriminate between them. Zheng, Yi et al. (2022) reported an increase of hemicellulose in Miscanthus grown on highly saline, alkaline soils compared to low saline, alkaline soils. In maize, salt stress did not result in alterations of xylose content, while an increase in arabinose only occurred in leaves but not in stems (Oliveira et al., 2020). Maksup et al. (2020) reported that in *Pennisetum purpureum* salinity-induced changes in hemicellulose depended on the genetic background and could not be generalized. However, our findings suggest a mostly consistent pattern in which xylose is marginally affected, but arabinose increased in a stress severity-dependent matter in *M. sinensis*. This means that although arabinose content increased in nearly all genotypes, it was much more pronounced under severe stress conditions and in genotypes that were more sensitive to salt.

The galacturonic acid, rhamnose and galactose levels all increased significantly under stress in a manner that was highly similar to alterations in arabinose content (Figure 2D-F). The substantial increase in rhamnose and galactose indicated that the additionally synthesized pectin would likely be enriched with substituted RG-I structures. It is reasonable to assume that at least part, but perhaps a substantial amount, of the additional arabinose that was synthesized during salt stress also belonged to the sidechains of the RG-I polymers. In several non-grass species, homogalacturonan accumulated together with increased neutral sugar substitutions attached to RG-I (Iraki et al., 1989; Corrêa-Ferreira et al., 2019; Vago et al., 2021). The negatively charged homogalacturonans are able to bind Na^{+}, which might fixate some of these ions inside the cell wall (Byrt et al., 2018; Corrêa-Ferreira et al., 2019). This could affect cell wall stiffness as it interferes with the binding of Ca^{2+} that is required for the formation of pectin crosslinks (Colin et al., 2023). It is proposed that the remodelling of the primary cell wall and the synthesis of branched polymers are essential to maintain cell wall integrity and handle perturbations in turgor pressure during salt stress (Jaramillo Roman, 2021; Colin et al., 2023). Our results indicate that genotypes that experienced the most stress due to a combination of treatment severity and genotypic sensitivity had undergone the most extensive cell wall remodelling in their stems. Since the experienced stress level determines the extent to which remodelling takes place, it is likely to contribute to salt tolerance, but does not explain the differences in sensitivity between genotypes in this study.

4.2 | Salt stress affects acid-induced lignin solubilization, subunit composition and hydroxycinnamic acids

Total lignin content remained relatively constant between treatments after the initial 4-week stress period, although it appeared that there were some variations between genotypes (Figure 3A). Other studies on Miscanthus reported that lignin content remained essentially unchanged in plants grown in saline environments (Stavridou, Webster and Robson, 2019; Zheng, Yi, et al., 2022). In contrast, in maize, salinity caused an upregulation of key genes within the lignin biosynthetic pathway, leading to increased lignin content that was more enriched with syringyl units (Oliveira et al., 2020). The authors reasoned that increased lignification could provide mechanical support and might create a barrier that prevents the influx of salt ions into the xylem vessels. Conversely, here, salt stress induced a relatively lower proportion of syringyl units in the lignin of three salt-sensitive genotypes (1, 4 and 7) after severe salt stress (Figure 4). In contrast, these structural modifications hardly took place in the similarly sensitive genotype 14, suggesting that such responses may be genotype-specific. A broader screening, also including less sensitive genotypes, would be required to further elucidate the effect of salt stress on the

lignin composition. Few other studies have addressed the effect of salinity on either lignin content or composition. In *Panicum miliaceum*, genes involved in the biosynthesis of both guaiacyl and syringyl lignin were upregulated, suggesting additional synthesis of both subunits, although the actual content was not measured (Y. H. Yuan et al., 2021). Studies on other grasses have reported both increased (Tiwari et al., 2018) and decreased (Kumar et al., 2018) lignin levels as a consequence of salt stress. Although there were slight variations in lignin content between genotypes, it seems unlikely that these could explain the large differences observed in tolerance.

The lignin content inside the plant cell walls typically increases gradually during plant maturation before reaching its plateau once senescence is completed (Chen et al., 2002; Da Costa et al., 2014; Crowe et al., 2017). Accordingly, for plants that were grown under control and moderate stress conditions, the lignin content indeed increased between the 4-week and 8-week timepoints. However, this increase was largely absent for plants that were initially grown under severe stress conditions (Table 1). Wang et al. (1997) showed that stems of salt-stressed *Atriplex prostrata* plants became less lignified and likely maintained a higher proportion of primary cell walls, proposing that reduced growth also slowed down maturation. In the current study, a delay in maturation was indicated as well by the reduced number of flowering plants under (formerly) stressed conditions at the end of the recovery period (data not shown). In the case of lignin content, the results after the recovery period showed that the initial response that was observed directly after the stress period is not necessarily predictive for the longer term. Moreover, it illustrates the importance of timing within a single study and might explain some of the contrasting results regarding the effects of abiotic stress on the cell wall in literature. Extending these observations to field conditions suggests that temporarily experiencing salt stress could lead to reduced lignification rates and, thereby, potentially result in lower lignin levels at the end of the growing season.

Regarding the hydroxycinnamic acids, there was a slight increase in FA and a considerable drop in *p*CA content (Figure 3E-F). Similar observations were reported in maize stems, although the increase in FA was far more substantial (~80%), where additional analysis suggested that FA increased solely in the form of arabinoxylan substitutions while the incorporation of *p*CA was reduced in lignin (Oliveira et al., 2020). An interesting observation was that for several genotypes, the severe stress conditions led to increased solubilization of lignin during the acid hydrolysis process, hinting at structural differences at the molecular level. Previous research has suggested that ASL is enriched in syringyl units and acid-induced lignin-carbohydrate condensation products (Yasuda, Fukushima and Kakehi, 2001; Matsushita et al., 2004). However, given the current results, it is unlikely that the increased ASL was related to the syringyl units as their incorporation decreased.

