International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Applications and Effects of Phytohormones on the Flower and Fruit Development of Pineapple (*Ananas comosus* L.)

Vences C. Valleser[1]*

1 Department of Horticulture, College of Agriculture, Central Mindanao University, Philippines

ARTICLE INFO

Article history:

Received: 28 January 2022,
Received in revised form: 17 April 2022,
Accepted: 1 May 2022

Article type:

Review article

Keywords:

Chemical substances,
Differentiation,
Fruit morphogenesis,
Plant regulators,
Reproductive development

ABSTRACT

Phytohormones are naturally-occurring substances produced by plants which promote vegetative and reproductive development and senescence. Similar to other crops, phytohormones affect the flowering and fruit development of pineapple. This writing provides an overview on the association and effects of phytohormones in pineapple flowering and fruit development. The effects of phytohormones are integrated and discussed in three sections: 1) flower induction in pineapple, 2) flower initiation and flowering in pineapple, and 3) pineapple fruit development. Based on the findings of multiple research, phytohormones involved in pineapple inflorescence initiation and inflorescence development include auxin, gibberellin, cytokinin, ethylene, and abscisic acid (ABA). Auxin, gibberellin and cytokinin are known as phytohormones that improve the fruit size and quality of pineapple. These sets of information can serve as a guide to commercial pineapple growers in formulating a low-cost input of technology for pineapple production.

Introduction

Philippines is among the top pineapple producing countries worldwide. The majority of the pineapple exported by the Philippines to other countries are produced in Bukidnon province. Pineapple production is generally managed by transnational companies since the crop needs substantial inputs, such as fertilizers, to provide proper nutrition. Similar to other crops, pineapple requires high amounts of nitrogen in the form of urea (Dayondon and Valleser, 2018; Haque et al., 2022) and potassium in the forms of potassium sulfate or potassium chloride. However, unlike other crops, pineapple can tolerate low levels of phosphorus (Valleser, 2019; Trejo et al., 2020). With the increasing prices of commercial fertilizers in the Philippines, there is a need to find alternatives to reduce the usage of commercial fertilizers, without a decrease in pineapple yield.

Phytohormones are naturally-occurring substances which regulate plant growth and development. Application of phytohormones in commercial pineapple production is limited only in forcing and ripening operations, aside from tissue culture. However, with the increasing prices of commercial fertilizers, the application of phytohormones can help reduce the amount of fertilizers to be applied in commercial pineapple fields. It is in this context that this paper reviews the potential use of phytohormones during the shift from the vegetative to the reproductive state of pineapple plants. The effects of phytohormones are integrated and discussed in three sections: 1) flower induction in pineapple, 2) flower initiation and flowering in pineapple, and 3) pineapple fruit development.

Flower induction in pineapple

Flowering induction is a crucial aspect of pineapple cultivation. Growers all around the world treat their plants with ethylene, or

* Corresponding author's email: vcvalleser@cmu.edu.ph

ethylene-releasing compounds, to obtain simultaneous flowering. This practice usually results in a homogenous pattern of flower and fruit development (Min and Bartholomew, 1996). Floral induction should only be performed on strong and well-developed plants, capable of producing fruits of appropriate size for sale and/or plantlets for use in new plantings. Fruit weight is directly related to plant vigor at the time of floral differentiation, although it also depends on the climatic conditions during its development. In the 'Pérola' variety, it is recommended to induce flowering in plants that have 'D' leaves with a minimum fresh weight of 80 g and a minimum length of 1.0 m, in order to obtain fruits weighing more than 1.5 kg (Reinhardt et al., 1987). Different pineapple varieties have different maturity indices. Fournier et al. (2007) found that at the time of forcing, leaf numbers were 62 for 'Flhoran 41', 55 for 'Smooth Cayenne' and 50 for 'MD2'. 'Flhoran' has more but smaller leaves than either 'Smooth Cayenne' or 'MD2'. In 'Flhoran 41', a D-leaf weight of 70 g is sufficient to assist in producing exportable fruits, whereas 80 g is the standard, usable amount for 'MD2' and 'Smooth Cayenne' (Fournier et al., 2007). Many studies have shown that fruit weight at harvest is highly correlated with plant weight (Py and Lossois, 1962; Wee et al., 1979), plant leaf number and even 'D' leaf weight measured at the time of forcing (Soler, 2008).

