Tropical Plant Biology (2024) 17:42–51
https://doi.org/10.1007/s12042-023-09351-z

RESEARCH

Influence of the Cellular Ultrastructure and Enzyme Activity of the Leaf Sheath on Spontaneous Defoliation in Sugarcane

Xin Hu[1,3] · Tiantian Wang[2] · Shaochun Liu[1,3] · Jiawen Guo[1,3] · Jingmei Dao[1,3] · Xinxin Gao[1,3] · Rudan Li[1,3] · Gaoyuan Liu[1,3]

Received: 3 September 2023 / Accepted: 6 November 2023 / Published online: 10 November 2023

Abstract
Spontaneous defoliation improves the harvesting efficiency and yield of sugarcane. Here, we investigated the ultrastructural changes and pectinase and cellulase activity in the third, fifth, and seventh leaf sheaths in four sugarcane varieties with varying spontaneous defoliation performance during maturation. At the early and middle stages of spontaneous defoliation, the cells in the abscission zones of the third, fifth, and seventh leaf sheaths were degrading. At the late stage, no complete organelles and hollow or broken spots in the cell walls were observed in the abscission zone cells of defoliation-prone varieties, while complete organelles and intact cell walls were present in the abscission zone cells at the same leaf positions in defoliation-resistant varieties. From the early to late stages, defoliation-prone varieties had higher pectinase activity in the abscission zones of the fifth and seventh leaf sheaths. At the early stage of defoliation, defoliation-prone varieties had significantly higher cellulase activity in the abscission zones of the third, fifth, and seventh leaf sheaths. Correlation analysis showed that the spontaneous defoliation rate was significantly positively correlated with pectinase activity in the leaf sheaths. In conclusion, the spontaneous defoliation of sugarcane was closely related to changes in cell morphology and pectinase activites in leaf sheaths.

Keywords Sugarcane · electron Microscope Observation · Pectinase · Cellulase · Spontaneous Defoliation

Introduction

Sugarcane (*Saccharum* spp. hybrids) is a major sugar crop around the world. Spontaneous defoliation is an important agronomic trait of sugarcane that varies greatly among varieties. For the production of sugarcane, the manual harvest of defoliation-resistant varieties requires a large amount of labor to strip leaves, resulting in high harvesting costs and low efficiency (Singh et al. 2011; Huang et al. 2018). The mechanized harvesting of defoliation-resistant varieties is also problematic due to their poor spontaneous defoliation performance, making it difficult to remove the sheaths from the stems and resulting in high impurities in the harvested sugarcane and lower yield of sugar. To improve harvesting efficiency, sugarcane plants in some countries are burned to remove the leaves before harvesting. However, burning sugarcane leaves produces large amounts of smoke and gases that are harmful to the surrounding environment. In addition, the dust produced by burning sugarcane leaves can also adversely affect the sugar production process (Cristale et al. 2012; Mugica-Alvarez et al. 2015). Therefore, studying how to improve the spontaneous defoliation performance of sugarcane is important for both manual and mechanized harvesting.

Plant organ abscission not only is of great biological significance for the reproduction and spread of plants but also is of important practical value in agricultural production. During long-term crop domestication, humans recognized the shedding characteristics of plant organs and selected against them. As a result, problems such as grain shedding

Communicated by Graham Bonnett.

✉ Shaochun Liu
ynliushch@163.com

[1] Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences, Kaiyuan 661699, China

[2] Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, China

[3] Yunnan Key Laboratory of Sugarcane Genetic Improvement, Kaiyuan 661699, China

and ear breaking in grasses (Poaceae), bell dropping in cotton, and premature pod shattering in leguminous crops were successfully resolved to reduce yield loss (Wang et al. 2009). In agricultural and horticultural production, mature seeds and fruits need to be shed through various artificial or natural means to ensure yield and quality and avoid economic loss (Geitmann 2018). Plant organ shedding mainly happens to flowers, leaves, fruits, seeds, leaf sheaths and lateral roots (Mao et al. 2000; Nakano et al. 2012; Botton et al. 2011; Zhou et al. 2012; Lewis et al. 2006). During organ shedding, a tissue with several layers of cells forms a site where an organ spontaneously detaches from the plant, this is called the abscission zone (Bleecker and Patterson 1997). The abscission zone in plants is generally composed of 5 to 50 layers of small, tightly packed, square cells with a dense cytoplasm that are capable of sensing abscission signals and undergoing cell separation, leading to organ detachment (McManus 2008). Flower and fruit abscission occurs due to the degradation of cell walls, which results from the disruption of the adhesion between cells caused by various enzymes such as cellulases, pectinases, peroxidases, polygalacturonases, xyloglucan endotransglucosylases/hydrolases, and expansins (Bar-Dror et al. 2011; Tariq et al. 2017; Roberts and Gonzalezcarranza 2009; Levine and Hall 2011; Djanaguiraman et al. 2010; Niederhuth et al. 2013). It were found that cellulase and pectinase are the primary enzymes involved in the sugarcane spontaneous defoliation (Fan et al. 2013; Li et al. 2015).

Sugarcane leaves are composed of blades and sheaths, and during spontaneous defoliation the detachment occurs mainly in the abscission zone of mature leaf sheaths. To date, there are few reports describing the in-depth study of sugarcane defoliation, and related knowledge is very limited compared with that in tomato, rice, and *Arabidopsis*. This study explored the relationship between spontaneous defoliation and the morphological and physiological characteristics of the cells in the leaf sheaths of sugarcane varieties with varied defoliation performances. The findings of this study will provide a reference for the improvement of the spontaneous defoliation performance of sugarcane at the cellular physiological level.