The strong correlations between several monosaccharides and ASL warrant the question of whether additional lignin-carbohydrate complexes were formed and if these could affect the lignin structure. The formation of lignin-carbohydrate complexes is usually considered to take place mainly between ferulic acid-substituted arabinoxylan and lignin (Hatfield, Rancour and Marita, 2017). The current results show a strong correlation between arabinose and ASL ($r = 0.9$). Although ferulic acid also increased, it was not fully proportional to the increase in the other two components (Figure S2). Additionally, it is notable that ASL also correlated significantly with the pectin-specific monosaccharides. Although both effects could be an independent response to the stress applied, Hu et al. (2021) also reported a positive correlation between galactose and ASL in rice and suggested the existence of linkages between these two components. The occurrence of such linkages seems plausible, as small amounts of pectin can be traced back to the secondary cell walls in grasses (Jeong, Nguyen and Lee, 2015; Bhatia et al., 2017), and both arabinose and galactose have been previously associated with the formation of pectin-lignin-complexes (Meshitsuka et al., 1982; Minor, 1982; Qin et al., 2018). Furthermore, glycome profiling in Miscanthus also indicated a tight association between pectin and lignin, which was mainly based on the occurrence of specific RG-I and arabinogalactan epitopes in lignin-rich extractives (De Souza et al., 2015; Da Costa et al., 2017). It is also of interest that the amount of water-extractable pectin decreased in salt-stressed plants (De Lima et al., 2014; Vago et al., 2021), implying that pectin somehow became more anchored into the cell walls. Although pectin-lignin linkages have thus far not been detected in mature plant stems, they potentially play a role in the onset of lignification in young plants (Kang et al., 2019). Nevertheless, additional research would be necessary to confirm if the proposed formation of pectin-lignin complexes takes place and could be causative for the increased ASL content in young and stressed plants.

4.3 | Salt stress leads to improved saccharification efficiency

Although salt stress reduced the amount of biomass and cellulose that would be available for enzymatic conversion, it remains interesting to evaluate the effect of the considerable changes the cell walls underwent from a quality perspective. First, it was clear that plants with lower AIL levels presented higher glucose conversion rates (Figure 6A). Many studies have described that higher lignin levels have a negative effect on enzymatic saccharification efficiency and that lowering its content should be one of the main targets for optimizing the composition of lignocellulose feedstocks (Li, Weng and Chapple, 2008; Lygin et al., 2011; van der Weijde, Kiesel, et al., 2017; Halpin, 2019). In this sense, it was interesting that exposure to salt stress resulted in lower lignin levels in all plants that were analyzed after the recovery period and thereby increased the glucose conversion rates compared to control plants (Figure 5B). Similarly, *M. x giganteus* that experienced continuous drought throughout the growing season had a lowered lignin content that reduced the biomass recalcitrance and could thereby partly compensate for its reduction in structural sugars and biomass yield (Hoover et al., 2018). The results after the recovery period indicate that a temporary period of abiotic stress could lead to lower lignin levels while the amount of cellulose within the cell wall was largely restored. Although it is unlikely this mechanism can be easily exploited in practice, it would mean that the disadvantage of less structural

sugars would not necessarily remain in every situation where abiotic stress takes place. However, more insight would be required on how the timing of abiotic stress events influences the physiological development and if this could lead to a lasting improvement in the cell wall composition. In contrast to AIL, it appeared that ASL correlated positively to glucose conversion (Figure 6B). It is important to note that the proportion of ASL showed a strong negative correlation with AIL, which was most apparent in the response of the genotypes that were sensitive to severe stress. Although the exact structural differences leading to increased ASL in stressed plants remain unknown, it is clear that this more labile form of lignin did not form an impenetrable barrier that limited glucose conversion. Previously, two studies on sugarcane already reported a positive effect of ASL on the enzymatic saccharification efficiency (Hodgson-Kratky et al., 2019; Chourasia et al., 2021).

Also, arabinose and the pectin-specific monosaccharides correlated positively to glucose conversion (Figure 6C-E). It has been commonly reported that higher arabinose levels tend to increase the enzymatic saccharification efficiency (Li et al., 2013; van der Weijde, Kiesel, et al., 2017; Brancourt-Hulmel et al., 2021). The positive effect has been mainly assigned to the arabinose substitutions that are present as side-chains of xylan, as their interaction with the cellulose microfibrils reduces the crystallinity of these structures (Li et al., 2013; Gao et al., 2020). A reduction in crystallinity enlarges the surface area of cellulose and thereby increases the enzymatic activity (Arantes and Saddler, 2010). Although in the current study, we cannot differentiate between hemicellulose and pectin-derived arabinose, it is still likely that this well-established mechanism explains part of the positive effect observed. Several studies have reported that pectin affects enzymatic saccharification efficiency in Miscanthus (De Souza et al., 2015; Wang et al., 2015; Cheng et al., 2018; Da Costa et al., 2019). The work by De Souza et al. (2015) claims that pectin contributes either positively or negatively depending on its structural interactions with the other cell wall components. However, two other studies describe a generally positive effect of higher pectin content, which was associated with increased galacturonic acid levels leading to a reduction in cellulose crystallinity (Wang et al., 2015; Cheng et al., 2018). Nevertheless, our results indicate a positive effect that was likely associated with branched RG-I structures, as indicated by the increased rhamnose and galactose levels. The importance of RG-I/arabinogalactans over homogalacturonan for saccharification efficiency was also observed in the leaves of mature Miscanthus plants, although neither appeared to be of significant importance in the stems (Da Costa et al., 2019). Grasses contain more primary cell walls in their earlier developmental stages, which are also associated with higher levels of arabinose and galactose (Rancour, Marita and Hatfield, 2012). In control and stressed plants a reduction in minor monosaccharides was already visible between the 4-week and 8-week timepoint, which also reduced the genotypic variation that was present. Even with the reduced variation, the minor monosaccharides were still positively correlated with saccharification at the 8-week timepoint. However, these correlations were much less pronounced than those observed for other treatments and timepoints. One possible explanation is that higher AIL levels in control plants at the 8-week timepoint severely restricted enzymatic saccharification across all genotypes. This restriction not only reduced variation but also led to a lower correlation between AIL and glucose conversion under these conditions ($r = -0.56$). We expect that this combination of high AIL levels and the lack of variation also partly masked the importance of the minor monosaccharides, which showed stronger correlations at other treatments and timepoints. Applying a pretreatment would increase the efficiency of the enzymatic saccharification process and create greater variation between genotypes, likely emphasizing the importance of different cell wall components again (Belmokhtar et al., 2017). In practice, the relevance of the minor monosaccharides would likely depend on whether substantial variation remains after senescence has taken place in mature plants under field conditions. Therefore, we propose additional germplasm screenings after senescence occurs to reveal the feasibility and potential of improving biomass quality by breeding for minor monosaccharides.

