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Effect of GA3 and Gly Plant Growth Regulators on Productivity and Sugar Content of Sugarcane

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Abstract: The use of plant growth regulators is one effective solution to improve sugarcane yields and sugar content in several countries. In this study, we examined the role of gibberellin acid (GA3) and glyphosate (Gly) plant growth regulators to determine the appropriate concentration of GA3 and Gly to increase the yield of sugarcane and sugar accumulation, respectively. The statistical results showed that GA3 was sprayed at 150 ppm to increment the actual yield by 19.94%; sucrose accumulation increased by 2.21%. With Gly treatment, although the yield decreased by 3.17%, sucrose accumulation increased by 11.27% compared to control trials. In this study, the combined concentration of 150 ppm of both GA3 and Gly gave the best results, for which sucrose accumulation increased from 2.21% to 10.74% and from 19.94% to 20.97% for actual yield. The results led to increased net income compared to the control. To address concerns about residues of plant growth regulators, residues of GA3 and Gly were evaluated after the sugarcane harvest using the HPLC and UV-vis methods, respectively. The analyzed results showed that their residues were lower than what is permitted in several countries. This showed the applicability of the study, on a large scale, to increase sucrose accumulation, productivity of sugarcane, and profit for farmers.

Keywords: plant growth regulators; gibberelin acid GA3; glyphosate; sucrose accumulation

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is an important crop in Vietnam that is grown on over 5% of the agricultural land. Globally, the total harvested area of sugarcane was approximately 27 million hectares in 2015/2016, which spread across more than 106 countries in the tropical and subtropical regions. Sugarcane farming is constrained due to unfavorable conditions, such as weeds, disease, pests, and/or numerous abiotic factors, such as drought or flood, which lead to yield stagnation [1]. Notably, the lower sugar content of cane causes lower procurement prices by sugar mills [1,2]. The problems faced in sugarcane cultivation have caused inability to adapt to the fast-growing demand for both sugar and ethanol in the global market. Therefore, the crisis in sugarcane production calls for alternative strategies, in terms of cultivating procedures and technologies, to break the slow yield and increase the profitability of sugarcane.

Despite huge efforts to develop new sugarcane varieties for commercial cultivation, the solution is to target the long-term rather than the short-term [2]. In this scenario, the application of plant

growth regulators becomes a positive step towards addressing the crisis facing sugarcane cultivation in the short-term [2,3]. Considering the potential of plant growth regulators, various countries with developed commercial agriculture, such as the United States and Brazil, European countries such as Germany, England, and Finland, and some countries in Asia such as India, China, Indonesia, and Thailand, have focused on promoting these regulatory agents to increase the output of agriculture [2–4]. The agent most frequently subject to sugarcane research is gibberellin acid (GA) [3]. Of the gibberellin A family, gibberellin A3 (GA3) is the most popular, at the commercial level, for agronomic as well as scientific research [2,4,5]. There is a long history of GA3 research evaluating the commercial field conditions in the sugarcane fields of Hawaii [3–6]. As a result, the application of GA3 showed a positive impact on some stages of development leading to an increase in cane yield compared to untreated controls. Various research programs revealed that GA3 had an effect on the activity of sucrose phosphosynthase, one of the major enzymes involved in the synthesis of sucrose [7], and promoted the proliferation of sink tissues, leading to an improved assimilation process in sugarcane [8–10]; economic efficiency was thereby enhanced. In addition, GA3 increased abiotic stress tolerance in crops like sugarcane that are grown in, for example, salinated environments [11]. However, GA3 proved that its function in the presence of invertase [12], by which sucrose is broken down into reducing sugar, further limited the phloem loading of sucrose in the sink. Along with productivity, the most important economic value of sugarcane is the sucrose concentration in the juice. Higher sucrose accumulation helps sugarcane farmers to procure a higher milling factory price [2]. Hence, this perturbed condition can be achieved by extra chemical ripening agents [13].

The cruciality of the chemical ripeners utilized was proven in long-term research of sugarcane fields [14]. Various studies of sugarcane in institutes in Brazil, Hawaii, and Australia revealed that the use of growth-inhibiting hormones such as 2,3,6-trichorobenzoic acid or ethephon and glyphosate (Gly) accelerated ripening and increased overall sucrose accumulation in cane [14]. Those growth-inhibiting hormones induced the proliferation of apical meristem, resulting in the unloading of sucrose. Therefore, higher sucrose content was loaded into sink tissues. Currently, the only Gly is registered in the US as a sugarcane ripener. This is the most widely used plant growth regulator in Hawaii, Texas, Florida, Guyana, Brazil, Guatemala, Jamaica, and Mauritius [14]. Several studies indicated that Gly-sprayed sugarcane significantly increased the sugar yield [15,16].

The aim of this study was to investigate the dual effects of a plant hormone stimulating plant growth and development (GA3) and a growth-inhibiting hormone (GLy) in sugarcane farming in the Tra Vinh Province, Vietnam. In order to reach the target, single treatments with GA3 and GLy were first conducted to assess the combined effect of GA3 and Gly on commercial sugar content, total fresh weight, and profitable ratoon sugarcane crops. In addition, examinations of GA3 and Gly residues on the treated sugarcane were performed to clarify concerns of the treatment.

