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Exogenous application of gibberellic acid on lateral bud emergence in pineapple propagated through stem cuttings

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Abstract

The effects of gibberellic acid (GA_3) on lateral bud emergence in pineapple stem is still not known. Hence, this study was conducted. 'MD2' pineapple stems were sectioned into vertical halves. The first half served as the control (untreated). Whereas the other halves were soaked in 500 ppm GA_3 for 2 hrs. The study was performed in two trials. Results in both trials show that untreated stem sections produced lateral buds after one week. In contrast, the GA_3 treatment prevented the development of buds. In trial 1, only 2 out of 18 stem sections (11.11%) soaked in 500 ppm of GA_3 produced buds after one week. In trial 2, GA_3-treated stem sections failed to produce buds. The average number of buds emergence per stem section and diameter of lateral buds were consistently better in untreated pineapple stem sections. These findings implied that the exogenous application of GA_3 could prevent the emergence of lateral buds in the pineapple stem.

1. Introduction

Gibberellic acid is an endogenous phytohormone that is involved in the regulation of the life cycle of plants, including seed germination, leaf expansion, stem elongation, floral induction and fruit maturation (Hedden and Kamiya, 1997). Effects of gibberellic acid at various developmental stages of the pineapple plant have been studied. Scherer *et al*. (2015) studied the effect of gibberellic acid in combination with an immersion system on the growth and acclimatization of micropropagated pineapple. Based on their results, the growth of micropropagated pineapple is better in the MS culture medium without GA_3. The effects of the levels of GA_3 in combination with other phytohormones were studied during inflorescence induction and development in 'Smooth Cayenne' pineapple by Liu *et al*. (2011). These results indicate that low levels of IAA, GA_3, and zeatin and high levels of ethylene, ABA and 2-iP facilitate inflorescence initiation. Whereas, high levels of IAA, GA_3 and zeatin and low levels of ethylene and ABA facilitate inflorescence development. Average fruit weight determines the number of fresh fruits that can be packed in a box and the number of boxes produced per unit of land area. Average fruit weight also provides information on the potential recovery of choice and other slice grades in the cannery. It has been reported that applications of gibberellin enhance the fruit size, weight and qualities in pineapple (Li *et al*., 2011; Suwandi *et al*., 2016). Exogenous application of GA_3 at 50 ppm increases the fruit weight of 'Comte de Paris' pineapple by 20.3% versus the untreated. The percentage of S phase (DNA replication) cells in treated fruits did not differ greatly from the control. The effect of GA_3 in pineapple fruit is more on cell surface enlargement rather than the increase in cell number. Further, GA_3 promotes fruit development in the transverse than in the longitudinal direction of pineapple fruit and increases vitamin C content (Li *et al*., 2011).

The effects of GA_3 on bud emergence in pineapple stem is not known at present. In other crops, dormancy can be induced through gibberellic acid treatment. For example, soaking white yam in gibberellic acid treatment (1000 ppm) for two hrs prevents the sprouting of tuberous roots (Rao and George, 1990). In another study, immersing yam tubers in a lower concentration of GA_3 solution (150 ppm) for 6 hrs after harvest controls sprouting during storage for 45 days (Nnodu and Alozie, 1992). This lower GA_3 solution is still effective and did not lose its sprout-controlling potency even after 36 hrs from the formulation. It is believed that gibberellins comprise substances that repress sprouting (Okagami and Tanno, 1988). However, the efficacy of GA_3 in controlling sprouts in potatoes seemed to be dependent on the concentration and cultivar. Lizarazo-Peña *et al*.

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(2020) reported that the dormancy of 'Diacol Capiro' potato is reduced by 18 days when soaked in 25 ppm GA_3 for 60 mins.