In conclusion, the general trends in salt stress-induced cell wall alterations were similar within stems of the most- and the least tolerant genotypes. However, the extent to which these alterations took place varied considerably as they were consistently more pronounced within the least tolerant genotypes. Therefore, it seems more probable that the alterations were adaptations to stress levels perceived differently by each genotype rather than a mechanism determining the differences in tolerance among genotypes. Interestingly, the induced compositional changes did enhance the enzymatic saccharification efficiency, and it would be of interest to investigate whether, through breeding, this more favourable composition could be realized in non-stressed plants.

AUTHOR CONTRIBUTIONS

Conceptualization: KvdC, MAH and LMT; Experimental analysis: KvdC, MAH, GvE, NK, BvL and AD; Statistical analysis: KvdC, OD and MP; Original draft preparation: KvdC, GvE and LMT; Reviewing and editing: all authors listed; Funding and acquisition: LMT. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

Jeroen Zonneveld and Geurt Versteeg provided technical assistance during the greenhouse experiments.

FUNDING INFORMATION

This research has received funding from the Bio-based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program under grant agreement No 745012 (GRACE).

DATA AVAILABILITY STATEMENT

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

ORCID

Luisa M. Trindade https://orcid.org/0000-0003-1541-2094

REFERENCES

An, P., Li, X., Zheng, Y., Matsuura, A., Abe, J., Eneji, A.E., Tanimoto, E. and Inanaga, S. (2014) 'Effects of NaCl on root growth and cell wall composition of two soya bean cultivars with contrasting salt tolerance', *Journal of Agronomy and Crop Science*, 200(3), pp. 212–218. Available at: https://doi.org/10.1111/jac.12060.

Arantes, V. and Saddler, J.N. (2010) 'Access to cellulose limits the efficiency of enzymatic hydrolysis: the role of amorphogenesis', *Biotechnology for Biofuels*, 3(1), p. 4. Available at: https://doi.org/10.1186/1754-6834-3-4.

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. and Hayat, S. (2020) 'Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance', *Plant Physiology and Biochemistry*, 156(July), pp. 64–77. Available at: https://doi.org/10.1016/j.plaphy.2020.08.042.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015) 'Fitting Linear Mixed-Effects Models Using lme4', *Journal of Statistical Software*, 67(1), pp. 1–48. Available at: https://doi.org/10.18637/jss.v067.i01.

Belmokhtar, N., Arnoult, S., Chabbert, B., Charpentier, J.P. and Brancourt-Hulmel, M. (2017) 'Saccharification performances of Miscanthus at the pilot and miniaturized assay scales: Genotype and year variabilities according to the biomass composition', *Frontiers in Plant Science*, 8(May), pp. 1–13. Available at: https://doi.org/10.3389/fpls.2017.00740.

Bhatia, R., Gallagher, J.A., Gomez, L.D. and Bosch, M. (2017) 'Genetic engineering of grass cell wall polysaccharides for biorefining', *Plant Biotechnology Journal*, 15(9), pp. 1071–1092. Available at: https://doi.org/10.1111/pbi.12764.

Brancourt-Hulmel, M., Arnoult, S., Cézard, L., El Hage, F., Gineau, E., Girones, J., Griveau, Y., Jacquemont, M.P., Jaffuel, S., Mignot, E., Mouille, G., Lapierre, C., Legée, F., Méchin, V., Navard, P., Vo, L.T.T. and Reymond, M. (2021) 'A Comparative Study of Maize and Miscanthus Regarding Cell-Wall Composition and Stem Anatomy for Conversion into Bioethanol and Polymer Composites', *Bioenergy Research* [Preprint]. Available at: https://doi.org/10.1007/s12155-020-10239-z.

Byrt, C.S., Munns, R., Burton, R.A., Gilliham, M. and Wege, S. (2018) 'Root cell wall solutions for crop plants in saline soils', *Plant Science*. Elsevier Ireland Ltd, pp. 47–55. Available at: https://doi.org/10.1016/j.plantsci.2017.12.012.

Carpita, N.C. (1996) 'Structure and biogenesis of the cell walls of grasses', *Annual Review of Plant Physiology and Plant Molecular Biology*, 47(1), pp. 445–476. Available at: https://doi.org/10.1146/annurev.arplant.47.1.445.

Chen, C.L., van der Schoot, H., Dehghan, S., Alvim Kamei, C.L., Schwarz, K.U., Meyer, H., Visser, R.G.F. and van der Linden, C.G. (2017) 'Genetic diversity of salt tolerance in Miscanthus', *Frontiers in Plant Science*, 8. Available at: https://doi.org/10.3389/fpls.2017.00187.