Materials and Methods

Plant Materials and Planting Conditions

Four sugarcane varieties, namely YZ03-194 and YZ01-1413 (defoliation-prone) as well as GT02-467 and YZ99-91 (defoliation-resistant), were selected for the study. These varieties have the same maturity time but different spontaneous defoliation performance. When the ratio of brix between the upper and lower internodes of the raw sugarcane stem is between 0.9 and 1 (be known as the maturity stage), starting from the fifth leaf below the most recent fully expanded leaf of defoliation-prone varieties, the angle between the sheath and stem increased, and the abscission zone connecting the sheath and stem at the base of the sheath spontaneously cracked, which allowed the leaf to fall off easily. In contrast, the leaf sheaths of defoliation-resistant varieties tightly wrapped stems, and the abscission zones that spontaneously cracked were at lower leaf positions (Fig. 1).

The sugarcane varieties were planted in the field in late February 2019. The soil surface (0–30 cm) had an organic matter content of 26.2 g•kg^{-1}, available nitrogen content of 117.7 mg•kg^{-1}, available phosphorus

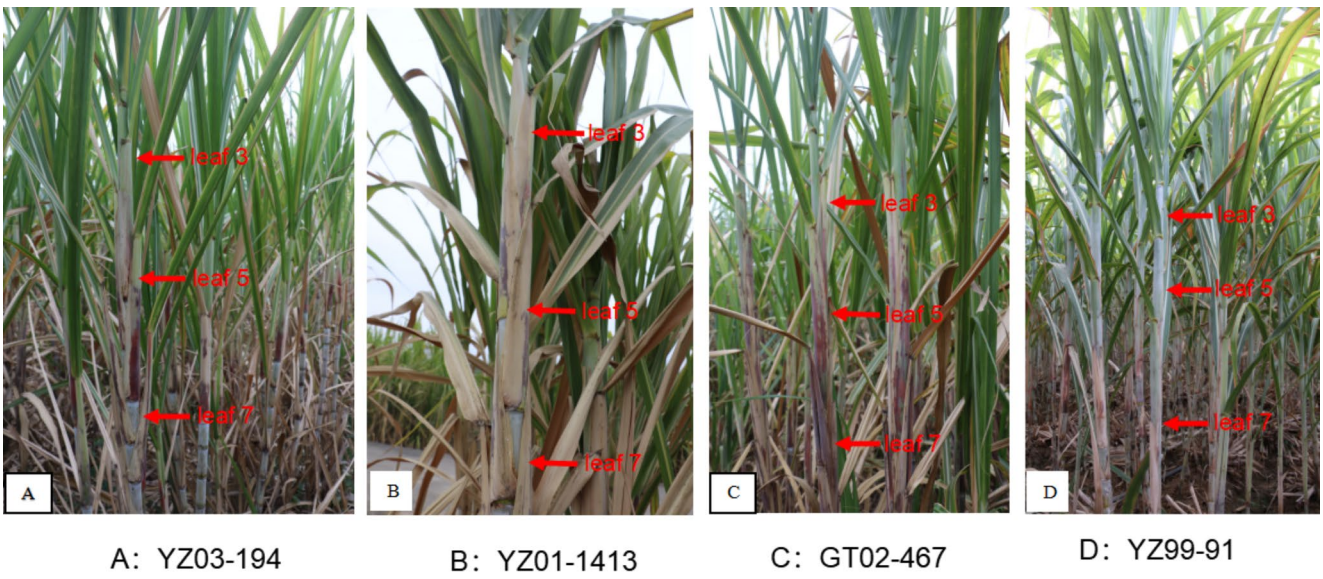

Fig. 1 Morphology of leaf sheaths in the studied sugarcane varieties

content of 27.5 mg•kg^{-1}, available potassium content of 115.2 mg•kg^{-1}, and pH of 5.7. The altitude of the experimental area was 1,055 m. During the experiment, the area had annual natural precipitation of 698 mm, 2,317 h of sunshine, an annual average temperature of 21.5 °C, and an average wind speed of 2.38 m•s^{-1}. The experiment adopted a randomized block design including three blocks. Each variety was planted in eight rows per block, with a row length of 6 m and an inter-row space of 1.2 m. Sugarcane plants were managed throughout the entire growth period according to the measures used for local sugarcane production to maintain their normal growth.

Sampling and Investigation

The sampling of sugarcane tissues began in late October when sugarcane plants started spontaneous defoliation, which was divided into three stages (the early, middle, and late stages). The sampling interval was 25–30 days. The leaf sheath tissues within 1 cm from the base line of the abscission zones connecting the stem and sheath of the third, fifth, and seventh leaf below the fully expanded leaf of normally growing sugarcane plants were sampled for the observation of ultrastructural morphology. The sampling included three replicates, once per variety in each of 3 blocks. The samples were frozen in liquid nitrogen immediately and then stored in a − 80 °C freezer for the determination of the pectinase and cellulase activity. Fifteen plants per plot were selected for investigating the spontaneous defoliation rate and the investigation was repeated three times (once per variety per block). The crack rate of leaf sheath is defined as the dehiscence length divided by the total length in the leaf sheaths around the cane stems where natural defoliation occurs. The crack rate of leaf that is not split open is set to 0%, while the crack rate of leaf that has fallen off is set to 100%.The leaves with ≥ 30% crack rate are easy detached by the winds, and are divided into shedding leaves. The spontaneous defoliation rate was calculated as follows: The spontaneous defoliation rate = (the number of leaf with ≥ 30% crack rate / the number of all leaf below the most recent fully expanded leaf) × 100%.

Observation of the Ultrastructure of the Cells in the Leaf Sheaths

Abscission zone tissues were isolated with a double-sided razor blade, and the third, fifth, and seventh leaf sheaths of each sample were randomly selected and used as three replicates for sectioning. The samples were immersed in 2.5% glutaraldehyde fixative for 24 h under vacuum, rinsed with 0.1 mol/L phosphate buffer (pH 7.0), fixed in 1% O_SO_4 fixative for 2 h, and rinsed three times in phosphate buffer for 15 min each time. The samples were then dehydrated sequentially in a gradient of 30%, 50%, 70%, 80%, 90%, 95%, and 100% ethanol, with dehydration in each concentration of ethanol for 15 min. After that, the samples were immersed in 100% acetone for 20 min to replace ethanol with acetone, and then infiltrated and embedded with Spurr low-viscosity embedding medium at 60 °C for 12 h. Ultrathin sections of the samples were made using a Leica EM UC7 Ultramicrotome, setting the thickness of section at 70 nm. Then, the sections were double-stained with uranyl acetate and lead citrate so as to increase the the contrast of light and shade under electron microscopy. The ultrastructure of cells was observed using an FEI transmission electron microscope (Taicnai G2 Spirit), with 80 kV accelerating voltage and 1100 times of magnification.

