FRUIT MORPHOLOGICAL RESPONSE OF F200 PINEAPPLE *Ananas comosus* (L.) merr AS INFLUENCED BY GIBBERELLIC ACID APPLICATION AND CALCIUM SUPPLEMENTS

Abstract.

The study investigated the influence of different levels of Gibberellic Acid (GA_3) applied alone or in combination with Calcium from field production and fruit growth development of F200 pineapple. Pineapple plants applied with GA_3 alone at any level from 100 to 300ppm and calcium by 10ml to 20ml/li delayed the days of maturity at 13 days and 2 days, respectively. Application of GA_3 increased fruit edible portion, number of fruitlets and width of center fruitlet (mm), fruit weight, and crown length at harvestable age. Fruits of pineapple plants sprayed with GA_3 at 300ppm were heavier than fruits of pineapple plants sprayed at 100ppm of GA_3 to about 2.54 kg and 1.52 kg with and without crown, respectively. The pineapple's crown length improved by combining the application of GA_3 at 300 ppm and calcium at 20ml/li.

Keywords: *Ananas comosus*, Gibberellic acid, Calcium, Fruit Morphology

Introduction

Pineapple (*Ananas comosus L.)*Merr belongs to Crassulacean Acid Metabolism (CAM) plants on the characteristic of carbon fixation, which features in the closing of stomata during the daytime and opens to fix CO2 at night to form malic acid with low pH, which disappears during the daytime. It has a larger vacuole on cells with deeper stomates and denser guard cells. CAM plants are drought-resistant plants with low CO2 compensation points that have a low light saturation point and lower photorespiration than compared with C3 plants (Domingo, 2016).

Increasing fruit size and quality improvement are basic factors in increasing yield and boxes produced per hectare in pineapple production. The fertilization program and nutritional status of the pineapple plant have a large influence on plant growth and, consequently, on yield and fruit quality (Malezieux and Bartholomew, 2003). To sustain growth and obtain good yields, it is important to provide adequate supplies of all nutrients in proper balance. But aside from fertilization, other means like the application of hormones to increase fruit growth and weight are important in production.

According to Vargas and Villalobos (2014), gibberellic acid 3 (GA_3) is well known for its ability to increase cell elongation and growth and could be used as an alternative for farmers to increase fruit size. In addition, effects on the expansion of the window or delaying crop maturation have been observed when the GA_3 is used. Increased harvest window or delaying the maturity could be used by producers for concentrating fruit volume in some periods where prices are higher or lower volumes in periods with excessive volume of fruit occurring as flowering Natural or NDF. They further stated that the development of the fruit takes about 21 to 25 weeks after flowering, with the optimum effect of GA_3 on the development of fruit observed especially when the application is made between 14 and 16 weeks after flowering (WAF) or between dry flower fruit immature. Multiple physiological processes can cause this behavior, but the size of the fruit and therefore movement of solutes and carbohydrates is produced during these particular stages of development of the fruit. Furthermore, the perception by the plant GA_3 could be more active during this developmental period. When plants are applied with GA_3, the cells and tissues increase and elongate, there is a possibility that produces bigger size in fruits but lower in weight and occurring of weakening in some tissues.

GA_3 application has been proven in some research findings of several crops cited by Li, et al., (2010) such as larger-sized fruit and improved fruit quality (Jackson 2003; Sharma and Singh 2009), maintaining cell expansion (Davis 2004; De Jong et al. 2009; Ozga and Dennis 2003), to enhance fruit growth in a wide variety of species such as Japanese pear (Zhang et al. 2005, 2007), litchi (Chang and Lin 2006) and grape (Casanova et al. 2009), improve the grape size and quality (Harrell & Williams, 1987; Dimovska et al., 2014;

Nampila et al., 2010; Kapłan et al., 2017); to obtain fruits of better quality (Gonzalez-Rossia et al. 2006; Sharma and Singh 2009), in blueberry (Vaccinium ashei) and some other fruits (Cano-Medrano and Darnell 1997).

Calcium being one of the elements with the key role in several plant structures like cell wall stability and plant structural strength can help the fruits to strengthen and avoid some physiological problems that affect the quality of fruits during the postharvest stage. Because of its role in binding cell wall substances (particularly pectin chains) and maintaining cell wall integrity in fruits, pre-harvest calcium nutrition has received considerable grower, and researcher, attention. Liquid calcium fertilizers and gypsum improve the firmness and storability of some fruits. The response to additional calcium is understandably more evident in acid soils as cited by Midmore, (2015).

The application of GA_3 and calcium together increased the fruit size, delayed fruit maturity, and strengthened the fruit tissue in resistance to bruises and quality problems in postharvest. There is the possibility that when fruits are applied with GA_3, they become weakened and more susceptible to bruises and quality deterioration. Calcium being the primary function is to provide more stronger cell wall and stronger tissues to become more resistant to any quality deterioration and improve the postharvest life. The objectives were to determine the influence of different levels of GA_3 applied alone or in combination with calcium from field production to differentiation, fruit growth and development of F200 pineapple.