Chen, L., Auh, C., Chen, F., Cheng, X., Aljoe, H., Dixon, R.A. and Wang, Z. (2002) 'Lignin deposition and associated changes in anatomy, enzyme activity, gene expression, and ruminal degradability in stems of tall fescue at different developmental stages', *Journal of Agricultural and Food Chemistry*, 50(20), pp. 5558–5565. Available at: https://doi.org/10.1021/jf020516x.

Cheng, S., Yu, H., Hu, M., Wu, Y., Cheng, L., Cai, Q., Tu, Y., Xia, T. and Peng, L. (2018) 'Miscanthus accessions distinctively accumulate cadmium for largely enhanced biomass enzymatic saccharification by increasing hemicellulose and pectin and reducing cellulose CrI and DP', *Bioresource Technology*, 263(February), pp. 67–74. Available at: https://doi.org/10.1016/j.biortech.2018.04.031.

Chourasia, V.R., Pandey, A., Pant, K.K. and Henry, R.J. (2021) 'Improving enzymatic digestibility of sugarcane bagasse from different varieties of sugarcane using deep eutectic solvent pretreatment', *Bioresource Technology*, 337(June), p. 125480. Available at: https://doi.org/10.1016/j.biortech.2021.125480.

Colin, L., Ruhnow, F., Zhu, J.K., Zhao, C., Zhao, Y. and Persson, S. (2023) 'The cell biology of primary cell walls during salt stress', *Plant Cell*. *American Society of Plant Biologists*, pp. 201–217. Available at: https://doi.org/10.1093/plcell/koac292.

Corrêa-Ferreira, M.L., Viudes, E.B., de Magalhães, P.M., Paixão de Santana Filho, A., Sassaki, G.L., Pacheco, A.C. and de Oliveira Petkowicz, C.L. (2019) 'Changes in the composition and structure of cell wall polysaccharides from Artemisia annua in response to salt stress', *Carbohydrate Research*, 483(April), p. 107753. Available at: https://doi.org/10.1016/j.carres.2019.107753.

Da Costa, R.M.F., Lee, S.J., Allison, G.G., Hazen, S.P., Winters, A. and Bosch, M. (2014) 'Genotype, development and tissue-derived variation of cell-wall properties in the lignocellulosic energy crop Miscanthus', *Annals of Botany*, 114(6), pp. 1265–1277. Available at: https://doi.org/10.1093/aob/mcu054.

Da Costa, R.M.F., Pattathil, S., Avci, U., Lee, S.J., Hazen, S.P., Winters, A., Hahn, M.G. and Bosch, M. (2017) 'A cell wall reference profile for Miscanthus bioenergy crops highlights compositional and structural variations associated with development and organ origin', *New Phytologist*, 213(4), pp. 1710–1725. Available at: https://doi.org/10.1111/nph.14306.

Da Costa, R.M.F., Pattathil, S., Avci, U., Winters, A., Hahn, M.G. and Bosch, M. (2019) 'Desirable plant cell wall traits for higher-quality Miscanthus lignocellulosic biomass', *Biotechnology for Biofuels*, 12(1), pp. 1–18. Available at: https://doi.org/10.1186/s13068-019-1426-7.

Crowe, J.D., Feringa, N., Pattathil, S., Merritt, B., Foster, C., Dines, D., Ong, R.G. and Hodge, D.B. (2017) 'Identification of developmental stage and anatomical fraction contributions to cell wall recalcitrance in switchgrass', *Biotechnology for Biofuels*, 10(1), pp. 1–16. Available at: https://doi.org/10.1186/s13068-017-0870-5.

van der Cruijsen, K., Al Hassan, M., van Erven, G., Dolstra, O. and Trindade, L.M. (2021) 'Breeding targets to improve biomass quality in Miscanthus', *Molecules*, 26(2), pp. 1–28. Available at: https://doi.org/10.3390/molecules26020254.

Daliakopoulos, I.N., Tsanis, I.K., Koutroulis, A., Kourgialas, N.N., Varouchakis, A.E., Karatzas, G.P. and Ritsema, C.J. (2016) 'The threat of soil salinity: A European scale review', *Science of the Total Environment*, 573, pp. 727–739. Available at: https://doi.org/10.1016/j.scitotenv.2016.08.177.

DiCara, C. and Gedan, K. (2023) 'Distinguishing the Effects of Stress Intensity and Stress Duration in Plant Responses to Salinity', *Plants*, 12(13). Available at: https://doi.org/10.3390/plants12132522.

van Erven, G., Hendrickx, P., Al Hassan, M., Beelen, B., op den Kamp, R., Keijsers, E., van der Cruijsen, K., Trindade, L.M., Harmsen, P.F.H. and van Peer, A.F. (2023) 'Plant Genotype and Fungal Strain Harmonization Improves Miscanthus sinensis Conversion by the White-Rot Fungus Ceriporiopsis subvermispora', *ACS Sustainable Chemistry and Engineering*, 11(17), pp. 6752–6764. Available at: https://doi.org/10.1021/acssuschemeng.3c00815.

van Erven, G., de Visser, R., Merkx, D.W.H., Strolenberg, W., de Gijsel, P., Gruppen, H. and Kabel, M.A. (2017) 'Quantification of Lignin and Its Structural Features in Plant Biomass Using 13C Lignin as Internal Standard for Pyrolysis-GC-SIM-MS', *Analytical Chemistry*, 89(20), pp. 10907–10916. Available at: https://doi.org/10.1021/acs.analchem.7b02632.

van Erven, G., de Visser, R., de Waard, P., van Berkel, W.J.H. and Kabel, M.A. (2019) 'Uniformly 13C Labeled Lignin Internal Standards for Quantitative Pyrolysis-GC-MS Analysis of Grass and Wood', *ACS Sustainable Chemistry and Engineering*, 7(24), pp. 20070–20076. Available at: https://doi.org/10.1021/acssuschemeng.9b05926.

