

Article

Spraying Lignin Inhibitors Increases the Enzymatic Saccharification of Maize for Ruminant Feeding

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Abstract

Improving forage biomass utilization is an important strategy for increasing the efficiency of ruminant production systems. Lignin limits the accessibility of structural polysaccharides to hydrolytic enzymes, reducing biomass deconstruction and nutrient utilization. Modulation of lignin biosynthesis during plant development has emerged as a promising approach to decrease cell wall recalcitrance and improve biomass digestibility. In this study, we evaluated the effects of two lignin biosynthesis modulators, piperonylic acid (PIP) and methylenedioxy cinnamic acid (MDCA), on maize biomass saccharification and plant development under field conditions. Maize plants received one or two foliar applications of each modulator, and saccharification was assessed in different plant organs. Neither treatment affected plant morphology, growth, or biomass productivity. Both modulators significantly increased biomass saccharification across multiple plant organs, with gains ranging from 31% to 72%. For whole-plant biomass, PIP increased saccharification by 62% and 74% after single and double applications, respectively, whereas MDCA increased saccharification by 42% and 76%. Although both compounds were effective, PIP showed a consistent performance following a single low-dose application. These results demonstrate the potential of lignin biosynthesis modulators to improve maize biomass digestibility without compromising agronomic performance, supporting their use as a potential strategy to enhance forage quality and livestock production efficiency.

Keywords: reducing sugar; saccharification; physiological engineering; lignocellulose; phenylpropanoid pathway; lignin biosynthesis modulators; piperonylic acid; methylenedioxy cinnamic acid

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1. Introduction

Livestock farming is one of the most important agricultural activities in Brazil, positioning the country as a key player in global beef production and trade. Based on USDA estimates for 2025, Brazil became the world's largest beef producer, surpassing the United States [1]. Cattle require a continuous supply of high-quality feed to maximize their potential for meat or milk production, which is essential for improving the efficiency

of livestock systems [2]. However, in many regions of Brazil, pasture production and quality fluctuate throughout the year due to climatic factors. As a result, preservation methods such as silage and haymaking are crucial for mitigating the seasonal effects on pasture production, providing an important alternative during periods of forage scarcity [3].

Among conserved forages, whole-plant maize silage (*Zea mays* L.) is the most used in ruminant production systems in Brazil [4] and globally [5,6] due to its high energy content, satisfactory productivity, and excellent silage compared to other forage sources. However, its biomass is only partially utilized as a nutritional energy source for ruminants. Herbivores' digestive enzymes hydrolyze the polysaccharides in the cell wall, releasing sugars that serve as nutrients. In addition to polysaccharides, lignocellulosic material can have varying concentrations of lignin, which occurs in association with structural carbohydrates such as cellulose, hemicellulose, and pectin [7], forming a matrix around them that limits the access of digestive enzymes [8]. Lignin is also a powerful adsorbent of enzymes, leading to a large fraction of enzymes becoming unavailable in the reaction medium [9,10]. Therefore, the nutritional value of maize forage is strongly influenced by the digestibility of its cell wall components, which directly affects nutrient availability and feed utilization efficiency.

Previous studies have shown that improvements in maize forage quality are not only associated with biomass production, but also with the proportion of digestible cell wall fractions available for ruminal fermentation [11]. In this context, lignin has been recognized as a major determinant of forage quality, since variations in lignin deposition and cell wall organization influence the extent to which structural carbohydrates can be utilized by ruminants [12–14]. Therefore, techniques capable of modifying the lignification process may enhance biomass digestibility and improve forage utilization efficiency by ruminants, representing a promising strategy for increasing the nutritional value of maize silage.

Lignin is a complex polymer formed by the polymerization of three main phenolic alcohols (*p*-coumaryl, coniferyl, and sinapyl alcohol), which are interlinked by highly resistant C-C and C-O-C bonds, making it resilient to both acidic and alkaline hydrolysis, as well as enzymatic digestion, including microbial and tissue enzymes from the gastrointestinal tract of herbivores. As forage plants mature, lignin concentration increases, exacerbating its negative impact on cell wall utilization in the rumen [15–17]. Consequently, the recalcitrance of biomass to enzymatic digestion is strongly related to the presence of lignin. In grasses, this recalcitrance is also associated with hydroxycinnamic acids, such as ferulic acid (FA) and *p*-coumaric acid [18]. FA, along with other phenolic compounds, plays a crucial role in cross-linking cell wall polymers, thereby limiting the attack of polysaccharides [19,20]. In vitro digestion studies indicate a negative correlation between cell wall saccharification and the degree of cross-linking promoted by ferulic acid [21,22]. Therefore, reducing the availability of ferulic acid in the cell wall by using specific chemical inhibitors of the phenylpropanoid pathway may improve the digestibility of lignocellulosic biomass.

The phenylpropanoid pathway is responsible for the synthesis of lignin as well as a wide variety of phenolic compounds such as pigments, antioxidants, signaling molecules and defense-related metabolites [23]. Several studies have demonstrated that inhibiting the transcription of genes encoding enzymes in the phenylpropanoid pathway leads to increased accessibility of digestive enzymes to the polysaccharides that make up lignocellulosic biomass. However, the reduction in lignin content often results in stunted plant growth [24,25] and can also affect other important pathways.