Materials and Methods

A field experiment from production to harvest was conducted in Brgy. Kablon, Tupi, South Cotabato Philippines. The site is slightly slope and within the highland agro environment located at the foot of Mt. Matutum with a topographical elevation of 870 above sea level. An established F200 variety of pineapple plots at maturity age ready for forcing at twelve (12) months from planting was used in this experiment. The 35 plants per treatment of about 5.33 square meters plot were used with a planting distance of 9 inches between hills and 18 inches between lines. The Split-Plot Design was used in this experiment with four replications. The GA_3 levels serve as the main plot while levels of calcium as the subplot and the treatments are as follows: The main plot is the GA_3 levels of 0ppm, 100ppm, 250, and 300ppm. The levels of calcium are 0 calcium, 5 ml/li, 10 ml/li, and 20 ml/li.

The Ryzup 40 SG granule formulation of gibberellic acid GA_3 40% (active ingredient) or 400g of GA_3 per 1000kg was used as a GA_3 source with a surfactant sticker incorporated into the product. GA_3 solution was prepared by weighing the Ryzup 40 SG to get the desired ppm by measuring the (gm) equivalent per liter of water in a solution per treatment based on the 40% active ingredient of the product. The amount of solution prepared was computed based on the 2400 liters of solution per hectare application rate in the commercial plantation. The rate of solution per treatment of 30 plants was 1.28 liters based on 75,000 hills per hectare population standard.

The Liquid Calcium Chelate (12% Ca) was used as a calcium source and applied through foliar application based on the treatment rates starting at 4 weeks after forcing (WAF) and the second application at 8 weeks after, and the application time was the same with GA_3. There was a 4-week interval on the first 2 applications and the last application was done at 4 weeks after the last GA_3 application. The passes of spraying and facing factor during spraying were also calibrated first same with the GA_3 procedure.

Days to Fruit Maturity. The fruit maturity with the period (in days) between the date of flowering of the plant and the day of maximum fruit harvest where the majority of fruits in treatment is at the color index of 4 to 5 (see figure 1) was gathered every week.

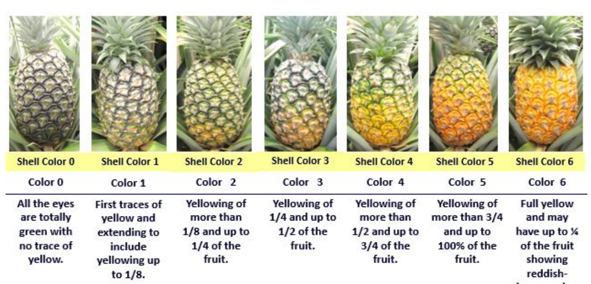

Figure 1. Shell color chart to determine the color index in F200 pineapple (Dull, 1971 Gortner et al., 1967).

Histological Observation. The histological observation started at 4 weeks to 8 weeks after GA3 application. This was performed by cutting the sample fruits longitudinally to measure the flesh portion size in the center of the cross-section of the fruit on both opposite sides. The center fruitlet of the fruit was also measured using the vernier caliper on both sides of the sliced fruit.

Crown Length (cm). The length of the crown was measured individually from 10 sample fruits harvested using the tape measure from the base up to the longest tip of the crown leaf.

Crown Weight (gram). The weight of the crown was also measured individually from 10 sample fruits harvested using the weighing scale.

Weight of 10 Sample Fruits (kg). The weight of 10 sample fruits was also obtained at the time of harvest. Sample fruits were taken in the middle row of the experimental plot.

Physiological Observation. The Normalized Difference Vegetation IndexNDVI was obtained at different stages during flowering and harvesting by the use of a handheld NDVI reader (Greenseeker Trimble, USA)

Fruit Calcium and Potassium Analysis (ppm). The fruit calcium and potassium levels of the fruit were analyzed by collecting the two (2) fruit samples placed in a plastic bag and brought to the laboratory for tissue analysis. The center flesh portion of the fruit was sliced and blended. Blended fruit flesh and juice were used for the analysis through microwave digestion and inductively coupled plasma-optical emission spectrometry (ICP-OES) method.

Statistical Analysis. Data were subjected to analyses of variance using Tukey's analysis.

Results and Discussion

Horticultural Traits

Days to Fruit Maturity. The number of days for pineapple fruit to mature in the field is presented in Table 1. The application of gibberellic acid and calcium at any level through foliar application significantly influenced the maturity of pineapple fruit. As observed, pineapple plants treated with GA_3 alone at any level delayed the fruit's maturation at around 13 days compared to untreated plants. Plants applied with GA_3 at any level in combination with different levels of calcium matured at 229-231 days. On calcium application alone without GA_3 combination, as calcium level increased by 10ml per liter and 20ml/li, maturity delays for about 1 to 3 days. Some studies on GA_3 reported its effect on fruit maturation as mentioned earlier by Suwandai, et al (2016) that the Gibberellin alone delayed the fruit maturity by 5 days of smooth Cayenne pineapple.