Determination of Pectinase Activity in the Leaf Sheaths

Three grams of abscission zone tissues were placed in a container with 40 mL 0.2 mol/L acetic acid-sodium acetate buffer (pH = 4.8), and then the container was shaken for 30 min in a 4–7 °C incubator. After shaking, the same buffer was added to reach a final volume of 100 mL. The solution was stored in the dark at 4 °C for 24 h and then centrifuged at 2795 *g* for 8 min. Next, 5 mL of the supernatant was taken and the volume was increased to 25 mL by adding acetic acid-sodium acetate buffer. The pectinase activity was determined according to the method used by Wang et al. (2007). During the determination of pectinase activity, 1 mg of D-galacturonic acid produced by 1 g pectinase in decomposing pectin for 1 h under the conditions of 48 °C and pH 4.8 was defined as 1 unit of pectinase activity. The determination was repeated three times (once per variety per block).

Determination of Cellulase Activity in the Leaf Sheaths

Three grams of leaf sheaths were ground on ice and then completely transferred into a 50-mL beaker. Forty milliliters of acetic acid-sodium acetate buffer (pH 5.5) was added and then stirred using a magnetic stirrer for 30 min. The buffer solution was added to reach a final volume of 50 mL and then the extract was stored at 4 °C in the dark for 24 h. After being shaken thoroughly, 50 mL of the extract was transferred into a centrifuge tube and centrifuged at 3,000 rpm for 3 min. The supernatant was used as the crude cellulase extract and stored at 4 °C. Cellulase activity was determined based on the method used by Hao and Liu (2001). Carboxymethyl cellulose was used as a substrate in the reaction system to produce reducing sugar that was measured using

the 3, 5-dinitrosalicylic acid colorimetric method. One milligram reducing sugar produced by 1 g cellulose in 1 h was defined as 1 unit of cellulase activity. The determination was repeated three times (once per variety per block).

Data Analysis

Experimental data were organized and analyzed using Microsoft Excel 2007, and one-way analysis of variance (ANOVA) and correlation analysis were performed using SPSS 21 statistical software. The enzyme activities at early, mid and late stage were combined to analyse their relation with spontaneous defoliation rate.

Results

Changes in the Ultrastructural Morphology of the Cells in the Leaf Sheaths

As shown in Fig. 2, in the spontaneous defoliation process the cells in the abscission zones of the third, fifth, and seventh leaf sheaths of all varieties degraded from the early to late stages of defoliation. At the early and middle stages of defoliation, there was no significant difference in the organelle degradation in the leaf sheath cells between defoliation-prone and defoliation-resistant varieties. At the late stage of defoliation, the organelles of the cells in the abscission zones of the third, fifth, and seventh leaf sheaths of defoliation-prone varieties were all degrading and cells with complete organelles were not observed, while the cells in the abscission zones of the same leaf positions of defoliation-resistant varieties showed complete and abundant organelles. There was a significant difference in the cell wall morphology of the abscission zone cells between the varieties with different defoliation performances from the early to the late stage of defoliation (Fig. 2A). Multiple cells with hollow spots in the cell wall were present in the abscission zones of the third leaf sheath of defoliation-prone varieties YZ03-194 and YZ01-1413. For the abscission zone of the fifth and seventh leaf sheath, with the decline of leaf position and the increase of leaf age, the cells with ruptured cell walls and broken spots in the cell wall were present. In contrast, at the same stage, cells with either hollow or broken spots in the cell wall in the abscission zones of the third, fifth, and seventh leaf sheaths of defoliation-resistant varieties GT02-467 and YZ99-91 were not observed. At the middle stage of defoliation (Fig. 2B), cells with broken spots in the cell wall were present in the abscission zone of the third leaf sheath of the defoliation-prone variety YZ03-194. The cell wall rupture became more severe and the number of cells with broken spots in the cell wall was significantly increased in the abscission zone of the fifth leaf sheath of the defoliation-prone varieties YZ03-194 and YZ01-1413. Cells with hollow or broken spots in the cell wall were also present in the abscission zone of the seventh leaf sheath of YZ03-194 and YZ01-1413. In contrast, cells with hollow or broken spots in the cell wall were only present in the abscission zones of the fifth leaf sheath but not in that of the third and seventh leaf sheaths of defoliation-resistant varieties GT02-467 and YZ99-91. At the late stage of defoliation (Fig. 2C), cells with broken spots in the cell wall were present in the abscission zones of the third, fifth, and seventh leaf sheaths of the defoliation-prone varieties YZ03-194 and YZ01-1413. Only a small number of cells with broken spots in the cell wall were observed in the abscission zone of the seventh leaf sheath of defoliation-resistant variety GT02-467, and cells with either hollow or broken spots in the cell wall were not found in the abscission zones of the third and fifth leaf sheaths of this variety.