Fox, J. and Weisberg, S. (2018) An R companion to applied regression. *Sage* publications.

Le Gall, H., Philippe, F., Domon, J.-M., Gillet, F., Pelloux, J. and Rayon, C. (2015) 'Cell Wall Metabolism in Response to Abiotic Stress', *Plants*, 4(1), pp. 112–166. Available at: https://doi.org/10.3390/plants4010112.

Gao, Y., Lipton, A.S., Wittmer, Y., Murray, D.T. and Mortimer, J.C. (2020) 'A grass-specific cellulose–xylan interaction dominates in sorghum secondary cell walls', *Nature Communications*, 11(1), pp. 1–10. Available at: https://doi.org/10.1038/s41467-020-19837-z.

Halpin, C. (2019) 'Lignin engineering to improve saccharification and digestibility in grasses', *Current Opinion in Biotechnology*, 56, pp. 223–229. Available at: https://doi.org/10.1016/j.copbio.2019.02.013.

Hatfield, R.D., Rancour, D.M. and Marita, J.M. (2017) 'Grass cell walls: A story of cross-linking', *Frontiers in Plant Science*, 7(January). Available at: https://doi.org/10.3389/fpls.2016.02056.

Hayat, K., Bundschuh, J., Jan, F., Menhas, S., Hayat, S., Haq, F., Shah, M.A., Chaudhary, H.J. and Ullah, A. (2020) 'Technology Combating soil salinity with combining saline agriculture and phytomanagement with salt-accumulating plants', *Critical Reviews in Environmental Science and Technology*, 50(11), pp. 1085–1115. Available at: https://doi.org/10.1080/10643389.2019.1646087.

He, L., Wang, C., Shi, H., Zhou, W., Zhang, Q. and Chen, X. (2019) 'Combination of steam explosion pretreatment and anaerobic alkalization treatment to improve enzymatic hydrolysis of Hippophae rhamnoides', *Bioresource Technology*, 289, p. 121693. Available at: https://doi.org/https://doi.org/10.1016/j.biortech.2019.121693.

Hodgson-Kratky, K., Papa, G., Rodriguez, A., Stavila, V., Simmons, B., Botha, F., Furtado, A. and Henry, R. (2019) 'Relationship between sugarcane culm and leaf biomass composition and saccharification efficiency', *Biotechnology for Biofuels*, 12(1), pp. 1–18. Available at: https://doi.org/10.1186/s13068-019-1588-3.

Hoover, A., Emerson, R., Ray, A., Stevens, D., Morgan, S., Cortez, M., Kallenbach, R., Sousek, M., Farris, R. and Daubaras, D. (2018) 'Impact of Drought on Chemical Composition and Sugar Yields From Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Miscanthus, a Tall Fescue Mixture, and Switchgrass', *Frontiers in Energy Research*, 6(June), pp. 1–15. Available at: https://doi.org/10.3389/fenrg.2018.00054.

Hu, Z., Wang, Youmei, Liu, J., Li, Y., Wang, Yanting, Huang, J., Ai, Y., Chen, P., He, Y., Aftab, M.N., Wang, L. and Peng, L. (2021) 'Integrated NIRS and QTL assays reveal minor mannose and galactose as contrast lignocellulose factors for biomass enzymatic saccharification in rice', *Biotechnology for Biofuels*, 14(1), pp. 1–13. Available at: https://doi.org/10.1186/s13068-021-01987-x.

Huang, C., Jiang, X., Shen, X., Hu, J., Tang, W., Wu, X., Ragauskas, A., Jameel, H., Meng, X. and Yong, Q. (2022) 'Lignin-enzyme interaction: A roadblock for efficient enzymatic hydrolysis of lignocellulosics', *Renewable and Sustainable Energy Reviews*, 154(October 2021), p. 111822. Available at: https://doi.org/10.1016/j.rser.2021.111822.

Iraki, N.M., Singh, N., Bressan, R.A. and Carpita, N.C. (1989) 'Cell Walls of Tobacco Cells and Changes in Composition Associated with Reduced Growth upon Adaptation to Water and Saline Stress 1', *Plant Physiology*, 91(1), pp. 48–53. Available at: https://doi.org/10.1104/pp.91.1.48.

Isayenkov, S. V. and Maathuis, F.J.M. (2019) 'Plant salinity stress: Many unanswered questions remain', *Frontiers in Plant Science*, 10(February). Available at: https://doi.org/10.3389/fpls.2019.00080.

Ivushkin, K., Bartholomeus, H., Bregt, A.K., Pulatov, A., Kempen, B. and de Sousa, L. (2019) 'Global mapping of soil salinity change', *Remote Sensing of Environment*, 231(December 2018), p. 111260. Available at: https://doi.org/10.1016/j.rse.2019.111260.

Jaramillo Roman, V. (2021) *Salt tolerance strategies of the ancient Andean crop quinoa*, *Wageningen University: Wageningen*, The Netherlands. Available at: https://doi.org/10.18174/535249.

Jeong, H.Y., Nguyen, H.P. and Lee, C. (2015) 'Genome-wide identification and expression analysis of rice pectin methylesterases: Implication of functional roles of pectin modification in rice physiology', *Journal of Plant Physiology*, 183, pp. 23–29. Available at: https://doi.org/10.1016/j.jplph.2015.05.001.

Kang, X., Kirui, A., Dickwella Widanage, M.C., Mentink-Vigier, F., Cosgrove, D.J. and Wang, T. (2019) 'Lignin-polysaccharide interactions in plant secondary cell walls revealed by solid-state NMR', *Nature Communications*, 10(1), pp. 1–10. Available at: https://doi.org/10.1038/s41467-018-08252-0.

