Relationship between endogenous ethylene production and natural defoliation traits during the maturation of sugarcane

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Abstract
The relationship between endogenous ethylene production and natural defoliation rate was examined in sugarcane cultivars with different natural defoliation traits. Ethylene production was examined at different positions on leaf sheaths and leaf scars at various sugarcane maturation stages using gas chromatography as an external standard method. During the sugarcane maturation process, ethylene production was greatest in the 2nd leaf sheath scar, followed by the 5th and 10th, in that order. It was also greatest during the early maturation stage, followed by the mid-maturation and harvest stages. Ethylene production of the leaf sheaths and leaf scars differed significantly among the sugarcane cultivars. Cultivars that defoliate easily produced significantly more ethylene than those that do not defoliate easily. The natural defoliation rates was greatest in the harvest stage, followed by mid-maturation and early maturation stage. Correlation analysis indicated that ethylene production was positively correlated with natural defoliation rates, particularly during early- and mid-maturation stages.

Key words: sugarcane maturity period, sheath and leaf scars, endogenous ethylene production, natural defoliation rate.

1. INTRODUCTION

Sugarcane (Saccharum spp.) is the most important global sugar crop, accounting for 65% of the world’s sugar production. China is one of the world’s major sugar producers. In 2010, 166.7 million hectares were planted to sugarcane in China, and the annual national sugar production was 10.08 million tons. The sugarcane planting areas in China are located mainly in the tropics and subtropics south of 24 degree. In raw sugar production, natural defoliation at the maturation stage affects the efficiency of the sugarcane harvesting process, especially in countries growing mountain sugarcane. Few machines can be used for harvesting sugarcane in China because this crop is planted mainly on hillsides. In manual sugarcane processing, manual peeling of the leaves accounts for 65% of the entire labor involved in the harvest process (Shukla et al., 1991; Singh et al., 2011; Srivastava & Kishan, 1990).

Sugarcane leaves are composed of a leaf blade and leaf sheath. During the maturation period, mature leaf sheaths naturally pull away from sugarcane stems and defoliate; however, different sugarcane cultivars have shown considerable variation in the rate of natural defoliation. Some high-yielding sugarcane clones are often eliminated from agriculture practice due to their poor natural defoliation traits. In recent years, in part because of rising labor costs, the breeding of cultivars with improved defoliation traits and research into agronomic technology for the regulation of defoliation have gained increasing interest.

International and domestic studies have indicated that ethylene plays an important role in the shedding process of plant organs and as a natural regulator of plant organ defoliation (Patterson, 2001; Taylor & Whitelaw, 2001). The abscission layer cells in the leaf sheath can regulate endogenous ethylene production to induce the expression of genes related to shedding by sensing signals of defoliation (Goto et al., 1980; Lu et al., 1997; Schupp & Greene, 2004; Uthaichay et al., 2007). Ethylene can improve enzyme degradation activity in plant cell walls in the organ abscission layer, and the degrading enzymes accumulate in the cell wall, promoting degradation of the cell wall and middle lamella, which causes abscission (Campillo & Bennett 1996; González-Carranza et al., 1998; Mishra et al., 2008). The production of ethylene significantly affects plant organ shedding. In citrus fruits during the early stage of abscission, ethylene promotes organ shedding at low concentrations but inhibits it at high
concentrations (Abeles et al., 1992; Plummer et al., 1991). Spraying low concentrations of ethylene on bean leaves can artificially induce bean leaf shedding (Jackson & Osborne, 1970). In addition, the application of appropriate concentrations of the ethylene biosynthesis inhibitor AVG (Aminoethoxyvinylglycin) to apple trees caused a reduction in fruit ethylene production and a significant delay in fruit shedding (Schupp & Greene, 2004). However, the spraying of high quantities of ethylene onto citrus fruits has little effect on fruit stalk abscission (Patterson & Bleecker, 2004). The effect of exogenous ethylene has been studied on sugarcane tillering (Mishra et al., 2014), sugar accumulation (Fong Chong et al., 2010; Wang et al., 2008), and drought resistance (Ye et al., 2005), but no previous studies on the regulation of sugarcane defoliation have been reported. Information on the relationship between endogenous ethylene production and intrinsic natural defoliation traits relevant to sugarcane maturation is of great significance.