Changes of Pectinase Activity in the Abscission zone of Sugarcane Leaf Sheaths

As shown in Fig. 3, during defoliation there were significant differences in pectinase activity in the abscission zones of the same leaf positions between the four sugarcane varieties. At the early stage of defoliation, the abscission zones of the third leaf sheath of YZ03-194, YZ01-1413, and GT02-467 had significantly higher pectinase activity than that of YZ99-91; the abscission zones of the fifth leaf sheath in YZ03-194 and YZ01-1413 had significantly higher pectinase activity compared to GT02-467 and YZ99-91; the abscission zones of the seventh leaf sheath of YZ03-194 and YZ01-1413 had significantly higher pectinase activity compared to GT02-467 and YZ99-91; and the abscission zone of the seventh leaf sheath in GT02-467 had significantly higher pectinase activity than those in YZ99-91. In the middle stage of defoliation, the abscission zones of the third and seventh leaf sheaths of YZ03-194 and YZ01-1413 had significantly higher pectinase activity than those of GT02-467 and YZ99-91, and at the same leaf position the abscission zone of YZ01-1413 had significantly higher pectinase activity than that of GT02-467, while the difference in pectinase activity in the abscission zone between GT02-467 and YZ99-91 was non-significant; at the fifth leaf position, the abscission zones in YZ03-194 and YZ01-1413 had significantly higher pectinase activity than those in GT02-467, the abscission zone of GT02-467 had significantly higher pectinase activity than that in YZ99-91, and there was no significant difference in the pectinase activity in the abscission zones between YZ03-194 and YZ01-1413. At the late stage of defoliation, there was no significant difference in the pectinase activity in the abscission zones of the third

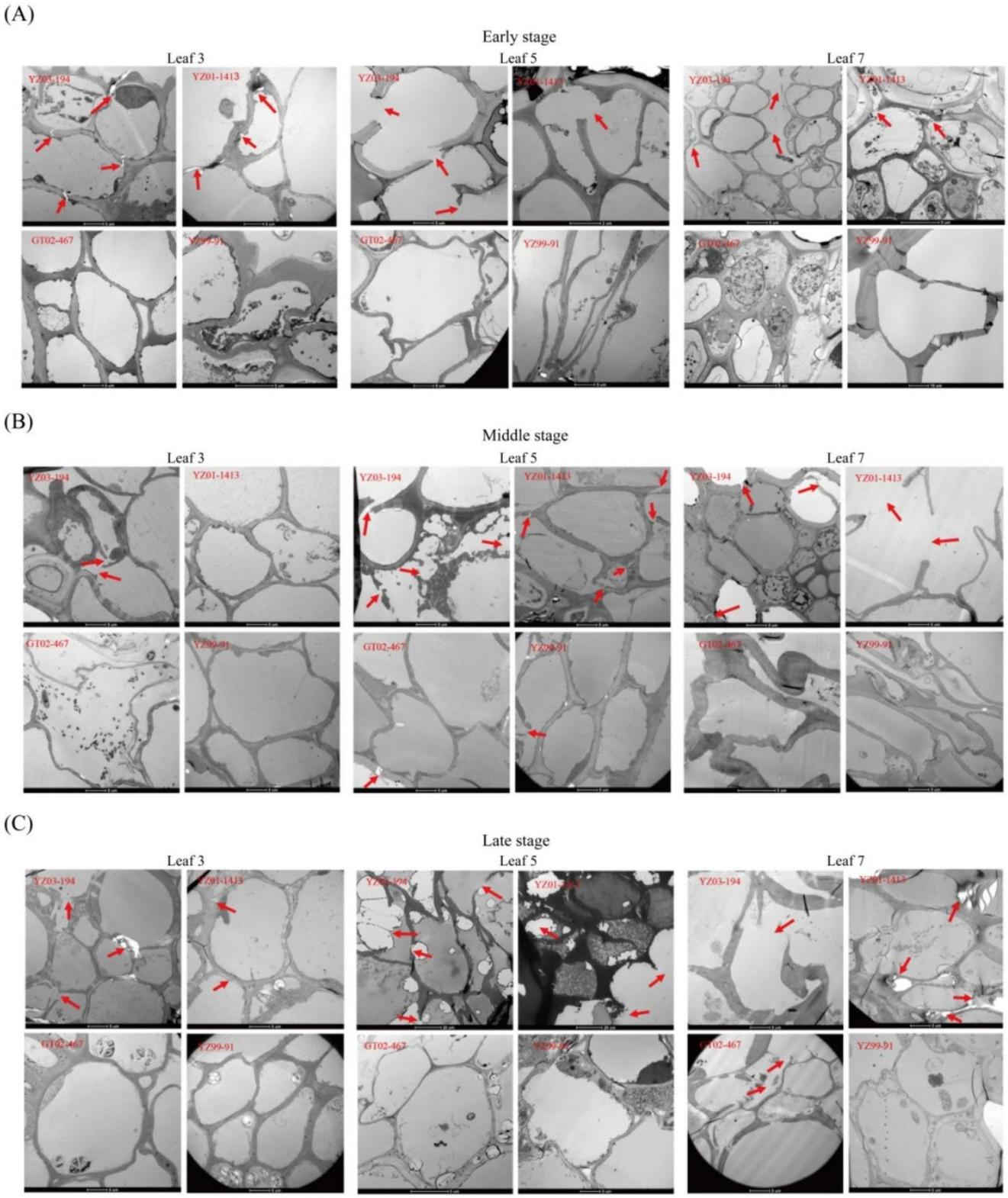

Fig. 2 Ultrastructural changes in the cells in the sugarcane leaf sheath. **A**: Early stage, **B**: Middle stage, **C**: Late stage. A, B, and C show the ultrastructure of the cells in the leaf sheath at the early, middle, and late stages of defoliation, respectively. Arrows indicate hollow or broken spots in the cell wall