Weekly Fruit maturity Spread. Fruits were observed and found to have different maturity spreads (Table 1 & Figure 3). On the effect of GA alone, a significant result was observed from 29 to 32 weeks after forcing. Untreated plants were found to mature early starting at 28 weeks after forcing. As observed further, the percentage of fruits that mature significantly differed among levels of GA_3. As evident in the number of fruits matured in the weekly spread result, as the rate of GA_3 decreases, fruit maturity development hastens.

Table 1. Days to maturity and weekly percent (%) maturity spread of F200 pineapple fruit applied with varying levels of GA_3 and calcium.

GA_3 level (ppm)	Days to Maturity	% Fruit Maturity Spread at Weeks after Forcing					
		28 WAF	29 WAF	30 WAF	31 WAF	32 WAF	33 WAF
GA_3 *(untreated)*	218.31	0.63	18.75^a	52.71^a	74.58^a	88.75^a	97.20
GA_3 *(100 ppm)*	231.00	0.00	3.54^b	15.42^b	37.5^b	65.42^b	94.83
GA_3 *(250 ppm)*	230.56	0.21	2.29^b	12.92^b	26.46^b	61.25^b	95.26
GA_3 *(300 ppm)*	231.00	0.00	0.21^b	5.00^b	18.13^c	41.04^c	94.40
Calcium level (ml/li) sub plot							
Calcium *(untreated)*	227.50	0.63	3.54	15.21	42.71	57.92	95.04
Calcium *(5ml/li)*	227.50	0.00	7.71	25.63	37.92	64.79	96.34
Calcium *(10ml/li)*	227.50	0.21	7.08	22.29	36.88	69.38	95.26
Calcium *(20ml/li)*	228.38	0.00	6.46	22.92	39.17	64.38	95.04
GA_3 with Calcium Interaction							
GA_3 *(untreated)* + Calcium *(untreated)*	217.00	1.67	10.83	31.67	75.53^a	88.33	98.28
GA_3 *(untreated)* + Calcium *(5ml/li)*	217.00	0.00	22.50	65.83	75.00^a	88.33	96.55
GA_3 *(untreated)* + Calcium *(10ml/li)*	218.75	0.83	23.33	60.00	79.17^a	93.33	98.28
GA_3 *(untreated)* + Calcium *(20ml/li)*	220.50	0.00	18.33	53.33	68.33^a	85.00	95.69
GA_3 *(100 ppm)* + Calcium *(untreated)*	231.00	0.00	3.33	12.50	53.33^b	61.67	91.38
GA_3 *(100 ppm)* + Calcium *(5ml/li)*	231.00	0.00	5.00	20.00	38.33^{cd}	69.17	95.69
GA_3 *(100 ppm)* + Calcium *(10ml/li)*	231.00	0.00	4.17	16.67	29.17^{cde}	67.50	96.55
GA_3 *(100 ppm)* + Calcium *(20ml/li)*	231.00	0.00	1.67	12.50	29.17^{cde}	63.33	95.69
GA_3 *(250 ppm)* + Calcium *(untreated)*	231.00	0.83	0.00	11.67	24.17^{de}	45.00	93.97
GA_3 *(250 ppm)* + Calcium *(5ml/li)*	231.00	0.00	2.50	12.50	22.50^e	60.00	96.55
GA_3 *(250 ppm)* + Calcium *(10ml/li)*	229.25	0.00	0.83	7.50	20.00^e	74.17	93.10
GA_3 *(250 ppm)* + Calcium *(20ml/li)*	231.00	0.00	5.83	20.00	39.17^{bc}	65.83	97.41
GA_3 *(300 ppm)* + Calcium *(untreated)*	231.00	0.00	0.00	5.00	17.50^e	36.67	96.55
GA_3 *(300 ppm)* + Calcium *(5ml/li)*	231.00	0.00	0.83	4.17	15.83^e	41.67	96.55
GA_3 *(300 ppm)* + Calcium *(10ml/li)*	231.00	0.00	0.00	5.00	19.17^e	42.50	93.10
GA_3 *(300 ppm)* + Calcium *(20ml/li)*	231.00	0.00	0.00	5.83	20.00^e	43.33	91.38
CV (%) *main plot*	2.6	565.8	113.4	83.3	28.2	33.2	3
CV (%) *interaction*	1.4	432.2	77.1	60.2	25.8	17.9	4.1

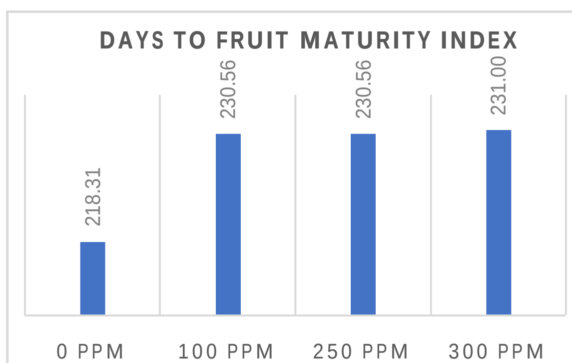

Figure 2. Days to fruit maturity index of pineapple fruit in the field applied with ppm levels of GA_3.

