Influences of trinexapac-ethyl on development and sugar content of sorghum bicolor

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Abstract Result showed that no-interactions among the cultivars. The different trinexapac-ethyl doses and different periods of pre-harvest weeks provided significantly different sweet sorghum growth and yield at the p < 0.05. The control treatment provided the highest growth but low sugar yield. The trinexapac-ethyl ripener affected the highest growth and sugar yield when applied at 0.05 ppm and 1 pre-harvest week. On the other hand, trinexapac-ethyl (0.20 ppm) level inhibited the growth of sweet sorghum when applied at 5 pre-harvest weeks. At 0.05 ppm dose and 1 pre-harvest week, the chemical ripener provided the tallest plant height, biggest stalk diameter, juice extract, and sugar yields. Based on these findings, we recommended to apply the trinexapac-ethyl at 0.05 ppm dose by spraying 1 week before harvest. The sweet sorghum variety is recommended to be KKU 40 cultivar.

Keywords: sweet sorghum, trinexapac-ethyl, yield, growth

Introduction

Biofuel production has derived from as sugar-cane, sugar beet, wheat, and sweet sorghum that becomes a new renewable production of energy from plant materials or organic matters, and an alternative to fossil fuel which is caused the global warming by increasing the amount of carbon dioxide in the atmosphere (Dar et al., 2018). Sweet sorghum crop has been recommended for the production of ethanol which is mixed with oil into a gasoline product (Almodares and Hadi, 2009). Sweet sorghum adapts well to any global environments. It is grown fertilely in three continents: America, Africa, and Asia (Rao et al., 2013). It is a short duration crop that can be harvested in about four months, whereas sugarcane is harvested in one year. Sweet sorghum is an industrial crop that can be grown with a lower cost than corn, wheat and sugar beet (Regassa and Wortmann, 2014 and Mathur et al., 2017). Tew and Cobill (2006) reported that cultivars and days of harvest were correlated to total juice

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extract yield and composition sugar yield of sweet sorghum. Sugar yield increased during its growth stage but decreased with limited soil water and crowded plant population (Dar et al. 2018). Good characteristics of sweet sorghum for energy production are Brix degree of 13–24%, sucrose content of 7.2–15.5%, total stalk sugar yield of 12.0 Mg ha⁻¹, stalk fresh yield of 24-120 Mg ha⁻¹ and biomass yield of 36-140 t ha⁻¹ (Regassa and Wortmann, 2014).

Chemical ripeners cause accumulation of sugar content in the internodes of sorghum and sugarcane at their pre-harvest stage after the ripeners were sprayed onto them (Dalley and Richard, 2010). Their primary purpose is for making sorghum or sugarcane get riped quickly, shortening the pre-harvesting stage duration (Faria et al., 2014). Trinexapac-ethyl (C₁₃H₁₆O₅) is a plant growth regulator commonly used for regulating the growth of cereal and horticultural crops. It is used as an anti-lodging agent for wheat and turf grasses (Matysiak, 2006) and for inhibiting vegetative growth of peanut, potato, and bean (Correia and Leite, 2012) as well as a chemical ripener for sugarcane (Spauhnhorst et al., 2019 and Van Heerden, 2014). In addition to these, trinexapac-ethyl is used for controlling the growth of grasses. In this regard, (Orgeron et al., 2013) Trinexapac-ethyl blocks gibberellin synthesis by inhibiting gibberellin acid 20 (GA₂₀) hydroxylation to gibberellin acid1 (GA₁). After trinexapac-ethyl is sprayed on the leaves, 3β-hydroxylase enzyme in the plant’s main biosynthesis pathway of GA₁ is inhibited (Rademacher, 2000). The outcome is that the sugar content of sugarcane crop increases. Van Heerden et al. (2015) found that trinexapac-ethyl could increase sugar yield but reduce internode elongation and leaf growth.

The objective of this study was to investigate the effects on growth, development and total sugar yield of two sweet sorghum cultivars at harvesting stage there after sprayed with trinexapae-ethyl at pre-harvesting times.