2. Materials and Methods

2.1. Material

The gibberellin GA3 basis (HPLC) (90%) used for analysis was purchased from Merck (Darmstadt, Germany), whereas the GA3 used in the field was obtained from Hifield—Ag Chem, India Private Limited (Maharashtra, India). Glyphosate (Gly) was ordered from Merck (Darmstadt, Germany) for the analytic standard and from AI Agrochem Joint Stock Company (Hochiminh, Vietnam) for the cane fields. CH03, the water absorbing agents, originated from IAMS (Hochiminh, Vietnam). Fluorrenilmetilloxicarbonilo chloride (FMOC–Cl) came from Merck (Darmstadt, Germany). Moreover, chemical solvents for analysis, such as methanol, acetonitrile, and phosphoric acid, were Fisher's HPLC grade solvents. Other chemical agents were supplied by Scharlau (Barcelona, Spain) or by Viet My Chemical Distribution Join Stock Company (Hochiminh, Vietnam).

2.2. Experimental Design

The study was conducted at Tra Cu District, Tra Vinh Province, Vietnam. The experiments were carried out in the dry season from March 2018. The study was assessed on commercial varieties of sugarcane K95-84, originating from Thai Lan, which were popular in the Tra Vinh Province. Each pot experiment was in 50 m^2. Three studies were performed with different treatments: (1) single treatments with 4 level concentration of GA3, (2) single treatments with various concentrations of Gly ranging from 0 to 200 ppm (4 levels), and (3) the combined treatment of GA3/GLy and GA3/CH03 with 4 concentrations of GA3. GA3 was dissolved in 5 mL ethanol and then diluted with water to reach the defined concentrations (0 ppm, 50 ppm, 100 ppm, 150 ppm) in 25 L. A total of 10 mL, 15 mL, and 20 mL of the stock solution of Gly (250 g/L) was dissolved in water up to 25 L to prepare 100 ppm, 150 ppm, and 200 ppm, respectively. Each experimental pot was sprayed with 25 L of prepared GA3 or/and Gly (50 mL/cane). In all treatments, GA3 was sprayed after 120 days of planting and Gly was applied for 45–60 days before harvest. All the experiments followed a randomized complete block design with four replications, each containing 4 treatment plots as four concentrations. The distance between the margin of each block was 1.4 m. All blocks received the same amount as well as type of fertilizer (recommended doses of N, P, and K were 16:8:14 $Kgha^{-1}$) at the same time. Application of insecticides was made as per recommendations for the region. The plants in all the treatments were free of pests and diseases during the experiments. At the determined time, all canes were collected to evaluate and compare the effect of treatment on internode length (cm), stem length (m), diameter (cm), weight, commercial cane sugar (CCS), sugarcane productivity (kg), and net-income (VND).

The calculation of commercial cane sugar was followed the quality standards of Quy chuẩn kỹ thuật Vietnam (QCVN) 01-98/2012/BNNPTNT set by the Vietnam Agriculture Ministry. Briefly, 50 canes were randomly selected in each experimental trial. All canes were cut at ground level, and then all leaves were removed before washing and weighting to get the initial mass (P). After they were passed through the cane crusher, the juice was filtered and then separated for 2 measurements (brix and Pol). To determinate the brix level, a refractometer (RHB0-90, Total Meter, Fujian, China) was used. The meter was set to zero using Milli Q water prior to obtaining a Brix measurement for each sugar solution. The Pol level of cane juice was measured using a polarimeter (AA 65, OA index, Hackettstown, NJ, USA) at 589.44 nm. Calibration of the polarimeter was performed using 500 mL solution mixture of lead (II) acetate (330 g) and lead (II) oxide (110 g). Prior to recording Pol, the Pol tube was rinsed with a large volume (200 mL) of a sample. The remained bagasse was softened and then used to identify the percentage of fiber (F%). Finally, based on the value of Brix, Pol, and cane fiber content, the value of CCS was calculated as the below formula:

$$\mathrm{CCS} = \frac{3}{2} \times \mathrm{Pol} \times \left(1 - \frac{5 + \mathrm{F}}{100}\right) - \frac{1}{2} \times \mathrm{Brix} \times \left(1 - \frac{3 + \mathrm{F}}{100}\right) \quad (1)$$

Following the Vietnamese sugar mill's scale, the procurement price of cane is approximately 1 million VND/ton, applied to sugarcane of 10 CCS. The predicted price was calculated as:

$$\text{Predicted prices} = \frac{\mathrm{CCS}}{10} \times 1,000,000\ (\mathrm{VND}) \quad (2)$$

The net income was figured out by deducting the costs for harvesting and planting from the gross income (Gross income (VND) = predicted prices × productivity). The cost of harvesting was around VND 100,000 per ton at the time of study. The total input cost for 50 m^2 was 298,000 VND, including seed, fertilizers, water, and labor. In cases where regulators were used, other costs were applied, such as the price of GA3 (80 VND/ppm), Gly (50 VND/ppm), CH03 (38,000 VND/50 m^2), and labor (600,000 VND/200 L/time). These prices were based on the standard level in the Tra Vinh Province, Vietnam.

2.3. Method for Determining GA3 Residue in Sugarcane

HPLC performed on a PerkinElmer (USA) was used to detect GA3 residues in cane juice. The stationary phase was C18 (5 µm), 4.6 × 250 mm. The mobile phase was the mixture of 0.1% phosphoric acid and acetonitrile (Table 1). The flow rate was controlled at 1.0 mL min^{-1}.

Table 1. The solvent program runs HPLC chromatograph.