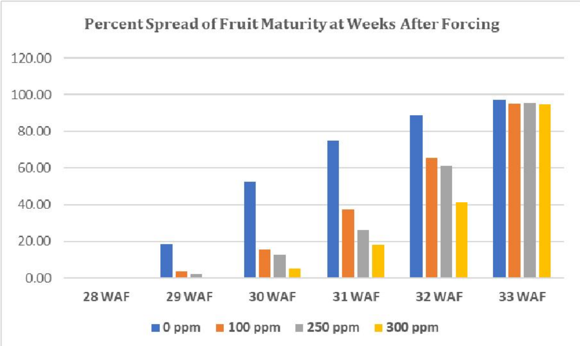

Figure 3. Percent spread of fruit maturity index of pineapple fruit in the field applied with ppm levels of GA_3.

Statistically no significant effect on calcium application on pineapple from 28 to 33 weeks after forcing. On the interaction effect of GA_3 and calcium, a significant difference was observed only 31 weeks after forcing. Calcium application alone or even without calcium and GA3 application was found to have the higher percentage of fruit to mature within 68.33 to 79.17%. It was also observed that the GA_3 application was the influence in delaying maturity with calcium combination, it was found that the higher rate of GA_3 from 250 to 300ppm with calcium was found to have a lower percentage of fruit to mature between 15 to 39% comparable to GA_3 application at 100ppm with calcium at 10 to 20ml/li. However, the untreated application of calcium with the combination of GA_3 at 100ppm was found a little higher percentage of mature compared to GA_3 250ppm with Calcium at 20ml/li.

Limited reports in pineapple that GA_3 delayed the field maturity of fruits but a similar study has been tested in banana postharvest treatment that Cavendish banana applied with GA_3 increased the green life and delayed the ripening (Vargas and Lopez, 2011). In citrus fruits, gibberellins delay senescence, allowing the fruits to be left on the tree longer to extend the market period (Taiz and Zeiger, 2004).

Histological Observation

Fruit Core Diameter. Although the core diameter in pineapple fruit has not contributed much to the edible portion of the fruits, it contributed to the weight of the fruit, where the total fruit weight was influenced by the size of the flesh edible portion or even the core diameter. Aside from the weight of the whole fruit, the core of the pineapple fruit is also a source of juice, especially for those varieties intended for juice as a product.

Table 2 presents the fruit core diameter of F200 pineapple fruit at harvestable age as affected by GA_3 and calcium application. The statistical analysis revealed that no significant differences among plants applied with GA_3 levels and calcium supplements.

However, on the effect of GA_3 with calcium application, the GA_3, even with calcium, still influenced the core diameter size at 22 to 23 mm in harvestable age compared with other rates, including the untreated one, in which the core diameter size was 21 to 23 mm. The untreated GA_3 and 300 ppm rate with calcium at 20 ml was the highest core diameter size response.

The result of GA_3 application on increasing fruit weight may result from the increase of either flesh weight or core weight. In order to determine which of those areas the GA_3 affects, the cores of the fruit were also weighed. This measurement showed that the core weight increased to a lesser extent compared to the increase of the whole fruit weight (Li et al., 2011).

Fruit Edible Portion Diameter (Left and Right side of the fruit). The edible portion diameter of F200 pineapple is presented in Table 2. The edible portion of the fruit of the pineapple at harvestable age was wider as applied with GA_3 at different levels compared to untreated fruits. On the other hand, on the effect of calcium application alone and a combination of calcium and GA_3 application, comparable results were observed during the weeks of observation and at the harvestable stage.

Among the growth regulators, gibberellins performed each primary role in stimulating cell division, resulting in cell enlargement (Nickell, 1982). The significant effect of applying GA_3 in pineapple fruit, resulting in the enlargement of the left and right fruit edible portions, concludes its primary function of enhancing more cell enlargement of fruit.

Gibberellin increases both cell elongation and cell division, as evidenced by increases in cell length and cell number in response to applications of gibberellin. The present study reports the effects of different concentrations of GA_3 on the flesh weight and qualities of pineapple fruit. All the tested concentrations, except 5 mg l-1 of GA_3, significantly increased fruit flesh weight (Yun-He Li., et al., 2011). The fact that GA3 can increase fruit weight has also been reported in Japanese plums (Gonzalez-Rossia et al. 2006) and sweet cherries (Lenahan et al. 2006).

As concluded in the previous study review by Li et al. (2011), the three highest concentrations of GA_3 significantly increased fruit weight, which increased the flesh weight by 20.3% compared with the control. The increase in fruit volume and flesh weight under this GA_3 treatment was not due to the increase in cell number but a result of the increase of cell area in the fruit flesh. The result of increasing the side fruit edible portion of diameter in F200 pineapple fruit was closely related to the increase of cell area in the fruit flesh, resulting in the increase in fruit tissues.

Number of Fruitlets on Fruit. A greater number of fruitlets on fruit was observed on plants applied with the highest rate of GA_3 than on plants that had no GA_3 application (Table 2). Plants applied with GA_3 at 300 ppm were found to have the highest number of fruitlets on fruit, which is comparable with 250 ppm compared with the control untreated one at the harvestable stage of the fruits. No significant differences in calcium application were found, but it was found that calcium at the highest rate of application, 20 ml/l, was numerically observed with a greater number of fruitlets, both from 4 weeks and 8 weeks until the harvestable age.