Another strategy with the potential to reduce the negative impact of lignin on saccharification in forages is the application of enzymatic inhibitors. Piperonylic acid is a

quasi-irreversible inhibitor of the cinnamate 4-hydroxylase enzyme (C4H) in the phenylpropanoid pathway. Its application on young sugarcane plants (60 days after planting) increased saccharification of the bagasse from adult plants, harvested at 12 months, by up to 120% [26]. Similar results were also obtained with the application of methylenedioxycinnamic acid (MDCA), a competitive inhibitor of the 4-coenzyme A ligase enzyme (4-CL), and with daidzin (DZN), a competitive inhibitor of coniferaldehyde dehydrogenase (CALDH) in sugarcane, soybeans, and brachiaria [26]. Likewise, benzohydrazide was also successful in promoting saccharification in maize residues, with and without pretreatments [25]. Together, these studies demonstrate that targeting different enzymes within the phenylpropanoid pathway can reduce biomass recalcitrance and facilitate hydrolase activity. Since PIP and MDCA act on distinct enzymatic steps and have shown promising effects on lignification and biomass digestibility in several plant species, they represent attractive candidates for evaluating whether the benefits previously observed with benzohydrazide [25] can be extended to alternative lignification modulators in maize.

Despite these promising results, information regarding the performance of PIP and MDCA in maize cultivated under field conditions remains limited. Furthermore, the agronomic consequences of applying these compounds, including their effects on plant growth, morphology, and productivity, are still poorly understood. Therefore, this study evaluated the effects of these modulators on maize biomass saccharification under agronomic conditions, while also assessing their impact on morphological and yield-related traits. By generating data under field conditions, this work contributes to the evaluation of lignin modulation as a potential management strategy for improving the utilization of maize biomass destined for silage production.

2. Materials and Methods

2.1. Maize Planting and Application of Lignin Modulators

The experiment was conducted at the Iguatemi Experimental Farm—FEI/UEM during the 2022 and 2023 growing seasons. Each treatment consisted of six replicates, with plots measuring 27 m². Hybrid maize seeds of the “VT Pro3 2m91” variety were planted with 90 cm spacing between rows and 17 cm between plants (Figure 1). Twenty days after planting, the plots were uniformly sprayed with a foliar application of one of the lignin modulators per plot, 3 µM PIP (Sigma-Aldrich, St. Louis, MO, USA) or 2 mM MDCA (Sigma-Aldrich, St. Louis, MO, USA), each dissolved in 1% Aureo® (*v/v*) (Bayer, Belford Roxo, Brazil). Control plants were sprayed under the same conditions using a solution containing 1% Aureo® (*v/v*) without the addition of lignin modulator. The volume of spray applied to each plot was calculated to correspond to 150 L ha⁻¹, ensuring an equivalent application per unit area. In addition to single-application treatments, a set of treatments with two sequential applications was also performed, with the first application at 20 days after planting and the second at 45 days.