Kirui, A., Zhao, W., Deligey, F., Yang, H., Kang, X., Mentink-Vigier, F. and Wang, T. (2022) 'Carbohydrate-aromatic interface and molecular architecture of lignocellulose', *Nature Communications*, 13(1), p. 538. Available at: https://doi.org/10.1038/s41467-022-28165-3.

Kumar, Ashwani, Kumar, Arvind, Kumar, P., Lata, C. and Kumar, S. (2018) 'Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of marvel grass', *Indian Journal of Experimental Biology*, 56(8), pp. 573–581.

Landi, S., Hausman, J.F., Guerriero, G. and Esposito, S. (2017) 'Poaceae vs. Abiotic stress: Focus on drought and salt stress, recent insights and perspectives', *Frontiers in Plant Science*, 8(July), pp. 1–9. Available at: https://doi.org/10.3389/fpls.2017.01214.

Lenth, R. V (2023) 'emmeans: Estimated Marginal Means, aka Least-Squares Means'. Available at: https://CRAN.R-project.org/package=emmeans.

Lewandowski, I., Clifton-Brown, J., Trindade, L.M., Van Der Linden, G.C., Schwarz, K.U., Müller-Sämann, K., Anisimov, A., Chen, C.L., Dolstra, O., Donnison, I.S., Farrar, K., Fonteyne, S., Harding, G., Hastings, A., Huxley, L.M., Iqbal, Y., Khokhlov, N., Kiesel, A., Lootens, P., Meyer, H., Mos, M., Muylle, H., Nunn, C., Özgüven, M., Roldán-Ruiz, I., Schüle, H., Tarakanov, I., Der Weijde, T., Wagner, M., Xi, Q. and Kalinina, O. (2016) 'Progress on optimizing Miscanthus biomass production for the european bioeconomy: Results of the EU FP7 project OPTIMISC', *Frontiers in Plant Science*, 7, pp. 1–23. Available at: https://doi.org/10.3389/fpls.2016.01620.

Li, F., Ren, S., Zhang, W., Xu, Z., Xie, G., Chen, Y., Tu, Y., Li, Qing, Zhou, S., Li, Y., Tu, F., Liu, L., Wang, Y., Jiang, J., Qin, J., Li, S., Li, Qiwei, Jing, H.C., Zhou, F., Gutterson, N. and Peng, L. (2013) 'Arabinose substitution degree in xylan positively affects lignocellulose enzymatic digestibility after various NaOH/H2SO4 pretreatments in Miscanthus', *Bioresource Technology*, 130, pp. 629–637. Available at: https://doi.org/10.1016/j.biortech.2012.12.107.

Li, X., Weng, J.K. and Chapple, C. (2008) 'Improvement of biomass through lignin modification', *Plant Journal*, 54(4), pp. 569–581. Available at: https://doi.org/10.1111/j.1365-313X.2008.03457.x.

De Lima, R.B., Dos Santos, T.B., Vieira, L.G.E., De Lourdes Lúcio Ferrarese, M., Ferrarese-Filho, O., Donatti, L., Boeger, M.R.T. and De Oliveira Petkowicz, C.L. (2014) 'Salt stress alters the cell wall polysaccharides and anatomy of coffee (Coffea arabica L.) leaf cells', *Carbohydrate Polymers*, 112, pp. 686–694. Available at: https://doi.org/10.1016/j.carbpol.2014.06.042.

Lygin, A. V., Upton, J., Dohleman, F.G., Juvik, J., Zabotina, O.A., Widholm, J.M. and Lozovaya, V. V. (2011) 'Composition of cell wall phenolics and polysaccharides of the potential bioenergy crop -Miscanthus', *GCB Bioenergy*, 3(4), pp. 333–345. Available at: https://doi.org/10.1111/j.1757-1707.2011.01091.x.

Maksup, S., Sengsai, S., Laosuntisuk, K., Asayot, J. and Pongprayoon, W. (2020) 'Physiological responses and the expression of cellulose and lignin associated genes in Napier grass hybrids exposed to salt stress', *Acta Physiologiae Plantarum*, 42(7), pp. 1–12. Available at: https://doi.org/10.1007/s11738-020-03092-2.

Matsushita, Y., Kakehi, A., Miyawaki, S. and Yasuda, S. (2004) 'Formation and chemical structures of acid-soluble lignin II: Reaction of aromatic nuclei model compounds with xylan in the presence of a counterpart for condensation, and behavior of lignin model compounds with guaiacyl and syringyl nuclei in 72% sulfuric', *Journal of Wood Science*, 50(2), pp. 136–141. Available at: https://doi.org/10.1007/s10086-003-0543-9.

Meshitsuka, G., Lee, Z.Z., Nakano, J. and Eda, S. (1982) 'Studies on the Nature of Lignin - Carbohydrate Bonding', *Journal of Wood Chemistry and Technology*, 2(3), pp. 251–267. Available at: https://doi.org/10.1080/02773818208085134.

Minor, J.L. (1982) 'Chemical Linkage of Pine Polysaccharides to Lignin', *Journal of Wood Chemistry and Technology*, 2(1), pp. 1–16. Available at: https://doi.org/10.1080/02773818208085116.

Mohnen, D. (2008) 'Pectin structure and biosynthesis', *Current Opinion in Plant Biology*, 11(3), pp. 266–277. Available at: https://doi.org/10.1016/j.pbi.2008.03.006.

Oliveira, D.M., Mota, T.R., Salatta, F. V., Sinzker, R.C., Končitíková, R., Kopečný, D., Simister, R., Silva, M., Goeminne, G., Morreel, K., Rencoret, J., Gutiérrez, A., Tryfona, T., Marchiosi, R., Dupree, P., del Río, J.C., Boerjan, W., McQueen-Mason, S.J., Gomez, L.D., Ferrarese-Filho, O. and dos Santos, W.D. (2020) 'Cell wall remodeling under salt stress: Insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize', *Plant Cell and Environment*, 43(9), pp. 2172–2191. Available at: https://doi.org/10.1111/pce.13805.