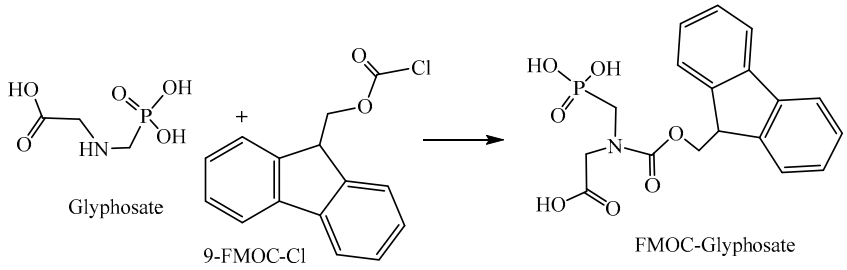
Time (min)	%A	%B
1	80	20
3	80	20
7	65	35
15	80	20

GA3 extraction: The preparation of sugarcane juice samples was analyzed in accordance with National Technical Regulation QCVN 01–98: 2012/BNNPTNT or in some previous reports [17,18]. Four samples of 10 mL of sugarcane juice were prepared, with 3 sugarcane samples spraying GA3 and 1 sample without GA3 during cultivation. The juice was adjusted to pH 3.0 with hydrochloric acid 0.1 M. A total of 3.0 mL of the solution was transferred to a glass tube and then diluted 2 times with ethyl acetate. The mixture was well-shaken for 4 min and centrifuged for 30 min. Supernatant was separated from the residue. The ethyl acetate solution in the upper layer was transferred into another tube containing anhydrous sodium sulfate in order to remove the water. The tube was shaken again using a vortex for 1 min and then centrifuged for 2 min. The solution was transferred into another tube and then evaporated using a vacuum rotary evaporator at 35 °C to remove all ethyl acetate. An aliquot of 3.0 mL of mobile phase was added into the tube to dissolve GA3. The sample solution was filtered through a 0.22 µm membrane filter for analyzing HPLC.

2.4. Method for Determining Gly Residues in Sugarcane

Gly extraction: Sugarcane juice samples were centrifuged at 5000 rpm (13,975× *g*) for 15 min to obtain a solution of sugarcane. Methanol was added to the solution at a ratio of 1:1 (*v*/*v*). Precipitate was filtered out of the mixture. The Gly-extracted solution was then filtered through the 0.22 µm membrane.

Derivatization of Gly: Because Gly did not show clear absorbance in the UV range, in this study, FMOC–Cl was incorporated with Gly as per Felton's protocol. [19]. However, the solution of FMOC–Cl has a short half-life [20]; therefore, the stock solution of FMOC–CL was freshly storaged before measurement. Before analysis, a known concentration of Gly with FMOC–Cl was used to identify the maximum absorbance in the range 190–1200 nm as well as the concentration of FMOC–Cl using spectrophotometer UV-vis 1800 SHIMAZU. The derivatizing reagent was prepared by dissolving 100 µg of FMOC–Cl in 10 mL acetonitrile. Borate buffer solution (200 mmol·L^{-1}) was firstly prepared by dissolving 123.7 mg boric acid in 10 mL distilled water, and its pH was adjusted to 9 with 200 mmol·L^{-1} sodium hydroxide solution. In 15 mL plastic centrifuge tubes, 3 mL of 0–20 µg.mL^{-1} Gly standard solutions were pipetted and followed by the addition of 1.5 mL of borate buffer and 3 mL FMOC–Cl solution. The mixture was then homogenized for 5 min by manual shaking. The derivatization reaction was maintained at 30 °C for 2 h, as seen in Scheme 1. The excess amount of FMOC–Cl and FMOC–OH was removed by extracting the reaction mixture thrice with diethyl ether (3 × 4 mL). The upper organic layer was removed, while the lower aqueous layer, which contained the Gly–FMOC product, was withdrawn and quantified using a UV-visible spectrophotometer at 265 nm wavelength. The obtained results were used to construct a standard equation. For the Gly-extracted solution, the same process was conducted. Regarding the standard equation, the amount of Gly residue in extracted solution was determined.



Scheme 1. Reaction between glyphosate (Gly) and fluorrenilmetilloxicarbonilo chloride (FMOC-Cl).

2.5. Statistical Analysis

Analysis of variance was done using MstatC computer software following Dosbox (0.74.2). All experiments in this study were collected at least three times, and the data are represented as the mean ± standard deviation (SD). Statistical analyses were performed using analysis of variance (ANOVA). Statistical significance was established at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Gibberellin Acid GA3 on the Production of Sugarcane