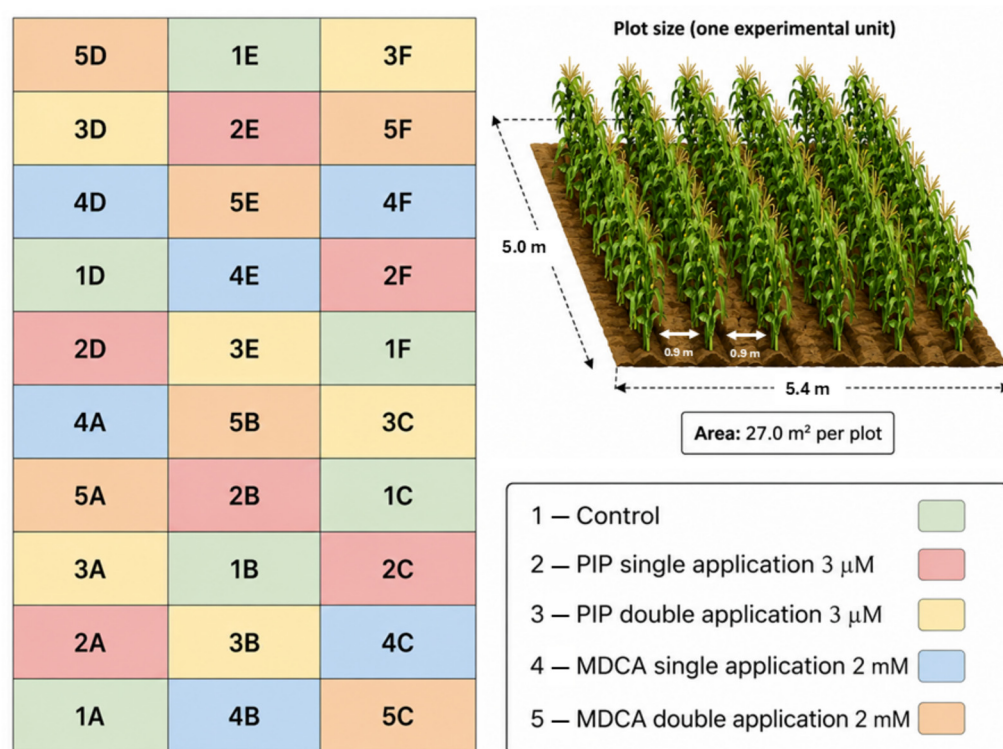


Figure 1. Experimental design used in field trials. The experiment was arranged in a randomized complete block design with 30 plots (10 rows $\times$ 3 columns). Each plot measured 5.4 m $\times$ 5.0 m (27 m²). Colors represent the five experimental treatments: Control (1), PIP single application (2), PIP double application (3), MDCA single application (4), and MDCA double application (5). Letters A–F indicate the six replicates of each treatment. Each colored square corresponds to one experimental plot.

2.2. Phytometric Assessments of the Plants Treated with PIP and MDCA

In each plot, ten plants were selected during cultivation for in situ, non-destructive measurements of plant height and culm diameter, with evaluations performed at 45 and 115 days after sowing. These measurements were carried out directly in the field, without plant harvesting, and were applied consistently across all treatments, regardless of whether plants received a single application (at 20 days) or sequential applications (at 20 and 45 days after planting). For biomass sampling, a single row in each plot was carefully chosen, avoiding border plants. A 3 m linear perimeter was measured along each selected row, and all plants within this area were harvested at 2/3 of kernel milk line stage, and the total fresh weight and number of plants were recorded. Then, three plants from each experimental plot were randomly selected and labelled for detailed phytometric analysis. Plant height and mean culm diameter were measured, with culms classified into upper and bottom sections. Plant components, including culms, leaves, cornhusks, grains, and cobs, were separated, stored in paper bags, and weighed to determine fresh weight. Samples were then dried in an oven at 60 °C until constant weight, after which dry matter was recorded. For biomass and yield-related traits, experimental plots were considered biological replicates ($n = 6$).

2.3. Saccharification Analysis

For saccharification analysis, maize plants were divided into culms (bottom and upper), leaves, cornhusk, corncob, and whole plants. From each sample, 200 mg of ground biomass was subjected to seven successive extraction cycles with 80% ethanol in a Soxhlet apparatus at 80 °C, and the material was subsequently dried in an oven at 60 °C. To ensure

the complete removal of soluble sugars, the pellet was repeatedly resuspended, agitated, and centrifuged, with soluble sugar content determined using the phenol–sulfuric acid method [27]. The remaining material was dried at 60 °C and considered alcohol-insoluble residue (AIR).

For enzymatic saccharification, 15 mg of AIR was transferred to a 1.5 mL screw-cap Eppendorf tube and vortexed with 0.9 mL of 50 mM sodium acetate buffer (pH 5.0) and 10 µL of 2% sodium azide, followed by the addition of a commercial cellulase cocktail for a final concentration of 20 U mL⁻¹. The digestion was done in a dry bath at 50 °C for 24 h, and a control test without enzymes was included to check for any natural sugar release. Aliquots were collected after 4 and 24 h of incubation, and the supernatant was analyzed for reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method [28]. Absorbance was measured at 540 nm, and the amount of reducing sugars released was expressed as mg g⁻¹ of alcohol-insoluble residue (AIR).

2.4. Statistical Analysis

For morphological, yield-related, and biomass analyses, in situ measurements were performed using ten plants per treatment (n = 10), whereas harvested plant traits were analyzed considering six experimental plots as biological replicates (n = 6). Differences between treated plants and the untreated control were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Additionally, morphological, yield-related, and biomass traits were analyzed using two-way ANOVA to evaluate the effects of lignin inhibitor (MDCA and PIP), number of applications (single and double application), and their interaction.

Saccharification data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Statistical analyses were performed using GraphPad Prism® version 6.0 (GraphPad Software Inc., San Diego, CA, USA) and OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA). Data were expressed as mean ± standard deviation (SD). Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results

The treatments with PIP and MDCA at the tested doses did not cause negative changes in plant morphology, architecture, stability, or other agronomically relevant traits (Figure 2, Tables S1 and S2 in the Supplementary Materials). While the heatmap illustrates relative variations among treatments compared with the control, these variations remain within the range of experimental variability, and no statistically significant differences were detected for any evaluated biometric parameter (Figure 2, Table S1 in the Supplementary Materials). Furthermore, no significant effects associated with treatment, number of applications, or their interaction were observed among the evaluated conditions (Table S2 in the Supplementary Materials). Although minor variations related to the control were observed for some plant architecture and biomass-related traits, these responses were not consistent across treatments and were not statistically supported (Figure 2). Overall, the absence of a clear pattern of growth inhibition or morphological impairment indicates that the lignin modulators did not impose detectable developmental penalties under field conditions. Representative photographs of the experimental plants further support the absence of visible differences in growth, architecture, or lodging symptoms among treatments (Figures S1 and S2 in the Supplementary Materials).