Statistical results revealed also that there was no significant interaction between GA_3 and calcium application. However, at harvestable age, it was observed that the untreated GA_3 with untreated calcium and with 5 to 10 ml/li of calcium were found to have a lesser number of fruitlets on fruit. The highest number of fruitlets was observed on those plants applied with calcium at 20 ml/li and comparable to all treatment combinations with GA_3 and calcium.

Pineapple fruit is a collection of many individual fruitlets fused with one another and to the core (Smith and Harris 1995). In the number of fruitlets on fruit, the increase in the number of fruitlets obviously increases the fruit size morphology as a whole. This result on the effect of GA_3 application on the number of fruitlets was similar to the result of the study conducted by Yun-He Li et al. (2011).

Width of Center Fruitlet. The effect of the application of GA3 on the size of the center fruitlet was noticed at the time of harvest. All GA_3-treated plants had wider fruitlets than those untreated with GA_3.

Statistically, there is no significant difference in calcium application and calcium with GA_3 combination on the width of center fruitlets in fruits of pineapple from 4 weeks, 8, and at harvestable age. This implied that the calcium application does not influence the growth and enlargement of pineapple fruitlets since the primary role of calcium is only to strengthen the cell wall and tissues in plants.

This result on the effect of GA_3 on the increase or enlargement of pineapple fruitlet width concluded its role in increasing the cell volume on fruit cell elongation and expansion. It is known that GA plays a key role in cell expansion (Matsuo et al., 2012). Gibberellin may not increase the number of cells but increases the cell volume of pineapple fruit (Yun-He Li et al., 2011).

Table 2. Histological observation data at harvestable age of F200 pineapple fruit as applied with varying levels of GA_3 and calcium.

Treatments	Fruit core diameter (mm)	Left Side Edible Portion (mm)	Right Side Edible Portion (mm)	Number of Fruitlets	Width of Center Fruitlet (mm)
GA3 level (ppm) main plot					
GA3 (0 ppm)	22.63	39.71b	39.28b	5.83c	24.63b
GA3 (100 ppm)	21.79	42.97a	41.33b	6.2b	26.09a
GA3 (150 ppm)	22.17	42.48a	42.33b	6.34ab	26.23a
GA3 (300 ppm)	23.09	42.99a	43.3ab	6.53a	26.24a
Calcium level (ml/Li) sub plot					
Ca (untreated)	22.46	41.96	41.79	6.24	25.77
Ca (5 ml/li)	22.45	41.27	40.24	6.13	25.53
Ca (10 ml/li)	22.21	42.65	42.09	6.20	26.12
Ca (20 ml/li)	22.55	42.27	42.13	6.34	25.78
GA3 with Calcium Interaction					
GA3 (0 ppm) + Ca (untreated)	22.32	39.71	39.80	5.63	25.25
GA3 (0 ppm) + Ca (5 ml/li)	21.47	39.59	38.14	5.79	24.47
GA3 (0 ppm) + Ca (10 ml/li)	22.95	39.75	39.33	5.79	24.18
GA3 (0 ppm) + Ca (20 ml/li)	23.77	39.78	39.83	6.13	24.62
GA3 (100 ppm) + Ca (untreated)	21.41	42.95	41.23	6.33	25.81
GA3 (100 ppm) + Ca (5 ml/li)	21.8	41.95	39.10	6	26.11
GA3 (100 ppm) + Ca (10 ml/li)	22.58	43.08	42.73	6.25	26.63
GA3 (100 ppm) + Ca (20 ml/li)	21.38	43.89	42.28	6.21	25.83
GA3 (250 ppm) + Ca (untreated)	23.18	42.50	41.77	6.42	26.24
GA3 (250 ppm) + Ca (5 ml/li)	22.63	41.88	41.49	6.13	26.14
GA3 (250 ppm) + Ca (10 ml/li)	21.11	43.83	42.65	6.17	26.71
GA3 (250 ppm) + Ca (20 ml/li)	21.75	41.73	43.43	6.67	25.84
GA3 (300 ppm) + Ca (untreated)	22.92	42.68	44.34	6.58	25.78
GA3 (300 ppm) + Ca (5 ml/li)	23.91	41.66	42.22	6.58	25.38
GA3 (300 ppm) + Ca (10 ml/li)	22.21	43.94	43.64	6.58	26.96
GA3 (300 ppm) + Ca (20 ml/li)	23.32	43.68	42.99	6.38	26.83
CV = (%) main plot	*8.8*	6	7.4	6.2	3.6
CV = (%) interaction	*7.7*	5.5	5.3	7.3	3.2

Fruit Weight of 10 Sample Fruits. The fruit weight of pineapple fruit is presented in Table 3. Application of GA_3 significantly increased the weight of pineapple fruit (with and without crown), particularly at a higher rate. Fruits of pineapple plants sprayed with GA_3 at 300 ppm were heavier than fruits of pineapple plants sprayed at 100 ppm of GA_3, to about 2.54 kg and 1.52 kg with and without a crown, respectively. Fruits of pineapple plants without GA_3 application revealed the lowest weight among treatments. On the other hand, calcium treatment alone did not improve the fruit weight of pineapple. On the interaction effect between GA_3 and calcium, no significant difference was observed among treatment combinations.