Estimated plant weight can also be an aid for decision-making when planning for forcing schedules. The relationship between plant weight at forcing and fruit weight at harvest is stable for a given cultivar within a particular environment, but the relationship developed for one geographical area may not be suitable for another area with a different average temperature. This is evident in the report of Fournier et al. (2006) that a 2.5 kg plant at forcing produced a 1.5 kg fruit at harvest in Tropical Cote d' Ivoire, while the ratio of plant weight at forcing to fruit weight at harvest was nearly 1:1 for 'Smooth Cayenne' in Hawaii.

De Poel and De Croylaan (2009b) reported that 'MD2' pineapple, at three months of age, after planting, reached physiological maturity and was susceptible to ethylene treatments even though they were not as yet large enough to produce a marketable fruit. The treatment for floral induction of pineapple using appropriate chemical substances as growth regulators or as phytohormones is a commonplace practice (Augusto and da Cunha, 2005). So far, several identified growth regulators have rendered yields efficient in initiating pineapple flowering. Among these products, the most commercially used are: α- naphthalene acetic acid (NAA), β- naphthalene acetic acid (BNA), indolebutyric acid (IBA), 2, 4- dichlorophenoxyacetic acid (2, 4- D), succinic acid, 2- ethylphosphonic acid (ethephon), ethylene (C_2H_4), acetylene (C_2H_2), calcium carbide (CaC_2), hydroxyethylhydrazine (HOH) and β- hydroxyethylhydrazine (BOH). However, only a few are practically used, such as ethylene, acetylene, calcium carbide and ethepon (Augusto and da Cunha, 2005).

Ethylene is normally applied to the plants at 11-12 months in the form of ethephon (Ethrel) at a rate of 25 to 100 ppm. Ethrel can be mixed with either urea or activated charcoal as a carrier, or it can be applied alone. Flower induction is triggered from 25 to 45 days, depending on the time of the year, size of plant, and temperature. The ethylene breaks down either in the plant cells or is volatilized into the air very quickly (NOSB Materials Database, 2007). Ethephon, an ethylene-releasing agent, has become the most popular active ingredient over the world as it may be used by large or small farms and the chemical can be applied during the day. It contributes to the slow release of ethylene from the plant. The main limitation of this technique is very poor efficiency in hot climatic condition (Fournier et al., 2007). In Bukidnon, Philippines, higher rates of Ethrel (800 to 1200 ppm) were effective for 'MD-2' pineapple (Valleser, 2018) since it compensated for losses during hot climatic conditions. It is also important to apply the ethylene treatment at the right moment. Thus, young plants can produce smaller fruits, while older plants are more subjected to natural flowering (Valleser, 2018), which disturbs flowering homogeneity (De Poel and De Croylaan, 2009a).

Calcium carbide (CaC_2) is a synthetic material made from limestone or quicklime mixed with crushed coke at high temperatures. When sprayed on the plants in water it forms acetylene gas which is a precursor to ethylene. The acetylene enters the plant and is transformed into ethylene in the cells, thereby triggering flowering (NOSB Materials Database, 2007). Studies by Maruthasalam et al. (2009) revealed that 1.0 % CaC_2 containing 0.5 % activated charcoal applied once, or 0.5% CaC_2 containing 0.5% activated charcoal applied twice, forced 'Tainon 17' pineapple.