In this study, four concentrations were conducted in order to compare the effectiveness of GA3 on the production of sugarcane based on several criteria, including the length of internode, the fresh weight, the CCS values and productivity, as well as the net income. In each independent variable, the experiment was laid out in a single-factor factorial randomized complete block design (RCBD model) with 4 levels of GA3 (0 ppm, 50 ppm, 100 ppm and 150 ppm) and 4 repetitions; thus, two-way analysis of variance (ANOVA-2 ways) was used for analysis following the least significant difference test (LSD) at 95% confidence level. Based on the aforementioned background, the best concentration of GA3 applied to sugarcane was 150 ppm [21]. In this study, concentration of GA3 was in the range of 0–150 ppm. As shown in Table 2, statistically significant difference was observed with all the evaluation criteria. Along with the increase in GA3 concentration, the growth of sugarcane has positive impacts. Increased GA3 concentration led to a substantial increase in the length of the internode ($F = 41.77$, $p < 0.05$). According to Schaffer, GA3 plays a critical role in controlling the growth and development of plants and is well known to enhance the photosynthetic efficiency of plants, resulting in an increase in the overproduction of sucrose from the leaf [22]. As a result, the sucrose loading into internodes is used for the mitosis process, which induces the increase of cell mass, leading to the elongation of internodes [23]. In Table 2, as the concentration of GA3 increased from 50 ppm to 150 ppm, the extension of translocation to internodes induced more internodal length by 112%, 115%, and 127%, respectively, against nontreated GA3, which was similar to another result. By the external application of GA3, sugarcane in the report of Moore (1980) [6] exhibited a significant bigger size of internodes than untreated GA3. The positive effect of GA3 on sugarcane internodes was also mentioned in the study of Rama Kant Rai (2017) [23]. In addition to the intensifying internodal length, the average stem length of the treated groups was higher compared to that of nontreated groups ($F = 31.06$, $p < 0.05$). The highest stem was in the treatment with 150 ppm, about 2.79 ± 0.04 m following the reduction in length from 2.67 ± 0.08 to 2.38 ± 0.07 m when the concentration of GA3 was reduced from 150 ppm to 0 ppm. In the same pattern with the fresh weight, the biomass of sugarcane in the 150 ppm GA3-treated group was 2.25 ± 0.01 kg, which was higher than the 24.3%, 11.94%, and 4.17% in the nontreated group (0 ppm), treated group with 50 ppm and 150 ppm, respectively ($F = 456.76$, $p < 0.05$). Due to the greatest values in stem length as well as in fresh weight, 50 m^2 of the trials with treatment of 150 ppm GA3 was harvested 499.3 ± 5.07 kg that was the highest productivity, about 104%, 113%, and 120% times as the yielding in nontreated groups, 50 ppm GA3 treated, and 100 ppm GA3 treated groups, respectively. Therefore, the net income of sugarcane in field 50 m^2 with 150 ppm GA3 was the highest,

169,192.5 VND (F = 77.06, $p < 0.05$). However, there was a remarkable increase in the commercial cane sugar (CCS) value, which is the most important criterion for evaluating the quality of sugarcane in Vietnam, between nontreated groups and those with external GA3 application (F = 35.72, $p < 0.05$), whereas no significant change was observed between the treated groups. It was similar as the report of Verma [24]. In comparison to GA3 level 0 ppm, possibly, the increase in internodes in the treated groups provided more sink tissues, leading to the expansion of space for sucrose accumulation [25]; consequently, CCS (%) was higher in treated canes. However, in order to increase the sink capacity through enlargement in internode size, a part of sugars from the site of synthesis in photosynthesis was used for the metabolism process, such as cell division rather than loading in the sinks [6]. In other words, the main role of GA3 in sugarcane is only in support of the development of sink tissues rather than in the enhancement of the CCS value.

Table 2. Effect of variety dose of GA3 on the different standard performance of production of sugarcane.

	0 ppm	50 ppm	100 ppm	150 ppm	CV (%)
Internode length (mm)	$101.2^{C} \pm 0.95$	$111.9^{B} \pm 0.49$	$116.0^{B} \pm 1.84$	$122.8^{A} \pm 3.82$	2.48
Stem length (m)	$2.38^{C} \pm 0.07$	$2.60^{B} \pm 0.04$	$2.67^{B} \pm 0.08$	$2.79^{A} \pm 0.04$	2.38
Diameter (mm)	$25.75^{C} \pm 0.21$	$26.31^{BC} \pm 0.51$	$27.03^{B} \pm 0.52$	$27.86^{A} \pm 0.21$	1.74
Fresh weight (1 stem/kg)	$1.81^{D} \pm 0.01$	$2.010^{C} \pm 0.02$	$2.160^{B} \pm 0.01$	$2.250^{A} \pm 0.03$	0.88
CCS (%)	$10.43^{B} \pm 0.02$	$10.66^{A} \pm 0.05$	$10.65^{A} \pm 0.01$	$10.66^{A} \pm 0.03$	0.36
Productivity (kg)/50 m^2	$416.3^{D} \pm 11.2$	$442.8^{C} \pm 3.11$	$479.5^{B} \pm 4.77$	$499.3^{A} \pm 5.07$	1.57
Net income (VND)/50 m^2	$94,315.75^{D} \pm 11710.74$	$122,438.25^{C} \pm 3316.08$	$153,518^{B} \pm 5077.74$	$169,192.5^{A} \pm 5041.95$	5.62

^{A,B,C,D} For a particular variable, means on the same row with different superscript, within the specified class variable, are significantly ($p < 0.05$, Duncan's Multiple Range test) different. CV = coefficient of variation; ± = standard deviation ($n = 4$, the number repeated trials).