Here, we studied the relationship between the amount of ethylene released by different leaf sheaths and leaf scars and the natural leaf defoliation rate during the maturation process of different sugarcane cultivars. The objective of this study was to determine the physiological index, which influences the defoliation traits of sugarcane and may provide information that will facilitate the development of agronomic techniques for promoting natural defoliation.

### 2. MATERIAL AND METHOD

**Sugarcane cultivars**

The easy-to-defoliate sugarcane cultivars CYZ03-194 and CMT69-421, and difficult-to-defoliate cultivars GT02-467 and CYZ99-91 were used in this study. Their leaf sheaths are of similar size and they reach maturity at the same time (maturity lasts from November to January). As shown in figure 1, the leaf sheaths and leaf scars of the easy-to-defoliate sugarcane cultivars cracked easily, but those of the difficult-to-defoliate cultivars did not. The characteristics of the experimental soil are as follows: 20.5 g kg$^{-1}$ organic matter, 1.64 g kg$^{-1}$ total nitrogen, 0.67 g kg$^{-1}$ total phosphorus, 13.7 g kg$^{-1}$ total potassium, 80.79 mg kg$^{-1}$ available nitrogen, 9.81 mg kg$^{-1}$ available phosphorus, 112.78 mg kg$^{-1}$ available potassium, and pH 6.0. The number of sugarcane buds was 120000/hm$^2$ and rows were spaced 1 m apart. Fertilizer was applied at the following rates: 345 kg N/hm$^2$, 108 kg P$_2$O$_5$/hm$^2$ and 110 kg K$_2$O/hm$^2$. The sugarcane was irrigated six times at a rate of 225 m$^3$/hm$^2$ each time. Sugarcane samples were collected during the early maturation stage (November 30, 2012), mid-maturation stage (December 30, 2012), and harvest stage (January 30, 2013). Ten plants were selected from each variety and the leaf scar tissues of each plant at the 2$^{nd}$, 5$^{th}$, and 10$^{th}$ leaves from the top visible hypertrophy ring were cut 1 cm above the leaf scar, and 2 cm of the leaf sheath was cut below the leaf scar. Samples were collected between 8-9:00 a.m. and were immediately placed into ethylene bottles and sealed. Ethylene was collected for 4 h and the amount of ethylene released was measured. The measurements were repeated three times. Total expanded leaves of each plant and abscised leaves were surveyed to calculate the defoliation rate for all sample plants. Defoliation rate = natural defoliation/total number of leaves × 100.

**Determination of ethylene production**

A Shimadzu 2014 gas chromatograph was used to determine ethylene production. The GC chromatographic conditions were as follows: RT-Q-BOND (30 m × 0.53 mm × 20 μm); inlet temperature: 100 °C; detector temperature:

![Figure 1. Abscission zone at the 10$^{th}$ leaf in four different cultivars.](image-url)
Ethylene production and defoliation of sugarcane


200 °C; carrier gas (N₂) flow rate: 1.8 mL/min; aid gas flow: air, 400 mL/min; hydrogen, 30 mL/min; makeup gas (N₂): 30 mL/min; split ratio: 5:1; oven temperature: 50 °C for 4 min. The external standard method was used; the retention time of the standard sample was used to assess quality, and the peak area was calculated to assess quantity and therefore overall ethylene production.

Data analysis

Test results were analyzed using Excel 2003 and SPSS 19.0 statistical software. The statistical method used was a single-factor analysis of variance (ANOVA), and LSD was used to test significance at a p<0.01 level.