Macropropagation of pineapple (*Ananas comosus*) through stem sectioning is one way of accelerating planting material production. These have been an essential source of planting material in clonal propagation before the introduction of tissue culturing (The Pineapple, 2009). Macropropagation of pineapple can be done through stem splitting or stem sectioning. The stems of mature pineapple plants can be prepared for fast multiplication after the harvest of the fruit. This classical technique allows the production of 20 to 40 plantlets per stem (Soler and Dole, 2006). However, when environmental condition is not favourable for plantlet growth, bud dormancy in pineapple stem sections is of advantage. Bud dormancy is a prevention against the stresses brought by unfavourable environmental conditions. It is thought that gibberellic acid treatment will also prevent or delay the emergence and development of shoots in pineapple stem cuttings similar to the findings on yam. Thus, this study aimed to evaluate the effect of GA_3 treatment on lateral bud development in pineapple stems.

2. Materials and methods

2.1 Sample plant preparation

This study was conducted at Lantapan, Bukidnon, Philippines from October to December 2021. There were two treatments in this experiment. Treatment 1 served as the control (without GA_3) and Treatment 2 samples were treated with 500 ppm GA_3.

'MD2' pineapple plants were collected from the newly cleared first "ratoon" pineapple field. Leaves were then trimmed (Figure 1). The first ratoon (Figure 2a) was detached from the stump of the mother plant and was utilized in the experiment. Deleafing was done, leaving only the stem of the pineapple (Figure 2b). Only those stems without lateral buds were selected. Stems with lateral buds were discarded. The apical and basal (below ground part) parts of the stem were cut off. Only the middle part of the pineapple stem was used (Figure 2b).

Figure 1. Collected ratoon pineapple plants used in the experiment.

The cleaned stem was cut into vertical halves (Figure 2c). Stem sections were then washed with tap water and allowed to dry for four hrs. The first half-sections served as control samples and the other half-sections were subjected to GA_3 treatment.

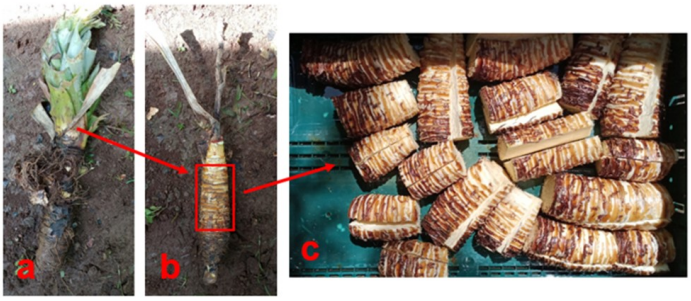

Figure 2. Preparation of pineapple stem sections. (a) ratoon was detached from the mother plants; (b) cleaned pineapple stem; (c) pineapple stems cut into vertical halves.

There were two trials conducted in this study. In the first trial, 18 stem sections were utilized for each treatment. Materials such as basin, pale, plastic containers, water, and knife were not sterilized in the first trial. In the second trial, only six stem sections were utilized for each treatment. Unlike the first trial, the materials used in the second trial were sterilized except for the pineapple stem sections. Stem sections in the second trial were also soaked in a 1000 ppm Ridomil Gold 480 SL (active ingredient: Metalaxyl-M and S-isomer) solution.

2.2 Gibberellic acid treatment and application

Berellex powder (95% GA_3) was used in this study as the source of GA_3. Berellex powder was dissolved first by mixing and stirring it in a small amount of 70% ethyl alcohol. A stock solution of 5000 ppm was then prepared by adding distilled water. Using the stock solution, 500 ppm was prepared for Treatment 2.

Pineapple stem sections were soaked for two hrs in distilled water for Treatment 1 (control) and in 500 ppm GA_3 solution (50% lower than the rate used by Rao and George (1990) in white yam) for Treatment 2 (Figure 3). After soaking, the water was drained and the stem sections were placed inside the plastic crate and allowed to dry for four hrs. The sections were then placed inside plastic containers containing a small amount of distilled water. Plastic containers with stem sections from different treatments were placed and maintained inside a room at ambient temperature and randomly arranged (Figure 4).