Materials and Methods

Study area description

The location of the study area was conducted at an experimental plot of the Faculty of Agricultural Technology, King’s Mongkut Institute of Technology Ladkrabang, Thailand. The plot was located at 13° 43’ 36.21” N, 100° 46' 48.454” E and 1.50 m. above mean sea level in Bangkok, Thailand. The experiment was done from July to December 2017. The soil belongs to Bangkok series with clay texture and slightly acidic (pH 6.2) which shown (details are in Table 1). The experiment was performed as 3 factors factorial experiment in Randomized Complete Block Design (RCBD) with three
replications. Factor A was two sweet sorghum cultivars; Factor B was four trinexapac-ethyl doses per square meter (0, 0.05, 0.10 and 0.20 ppm) and factor 3 was three application times (1, 3 and 5 pre-harvest weeks). The size of the sub-plots was 9 square meters.

**Crop husbandry**

Sorghum plants were grown with a row spacing of 60 cm and plant-to-plant spacing of 10 cm in moist soil. The seeds were placed at a depth of 25 mm from the soil surface, and the seed rate was 11 kg ha\(^{-1}\). Fertilizer were used as the following: 46 kg ha\(^{-1}\) of urea, 42 kg ha\(^{-1}\) of triple superphosphate, and 42 kg ha\(^{-1}\) of potassium sulphate. Furrow irrigation was applied every three days during the experiment. A herbicide was used as needed (one kg ha\(^{-1}\) of Atrazine). An insecticide Carbosulfan was sprayed at 15 days after germination. The weather conditions during the experiment are presented in Figure 1. The maximum and minimum temperatures were 32.85 and 24.36 °C, and the average amount of rainfall was 6.20 mm per month. The humidity and evaporation per month were 76.01 % and 3.98 mm, respectively.

![Figure 1](image1.png)

**Figure 1.** Weather conditions during the experiment: rainfall, maximum and minimum temperatures (A), and humidity and evaporation rate (B)

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>pH</th>
<th>EC (µS/cm)</th>
<th>OM (%)</th>
<th>Available nutrient (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>0-30</td>
<td>6.2</td>
<td>2.60</td>
<td>1.95</td>
<td>46.49</td>
</tr>
</tbody>
</table>

Table 1. Analysis of soil chemicals of Bangkok series soil
Data collection

Growth parameter data were recorded at the harvest stage. The stem height of the sweet sorghum stem was measured from the ground to the distal by using the method of Shukla et al. (2017). A digital vernier caliper (Mitutoyo model: 500-196-30, Japan) was used to measure the diameter of sorghum stem according to the method of Tsuchihashi and Goto (2004). The number of internodes was counted for each internode from the ground to the top of the stem. A leaf area meter (Li-Cor model: Li 3000, USA) was used as a standard device for measuring leaf area. The biological yield was determined as the weight of dry stem samples. The juice was extracted by using a sugarcane juice machine (a motor-operated 3-roller press). Degrees of Brix was determined with a digital refractometer. Total soluble sugar content (Liua et al., 2008) was estimated based on the following equation: \( y = 0.8111x - 0.37285 \) (\( y \) = total soluble sugar content (%); \( x \) = Brix of stem juice (%)).

Statistical analyses

All collected data were statistically analyzed with a program called SAS v.9.2 for Windows. The growth and yield results were subjected to a three-way analysis of variance (ANOVA). The experimental design was a 2x4x3 factorial in RCBD, and means were compared by the Duncan's multiple range test (DMRT) at \( p < 0.05 \).

Results

Plant height and Stem diameter

The plant heights and stem diameters of the two sweet sorghum cultivars were not significantly different \( p < 0.05 \), but the plant heights and stem diameters of sorghum treated with different trinexapac-ethyl doses or different application times were significantly different \( p < 0.05 \), as listed in table 2. A higher dose of trinexapac-ethyl (0.05-0.20 ppm) caused reduced plant height and stem diameter when compared to the control group (0 ppm), as listed in table 3. The application time at one week before harvesting provided the highest mean growth and stem diameter (268.58 cm and 22.39 mm, respectively), whereas the application time at three and five weeks before harvesting inhibited the growth of sweet sorghum, (253.08 cm and 18.54 mm, respectively, and 236.97 cm and 14.00 mm, respectively), as listed in table 3.
### Table 2. Plant height, stem diameter, number of internodes, leaf area, biological and juice extract yield, Brix degrees, and total soluble sugar content of two sweet sorghum cultivars at the harvesting stage as affected by different trinexapac-ethyl application doses and application times