The significant results of increasing fruit morphology of F200 pineapple fruits, such as left and right fruit edible portion diameter, number of side eye on fruit, and width of center side eye on fruit applied with GA_3 at different rates in the previous data, were found correlated with the weight of fruits, both with crown and without crown. This result confirmed the report of Suwandi et al. (2016) that gibberellin alone at 100 to 200 ppm can increase the fruit weight of pineapple. Li et al. (2011) also reported that the GA_3 significantly increased the fruit weight, which increased the flesh weight.

Gibberellin (GA) plays a role in increasing the source-sink relation or sink strength by activating enzymes involved in sugar metabolism. The partitioning of assimilates from source leaves is a key factor for fruit development, as any limitation of assimilating supply affects the final fruit size. Gibberellin is a PGR commonly used to increase fruit weight in various types of plants (Suwandi et al., 2016). A similar result was also reported by Gelmesa et al. (2010) in tomatoes, in which a significant increase in fruit size and weight due to 2,4-D and increased fruit number due to GA_3 spray contributed to increased fruit yield.

Crown Length. The data on the crown length of F200 pineapple at harvestable age was also presented in Table 3. Statistical analysis revealed that there were no significant differences between treatment applications of GA_3 and calcium levels on the crown length of pineapple at harvestable age. However, it was observed that GA_3 and calcium application on fruit have a trending influence on crown length development in height. As the rate of GA_3 and calcium increased, the longer crown length was observed compared with the untreated one.

The interaction effect between GA_3 and calcium application shows a significant result on crown length in pineapple. In general observation, the GA_3 applied fruit in pineapple with calcium combination at any rate was found with a longer crown compared with the untreated GA_3 in combination with calcium application at any level. The highest rate of GA_3 at 300ppm and calcium 20mk/li combination had the longest crown length observed comparable to GA_3 300ppm with calcium at 5ml/li which is significantly comparable to GA_3 at 250 and 100ppm with any level of calcium combination compared with the untreated GA_3 with any level of calcium application.

This significant result also confirmed the report of Suwandi et al. (2016) that the gibberellin can increase the crown length of pineapple with a positive effect on the quality of the pineapple planting materials. GA induces intensive stem growth in many rosette plants and dwarf mutants, stem elongation followed by increased cell division in the sub-apical meristem. GA first enhances cell division via an increase in cell wall elasticity by a mechanism that is unknown and different than auxin. When cells are large enough, they transition from the G1 to the S phase of the cell cycle (Sun, 2004). While calcium is an essential element, it was also involved in growth. As mentioned in the previous literature review, the cell growth or extension and division require a general degradation of the Ca-pectate cell wall material, a growth process triggered by auxin, which mediates the acidification of the cell wall region (Cleland et al. 1990). Calcium plays a central role in the formation of new cells that sustain new growth.

Table 3. Weight of ten (10) fruits with and without crown and crown length of F200 pineapple fruitafter application with varying levels of GA3 and calcium.

GA3 level (ppm) main plot	measurement of fruit appearance		
	weight of ten (10) fruits with crown (kg)	weight of ten (10) fruits without crown (kg)	Crown length (cm)
GA_3 *(untreated)*	11.14^{c}	9.77^{c}	21.34
GA_3 *(100 ppm)*	12.42^{b}	10.48^{bc}	24.53
GA_3 *(250 ppm)*	13.47^{ab}	11.24^{ab}	25.25
GA_3 *(300 ppm)*	13.68^{a}	11.29^{a}	26.25
Calcium level (ml/li) sub plot			
Calcium *(untreated)*	12.38	10.50	23.81
Calcium *(5ml/li)*	12.61	10.54	24.75
Calcium *(10ml/li)*	13.27	11.23	24.22
Calcium *(20ml/li)*	12.46	10.51	24.59
GA3 with Calcium Interaction			
GA_3 *(untreated)* + Calcium *(untreated)*	11.67	9.92	23.5^{cde}
GA_3 *(untreated)* + Calcium *(5ml/li)*	10.61	9.31	20.88^{ef}
GA_3 *(untreated)* + Calcium *(10ml/li)*	11.98	10.66	20.75^{ef}
GA_3 *(untreated)* + Calcium *(20ml/li)*	10.33	9.19	20.25^{f}
GA_3 *(100 ppm)* + Calcium *(untreated)*	11.51	9.70	24.38^{bcd}
GA_3 *(100 ppm)* + Calcium *(5ml/li)*	10.99	9.25	24.63^{bcd}
GA_3 *(100 ppm)* + Calcium *(10ml/li)*	13.70	11.42	25.38^{bcd}
GA_3 *(100 ppm)* + Calcium *(20ml/li)*	13.47	11.54	23.75^{bcde}
GA_3 *(250 ppm)* + Calcium *(untreated)*	12.73	10.91	23.00^{def}
GA_3 *(250 ppm)* + Calcium *(5ml/li)*	13.61	11.12	26.63^{abc}
GA_3 *(250 ppm)* + Calcium *(10ml/li)*	14.51	12.05	25.63^{abcd}
GA_3 *(250 ppm)* + Calcium *(20ml/li)*	13.03	10.87	25.75^{abcd}
GA_3 *(300 ppm)* + Calcium *(untreated)*	13.60	11.48	24.38^{bcd}
GA_3 *(300 ppm)* + Calcium *(5ml/li)*	15.22	12.48	26.88^{ab}
GA_3 *(300 ppm)* + Calcium *(10ml/li)*	12.88	10.77	25.13^{bcd}
GA_3 *(300 ppm)* + Calcium *(20ml/li)*	13.03	10.44	28.63^{a}
CV (%) *main plot*	10.5	9.2	12.6
CV (%) *interaction*	11.8	12.3	9