Gaseous ethylene injected into water with activated charcoal is the most widely-used technique on large scale farms with a high level of mechanization (Py et al., 1987). Night applications are required as the pineapple stomata through which the plants absorb the gas are closed during the day. From different research findings, it was found that the use of growth regulators in pineapple induced early-flowering and helped harvest fruits out of the season (Uddin et al., 1998). Effect of growth regulators on inducing flowers varied according to environmental conditions (Hussain et al., 2008). Early, uniform, year-round productions of pineapple were possible by the application of growth regulators, especially ethephon (Ethrel) and calcium carbide (Bose et al., 1983). Reports indicated that the efficacy of ethephon at lower concentration increased when urea, alkali calcium carbonate and sodium carbonate were added to it (Dass et al., 1976; Hussain et al., 2008). It was reported that adding a boron-containing compound to an Ethrel solution raised the pH and increased the speed of Ethrel degradation to ethylene. Regarding summer forcing in Australia, 2,500 to 3,000 liters of a solution containing 0.5% Borax or 0.25% Solubor, 5% urea, and 2.5 liters of Ethrel were applied per hectare (ISHS, 1995). In India, Dass et al. (1976) found that a 2% urea solution, which was adjusted to a pH of 9.0, and which contained 10 ppm ethephon, induced over 90% of plants to flower within 50-60 days of treatment, which had a better effect than the result of using α- naphthalene acetic acid-based products.

Ethrel had significant effects on flower induction and fruit development in pineapple. Mostly, pineapple growers use Ethrel in mixture with urea to induce flowering in pineapple. Ethephon, the active ingredient of ethrel, is degraded when reaching the internal plant tissues, thereby releasing ethylene, chlorate and phosphate ions. This degradation is enhanced when the pH of the solution is higher than four (within the alkaline range) (De Wilde, 1971; Augusto and da Cunha, 2005) because ethylene is stable in an aqueous solution with low values of pH (within the acidic range). The efficiency of ethephon at lower concentrations increases when urea, alkali-like calcium carbonate and sodium carbonate are added (Hussain et al., 2008). Malip (2010) recommended that ethrel and urea combination should remain at 240 ppm and 2% in order to obtain optimum flowering and desirable qualities for 'Maspine' pineapple in Malaysia. Bhowmick et al. (2009) reported that ethrel at 25 ppm showed better results on fruiting characteristics of 'Kew' pineapple in Cooch Behar District, West Bengal, India. In line with this, Suresh et al. (2009) found that maximum yield per hectare with crown (82.6 tons) of 'Kew' pineapple was obtained with the application of NAA at 10 ppm as a forcing solution.

Results of a study conducted by Liu et al. (2009) showed that for cultivars 'Comte de Paris', 'Tainung 20' and 'Pearl', the ethephon solution of 200 ppm was the best for flower initiation and induction in the warm season (from June to September) and 400 ppm in the cool season (from October to May), with a flowering rate of 100%. For 'Smooth Cayenne', the concentration was much higher with 400 ppm in the warm season and 600 ppm in the cool season. Regarding 'Tainung 13', 800 ppm was the optimum concentration. Both the fruit size and the fruit weight, as well as the D-leaf length, D-leaf weight and fruitlet number decreased significantly as ethephon concentration increased from 200 ppm to 400 ppm plus 1% urea. On the other hand, the addition of 0.4 % KH_2PO_4 in the 200 ppm ethephon solution increased the fruitlet number and fruit weight of 'Comte de Paris', which significantly increased yield.

Dass et al. (1976) reported that ethephon at 25 ppm in combination with urea (2%) and calcium carbonate (0.04 %) induced more than 90% of flowering after 50 days of treatment. Adding either urea or calcium carbonate increased the effectiveness of ethephon for the induction of flowering. The control plants started flowering 250 days after treatment and completed flowering at 340 days. Apart from earlier flowering, ethephon treatments resulted in uniform ripening, and harvests were completed within 10 days, whereas it continued for 70 days in the control harvests.

De Poel and De Croylaan (2009a) reported 'zeothene' as a flower induction treatment for pineapple. Zeothene released gaseous ethylene when it came into contact with water, which resulted in homogenous flowering, as a highly-desired feature on commercial farms. On the other hand, 'zeothene' treatment was more favourable to obtain a homogenous flowering when applied as a central cup than in whole plant spraying.