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean square</th>
<th>PH</th>
<th>SD</th>
<th>NI</th>
<th>LA</th>
<th>BY</th>
<th>JEY</th>
<th>Brix</th>
<th>Total SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>29.32ns</td>
<td>0.12ns</td>
<td>3.76ns</td>
<td>32,338.74ns</td>
<td>97,532.11ns</td>
<td>69,202.89ns</td>
<td>1.16ns</td>
<td>0.76ns</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>23</td>
<td>1,667.60*</td>
<td>53.75**</td>
<td>23.97**</td>
<td>2,336,678.83**</td>
<td>456,931.55**</td>
<td>779,817.24**</td>
<td>17.55**</td>
<td>11.55**</td>
<td></td>
</tr>
<tr>
<td>Cultivar (A)</td>
<td>1</td>
<td>897.82ns</td>
<td>13.40ns</td>
<td>9.03ns</td>
<td>224,595.15ns</td>
<td>321,785.05ns</td>
<td>150,883.55ns</td>
<td>3.22ns</td>
<td>2.12ns</td>
<td></td>
</tr>
<tr>
<td>Trinexapac-ethyl (B)</td>
<td>3</td>
<td>8,013.18*</td>
<td>117.84**</td>
<td>113.94**</td>
<td>5,569,913.29**</td>
<td>2,014,487.18**</td>
<td>2,816,184.00**</td>
<td>80.36**</td>
<td>52.88**</td>
<td></td>
</tr>
<tr>
<td>Pre-harvest weeks (C)</td>
<td>2</td>
<td>5,997.65*</td>
<td>422.29**</td>
<td>87.22**</td>
<td>16,340,996.27**</td>
<td>1,865,443.27**</td>
<td>3,930,410.88**</td>
<td>63.44**</td>
<td>41.74**</td>
<td></td>
</tr>
<tr>
<td>AxB</td>
<td>3</td>
<td>79.26ns</td>
<td>3.52ns</td>
<td>0.45ns</td>
<td>22,172.58ns</td>
<td>33,701.32ns</td>
<td>242,693.33ns</td>
<td>4.31ns</td>
<td>2.82ns</td>
<td></td>
</tr>
<tr>
<td>AxC</td>
<td>2</td>
<td>5.11ns</td>
<td>3.62ns</td>
<td>0.41ns</td>
<td>72,597.76ns</td>
<td>34,573.33ns</td>
<td>67,593.55ns</td>
<td>0.14ns</td>
<td>0.10ns</td>
<td></td>
</tr>
<tr>
<td>BxC</td>
<td>6</td>
<td>124.94ns</td>
<td>0.64ns</td>
<td>3.26ns</td>
<td>196,487.84ns</td>
<td>29,908.61ns</td>
<td>65,039.33ns</td>
<td>2.25ns</td>
<td>1.48ns</td>
<td></td>
</tr>
<tr>
<td>AxBxC</td>
<td>6</td>
<td>70.75ns</td>
<td>0.51ns</td>
<td>0.70ns</td>
<td>456,107.54ns</td>
<td>10,598.35ns</td>
<td>37,006.00ns</td>
<td>0.97ns</td>
<td>0.63ns</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>46</td>
<td>693.43</td>
<td>4.45</td>
<td>3.41</td>
<td>108,056.13</td>
<td>102,500.50</td>
<td>92,715.24</td>
<td>2.84</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>900.42</td>
<td>20.29</td>
<td>10.08</td>
<td>827,871.44</td>
<td>217,176.24</td>
<td>314,635.25</td>
<td>7.56</td>
<td>4.97</td>
<td></td>
</tr>
</tbody>
</table>

DMRT (0.05) (%): 
- (A) ns ns ns ns ns ns ns ns
- (B) 6.21 0.50 0.43 77.48 75.46 71.77 0.40 0.32
- (C) 5.37 0.43 0.38 67.10 65.35 62.15 0.34 0.28

C.V. (%) 10.41 10.52 10.57 15.42 14.93 13.10 10.17 10.47

PH = plant height; SD = stem diameter; NI = number of internodes; LA = leaf area; BY = biological yield; JEY = juice extract yield; Brix = Brix degree; and Total SS = total soluble sugar content.

ns = non-significant, * = significant at 0.05, and ** = significant at 0.01, respectively.