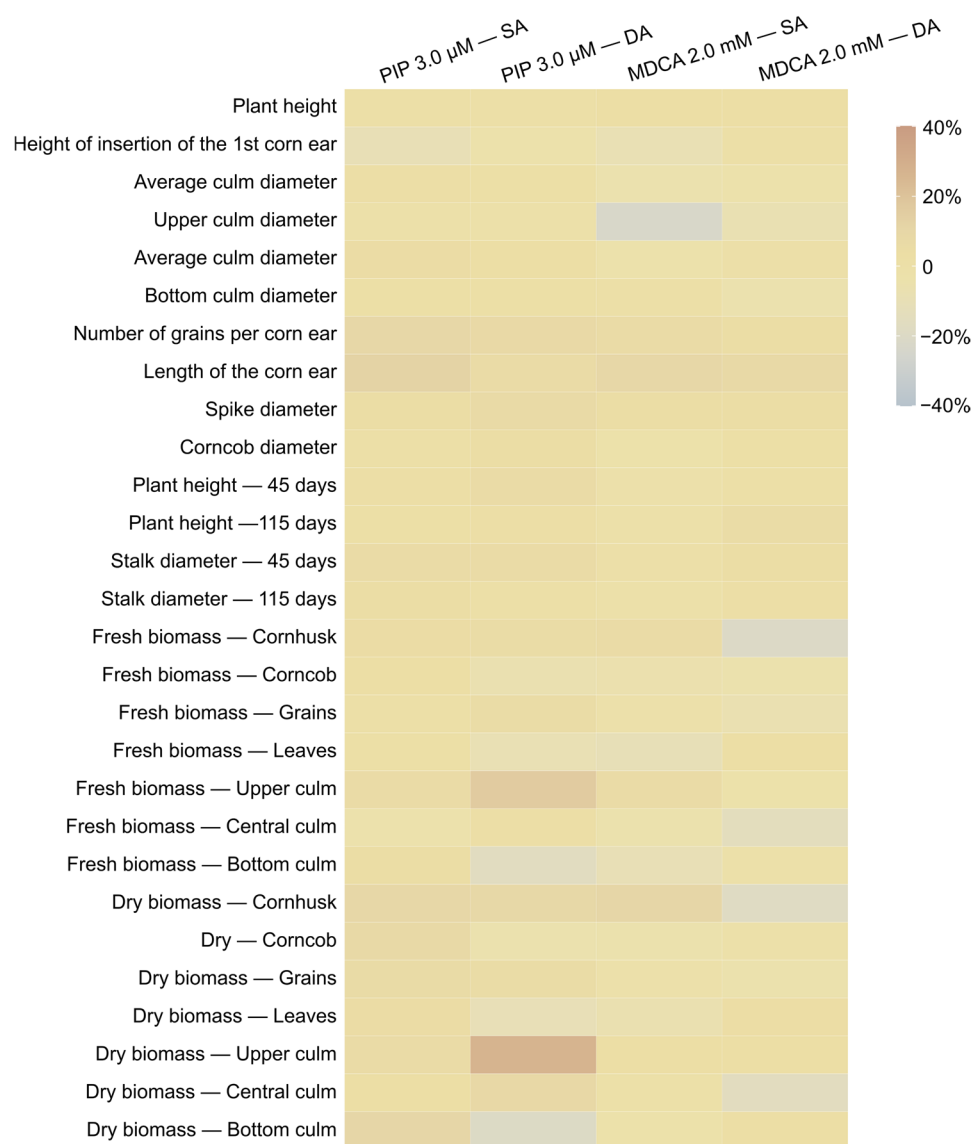


Figure 2. Heatmap of the effects of lignin modulators on the morphological traits of maize plants. MDCA stands for 3,4-methylenedioxycinnamic acid; PIP refers to piperonylic acid; SA indicates a single application; and DA denotes a double application. Colors represent the percentage variation in mean values of treated plants relative to untreated control plants. Statistical comparisons between treated and control plants were performed using one-way ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$). No significant differences were observed. For in situ measurements, values represent the mean of 10 plants ($n = 10$); for harvested plant traits, values represent the mean of six biological replicates (experimental plots; $n = 6$). The data underlying the heatmap are provided in Supplementary Table S1.

Saccharification assays were conducted on different plant organs, as these may exhibit distinct saccharification patterns. After 4 h of digestion, saccharification of the central part of the culm increased by 41% in plants subjected to a single PIP application and by 54% in plants subjected to a double PIP application (Figure 3).