3. RESULTS

Validation of ethylene GC detection methods

Ethylene gas chromatography measurements were conducted on a standard ethylene sample (>99.95%) and fresh sugarcane test samples. As shown in figure 2, the ethylene peak retention time was 3.350 min, the peak shape was good, there was no interference from other substances, and the time of the peak appearance was short and highly precise. The standard curve of ethylene concentration vs. the peak area is described using the following equation: \[ Y = 0.0068X - 3.2119 \] (Y, concentration; X, peak area), \[ R^2 = 0.9998, \] with an 88% recovery rate. The fresh sugarcane test tissue samples were measured six times in parallel to calculate the standard deviation (SD) and relative standard deviation (RSD). The average concentration of the six test samples was 0.0184 μL/L, with an SD of 0.00052 and an RSD of 2.24%. The data indicated a high ethylene correlation coefficient, high sample recovery, and considerable reproducibility, which meets the test requirements and indicates that this method is suitable for the quantitative analysis of sugarcane ethylene production.

Natural defoliation rate

During the maturity process, the natural defoliation rates of four sugarcane cultivars was greatest in the harvest stage, followed by mid-maturation and early maturation stage. As shown in figure 3, the natural defoliation rates differed significantly between the cultivars at different stages of maturation, It was greatest for the cultivar of GT02-467, followed by CYZ99-91, CMT69-421 and CYZ03-194. The natural defoliation rates increased as the maturation process progressed, and the high rates of defoliation differed significantly between stages. Specifically, the defoliation rate of CYZ03-194 at the harvest stage was 13.32% and 6.59% higher than the mid-maturation and early maturation stages, respectively; the defoliation rate of CMT69-421 at the harvest stage was 16.16% and 7.52% higher than the mid-maturation and early maturation stages, respectively; the defoliation rate of GT02-467 was 8.26% and 2.91% higher than the mid-maturation and early maturation stages, respectively; the defoliation rate of CYZ99-91 was 5.84% and 0.47% higher than the mid-maturation and early maturation stages, respectively. The magnitude of increase in defoliation rate from highest to lowest was CYZ03-194 > CMT69-421 > CYZ99-91 > GT02-467.

Ethylene production by leaf sheaths at the mature stage

During the process of maturation, ethylene production at the same leaf positions differed significantly between the four cultivars (Table 1). There was higher ethylene production in young leaves than in mature leaves. It was greatest in the 2nd leaf sheath scar, followed by the 5th and...
With respect to different cultivars and leaf locations, ethylene production of CYZ03-194 was greatest, followed by CMT69-421, GT02-467 and CYZ99-91, for the 2nd, 5th, and 10th leaves at the early maturation stage and for the 2nd and 5th leaves at the mid-maturation and harvest stages. Ethylene production was significantly higher in the easy-to-defoliate cultivars CYZ03-194 and CMT69-421 than in the difficult-to-defoliate cultivars GT02-467 and CYZ99-91 in the 2nd, 5th, and 10th leaves during the early maturation stage, in the 5th leaves during the mid-maturation stage, and in the 2nd and 5th leaves during the harvest stage. At the mid-maturation and harvest stages, ethylene production of the 10th leaves was below the detection limits for all four cultivars. At this stage, the sugarcane continued to mature, and the 10th leaf sheath tissues showed accelerated aging and became yellowish, which may have caused ethylene production to fall below the detection values. During the cane maturation process, less ethylene was released by the sheath at harvest stage than other times for all leaves except the 10th leaf.

### Ethylene production by leaf scars at the mature stage

During the cane maturation process, the release of ethylene by leaf scars followed the same trend as the leaf sheaths, i.e., there was a decline in the amount of ethylene released as the canes matured (Table 2). Ethylene production was significantly different between all four cultivars at the same leaf positions. Ethylene production of CYZ03-194 was greatest, followed by CMT69-421, GT02-467 and CYZ99-91 for all leaf scar positions and maturation stages, and the differences were significant. Ethylene production by the easy-to-defoliate cultivars CYZ03-194 and CMT69-421 was significantly higher than that of the difficult-to-defoliate cultivars GT02-467 and CYZ99-91. Young leaves produced significantly more ethylene compared with mature leaves. The results were similar to those recorded for leaf sheaths, which might be related to the degree of maturity and aging status of the leaf scar tissue.