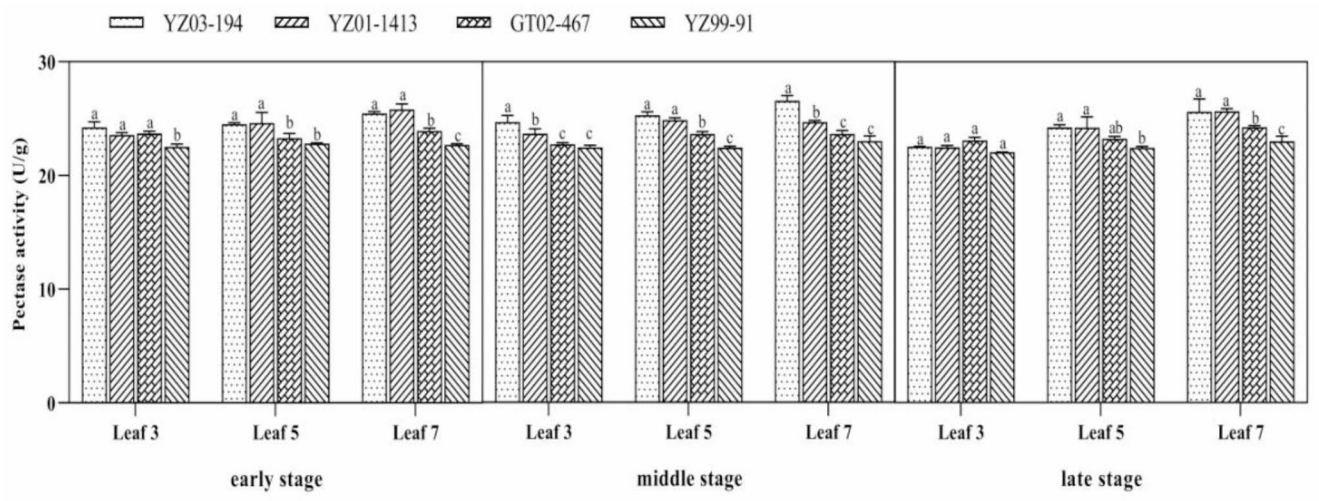

Fig. 3 Changes in pectinase activity in the abscission zone of sugarcane leaf sheaths. Different letters on the histogram represent significant differences at $p < 0.05$

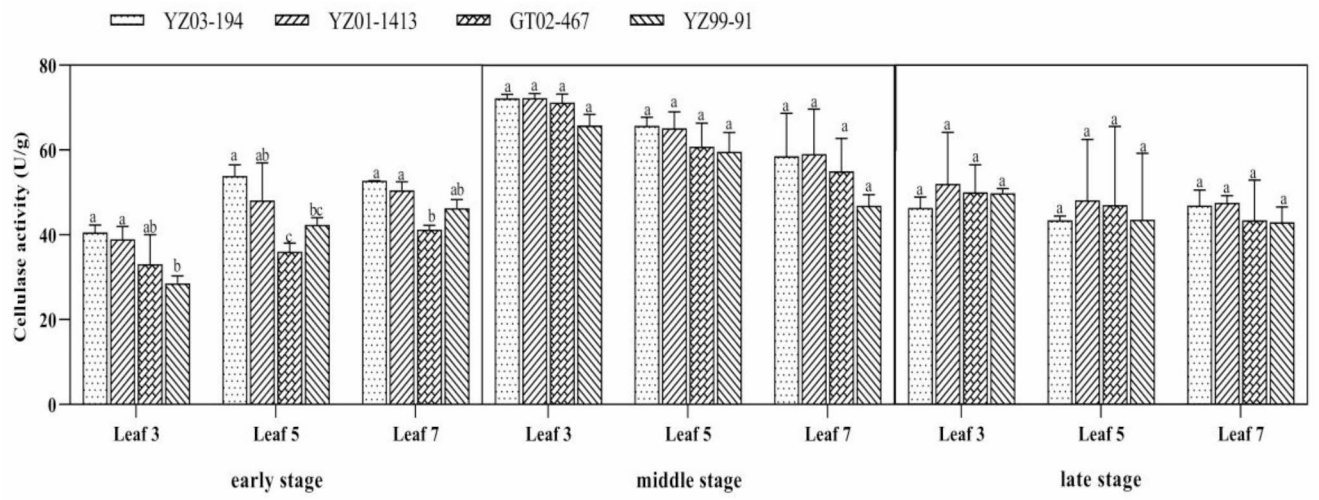

Fig. 4 Changes in cellulase activity in the abscission zone of sugarcane leaf sheaths. Different letters on the histogram represent significant differences at $p < 0.05$

leaf sheaths between the four varieties; the fifth leaf sheath of YZ03-194 and YZ01-1413 had significantly higher pectinase activities than that in YZ99-91; the abscission zones of the seventh leaf sheath of YZ03-194 and YZ01-1413 had significantly higher pectinase activity than that in GT02-467, and the abscission zone of the seventh leaf sheath in GT02-467 had significantly higher pectinase activity than that in YZ99-91.

Changes of Cellulase Activity in the Abscission Zone of Sugarcane Leaf Sheaths

As shown in Fig. 4, in the early stage of defoliation, the cellulase activities in the abscission zones of the third leaf sheath of YZ03-194, YZ01-1413, and GT02-467 were 1.42, 1.36, and 1.16 times of that in YZ99-91, respectively, with the abscission zones of the third leaf sheath in YZ03-194 and YZ01-1413 having significantly higher cellulase activity than that in YZ99-91; the cellulase activities in the abscission zones of the fifth leaf sheath in YZ03-194, YZ01-1413, and YZ99-91 were 1.49, 1.33, and 1.17 times of that in GT02-467, respectively, with YZ03-194 and YZ01-1413 having significantly higher cellulase activity in the leaf sheaths than GT02-467; and the cellulase activities of the abscission zones of the seventh leaf sheath in YZ03-194, YZ01-1413, and YZ99-91 were 1.28, 1.22, and 1.12 times of that in GT02-467, respectively, with YZ03-194 and YZ01-1413 having significantly higher cellulase activity in the leaf sheath than GT02-467. At the middle and late stages of defoliation, there was no significant difference in

cellulase activity in the leaf sheaths in the four varieties at the same leaf position among the third, fifth, and seventh leaves.