3.2. Effect of Gly on Sucrose Accumulation in Sugarcane

Although the GA3 application on sugarcane helped to improve its yield via stem elongation and diameter increment. In order to improve the effectiveness of sugarcane fields, the requisition of enhancing sucrose gradient should be considered [1,2,4]. In this study, Gly, a chemical ripening agent, was expected to increase early sucrose content, thereby increasing profitability. As shown in Figure 1a, the CCS value showed strong positive correlation with the application of Gly ($r^2 = 0.91$, F = 32.44, $p = 0.029 < 0.05$), ranging from 10.47 ± 0.03 to 12.15 ± 0.03% as Gly concentration increased. Due to the enhancement of the CCS value, the purchasing price was also increased from 1.05 ± 0.03 to 1.22 ± 0.03 million VND (Figure 1b). The function of Gly in the improvement of the sucrose content was also shown through an increase of sucrose content (1.6%) as well as recoverable sugar (1.5%) in treated sugarcane with Gly as compared to the control [26]. Gly is involved in the process of growth inhibition, causing the death of the apical bud of cane stalk and the inhibition of cell elongation; hence, the level of sucrose loading in sink tissue is improved, resulting in a higher CCS value [27]. However, average cane weight (Figure 1c) as well as internodal length (Figure 1d) decreased remarkably as the concentration of Gly increased from 0 ppm to 200 ppm. This was in total agreement with other research [28]. Gly induces the maturity of young upper internodes that enables improving the culm sucrose content compared to non-Gly treatments [29]. The culm sucrose is then directly translated to promote the ratios of sugar to fiber and finally enriches the sucrose content in cane juice [28]. Notwithstanding, the improvement in culm sucrose content is the action of Gly in stunted immaturity cane tissues and Gly treatment induces slowing of cane growth and reduces stalk weight, hence reducing millable cane yields.

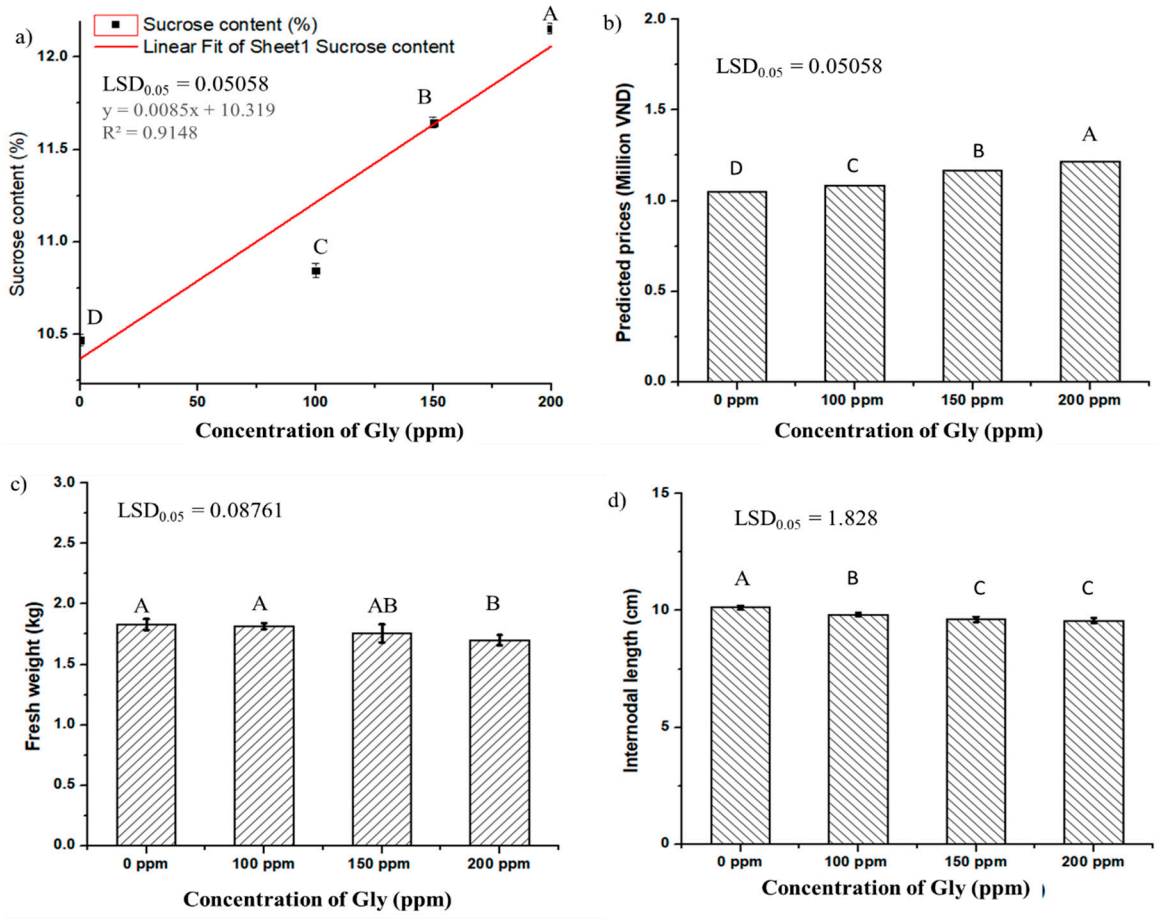


Figure 1. Effect of various Gly concentration on commercial cane sugar (CCS) value (**a**), purchase prices based on CCS value (**b**), (**c**) fresh weight for 1 sugarcane (kg) and internodal length of cane (**d**). Statistical differences between control and their corresponding treated samples were calculated by F-test at 95% level. The significant difference between variable means was presented by words A, B, C, and D following least significant difference (LSD) test, 95% confident level.