Physiological Observation. The Normalized Difference Vegetation Index (NDVI) was used to detect the chlorophyll content on leaves using the handheld crop sensor at the flowering and harvestable age of the pineapple.

Statistical analysis revealed no significant differences between treatment applied with GA_3 and calcium and even the interaction between the two factors on the chlorophyll content of leaves of F200 pineapple taken at flowering and harvestable age (Table 4). But differences of means between flowering and harvestable age varied on each chlorophyll content; pineapple plants at the flowering stage have numerically higher chlorophyll content of 0.82 to 0.85 compared with plants at harvestable age with 0.80 to 0.81 mean. Plant maturity affects the chlorophyll content in leaves, and this can affect the photosynthetic activity.

CAM species like pineapple have the biochemistry of C4 species and the anatomy of C3 species. Photosynthetic rates steadily decline with leaf age; in fact, leaf age is more of a factor in photosynthetic rates than light intensity. Older leaves of C4 species may exhibit photorespiration due to a diminished capacity to concentrate CO2 in the bundle sheath cells. When chlorophyll and RuBisCo content of senescing C4 leaves reaches 50% of normal mature leaves, photorespiration can approach that in C3 plants, as cited by Durner (2013).

Table 4. Normalized Difference Vegetation Index NDVI reading at flowering and harvestable stage of F200 pineapple fruit after application with varying levels of GA3 and calcium.

GA3 level (ppm) main plot	chlorophyll reading	
	flowering stage	harvestable stage
GA3 *(untreated)*	0.83	0.80
GA3 *(100 ppm)*	0.84	0.80
GA3 *(250 ppm)*	0.83	0.80
GA3 *(300 ppm)*	0.84	0.80
Calcium level (ml/li) sub plot		
Calcium *(untreated)*	0.83	0.80
Calcium *(5ml/li)*	0.83	0.81
Calcium *(10ml/li)*	0.84	0.81
Calcium *(20ml/li)*	0.84	0.80
GA3 with Calcium Interaction		
GA3 *(untreated)* + Calcium *(untreated)*	0.82	0.80
GA3 *(untreated)* + Calcium *(5ml/li)*	0.82	0.80
GA3 *(untreated)* + Calcium *(10ml/li)*	0.84	0.80
GA3 *(untreated)* + Calcium *(20ml/li)*	0.84	0.80
GA3 *(100 ppm)* + Calcium *(untreated)*	0.84	0.80
GA3 *(100 ppm)* + Calcium *(5ml/li)*	0.83	0.81
GA3 *(100 ppm)* + Calcium *(10ml/li)*	0.85	0.80
GA3 *(100 ppm)* + Calcium *(20ml/li)*	0.84	0.80
GA3 *(250 ppm)* + Calcium *(untreated)*	0.83	0.81
GA3 *(250 ppm)* + Calcium *(5ml/li)*	0.83	0.81
GA3 *(250 ppm)* + Calcium *(10ml/li)*	0.83	0.81
GA3 *(250 ppm)* + Calcium *(20ml/li)*	0.82	0.80
GA3 *(300 ppm)* + Calcium *(untreated)*	0.83	0.79
GA3 *(300 ppm)* + Calcium *(5ml/li)*	0.83	0.81
GA3 *(300 ppm)* + Calcium *(10ml/li)*	0.85	0.81
GA3 *(300 ppm)* + Calcium *(20ml/li)*	0.85	0.82
CV (%) *main plot*	2.5	2
CV (%) *interaction*	2.0	1.6

Fruit Calcium and Potassium Analysis. Calcium and potassium content in fruit revealed no statistically significant result between treatment means of GA_3 and calcium supplement application, and even in the interaction of the two factors (Table 5). GA_3 application in pineapple has no relationship with calcium uptake in fruit, and this result concluded that calcium content in relation to calcium supplement application was not affecting the calcium and potassium content on fruit at harvestable age. However, numerically, it was found that potassium content in fruit was higher on plants applied with GA_3 at 100 and 300 ppm + calcium of 20 ml/li with 974.80 ppm and 930.56 ppm.