Pancaldi, F. and Trindade, L.M. (2020) 'Marginal Lands to Grow Novel Bio-Based Crops: A Plant Breeding Perspective', *Frontiers in Plant Science*, 11, p. 227. Available at: https://doi.org/10.3389/fpls.2020.00227.

Pauly, M., Gille, S., Liu, L., Mansoori, N., de Souza, A., Schultink, A. and Xiong, G. (2013) 'Hemicellulose biosynthesis', *Planta*, 238(4), pp. 627–642. Available at: https://doi.org/10.1007/s00425-013-1921-1.

Petit, J., Gulisano, A., Dechesne, A. and Trindade, L.M. (2019) 'Phenotypic Variation of Cell Wall Composition and Stem Morphology in Hemp (Cannabis sativa L.): Optimization of Methods', *Frontiers in Plant Science*, 10(July). Available at: https://doi.org/10.3389/fpls.2019.00959.

Pettolino, F.A., Walsh, C., Fincher, G.B. and Bacic, A. (2012) 'Determining the polysaccharide composition of plant cell walls', *Nature Protocols*, 7(9), pp. 1590–1607. Available at: https://doi.org/10.1038/nprot.2012.081.

Qin, Z., Ma, Y.X., Liu, H.M., Qin, G.Y. and Wang, X. de (2018) 'Structural elucidation of lignin-carbohydrate complexes (LCCs) from Chinese quince (Chaenomeles sinensis) fruit', *International Journal of Biological Macromolecules*, 116, pp. 1240–1249. Available at: https://doi.org/10.1016/j.ijbiomac.2018.05.117.

Ralph, J., Lapierre, C. and Boerjan, W. (2019) 'Lignin structure and its engineering', *Current Opinion in Biotechnology*, 56, pp. 240–249. Available at: https://doi.org/10.1016/j.copbio.2019.02.019.

Rancour, D.M., Marita, J.M. and Hatfield, R.D. (2012) 'Cell wall composition throughout development for the model grass Brachypodium distachyon', *Frontiers in Plant Science*, 3(DEC), pp. 1–14. Available at: https://doi.org/10.3389/fpls.2012.00266.

Rengasamy, P. (2010) 'Soil processes affecting crop production in salt-affected soils', *Functional Plant Biology*, 37(7), pp. 613–620. Available at: https://doi.org/10.1071/FP09249.

Resch, M.G., Baker, J.O. and Decker, S.R. (2015) *Low Solids Enzymatic Saccharification of Lignocellulosic* Biomass:National Renewable Energy Laboratory Golden, CO.

Rui, Y. and Dinneny, J.R. (2020) 'A wall with integrity: surveillance and maintenance of the plant cell wall under stress', *New Phytologist*, 225(4), pp. 1428–1439. Available at: https://doi.org/10.1111/nph.16166.

Saini, J.K., Saini, R. and Tewari, L. (2015) 'Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments', *3 Biotech*, 5(4), pp. 337–353. Available at: https://doi.org/10.1007/s13205-014-0246-5.

Schäfer, J., Sattler, M., Iqbal, Y., Lewandowski, I. and Bunzel, M. (2019) 'Characterization of Miscanthus cell wall polymers', *GCB Bioenergy*, 11(1), pp. 191–205. Available at: https://doi.org/10.1111/gcbb.12538.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Crocker, D. (2008) 'Determination of structural carbohydrates and lignin in biomass', *Laboratory analytical procedure*, 1617(1), pp. 1–16.

Somerville, C. (2006) 'Cellulose Synthesis in Higher Plants', *Annual Review of Cell and Developmental Biology*, 22(1), pp. 53–78. Available at: https://doi.org/10.1146/annurev.cellbio.22.022206.160206.

De Souza, A.P., Kamei, C.L.A., Torres, A.F., Pattathil, S., Hahn, M.G., Trindade, L.M. and Buckeridge, M.S. (2015) 'How cell wall complexity influences saccharification efficiency in Miscanthus sinensis', *Journal of Experimental Botany*, 66(14), pp. 4351–4365. Available at: https://doi.org/10.1093/jxb/erv183.

Stavridou, E., Webster, R.J. and Robson, P.R.H. (2019) 'Novel Miscanthus genotypes selected for different drought tolerance phenotypes show enhanced tolerance across combinations of salinity and drought treatments', *Annals of Botany*, pp. 1–22. Available at: https://doi.org/10.1093/aob/mcz009.

Tang, Y., Wang, M., Cao, L., Dang, Z., Ruan, N., Wang, Y., Huang, Y., Wu, J., Zhang, M., Xu, Z., Chen, W., Li, F. and Xu, Q. (2022) 'OsUGE3-mediated cell wall polysaccharides accumulation improves biomass production, mechanical strength, and salt tolerance', *Plant Cell and Environment*, 45(8), pp. 2492–2507. Available at: https://doi.org/10.1111/pce.14359.

Tiwari, G., Duraivadivel, P., Sharma, S. and Hariprasad, P. (2018) '1-Amino-cyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of Panicum maximum Jacq. by mitigating drought and salt stress', *Scientific Reports*, 8(1), pp. 1–12. Available at: https://doi.org/10.1038/s41598-018-35565-3.

Tryfona, T., Bourdon, M., Delgado Marques, R., Busse-Wicher, M., Vilaplana, F., Stott, K. and Dupree, P. (2023) 'Grass xylan structural variation suggests functional specialization and distinctive interaction with cellulose and lignin', *Plant Journal*, 113(5), pp. 1004–1020. Available at: https://doi.org/10.1111/tpj.16096.