### Table 1. Ethylene production by leaf sheaths of different sugarcane cultivars

<table>
<thead>
<tr>
<th>Leaf positions</th>
<th>Clone</th>
<th>Ethylene production (nL·g⁻¹·h⁻¹×10⁻⁴)</th>
<th>Early stage</th>
<th>Mid stage</th>
<th>Harvest stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>CYZ03-194</td>
<td>255±3.21A</td>
<td>142±3.61A</td>
<td>107±1.53B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT69-421</td>
<td>243±1.52B</td>
<td>117±2.00B</td>
<td>113±1.00A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>227±1.73C</td>
<td>97±1.00C</td>
<td>88±2.00C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>93±2.15D</td>
<td>60±10.00D</td>
<td>53±2.52D</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>CYZ03-194</td>
<td>215±2.08A</td>
<td>139±2.65A</td>
<td>89±2.65B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT69-421</td>
<td>199±1.34B</td>
<td>109±1.00B</td>
<td>106±1.00A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>149±3.51C</td>
<td>85±1.00C</td>
<td>76±2.65C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>64±1.53D</td>
<td>50±1.73D</td>
<td>47±1.00D</td>
<td></td>
</tr>
<tr>
<td>10th</td>
<td>CYZ03-194</td>
<td>105±1.00A</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT69-421</td>
<td>68±1.00C</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>87±1.00B</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>29±1.00D</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Letters within a column indicate significance levels of 0.01.

### Table 2. Ethylene production by leaf scars of different sugarcane cultivars

<table>
<thead>
<tr>
<th>Leaf positions</th>
<th>Clone</th>
<th>Ethylene production (nL·g⁻¹·h⁻¹×10⁻⁴)</th>
<th>Early stage</th>
<th>Mid stage</th>
<th>Harvest stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>CYZ03-194</td>
<td>222±1.00A</td>
<td>108±1.00A</td>
<td>102±2.08A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT69-421</td>
<td>181±2.65B</td>
<td>97±1.00B</td>
<td>88±1.00B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>175±2.00B</td>
<td>84±1.00C</td>
<td>72±1.00C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>55±3.00C</td>
<td>50±1.73D</td>
<td>43±1.73D</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>CYZ03-194</td>
<td>191±1.00A</td>
<td>107±1.53A</td>
<td>91±8.50A</td>
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<tr>
<td></td>
<td>CMT69-421</td>
<td>168±1.00B</td>
<td>96±2.65B</td>
<td>88±1.53B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>140±2.65C</td>
<td>76±1.53C</td>
<td>56±3.21C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>53±1.73D</td>
<td>46±1.00D</td>
<td>38±1.00C</td>
<td></td>
</tr>
<tr>
<td>10th</td>
<td>CYZ03-194</td>
<td>73±1.00A</td>
<td>58±1.00A</td>
<td>43±1.00A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT69-421</td>
<td>54±1.73B</td>
<td>38±1.00B</td>
<td>28±1.00B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT02-467</td>
<td>29±1.00C</td>
<td>25±1.00C</td>
<td>21±1.73C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYZ99-91</td>
<td>14±1.00D</td>
<td>13±2.00D</td>
<td>9.2±0.17D</td>
<td></td>
</tr>
</tbody>
</table>

Letters within a column indicate a significance level of 0.01.
Correlation analysis between ethylene production by leaf sheaths and leaf scars and defoliation rate during maturation

Ethylene production was evaluated in four sugarcane cultivars during the same period in the maturation process. The corresponding natural defoliation rate was used for correlation analysis, which showed that ethylene production of different leaf sheaths and leaf scars were positively correlated with the natural defoliation rate. During the early maturation and mid-maturation stages, ethylene produced by leaf sheaths and leaf scars was significantly positively correlated with the natural defoliation rate (Table 3). At the harvest stage, ethylene production from the leaf sheath and leaf scars was not significantly related to the defoliation rates. This may be because both sheaths and scars mature during the harvest stage when ethylene production declines.