2.3 Data analysis

The following data were gathered: a) number of stem sections with developed lateral buds- this was gathered by counting the pineapple stem sections with lateral buds

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Figure 3. Soaking pineapple stem sections in treatments.

Figure 4. Experiment setup in Experiment 1.

(measuring at least 1 mm in diameter and already visible); b) number of lateral buds developed per stem section- this was determined using the equation: ∑ lateral buds developed/number of stem section with developed lateral buds; and c) diameter of developed lateral buds- measurement was done using a pre-calibrated measuring device. These data were gathered at weekly intervals.

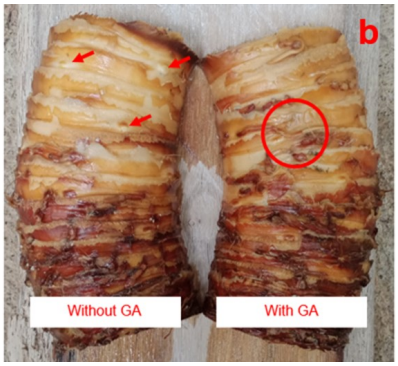
3. Results

3.1 Pineapple stem section with developed lateral buds

Results in both trials (Tables 1 and 2) show that all untreated stem sections produced buds. In contrast, the GA_3 treatment prevented the development of buds. In trial 1 (Table 1), only 2 out of 18 stem sections (11.11%) soaked in 500 ppm of GA_3 produced buds after one week. In trial 2 (Table 2), GA_3-treated stem sections failed to produce buds.

Buds were originated on the leaf scar (Figures 5a and 5b). Data on the number of buds developed for trials 1 and 2 were only taken one week after treatment (WAT). GA_3-treated pineapple stem sections began to rot after 1 WAT (Figure 5b). The fungal infection then commenced at 2 WAT (Figure 6). The fungal infection also spread to untreated stem sections. The GA_3-treated stem sections were completely dried up and shrank at 4 WAT (Figure 7).

(a) Trial 1: Bud emerged in the pineapple stem section without GA_3 treatment after one week



(a) Trial 2: emerged bud (pointed by red arrow) in the pineapple stem section without GA_3 treatment after one week (left) and the pineapple stem section started to rot (encircled) in 500 ppm GA_3 treatment (right).

Figure 5. Pineapple stem sections one week after treatment application.

Figure 6. Pineapple stem sections infected by pathogen; note the severity of mycelial infection in GA_3-treated pineapple stem sections.

Table 1. Stem section with developed buds, number of buds developed per stem section and diameter of buds observed in the first trial at 1 WAT.

Treatment	Number of stem section with developed buds	Average number of buds developed per stem section	Average diameter of buds (cm)
Without GA	18/18 = 100%	2.17	0.70
With GA (500 ppm)	2/18 = 11.11%	1.00	0.20

Table 2. Stem section with developed buds, number of buds developed per stem section and diameter of buds observed in the second trial at 1 WAT

Treatment	Number of stem section with developed buds	Average number of buds developed per stem section	Average diameter of buds (cm)
Without GA	6/6 = 100%	2.50	0.85
With GA (500 ppm)	0/6 = 0%	0.00	--

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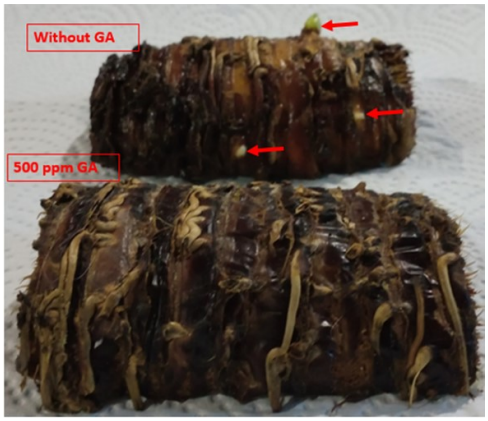

Figure 7. Shoots emerged in the untreated (control) pineapple stem section (upper photo), whereas GA_3-treated stem section shrunk and dried up (lower photo) at 4 WAT.