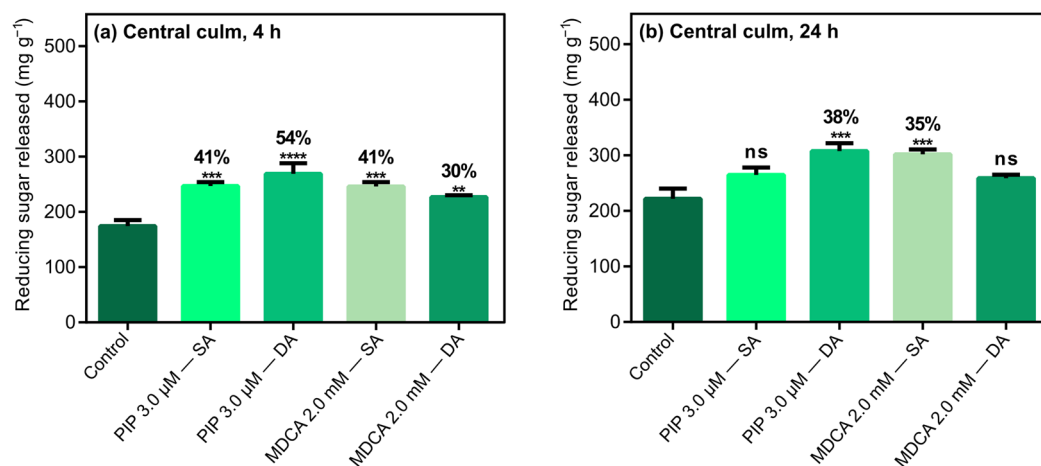


Figure 3. Enzymatic hydrolysis assays for central maize culm. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxybenzoic acid. Bars marked with asterisks indicate significant results (**) ($p < 0.01$), (***) ($p < 0.001$), and (****) ($p < 0.0001$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

A single spray of MDCA increased culm saccharification by 41% and 35% after 4 and 24 h of digestion, respectively. The bottom culm had a notable rise in saccharification after 24 h of treatment with both PIP (31%) and MDCA (38%) (Figure 4).

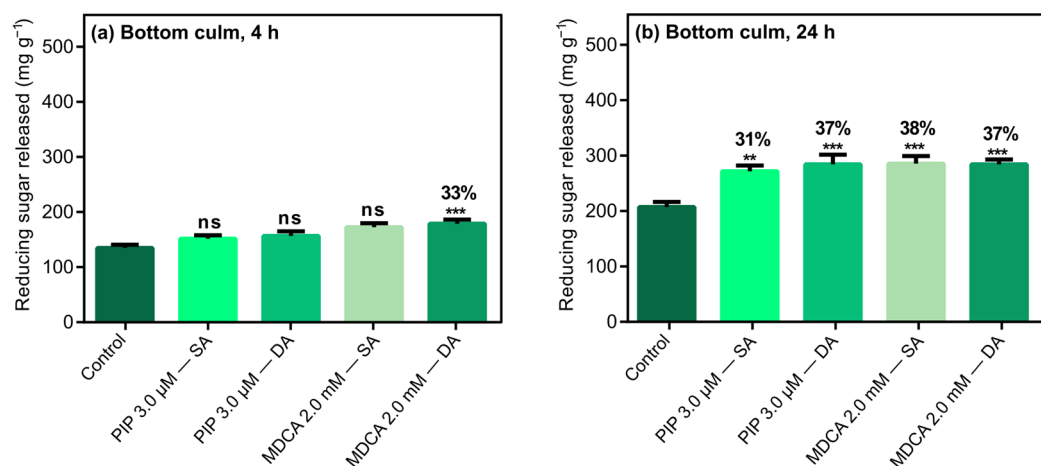


Figure 4. Enzymatic hydrolysis assays for maize culm base. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxybenzoic acid. Bars marked with asterisks indicate significant results (**) ($p < 0.01$) and (***) ($p < 0.001$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

After a 4 h digestion, we observed an increase of 47% and 60% of cornhusk in single and double PIP-spraying treatments, respectively (Figure 5). For MDCA-treated plants, saccharification increased by 52% following a single application, reaching 72% following a double application (Figure 5). In contrast, after 24 h of reaction, a significant 53% increase was observed only in PIP-treated plants following a single application.

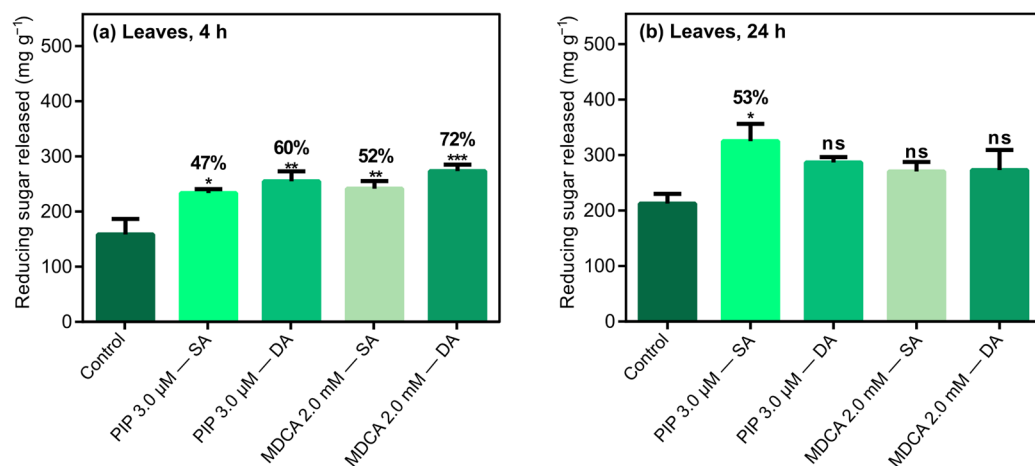


Figure 5. Enzymatic hydrolysis assays for maize leaves. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxycinnamic acid. Bars marked with asterisks indicate significant results (*) ($p < 0.05$), (**) ($p < 0.01$), and (***) ($p < 0.001$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

Single and double applications of PIP increased the reducing sugar release after 24 h of saccharification of cornhusk by 34% and 43%, respectively (Figure 5). In turn, treatments with MDCA increased cornhusk saccharification only after double applications, leading to increases of 37% after 4 h of digestion and 49% after 24 h of digestion (Figure 6).