Changes in the Spontaneous Defoliation Rate of Sugarcane

As shown in Fig. 5, during spontaneous defoliation, the abscission zones of the mature leaf sheaths spontaneously cracked one after another, leading to defoliation. From the early to late stages of defoliation, the spontaneous defoliation rates in the four varieties showed a regular upward trend, and for each stage the order of the varieties based on their spontaneous defoliation rates was YZ03-194 > YZ01-1413 > GT02-467 > YZ99-91. At the early stage of defoliation, the spontaneous defoliation rates in YZ03-194, YZ01-1413, and GT02-467 were 5.38, 4.62, and 1.92 times of that in YZ99-91, respectively. At the middle stage of defoliation, the spontaneous defoliation rates of YZ03-194, YZ01-1413, and GT02-467 were 4.46, 4.16, and 1.52 times of that in YZ99-91, respectively. At the late stage of defoliation, the spontaneous defoliation rates of YZ03-194, YZ01-1413, and GT02-467 were 3.85, 3.58, and 1.49 times of that in YZ99-91, respectively. Overall, YZ03-194 and YZ01-1413 had higher spontaneous defoliation rates and GT02-467 and YZ99-91 had lower spontaneous defoliation rates. The differences in the spontaneous defoliation rates between the varieties showed a decreasing trend over time.

Correlation between the Spontaneous Defoliation Rate and the Enzyme Activity in Sugarcane Leaf Sheaths

Correlation analysis (Table 1) showed that in the process of sugarcane spontaneous defoliation, the pectinase activity in the abscission zones of the fifth ($R = 0.772$, $p < 0.00001$) and seventh ($R = 0.805$, $p < 0.0001$) leaf sheaths had a significant positive correlation with the spontaneous defoliation rate, and the cellulase activity in the abscission zone of the seventh leaf sheath had a significant positive correlation with the spontaneous defoliation rate ($R = 0.396$, $p = 0.017$). There was a significant positive correlation between the pectinase activity in the third, fifth, and seventh leaf sheaths, and there was also a significant positive correlation between the cellulase activities in the third, fifth, and seventh leaf sheaths. The cellulase activity in the abscission zone of the seventh leaf sheath was correlated with the pectinase activity in the abscission zones of the third, fifth, and seventh leaf sheaths, whose correlation coefficient were 0.436, 0.584 and 0.367, respectively. There was a positive correlation between cellulase activity and pectinase activity in the abscission zone of the fifth leaf sheath ($R = 0.339$, $p < 0.05$).

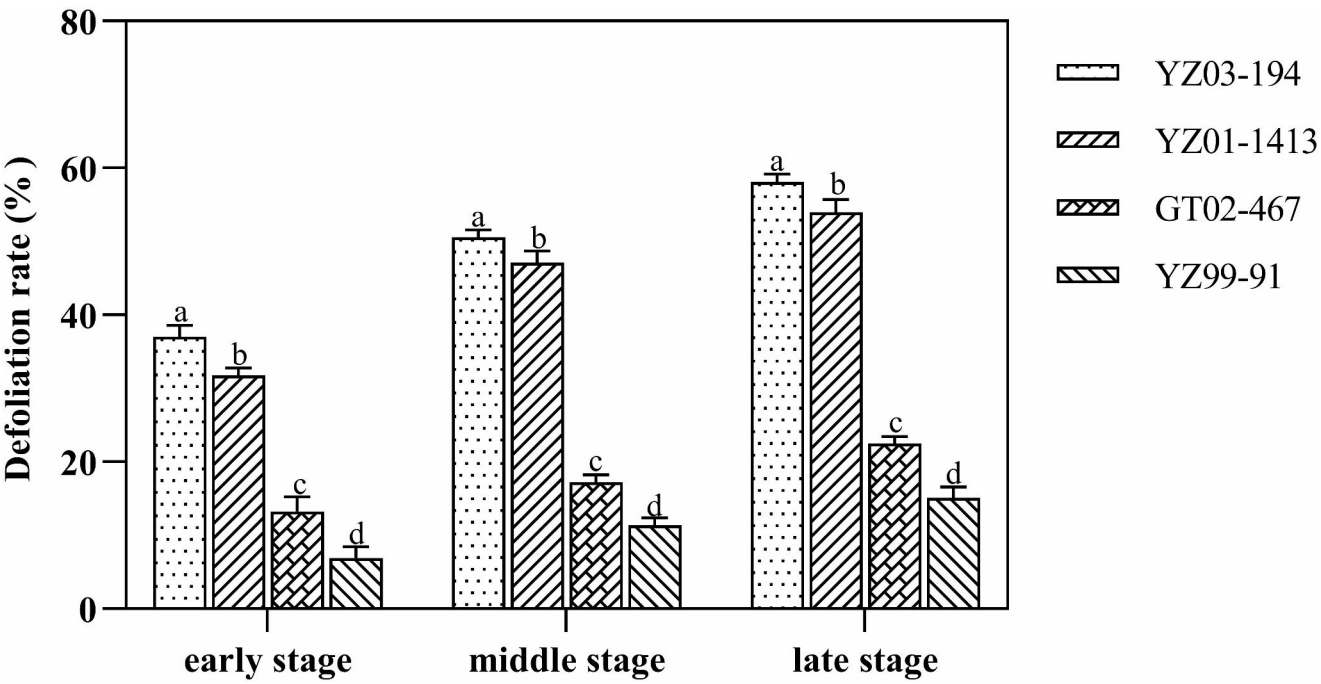

Fig. 5 Spontaneous defoliation rate of four sugarcane varieties at different stages of defoliation. Different letters on the histogram represent significant differences at $p < 0.05$

Table 1 Correlation between enzyme activity and the spontaneous defoliation rate in sugarcane

	Pectinase activity (leaf 3)	Pectinase activity (leaf 5)	Pectinase activity (leaf 7)	Cellulase activity (leaf 3)	Cellulase activity (leaf 5)	Cellulase activity (leaf 7)	Defoliation rate
Pectinase activity (leaf 3)	1						
Pectinase activity (leaf 5)	0.652**	1					
Pectinase activity (leaf 7)	0.573**	0.833**	1				
Cellulase activity (leaf 3)	0.103	0.227	0.120	1			
Cellulase activity (leaf 5)	0.304	0.339*	0.189	0.632**	1		
Cellulase activity (leaf 7)	0.436**	0.584**	0.367*	0.516**	0.448**	1	
Defoliation rate	0.324	0.772**	0.805**	0.301	0.253	0.396*	1