3.3. Dual Effect of GA3 and Gly on Sugarcane

As shown in Figure 1c,d, there were nonsignificant differences in fresh weight of sugarcane in Gly treatment from 0–150 ppm, and nonsignificance was detected in the length of internodes in treatment with 150 ppm Gly and 200 ppm Gly. The highest fresh weight was in the cane experiment with the Gly concentration that was under 150 ppm ($F = 4.82$, $p < 0.05$), whereas the longest internode was in the control. The internodal length reduced with the increase of Gly treatment ($F = 19.71$, $p < 0.05$). However, the purchase prices based on CCS value were highest in the pot experiment with 200 ppm Gly and reduced following reduction in the dosage of Gly application (Figure 1b). Therefore, a dosage of 150 ppm Gly was used to test the dual effect with GA3 treatment. In addition, the experiments were conducted in dry season at the Tra Vinh Province, in which the humidity level was 47–48% without rainfall. Water-absorbing CH03 agents (the product was made by IAMS, Hochiminh, Vietnam) were also used to test the effectiveness of these experiments. Marques reported that the utilized water-absorbing agents led to an increase of the number of tillers of cane, resulting in a positive increase of gross income per hectare [30]. This investigation was based on a balanced 4 (four concentrations of GA3)-by-3 (three treatments) factorial design (two-factor design) that was repeated three times under four criteria (CCS values, fresh weight, cost of production, and net income).

It was noted that there was a highly significant difference ($p < 0.05$) in body weight gain among different treatments following the increase of concentration of GA3. In the case of CCS, the sugar content in the combined treatments (Gly and GA3) showed an improvement ($F = 1066.2$, $p < 0.05$) in

most of concentrations of GA3 (F = 614.4, $p < 0.05$), except from that of 50 ppm GA3 (Figure 2a). It was totally consistent with the study in the single treatment. The major concern regarding Gly was the loss of cane yield. Surprisingly, in the combined Gly and GA3, the fresh weight of canes was similar between single GA3 treatment and combined Gly/GA3 (Figure 2b). Figure 2c indicates that although the production cost of GA3/Gly treatment was higher than that of a single GA3 application (F = 69.19, $p < 0.05$), because of the higher CCS values, the net income in GA3/Gly pot experiments (Figure 2d) was remarkably higher than that in single GA3 treatment (F = 144, $p < 0.05$). In addition, nothing of significance was assessed by analyzing CCS values in both single GA3 and GA3/CH03 (Figure 2a). Moreover, the obtained results from GA3/CH03 indicated that combination of CH03 with gibberellic acid induced an insignificant increment in cane yield as well as the quality of cane via the improvement of the sugarcane growth.