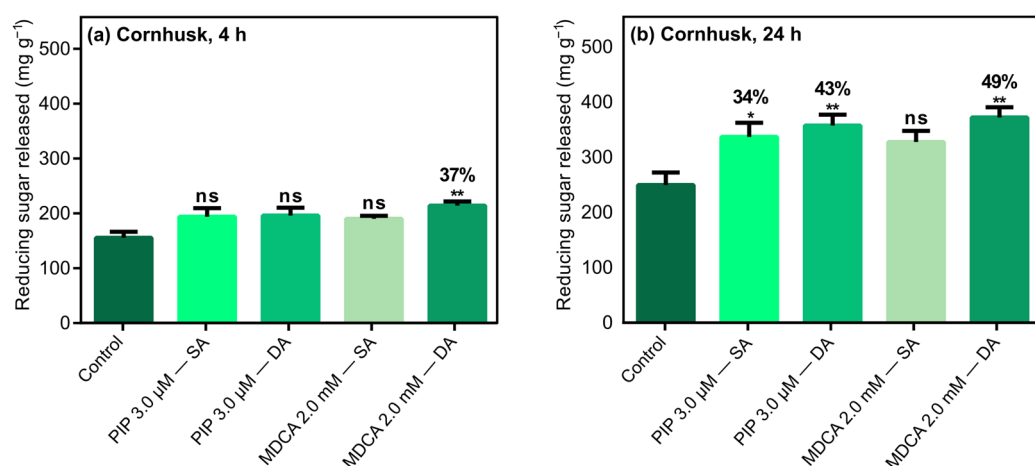


Figure 6. Enzymatic hydrolysis assays for cornhusk. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxycinnamic acid. Bars marked with asterisks indicate significant results (*) ($p < 0.05$), and (**) ($p < 0.01$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

When analyzing the entire plant, the increases in saccharification for single and double applications of PIP were quite similar. Specifically, single applications resulted in increases of 32% at 4 h and 62% at 24 h, while double applications led to increases of 34% at 4 h and 74% at 24 h (Figure 7).

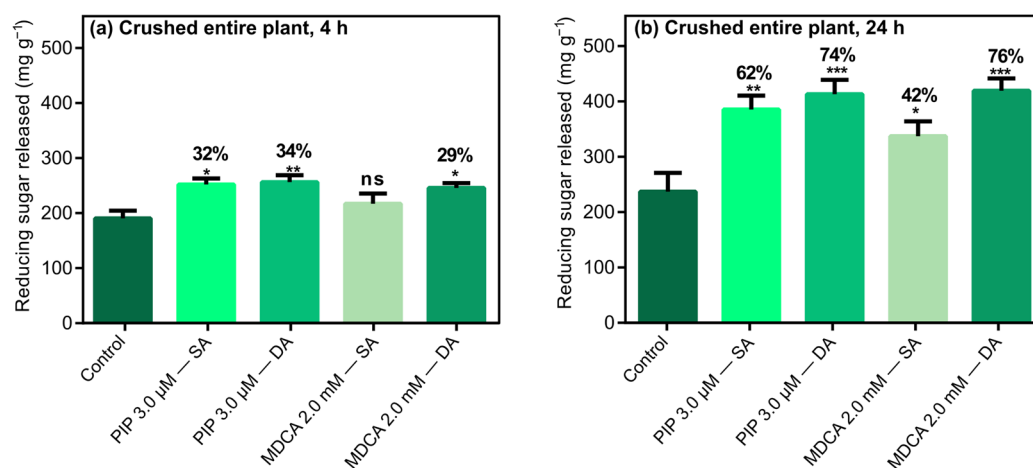


Figure 7. Enzymatic hydrolysis assays for the entire crushed maize plant. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxy cinnamic acid. Bars marked with asterisks indicate significant results (*) ($p < 0.05$), (**) ($p < 0.01$), and (***) ($p < 0.001$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

For MDCA treatments, significant improvements in saccharification were achieved only with double applications but reached the highest value: 76% more reducing sugars produced after 24 h of digestion (Figure 6b). Finally, in the corncob, only a slight increase was observed with the MDCA double-spraying treatment (Figure 8).