Note: * indicates $p < 0.05$, and ** indicates $p < 0.01$

Discussion

During the growth and development of sugarcane, spontaneous cracking occurs at the leaf sheath of aging leaves, leading to leaf shedding. From the early to late stages of defoliation, there were significant differences between the spontaneous defoliation rates in four sugarcane varieties, and the spontaneous defoliation rate of each variety increased over time. In cotton, soybean, and tomato, defoliation is closely related to the formation and cracking of abscission zones, and differences in the regulation of cell growth in abscission zones in different varieties lead to the differences in their defoliation performance (Mishra et al. 2008; Jáquez-Gutiérre et al. 2019; Woo et al. 2019). In this study, cells in the abscission zones of the third, fifth, and seventh leaf sheaths of all varieties undergo degradation from early to late stages. At early and middle stages, no significant difference in organelle degradation is observed between defoliation-prone and defoliation-resistant varieties. However, during late-stage defoliation, organelles in the abscission zone cells of defoliation-prone varieties degrade completely, while those in defoliation-resistant varieties remain intact. Significant differences in cell wall morphology exist between varieties with different defoliation performances throughout the process. Defoliation-prone varieties exhibit multiple cells with hollow spots in the cell wall, whereas defoliation-resistant varieties do not show such abnormalities.

The plant cell wall is a complex network structure mainly composed of polysaccharides such as cellulose, hemicelluloses, and pectin (Carpita 1996). Changes in the composition and structure of the cell wall play a crucial role in plant growth and development and in the response of a plant to external stresses (Tenhaken 2015). The direct cause of flower and fruit drop in fruit crops is the degradation of the cell wall, which results from the breakdown of cell adhesion due to the action of various enzymes such as cellulase, pectinase, peroxidase, polygalacturonase, xyloglucan endotransglucosylases/hydrolases, and expansins (Bar-Dror et al. 2011; Tariq et al. 2017; Roberts and Gonzalez-Carranza 2009; Levine and Hall 2011; Djanaguiraman et al. 2010; Niederhuth et al. 2013). Pectin is synthesized in the Golgi apparatus, methylated by methyltransferases, and transported to the cell wall via vesicular transport (Ibar and Orellana 2007). In the cell wall, pectin is demethylated by pectin methylesterase (Sénéchal et al. 2014). Changes in the degree of methylation of pectin affect the cross-linking between pectin and other cell wall components, thereby affecting the mechanical strength, fluidity, and other properties of the cell wall (Burton et al. 2010). During plant organ abscission, polygalacturonase and cellulase exhibit high levels of activity in the abscission zone (Sundaresan et al. 2020). In the present study, the pectinase activity in the abscission zones of the fifth and seventh leaf sheaths in the defoliation-prone varieties YZ03-194 and YZ01-1413 was significantly higher compared to the defoliation-resistant varieties GT02-467 and YZ99-91 from the early to late stages of defoliation. At the early stage of defoliation, the cellulose activity in the abscission zones of the third leaf sheath in the defoliation-prone varieties YZ03-194 and YZ01-1413 was significantly higher than that of the defoliation-resistant variety YZ99-91. The cellulase activity in the abscission zones of the fifth and seventh leaf sheaths in YZ03-194 and YZ01-1413 was also significantly higher than that in GT02-467. From the middle to late defoliation stages, there was no significant difference in the cellulase activity in the abscission zones of the same leaf sheaths in four varieties. This result indicated that pectinase was involved in the physiological events promoting cell degradation in leaf sheaths from the early to late stages of defoliation, while the promoting effect of cellulose was concentrated at the early stage of defoliation. The cell wall morphology in the third, fifth and seventh leaf sheaths was significantly altered in the defoliation susceptible varieties, but no significant difference was observed in the defoliation resistant varieties. This was consistent with pectinase activity modification in different resistant cultivars, suggesting that the ultrastructural changes was influenced by pectinase and age together. Moreover, correlation analysis (Table 1) showed that pectinase activity in the abscission

zones exhibited a significant positive correlation with defoliation rate, indicating this enzyme may play an important role during sugarcane spontaneous defoliation. On the other hand, weak correlations were found between cellulase activity and defoliation rate, which meant this enzyme didn't have great influence on the sugarcane leaf abscission. These results were not entirely consistent with previous researches (Burton et al. 2010; Sundaresan et al. 2020). It suggests that the pectinase and cellulase activity exhibits very different impacts on the leaf abscission in different plants. It was found that the length and angle of the sheaths ruptred from stalk, defoliation force and rate were the major contribution phenotype for quantitative assessment of sugarcane defoliation (Huang et al. 2018). However, these traits are correlated and not easy to be evaluated accurately. In addition to phenotypic identification, joint analysis of molecular indicator should provide a better assessment of sugarcane defoliation. Therefore, the pectinase activity can be used as a reference index for breeding and evaluating the difficulty of natural defoliation traits in sugarcane varieties.

Author Contributions Author Contributions: Conceptualization, SL and JG; methodology, XH; validation, SL and JG; formal analysis, XH; investigation, TW, XH, XG, JD, RL and GL; data curation, XH and TW; writing—original draft preparation, SL and XH; writing—review and editing, SLand XH; supervision, SL; project administration, SL and JG; funding acquisition, SL All authors have read and agreed to the published version of the manuscript.

Funding This work was supported by National Natural Science Foundation of China (grant number 31960392).