3.2 Number of buds developed per stem section

In the first trial (Table 1), the control treatment resulted in the production of 2.17 buds per stem section compared to the GA_3 treatment which resulted in an average of only 1 developed bud per stem section. In trial 2 (Table 2), control treatment resulted in the production of 2.50 buds per stem section whereas stem sections subjected to GA_3 treatment failed to produce buds.

3.3 Diameter of developed buds

The diameter of developed buds in the first trial (Table 1) was wider in untreated stem sections (0.7 cm) compared to 0.2 cm in GA_3-treated stem sections. In trial 2, the diameter of developed buds in untreated stem sections was 0.85 cm (Table 2).

4. Discussion

The stem is the storage reservoir of the pineapple plant. Pineapple plant stems has high starch content (77.78%). The lignocellulosic composition of the pineapple plant stem consists of 46.15% hemicellulose, 31.86% cellulose, and 18.60% lignin (Chu *et al*., 2021). The starch reserve of pineapple stems is needed for the development of lateral buds. Lateral buds will then develop into ratoon suckers. These have been an essential source of planting material in clonal propagation before the introduction of tissue culturing (The Pineapple, 2009). The extent to which suckers develop during the reproductive phase is highly dependent on the presence of starch reserves in the stem (Hepton, 2003).

Tables 1 and 2 imply that GA_3 treatment prevents the formation of lateral buds in 'MD2' pineapple stem. These findings are comparable to the results of studies conducted on potato and yam of which gibberellic acid treatment suppressed shoots from sprouting. The treatment of potato tubers with 150 µmol dm^{-3} gibberellic acid stimulates starch breakdown and hexose accumulation in tuber tissues (Davies and Viola, 1988). Soaking white yam in gibberellic acid treatment (1000 ppm) for 2 hrs prevents the sprouting of tuberous roots (Rao and George, 1990). In another study, immersing yam tubers in a lower concentration of GA_3 solution (150 ppm) for 6 hrs after harvest controls sprouting during storage for 45 days (Nnodu and Alozie, 1992). This low GA_3 solution is still effective and did not lose its sprout-controlling potency even after 36 hrs from the formulation. It is believed that gibberellins comprise substances that repress sprouting (Okagami and Tanno, 1988).

In this study, soaking pineapple stem sections in 500 ppm GA_3 for 2 hrs not only prevent lateral bud development. It causes also the stem section to rot after one week after treatment. Subsequently, fungal infection occurs. The oomycete *Phytophthora palmivora* is the causal pathogen of pineapple "heart rot" disease. Hence, in the first trial, it was thought that *P. palmivora* initiated the rotting. But, during the second trial, the signs of the disease were still observed in the pineapple stem. The fungal infection might just be a secondary effect. Zhang *et al*. (2016) reported that gibberellin upregulates the PPO enzyme which led to pineapple fruit flesh browning. In this study, it is hypothesized that primarily, polyphenol oxidase (PPO) enzyme must have been activated through GA_3 treatment which caused the stem to rot. Based on visual observation (not supported by figure), browning of the stele portion of pineapple stem was evident prior to rotting incidence. Otherwise, the moisture inside containers where pineapple stem sections were placed could be too high. Soler and Dole (2006) mentioned that too much moisture could lead to the rotting of pineapple stem sections.

5. Conclusion

Lateral bud formation in pineapple stems is prevented in one week after treatment with GA_3. In contrast, the development of lateral buds can be observed one week after treatment in untreated (control) pineapple stem sections. Whether GA_3 can suppress or only delay lateral bud emergence in pineapple stems is still unknown since the pineapple stem section began to rot after one week. In order to determine the duration of the efficacy of GA_3 in preventing lateral bud formation, a follow-up study using varying levels of GA_3 is recommended.

Conflict of interest

The author declares no conflict of interest.

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