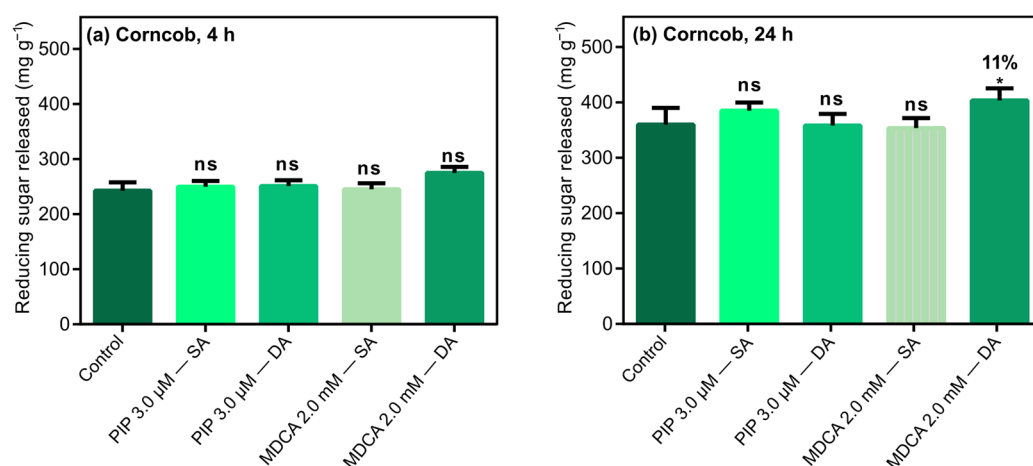


Figure 8. Enzymatic hydrolysis assays for maize corncob. (a) Total reducing sugar released after 4 h (DNS method). (b) Total reducing sugar released after 24 h (DNS method). SA, single application; DA, double application; PIP, piperonylic acid; MDCA, methylenedioxycinnamic acid. Bars marked with asterisk indicate significant results (*) ($p < 0.05$) according to Dunnett's statistical test. Values are expressed as mean $\pm$ SEM ($n = 6$). ns = not significant.

4. Discussion

This study demonstrates that the foliar application of phenylpropanoid pathway inhibitors can effectively enhance the saccharification of maize biomass under agronomic conditions without compromising plant growth, morphology, or yield-related traits. Both PIP and MDCA promoted consistent increases in reducing sugar release across multiple plant organs, highlighting their capacity to reduce lignocellulosic recalcitrance in a field-relevant context. Previous studies have shown that inhibition of key enzymes in the phenylpropanoid pathway may improve lignocellulose saccharification without changing

the net content of lignin. Such improvements are due to modifications in the monomeric composition, cross-linking patterns with polysaccharides and redistribution of lignin among different tissues [18,19]. Such alterations are thought to contribute to reducing biomass recalcitrance by increasing the accessibility of structural polysaccharides to hydrolytic enzymes. Studies with PIP suggest that it presents two distinct targets. Besides inhibiting cinnamyl 4-hydroxylase (C4H), PIP inhibits GH3 enzymes responsible for the inactivation of AIA. Inactivating the auxin turnover pathway causes a rapid increase in endogenous IAA and disrupts normal phytohormone homeostasis [29]. These activities can trigger the long-lasting remodeling of the cell wall, which may further contribute to improved enzymatic accessibility to cell wall polysaccharides [30]. Another study that examined the effect of MDCA on lignin distribution in different tissues of sugarcane culm showed that treatment with MDCA increased lignin and hydroxycinnamate content in vessels and fibers, but reduced lignin and phenolic acids in parenchymatic cell walls [21]. Treatments with both PIP and MDCA consistently increased saccharification of lignocellulosic biomass in sugarcane, soybean, brachiaria [26], energy cane [31], and barley [32].

A notable aspect of the present approach is its contrast with genetic strategies aimed at reducing lignin recalcitrance. Downregulation of genes involved in the phenylpropanoid pathway has frequently been associated with increased saccharification and improved cell wall accessibility [33,34]; however, these modifications often result in growth penalties, altered plant morphology, or reduced biomass accumulation [24,25]. In contrast, the transient application of PIP and MDCA during plant development increased saccharification without affecting agronomic performance, suggesting that chemical modulation may provide a more flexible means of influencing lignocellulosic recalcitrance while maintaining normal crop development. Furthermore, because these compounds can be applied to commercially available cultivars using conventional spraying practices, this strategy may offer practical advantages over approaches requiring permanent genetic modification.

Our study demonstrated that foliar applications of PIP and MDCA enhanced the saccharification of maize biomass intended for silage. This finding is particularly relevant because lignin-associated recalcitrance remains one of the main factors limiting accessibility of structural carbohydrates in forage biomass [8,15,35]. Similarly to what we observed here for PIP and MDCA treatments, spraying maize plants with benzohydrazide increased maize lignocellulosic biomass saccharification *in vitro* [25]. Subsequent tests have shown that such improvement in biomass saccharification was reflected in the higher feeding value of corn silage for growing heifers. Feed efficiency increased by 11% under practical production conditions [36]. These findings provide additional support for the biological relevance of improving cell wall accessibility through chemical modulation of lignification. Although saccharification assays do not fully replicate the complexity of ruminal digestion, the consistent increases observed here with PIP and MDCA suggest that these compounds may also contribute to improved forage utilization and deserve further evaluation under silage production and animal feeding conditions.