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Bar-Dror T, Dermastia M, Kladnik A, Znidaric MT, Novak MP, Meir S, Burd S, Philosoph-Hadas S, Ori N, Sonego L, Martin BD, Lers A (2011) Programmed cell death occurs asymmetrically during abscission in tomato. Plant Cell 23(11):4146–4163

Bleecker AB, Patterson SE (1997) Last exit: senescence, abscission, and meristem arrest in *Arabidopsis*. Plant Cell 9:1169–1179

Botton A, Eccher G, Forcato C, Ferrarini A, Begheldo M, Zermiani M, Moscatello S, Battistelli A, Velasco R, Ruperti B, Ramina A (2011) Signaling pathways mediating the induction of apple fruitlet abscission. Plant Physiol 155:185–208

Burton RA, Gidley MJ, Fincher GB (2010) Heterogeneity in the chemistry, structure and function of plant cell walls. Nat Chem Biol 6(10):724–732

Carpita NC (1996) Structure and biogenesis of the cell walls of grasses. Annu Rev Plant Physiol Plant Mol Biol 47:445–476

Cristale J, Silva FS, Zocolo GJ, Marchi MRR (2012) Influence of sugarcane burning on indoor/outdoor PAH air pollution in Brazil. Environ Pollut 169:210–216

Djanaguiraman M, Sheeba JA, Devi DD, Bangarusamy U, Prasad PVV (2010) Nitrophenolates spray can alter boll abscission rate in cotton through enhanced peroxidase activity and increased ascorbate and phenolics levels. J Plant Physiol 167(1):1–9

Fan X, Liu SC, Gao XX, Dao JM, Deng J (2013) Effect of cellulose activity on abscission rate of sugarcane (*Saccharum officinarum* L.) leaf sheath in mature period. Plant Physiol J 49(11):1228–

Geitmann A (2018) Bracing for Abscission. Cell 173(6):1320

Hao JJ, Liu YJ (2001) Plant physiology experiment. Liaoning Science-and Techinology Publishing House, Shenyang, pp 9–13

Huang Y, Shang H, Xu Y, Jiang H, Xu S, Zhang M (2018) Quantitative evaluation of variation in defoliation traits among sugarcane genotypes. PLoS ONE 13(5):e0196071

Ibar C, Orellana A (2007) The import of S-adenosylmethionine into the golgi apparatus is required for the methylation of homogalacturonan. Plant Physiol 145(2):504–512

Jáquez-Gutiérrez M, Atarés A, Pineda B, Angarita P, Ribelles C, García-Sogo B, Sánchez-López J, Capel C, Yuste-Lisbona FJ, Lozano R, Moreno V (2019) Phenotypic and genetic characterization of tomato mutants provides new insights into leaf development and its relationship to agronomic traits. BMC Plant Biol 19(1):1–24

Levine E, Hall FR (2011) Pectinases and cellulases from plum Curculio larvae: possible causes of apple and plum fruit abscission. Entomol Exp Appl 23(3):259–268

Lewis MW, Leslie ME, Liljegren SJ (2006) Plant separation:50 ways to leave your mother. Curr Opin Plant Biol 9:59–65

Li RD, Fang ZC, Liu SC, Gao XX, Fan X, Dao JM, Zhang YB, Guo JW, Deng J (2015) Correlation analysis of leaf pectinase activity and natural defoliation traits during the maturation stage in sugarcane (*Saccharum offi cinarum*). Plant Physiol J 51(6):887–892

Mao L, Begum D, Hueywen C, Budiman MA, Szymkowlak EJ (2000) Jointless is a MADS-box gene controlling tomato flower abscission zone development. Nature 406:910–913

McManus MT (2008) Further examination of abscission zone cells as ethylene target cells in higher plants. Ann Bot-London 101(2):285–292

Mishra A, Khare S, Trivedi PK, Nath P (2008) Ethylene induced cotton leaf abscission is associated with higher expression of

cellulase (GhCel1) and increased activities of ethylene biosynthesis enzymes in abscission zone. Plant Physio Biochem 46(1):54–63

Mugica-Alvarez V, Santiago-de la Rosa N, Figueroa-Lara J, Flores-Rodriguez J, Torres-Rodriguez M, Magana-Reyes M (2015) Emissions of PAHs derived from sugarcane burming and processing in Chiapas and Morelos Mexico. Sci Total Environ 527:474–482

Nakano T, Kimbara J, Fujisawa M, Kitagawa M, Ihashi N, Maeda H, Kasumi T, Ito Y (2012) MACROCALYX and JOINTLESS interact in the transcriptional egulation of tomato fruit abscission zone development. Plant Physiol 158(1):439–450

Niederhuth CE, Patharkar OR, Walker JC (2013) Transcriptional profiling of the Arabidopsis abscission mutant hae hsl2 by RNA-Seq. BMC Genomics 14(1):1–12

Roberts JA, Gonzalez-carranza ZH (2009) Pectinase functions in abscission. Stewart Postharvest Rev 5(1):1–4

Sénéchal F, Wattier C, Rustérucci C, Pelloux J (2014) Homogalac-Turonan-modifying enzymes: structure, expression, and roles in plants. J Exp Bot 65(18):5125–5160

Singh J, Singh AK, Sharma MP, Singh PR, Srivastava AC (2011) Mechanization of sugarcane cultivation in India. SugarTech 13(4):310–314

Sundaresan S, Philosoph-Hadas S, Riov J, Salim S, Meir S (2020) Expression kinetics of regulatory genes involved in the vesicle trafficking processes operating in tomato flower abscission zone cells during Pedicel Abscission. Life 10(11):273

Tariq M, Yasmeen A, Ahmad S, Hussain N, Afzal M, Hasanuzzaman M (2017) Shedding of fruiting structures in cotton: factors, compensation and prevention. Trop Subtrop Agroecosyst 20(2):251–252

Tenhaken R (2015) Cell wall remodeling under abiotic stress. Front Plant Sci 5:771

Wang XM, Wu WL, Yan LF, Li WL, Qu YW (2007) Study on the spectrophotometric analysis of peclinase activity. Sci Technol Food Ind 28(5):227–229

Wang X, Chen XB, Li AL, Mao L (2009) Advances in molecular biology study of plant organ abscission. Acta Agron Sin 35(3):381–387

Woo HR, Kim HJ, Lim PO, Nam HG (2019) Leaf senescence: systems and dynamics aspects. Annu Rev Plant Biol 70:347–376

Zhou Y, Lu DF, Li CY, Luo JH, Zhu BF, Zhu JJ, Shangguan YY, Wang ZX, Sang T, Zhou B, Han B (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor SHATTERING ABORTION1. Plant Cell 24(3):1034–1048