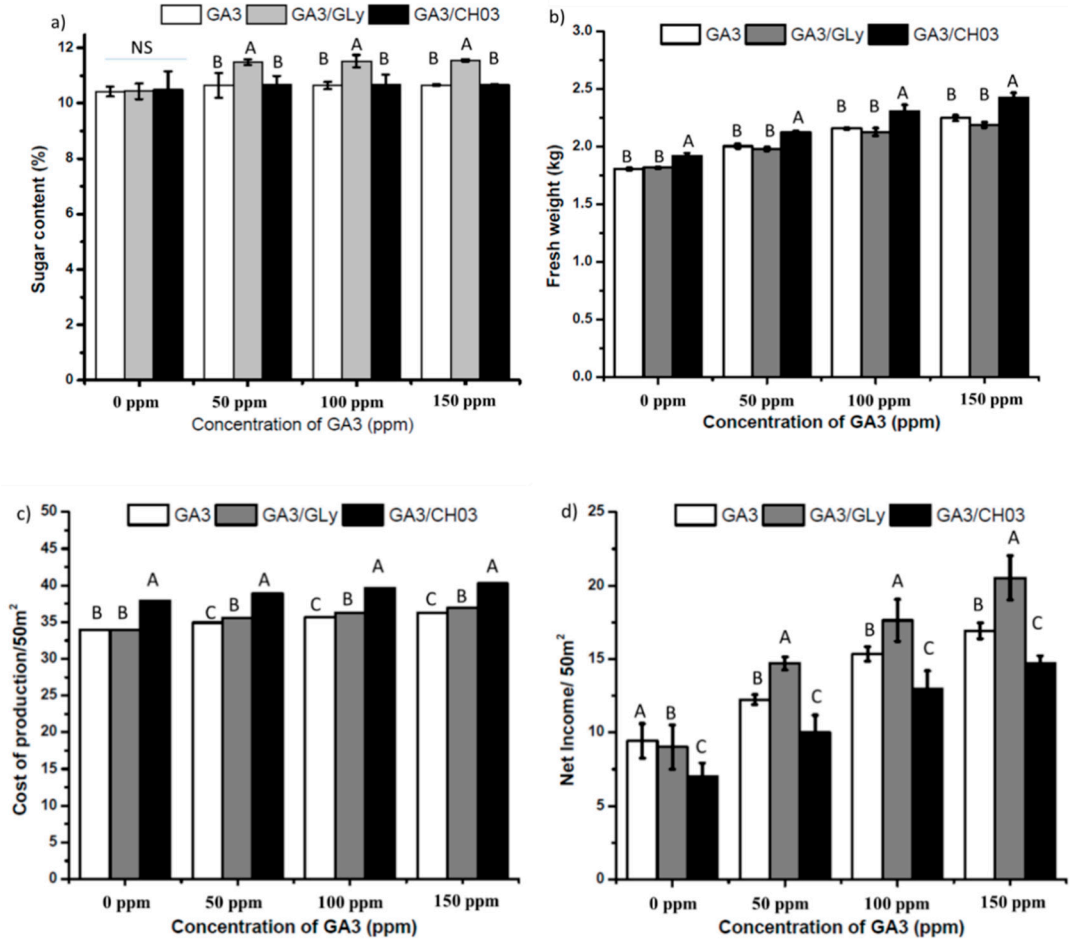


Figure 2. Effect of GA3 concentration on: setts treatment with Gly 150 ppm (GA3/Gly) and Humectants CH03 (GA3/CH03) compared to single treatment (GA3) in the aspect of sugar content (**a**), fresh weight for 1 sugarcane (kg) (**b**), cost of production/50 m^2 (**c**) and net income/50 m^2 (**d**). Errors bars indicate ± SD from four independent experiments. Statistical differences between control and their corresponding treated samples were calculated by F-test at 95% level. The significant difference between variable means was presented by words A, B, C, and D following the LSD test, 95% confident level, whereas NS means nonsignificant difference.

Furthermore, in the comparison between single-treated GLy at 150 ppm and a combination with the various concentrations of GA3, the internodal length was enhanced with the increase of GA3 concentration, from 112 ± 0.05% to 121 ± 0.018% with respect to single Gly (150 ppm); consequently, the productivity also had a positive correlation with the increase of GA3 in the combined method, presented in Figure 3. However, no significant difference ($p > 0.05$) was noted among the 4 treatments

in CCS values. Altogether, GA3 could not be involved in the improvement of accumulated sucrose loading into the sink rather than involved in enhancing the sink capacity. Thus, the crisis in sugarcane production could be solved completely by leveraging the dual GA3/Gly treatment.

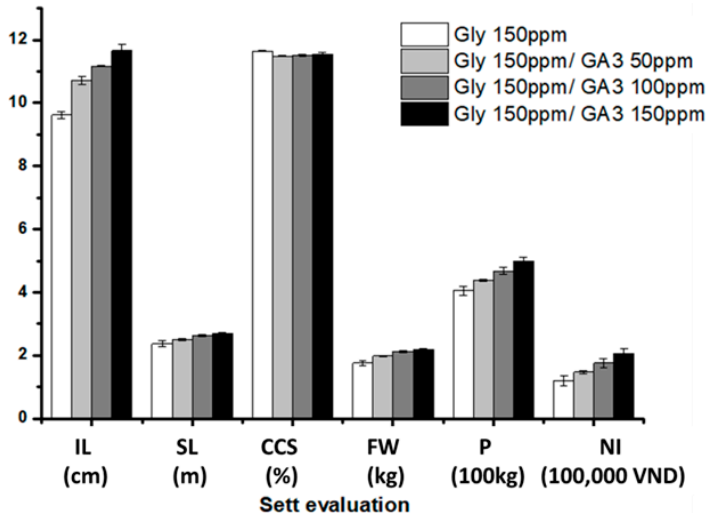


Figure 3. The effect of various treated GA3 concentration (at the treated 150 ppm Gly concentrtion) on sugarcane. Errors bars indicate ± SD from four independent experiments. IL = internodal length (cm), SL = stem length (m), CCS = commercial cane sugar (%), FW = fresh weight (kg), P = productivity (100 kg), NI = net income (100,000 VND).

3.4. Evaluation of GA3 and Gly Residues in Sugarcane

Currently, the concern about the residues of plant growth regulators in food has been going up. Various studies on animals such as mice have shown that residues of GA3 in diet derived from the consumption of plants treated with GA3 induced adverse effects [31]. Food containing GA3 affected the action of estrogen hormone in female mice [32]. Neonatal female mice produced a similar amount of hormone in adulthood; thus, the period of puberty was reduced. In order words, human beings may suffer side effects from residues of GA3 in their diets. Therefore, the application of GA3 should be controlled in order to enjoy its advantages without its harmful effects on end users. Although 254 nm is the standard in UV monitoring, GA3 was not absorbed at this wavelength, similarly with another study [33] After examination, the measurements were monitored at 206 nm. The optimization of the flow rate was 1 mL/min that could be good for GA3 under a gradient mode of elution (Table 1). In HPLC chromatogram, GA3 showed the remaining time to be 7.34 min (Figure 4a). The constructed method was sensitive to detect GA3 with lower limit of detection (LOD = 0.55 µg/mL) as well as limit of quantitation (LOQ = 1.80 µg/mL). In addition, the method was validated for linearity, precision, accuracy, LOD, and LOQ. The percentage relative standard deviation (% RSD) was lower than 2%. The calibration curve for GA3 was prepared in a range of 0–250 mg/mL that exposed the perfect best fit in line with a correlation coefficient (r^2) of 0.9998 (Figure 4b). Thus, the HPLC program can be of acceptable precision. Furthermore, in Figure 4a, the best positive correlation existed between the peak area and the concentration of GA3 within the concentration range. Through adding the standard solution methods, the percentage of recovery of GA3 was well within the range of 90–107%. These results suggested the suitability of the HPLC program for quantitative analysis of GA3. For detection residues GA3 in cane juice, the sugarcane in pot experiments was in a miller to obtain their juice. Ethyl acetate was used to extract GA3 contained in cane juice [17,18]. However, the concentration of GA3 was under the limits of detection and quantitation (Table 3); thus, it could be concluded that spraying GA3 in a range from 50 to 150 ppm might comprise a safe dosage.