The comparison between single (20 days after emergence) and double (20 and 45 days) applications revealed no consistent advantage of double application, suggesting that modulation of the phenylpropanoid pathway during early plant development may be sufficient to promote the observed gains in saccharification. From a practical perspective, this result is encouraging because it indicates that the technology could potentially be integrated into existing crop management systems through a single foliar application, simplifying field implementation and reducing operational costs. Moreover, the effectiveness of a transient intervention may help minimize the risk of undesirable impacts commonly associated with more persistent modifications of lignin biosynthesis.

Nevertheless, because the phenylpropanoid pathway is also involved in plant defense, stress responses, and plant–microbe interactions, additional studies are needed to evaluate the broader biological effects of this approach under different environmental and agronomic conditions. The toxicity of this class of compounds is considered very low and plants that naturally produce PIP such as *Piper umbellatum* and MDCA such as paracoto tree are used as medicinal plants. The maximum tolerated dose of *P. umbellatum* water extract administrated by oral route was determined to be 1000 mg kg^{−1} [37]. Considering the maximum dose used in our tests (MDCA 146 g ha^{−1}), the total MDCA ingested over 36 months would be about 10% of the MTD. However, we were unable to find even traces of MDCA in extracts of plants harvested 3 days after the treatment. PIP doses were three orders of magnitude lower. Nonetheless, future studies must investigate the persistence of these compounds in plant tissues, their fate during ensiling, and other regulatory aspects relevant to commercial application.

The differential response among plant organs with leaves and culm generally showing greater increases in saccharification, especially during the early stages of hydrolysis (4 h), may be related to differences in cell wall composition and architecture [7], tissue metabolic activity, or inhibitor distribution [38]. Although the molecular mechanisms are not yet fully elucidated, it is plausible that inhibition of key enzymes such as C4H or 4-CL [26] alters metabolic flux through the phenylpropanoid pathway, thereby modifying lignin composition and reducing ferulic acid cross-links with polysaccharides [21]. Even modest changes may increase accessibility to hydrolytic enzymes, such as cellulases and hemicellulases, resulting in enhanced release of reducing sugars [39,40].

The practical implications of this study go beyond animal nutrition. Improving fiber digestibility without penalizing plant growth offers pathways to more efficient and sustainable production systems [40,41]. Increased forage utilization efficiency can reduce methane emissions per unit of animal product [35], helping to mitigate the environmental impact of livestock systems [42]. While the relationship between lignin content and ruminal methanogenesis is complex [43,44], this remains a promising area for further investigation.

Finally, cell wall manipulation may also influence broader ecological processes, such as the formation of stable organic matter in the soil and carbon cycling dynamics. Changes in biomass composition, particularly in lignin quantity and structure, can affect the decomposition rate of plant residues and favor the accumulation of more persistent forms of carbon in soil [45,46]. Although these effects were not evaluated in the present study, they represent a promising field for future research.

5. Conclusions

Foliar application of inhibitors of the phenylpropanoid pathway enhanced the saccharification of maize biomass without compromising plant growth or yield. These findings validate a promising non-transgenic strategy to overcome lignocellulosic recalcitrance in forage crops. PIP was especially effective, achieving high levels of biomass digestibility with a single, low-dose application, highlighting its feasibility for large-scale adoption. The proposed approach combines agronomic viability with improved biomass deconstruction potential, highlighting the promise of transient phenylpropanoid pathway modulation as a practical strategy to reduce lignocellulosic recalcitrance in forage crops. Furthermore, it paves the way for future research into the broader ecological impacts of cell wall modulation, including carbon cycling and soil sustainability.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16141502/s1>, Figure S1: Representative photograph of the experimental maize field (vertical view). Figure S2: Representative photograph

of the experimental maize field (horizontal view). Table S1: Morphological, yield-related, and biomass traits of maize plants subjected to foliar application of phenylpropanoid pathway modulators. Values are presented as mean $\pm$ SD ($n = 6$). Table S2: Two-way ANOVA analysis of morphological, yield-related, and biomass traits of maize plants subjected to phenylpropanoid pathway modulators. The effects of lignin modulator (MDCA and PIP), number of applications (single and double application), and their interaction were evaluated using two-way ANOVA. When applicable, multiple comparisons were performed using Holm–Sidak test ($p < 0.05$). Values represent F statistics and p -values for each source of variation. For in situ measurements, $n = 10$ plants; for harvested plant traits, $n = 6$ biological replicates (plots).

Author Contributions: Modulator application, W.M.C., G.M.C. and R.R.d.R.; sample collection and preparation, G.M.C., R.R.d.R., E.S.B., K.Z., J.M.B. and A.F.S.; saccharification assays, W.M.C.; phytometric analyses, W.M.C., G.M.C., R.R.d.R., E.S.B., K.Z., J.M.B. and A.F.S.; data curation, W.M.C.; writing—original draft preparation, W.M.C.; writing—review and editing, W.M.C., R.P.C., R.M., J.L.P.D., O.F.-F. and W.D.d.S.; supervision, W.D.d.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

PIP	Piperonylic acid
MDCA	Methylenedioxycinnamic acid

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