Calcium nutrition has a relationship with potassium in fruit in pineapple. Calcium supply affected fruit aroma, possibly because higher levels of Ca could interfere with the absorption of K, so the effect is probably not specific. Higher levels of Ca in the fruit are also associated with reduced incidence of storage disorders (Wilson Wijeratnam et al., 1996; Selvarajah et al., 1998).

Table 5. Fruit calcium and potassium content on fruits (ppm) of F200 pineapple fruit after application with varying levels of GA3 and calcium.

GA3 level (ppm) main plot	Fruit Ca and K analysis	
	Ca (ppm)	K (ppm)
GA3 *(untreated)*	40.37	853.29
GA3 *(100 ppm)*	25.56	884.58
GA3 *(250 ppm)*	7.49	841.38
GA3 *(300 ppm)*	23.85	836.10
Calcium level (ml/li) sub plot		
Calcium *(untreated)*	25.82	867.87
Calcium *(5ml/li)*	14.90	870.81
Calcium *(10ml/li)*	37.41	797.20
Calcium *(20ml/li)*	19.15	879.47
GA3 with Calcium Interaction		
GA3 *(untreated)* + Calcium *(untreated)*	35.98	978.82
GA3 *(untreated)* + Calcium *(5ml/li)*	39.16	857.67
GA3 *(untreated)* + Calcium *(10ml/li)*	83.51	800.20
GA3 *(untreated)* + Calcium *(20ml/li)*	2.86	776.48
GA3 *(100 ppm)* + Calcium *(untreated)*	47.59	847.37
GA3 *(100 ppm)* + Calcium *(5ml/li)*	12.22	941.22
GA3 *(100 ppm)* + Calcium *(10ml/li)*	27.80	774.92
GA3 *(100 ppm)* + Calcium *(20ml/li)*	14.62	974.80
GA3 *(250 ppm)* + Calcium *(untreated)*	0.91	861.21
GA3 *(250 ppm)* + Calcium *(5ml/li)*	8.20	886.59
GA3 *(250 ppm)* + Calcium *(10ml/li)*	9.59	781.68
GA3 *(250 ppm)* + Calcium *(20ml/li)*	11.27	836.06
GA3 *(300 ppm)* + Calcium *(untreated)*	18.81	784.07
GA3 *(300 ppm)* + Calcium *(5ml/li)*	0.00	797.76
GA3 *(300 ppm)* + Calcium *(10ml/li)*	28.74	832.01
GA3 *(300 ppm)* + Calcium *(20ml/li)*	47.86	930.56
CV (%) *main plot*	216.7	13.7
CV (%) *interaction*	208.5	15.1

In summary, GA_3 and calcium application at any level significantly influence the time of maturity of pineapple fruit. As observed, pineapple plants applied with GA_3 alone at any level at 100 to 300 ppm delayed the days to maturity to 13 days longer than the untreated plants. Pineapple plants treated with GA_3 at any level in combination with different levels of calcium matured at 229-231 days. As the calcium level increased by 10 to 20 ml/li, maturity was delayed for about 2 days.

A greater number of fruits from plants untreated with GA_3 were found to mature early, starting at 28 weeks after forcing. As evident in the number of fruits matured in the weekly spread result, as the rate of GA_3 decreases, fruit maturity development hastens. On the interaction effect of GA_3 and calcium, a significant difference was observed only 31 weeks after forcing. Calcium application alone or even without calcium and GA_3 application was found to have a higher percentage of fruit to mature within 68.33% to 79.17%. It was also observed that the GA_3 application with calcium combination influenced delaying fruit maturity.

Application of GA_3 at any level significantly influences the histological development of pineapple, such as fruit edible portion diameter, number of fruitlets on fruit, and width of center fruitlet, mostly at harvestable age. No significant result on calcium application and even on the combination of treatments. Application of GA_3 significantly increased the weight of pineapple fruit (with and without crown), particularly at the higher rate. Fruits of pineapple plants sprayed with GA_3 at 300 ppm were heavier than fruits of pineapple plants sprayed at 100 ppm of GA_3, to about 2.54 kg and 1.52 kg with and without a crown, respectively. Fruits of pineapple plants without GA_3 application revealed the lowest weight among treatments. Calcium alone and the interaction between GA_3 and calcium have no significant effect observed. No significant differences between treatment application of GA_3 and calcium levels on crown length of pineapple at harvestable age.

GA_3 applied fruit in pineapple with a calcium combination at any rate produced a longer crown compared with the pure calcium application alone at any level. The highest rate of GA_3 at 300 ppm and calcium 20 ml/li combination had the longest crown length observed.

Comparable chlorophyll content on leaves at flowering and at the harvestable stage in all treatments of GA_3 and calcium. Fruit calcium and potassium content at harvestable age in all treatments were also the same.

Conclusion

In conclusion, the application of GA_3 alone, even at 100 ppm, delayed the fruit maturity. GA_3 increased the histological size and morphology of fruits, such as the edible portion diameter, number of fruitlets, and width of center fruitlets at harvestable age. Horticultural traits of F200 pineapple were not improved by calcium or in combination with GA_3 application. The application of GA_3 from a lower rate of 100 ppm improved the weight of the pineapple fruit but was even heavier at a higher rate of 300 ppm.

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1.

2.

3.

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