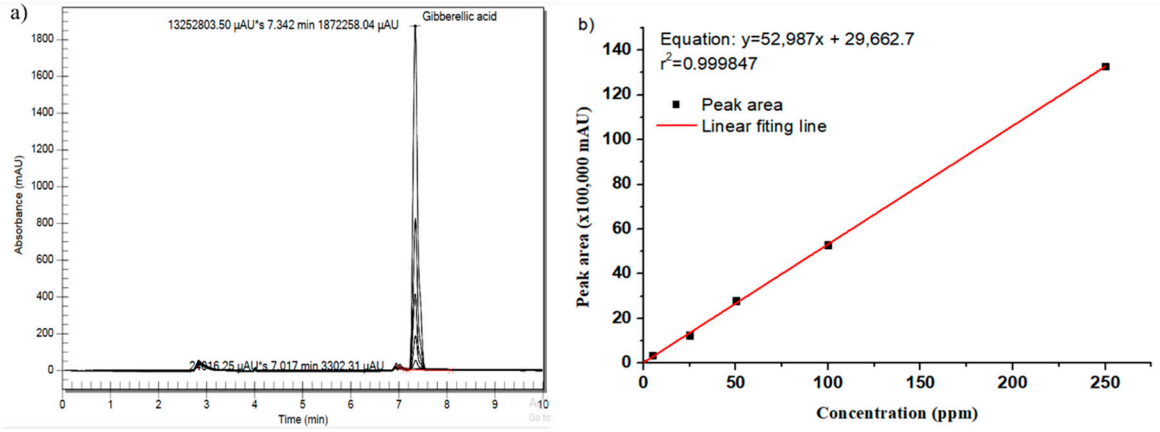


Figure 4. Absorbance of GA3 samples in different concentrations (**a**) and its calibration curve (**b**).

Currently, due to the concerns related to a probable carcinogenic effect linked to exposure, glyphosate is being forbidden in some countries. However, the function of GLy on recoverable sugar has been proven in a long history of sugarcane studies [34–36]. Although the application of Gly causes a remarkable reduction in productivity, farmers and mill workers have expressed their satisfaction regarding the economic advantages of the increment of sugar yield [37,38]. Therefore, the detection of trace amounts of Gly in cane juice after application is very important in order to determine the future of the use of this herbicide in agricultural fields. Gly has high polarity, with the added feature of forming ionic species and no chromophores groups [19]. Thus, the determination of this compound often requires additional processes that may allow quantification through chromatographic methods. It is known that there are several studies on the determination of Gly in various types of samples, such as urine and breast milk [19,39]. In this study, the development of analytical methods for Gly was based on UV-Vis measurement. The detection method of Gly was built via formation of Gly-FMOC from fluorrenilmetilloxicarbonilo chloride (FMOC–Cl) and Gly (Scheme 1). By using UV-vis, Gly had 0.22 µg/mL in LOD, whereas LOQ was 0.74 µg/mL. Furthermore, all data of standard solution in a range of 0–10 µg/mL were best fitted in the line with the correlation coefficient: r^2 = 0.9994. It was shown that the quantification of Gly could be done well with this method. Although qualification of the traces of Gly in cane fiber, cane roots, as well as cane shoot was not involved in this study, studies on the persistence of Gly in crops revealed that the accumulation of Gly in these parts of a crop was below the limit of detection [40–42]. More interestingly, application of GLy on sugar beet only found trace amounts of glyphosate residue in its shoots and roots during two weeks' treatment [43]. As shown in Table 3, all cane juice from the Gly-sprayed experiments had its residue in a range of 1.05 to 1.06, which is under the limit as per the regulations of the Ministry of Health (Gly ≤ 2 µg/mL). Thus, the dual application of 150 ppm (30 g a.e/ha) Gly and 150 ppm (30 g a.i/ha) could be the best combination for both health as well as economic concerns.

Table 3. Results of residue analysis of GA3 and Gly in sugarcane after the trials of experiment 3.

Sample	NT1		NT2		NT3		NT4		Residue
GA3 (µg/mL)	ND		ND		ND		ND		ND
Gly (µg/mL)	1.05	1.06	1.05	1.05	1.06	1.06	1.07	1.06	1.06 ± 0.01

4. Conclusions

The major problems related to sugarcane around the world are the lower productivity because of the physiobiochemical constraints and the adverse climatic conditions during the growth and ripening process. Cane yield as well as sugar content are directly impacted by many factors related to the gene of cane, quality of agriculture technology, as well as climate. Therefore, the application of plant

growth regulators becomes the greatest approach to overcoming these constraints in the short-term. In this study, at the Tra Vinh Province, the combination of GA3 and Gly resulted in the enhancement of cane yield as well as sucrose accumulation; consequently leading to positive advancements in net income in sugarcane fields ($p < 0.05$). GA3 efficacy was demonstrated in improving strength of sink used to load sugar, whereas Gly accelerated the cane maturation process, which favored the promotion of the accumulated sugar into stalks. Surprisingly, the residue of GA3 in the cane juice of all treatments was under the limits of detection, while the concentration of Gly in cane juice was under what the regulations by the Vietnam Ministry of Health determine as acceptable. These obtained results suggested that dual application (Gly and GA3) on sugarcane could be an important agrotechnology for cane growers due to its multiple benefits.

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