Means followed by different letters are statistically different according to Duncan's multiple range test (DMRT).
Table 3. Plant height, stem diameter, number of internodes, leaf area, biological and juice extract yield, Brix degrees, and total soluble sugar content of two sweet sorghum cultivars at the harvesting stage as affected by different trinexapac-ethyl application doses and application times.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PH (cm)</th>
<th>SD (mm)</th>
<th>NI (internodes plant)</th>
<th>LA (m²)</th>
<th>BY (Kg rai⁻¹)</th>
<th>JEY (l rai⁻¹)</th>
<th>Brix (%)</th>
<th>Total SS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KKU 40</td>
<td>256.41</td>
<td>18.74</td>
<td>17.83</td>
<td>2,187.98</td>
<td>2,210.74</td>
<td>2,369.89</td>
<td>16.77</td>
<td>13.23</td>
</tr>
<tr>
<td>Cowley</td>
<td>249.35</td>
<td>17.88</td>
<td>17.12</td>
<td>2,076.30</td>
<td>2,077.04</td>
<td>2,278.33</td>
<td>16.34</td>
<td>12.88</td>
</tr>
<tr>
<td>trinexapac-ethyl concentrations (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 ppm (control)</td>
<td>278.12A</td>
<td>20.85A</td>
<td>20.58A</td>
<td>2,834.64A</td>
<td>2,484.81A</td>
<td>2,783.77A</td>
<td>14.10D</td>
<td>11.06D</td>
</tr>
<tr>
<td>0.05 ppm</td>
<td>260.04B</td>
<td>19.19B</td>
<td>18.25B</td>
<td>2,301.05B</td>
<td>2,318.88A</td>
<td>2,499.78B</td>
<td>18.85A</td>
<td>14.92A</td>
</tr>
<tr>
<td>0.10 ppm</td>
<td>244.47BC</td>
<td>18.42B</td>
<td>16.33C</td>
<td>1,811.75C</td>
<td>2,052.96B</td>
<td>2119.11C</td>
<td>17.66B</td>
<td>13.95B</td>
</tr>
<tr>
<td>0.20 ppm</td>
<td>228.87C</td>
<td>14.79C</td>
<td>14.75D</td>
<td>1,581.10D</td>
<td>1,718.89C</td>
<td>1893.78D</td>
<td>15.63C</td>
<td>12.30C</td>
</tr>
<tr>
<td>pre-harvest weeks (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>268.58a</td>
<td>22.39a</td>
<td>19.37a</td>
<td>2,945.08a</td>
<td>2,419.72a</td>
<td>2,746.50a</td>
<td>18.09a</td>
<td>14.30a</td>
</tr>
<tr>
<td>3 weeks</td>
<td>253.08b</td>
<td>18.54b</td>
<td>17.50b</td>
<td>2,156.03b</td>
<td>2,149.72b</td>
<td>2,286.00b</td>
<td>16.74b</td>
<td>13.20b</td>
</tr>
<tr>
<td>5 weeks</td>
<td>236.97c</td>
<td>14.00c</td>
<td>15.56c</td>
<td>1,295.30c</td>
<td>1,862.2c</td>
<td>1,939.83c</td>
<td>14.85c</td>
<td>11.67c</td>
</tr>
</tbody>
</table>

PH = plant height; SD = stem diameter; NI = number of internodes; LA = leaf area; BY = biological yield; JEY = juice extract yield; Brix = brix degree; and Total SS = total soluble sugar content.

Means followed by different letters are statistically different according to Duncan's multiple range test (DMRT).
Number of internodes and leaf area

Factor A (two sweet sorghum cultivars) did not significantly affect the number of internodes \((p < 0.05)\), as listed in Table 2. Factor B (four different Trinexapac-ethyl concentrations doses) and Factor C (three different application times) significantly affect the number of internodes and leaf area \((p < 0.05)\), as shown that in table 2. The number of internodes and leaf area of sweet sorghum grown normally in the control treatment was 20.58 internodes plant\(^{-1}\) and 2,834.64 mm\(^2\), respectively, whereas they were significantly lower in Factor B treatments (trinexapac-ethyl spraying at 0.05-0.20 ppm), as shown in Table 3. Similarly, Factor C treatments exhibited significant differences. The application time at one week before harvesting resulted in 9.37 internodes plant\(^{-1}\) and 2,945.08 mm\(^2\) leaf area, while the application time at three weeks before harvesting resulted in 17.50 internodes plant\(^{-1}\) and 2,156.03 mm\(^2\) leaf area, and the application time at five weeks before harvesting resulted in 15.56 internodes plant\(^{-1}\) and 1,295.30 mm\(^2\) leaf area.