Uddin, M.N., Hanstein, S., Leubner, R. and Schubert, S. (2013) 'Leaf Cell-Wall Components as Influenced in the First Phase of Salt Stress in Three Maize (Zea mays L.) Hybrids Differing in Salt Resistance', *Journal of Agronomy and Crop Science*, 199(6), pp. 405–415. Available at: https://doi.org/10.1111/jac.12031.

Vago, M.E., Jaurena, G., Estevez, J.M., Castro, M.A., Zavala, J.A. and Ciancia, M. (2021) 'Salt stress on Lotus tenuis triggers cell wall polysaccharide changes affecting their digestibility by ruminants', *Plant Physiology and Biochemistry*, 166(February), pp. 405–415. Available at: https://doi.org/10.1016/j.plaphy.2021.05.049.

Wang, L.W., Showalter, A.M. and Ungar, I.A. (1997) 'Effect of salinity on growth, ion content, and cell wall chemistry in Atriplex prostrata (Chenopodiaceae)', *American Journal of Botany*, 84(9), pp. 1247–1255. Available at: https://doi.org/10.2307/2446049.

Wang, T., McFarlane, H.E. and Persson, S. (2016) 'The impact of abiotic factors on cellulose synthesis', *Journal of Experimental Botany*, 67(2), pp. 543–552. Available at: https://doi.org/10.1093/jxb/erv488.

Wang, Yanting, Huang, J., Li, Y., Xiong, K., Wang, Youmei, Li, F., Liu, M., Wu, Z., Tu, Y. and Peng, L. (2015) 'Ammonium oxalate-extractable uronic acids positively affect biomass enzymatic digestibility by reducing lignocellulose crystallinity in Miscanthus', *Bioresource Technology*, 196, pp. 391–398. Available at: https://doi.org/10.1016/j.biortech.2015.07.099.

van der Weijde, T., Huxley, L.M., Hawkins, S., Sembiring, E.H., Farrar, K., Dolstra, O., Visser, R.G.F. and Trindade, L.M. (2017) 'Impact of drought stress on growth and quality of Miscanthus for biofuel production', *GCB Bioenergy*, 9(4), pp. 770–782. Available at: https://doi.org/10.1111/gcbb.12382.

van der Weijde, T., Kiesel, A., Iqbal, Y., Muylle, H., Dolstra, O., Visser, R.G.F., Lewandowski, I. and Trindade, L.M. (2017) 'Evaluation of Miscanthus sinensis biomass quality as feedstock for conversion into different bioenergy products', *GCB Bioenergy*, 9(1), pp. 176–190. Available at: https://doi.org/10.1111/gcbb.12355.

van der Weijde, T., Torres, A.F., Dolstra, O., Dechesne, A., Visser, R.G.F. and Trindade, L.M. (2016) 'Impact of Different Lignin Fractions on Saccharification Efficiency in Diverse Species of the Bioenergy Crop Miscanthus', *Bioenergy Research*, 9(1), pp. 146–156. Available at: https://doi.org/10.1007/s12155-015-9669-z.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-VerlagNew York. Available at: https://ggplot2.tidyverse.org.

Yasuda, S., Fukushima, K. and Kakehi, A. (2001) 'Formation and chemical structures of acid-soluble lignin I: Sulfuric acid treatment time and acid-soluble lignin content of hardwood', *Journal of Wood Science*, 47(1), pp. 69–72. Available at: https://doi.org/10.1007/BF00776648.

Yuan, Y., Liu, C., Gao, Y., Ma, Q., Yang, Q. and Feng, B. (2021) 'Proso millet (Panicum miliaceum L.): A potential crop to meet demand scenario for

sustainable saline agriculture', *Journal of Environmental Management*, 296(June), p. 113216. Available at: https://doi.org/10.1016/j.jenvman.2021.113216.

Yuan, Y.H., Li, J., Ma, H.C., Yang, Q.H., Liu, C.J. and Feng, B.L. (2021) 'Salt-tolerant broomcorn millet (Panicum miliaceum L.) resists salt stress via modulation of cell wall biosynthesis and Na+ balance', *Land Degradation and Development*, 32(1), pp. 518–532. Available at: https://doi.org/10.1002/ldr.3717.

Zhao, C., Zhang, H., Song, C., Zhu, J.-K. and Shabala, S. (2020) 'Mechanisms of Plant Responses and Adaptation to Soil Salinity', *The Innovation*, 1(1), p. 100017. Available at: https://doi.org/https://doi.org/10.1016/j.xinn.2020.100017.

Zheng, C., Xiao, L., Iqbal, Y., Sun, G., Feng, H., Liu, F., Duan, M. and Yi, Z. (2022) 'Miscanthus interspecific hybrids exceed the biomass yield and quality of their parents in the saline–alkaline Yellow River delta', *Food and Energy Security*, 11(1), pp. 1–14. Available at: https://doi.org/10.1002/fes3.347.

Zheng, C., Yi, Z., Xiao, L., Sun, G., Li, M., Xue, S., Peng, X., Duan, M. and Chen, Z. (2022) 'The performance of Miscanthus hybrids in saline-alkaline soil', *Frontiers in Plant Science*, 13. Available at: https://doi.org/10.3389/fpls.2022.921824.

Zörb, C., Geilfus, C.M. and Dietz, K.J. (2019) 'Salinity and crop yield', *Plant Biology*, 21, pp. 31–38. Available at: https://doi.org/10.1111/plb.12884.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: van der Cruijsen, K., Al Hassan, M., van Erven, G., Kollerie, N., van Lent, B., Dechesne, A. et al. (2024) Salt stress alters the cell wall components and structure in *Miscanthus sinensis* stems. *Physiologia Plantarum*, 176(4), e14430. Available from: https://doi.org/10.1111/ppl.14430