4. DISCUSSION

The shedding of plant organs is a complex physiological process. During abscission, an abscission zone is formed at the base area and hormones, enzymes, and other regulatory substances complete the process of leaf shedding (Hadfield & Bennett, 1998; Kalaitzis et al., 1997; McManus, 2008; Tabuchi et al., 2000). Ethylene is one of the most important hormones involved in the regulation of plant organ shedding. This hormone acts directly on the genes encoding cellulase and polygalacturonase, which are closely related to shedding. In addition, ethylene can regulate and control the genes encoding glutathione S-transferase, actin-related proteins, and the light-harvesting complex, which are closely correlated with resistance in sugarcane plants.

Numerous studies have shown that ethylene is an important regulatory hormone affecting the shedding of plant organs. In previous work, spraying 20 μL/L ethylene on tomato pedicellariae significantly improved the abscission rate (Wei et al., 2011). At all levels of maturity in the current study, more ethylene was produced by the leaf sheaths and leaf scars of easy-to-defoliate cultivars than by those of difficult-to-defoliate cultivars. During maturation, ethylene production by leaf sheaths and leaf scars of different cultivars was positively correlated with the defoliation rate; these correlations were significant during the early maturation and mid-maturation stages (Table 3). This finding is consistent with results reported for tomatoes, mung beans, and apples (Decoteau & Craker, 1987; Michael, 1985; Wang et al., 2003) and suggests that increased endogenous ethylene production of leaf sheaths and leaf scars helps to regulate and control the defoliation rate. Previous studies in citrus fruits have shown ethylene promotes shedding at low concentrations but inhibits it at high concentrations (Abeles et al., 1992; Plummer et al., 1991). Ethylene production by mature leaf sheaths and leaf scars showed a gradual declining trend. Similarly, natural defoliation only occurred at relatively low leaf positions with low ethylene production and did not occur at all at the high leaf positions where there was greater ethylene production. This suggests that high concentrations of ethylene have no effect on defoliation, but these results are limited to endogenous ethylene. It is necessary to conduct experiments to validate the role of exogenous ethylene and to clearly quantify the role of ethylene in the regulation of cane defoliation traits.

Previous studies have shown that ethylene is produced in all living plant cells (Uheda & Nakamura, 2000). More ethylene was produced by cucumber leaves at higher positions compared with lower positions (Zheng et al., 2003). In the current study, more ethylene was emitted by young leaf sheaths and scars than by mature leaf sheaths. Later, during the mid-maturation stage, ethylene production by the 10th leaf sheath was below the detection limit. This may be because the upper leaf sheath tissues are younger and contain more living cells. Auxins can inhibit the shedding of plant organs (Abebie et al., 2008), and there is antagonism between auxin and ethylene. Young leaves have a higher auxin content than mature leaves, which is why the abscission layer cells of young leaves are not as sensitive to ethylene compared with mature leaves (Meir et al., 2010). During the maturation process in the current study, natural cane defoliation always occurred in mature leaves, and there was significantly more ethylene production by young leaves than mature leaves; however, no defoliation was observed in the young leaf sheaths. It is possible that the auxin content of the upper young leaves was higher than the mature leaves.

5. CONCLUSION

There was a close correlation between the natural defoliation rate and endogenous ethylene production by leaf sheaths and leaf scars of mature sugarcane. An appropriate level of endogenous ethylene production was the physiological basis for the increased rate of natural defoliation, and the natural cane defoliation always occurred at the early stages of maturation. With the use of agronomic technology, exogenous ethylene may be suitable for regulating defoliation in sugarcane. Promoting endogenous ethylene production

Table 3. Correlation analysis of ethylene produced by leaf sheaths and leaf scars and defoliation rates

<table>
<thead>
<tr>
<th>Stages</th>
<th>Leaf sheath</th>
<th>Leaf scar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>Early stage</td>
<td>0.843</td>
<td>0.009</td>
</tr>
<tr>
<td>Mid stage</td>
<td>0.862</td>
<td>0.006</td>
</tr>
<tr>
<td>Harvest stage</td>
<td>0.331</td>
<td>0.424</td>
</tr>
</tbody>
</table>

r, correlation coefficient.
in an appropriate range during the early- and mid-stages of maturation can improve the natural defoliation rate of sugarcane. This may help solve the problem of defoliation in sugarcane harvesting.

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