Biological and juice extract yields

Factor A (two sorghum cultivars) did not significantly affect the biological and juice extract yield \((p < 0.05)\), whereas Factor B (four plant growth regulator doses) significantly affected them as well as Factor C (three different application times) as listed in Table 2. The biological and juice extract yields provided by the control were 2,484.81 Kg rai\(^{-1}\) and 2,783.77 l rai\(^{-1}\), respectively, while those provided by and the plant growth regulator at 0.02 ppm were 1,718.89 Kg rai\(^{-1}\) and 1893.78 l rai\(^{-1}\), respectively, as listed in Table 3. For Factor C (three different application times), the biological and juice extract yields were 2,419.72 Kg rai\(^{-1}\) and 2,746.50 l rai\(^{-1}\), respectively, when the plant growth regulator was applied at one week before harvesting, 2,149.72 Kg rai\(^{-1}\) and 2,286.00 l rai\(^{-1}\), respectively, when applied three weeks before harvesting, 1,862.20 Kg rai\(^{-1}\) and 1,939.83 l rai\(^{-1}\), respectively, when applied five weeks before harvesting.

Brix degrees and total soluble sugar content

There were no significant differences \((p < 0.05)\) in the Brix degrees and total soluble sugar content of the two sweet sorghum cultivars (Factor A), as listed in table 2, whereas applications of Factor B (four plant growth regulator doses) and C (three different application times) resulted in significant differences at \(p < 0.05\). The Brix degree of sorghum in the control group was significantly lower than that in the group treated with trinexapac-ethyl at 0.05-
0.20 ppm, as listed in Table 3. The Brix degree and total soluble sugar content of sweet sorghum applied with the plant growth regulator at one week before harvesting were 18.09 % and 14.30 %, respectively, at three weeks before harvesting was 16.74 % and 13.20 %, respectively, and at five weeks before harvesting were 14.85 % and 11.67 %, respectively.

Discussion

The growth parameters and sugar contents of sweet sorghum from treatments and control, were significantly differed. Those differences might come from the differences in the genes, breeding, and cultivars (Almodares and Hadi, 2009). That review paper stated that the aim of sweet sorghum development was to increase its growth parameter and sugar content with a focus on making it a feedstock for ethanol production. The effects investigated in the development were concerned the effect of crop management such as water (Yanaso and Detpiratmongkol, 2009), fertilizer (Almodares and Hoseini, 2016), and irrigation (Vannavong and Detpiratmongkol, 2008). The effect of weather such as temperature, day length, longitude, latitude, and elevation above mean sea level. The effect of crop systems such as crop rotation (Palumbo et al., 2014). It was found in this study that KKU 40 cultivar had better growth parameters (plant height, stem diameter, number of internodes, leaf area, biological and juice extract yield) and sugar content than Cowley cultivar, though not significantly ($p < 0.05$), which was not surprising because KKU 40 has been developed as a hybrid variety with good agronomic traits for planting in Thailand.

This study investigated applications of four different trinexapac-ethyl doses and three different application times harvesting that found the different doses, and provided significant differences in the development of stalk and internodes of sweet sorghum. Kingston and Rixon (2007) found that trinexapac-ethyl at 200 g ha$^{-1}$ sprayed on sugarcane at 6-10 weeks before harvesting resulted in increased a higher sucrose level than that of the control, in agreement with the findings by Adams et al. (1991 and 1992) that the regulator reduced the length of internodes and stem. The plant growth regulator was also found to inhibit the growth of turfgrass compared to the control (Czeluściński et al., 2017). Furthermore, a study reported that trinexapac-ethyl at 200 g ha$^{-1}$ provided a higher sucrose yield content than the control (Spaunhorst et al., 2019).

In this study, trinexapac-ethyl was found to decrease vegetative growth and increase sugar yield content. Application of the plant growth regulator at five weeks before harvesting inhibited cell division and vegetative growth (plant height, stem diameter, number of internodes, leaf area, biological and
juice extract yield) because of increased gibberellin acid (GA$_1$), in agreement with the findings by Van Heerden _et al._ (2014). Increased GA$_1$ would be decreased cell volume, elongation of leaf cells, and shoot cell division cell of grasses crops (Van Heerden _et al._, 2014 and 2015). Application of the plant growth regulator at 0.05 ppm and one week before harvesting showed very effective at ripening sorghum, increasing juice sugar yield of up to 85% at harvesting (Resende _et al._, 2000).

Our recommendation to farmers is for them to spray trinexapac-ethyl at a 0.05 ppm dose and 1 week before harvesting for better sorghum growth and sugar content. Moreover, KKU 40 cultivar is a good cultivar that is well adapted to the environmental conditions in Thailand.

**Acknowledgments**

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