Pineapple flowering can occur naturally. Its extent can be related to climatic factors (e.g. photoperiod, temperature, solar radiation), and can be modified artificially with the use of chemical substances (plant growth regulators). Naturally differentiated flowering (NDF) of pineapple is a serious problem in commercial pineapple plantations. The problem occurs at cooler temperatures < 20oC, shorter day-lengths and when plants reach 2.0 kg weight. In such circumstances, the incidence of NDF can be high, resulting in off-schedule fruiting and harvesting. Thus, a survey was conducted when minimum temperature (< 20oC) occurred for five consecutive days in fields with 2.0 kg plant weight and/or when the plants were 10-11 months old (below 2 kg) during the onset of the cold months. Different pineapple varieties respond differently to natural flowering. Smooth Cayenne is

considered to be reasonably resistant to natural flower initiation, although low levels can be experienced throughout the year, particularly in the May-August period. Most other varieties are more susceptible during periods of very hot weather (December-January) or when temperatures are around 20oC or lower (May-August). The time of planting, size of planting material and plant vigor are all important to minimize the incidence. Irrigation is useful for ensuring good plant vigor. "MD2" is extremely susceptible and it was assumed doubtful that cultural techniques can be sufficient to keep the incidence at a commercially tolerable level in Southeast Queensland. Regarding MD2, on a winter plant crop in Southeast Queensland, the plants usually experienced 50% or more natural flower initiation in the May-August period (https://www.daf.qld.gov.au/__data/assets/pdf_file/0005/51449/Ch-7-Fresh-Fruit-Varieties.pdf).
Pineapple flowering was also affected by season. Ethrel was applied in September on 300-day-old plants, and this induced the flowering 146 days earlier in off-season, compared to those in the control. Ethrel-treated plots in September showed maximum flowering (84%), followed by ethrel applied in November on 360-day-old plants. Plants treated in September took minimum duration (520 days) while control plots took maximum crop duration (613 days) for ripening. The size of fruits increased as the plants aged at forcing. The highest fruit yield (31.275ton ha-1) was produced by control plots and the lowest was produced from September forcing (Hussain et al., 2008).
Pineapple flowering can be avoided or delayed, if (1) the vegetative growth of the plant is reduced so that it will not be able to flower at the time when the weather conditions become favorable for natural flowering; (2) the vegetative growth rate of the plant is increased due to the use of such production factors as nitrogen fertilization and irrigation; and (3) the biosynthesis and action of ethylene in the plant are inhibited or blocked, with the use of some chemical substances (Augusto et al., 2004) such as aviglycine (Wang et al., 2007). Previously, a mixture of naphthalene and indole compounds such as α-naphthaleneacetic (2000 ppm), β-naphthoxyacetic (1000 ppm), indole acetic (200 ppm) and indolebutyric (100 ppm) acids, and amides (50 ppm) and alkali metal salts (30 ppm) could hasten or delay the flowering of pineapple. At higher concentration (100-1000 ppm) of the mixture, it delayed differentiation, flowering and fruit maturation. Nonetheless, lower concentrations (5-60 ppm) resulted in precocious differentiation, flowering and fruit maturation (Mehrlich, 1948).
To check whether a pineapple plant responded to flower induction treatment, a common practice in commercial pineapple plantations known as "differentiation survey" is conducted. Within a pineapple block (in mechanized commercial pineapple plantation, a pineapple block measures 33 meters in width and is usually composed of 24 beds), 20 plants are selected within various beds (i.e. 1st, 5th, 9th, 13th, 17th, 21st and 24th beds) in a block. Selected plants are then uprooted. Plants are inverted and using a sharp knife it will be cut across vertically into halves. If the plants did not respond to the flowering induction treatment, the growing point remains rounded and the pineapple plant is said to be at the flowering stage when the growing point becomes pointed (Fig. 1).

Flower initiation and flowering in pineapple

Pineapple plants require careful management in terms of morphological and physiological aspects, which facilitate the understanding of its flowering mechanisms. Among those aspects are the features of the apical meristem, the segment that differentiates into leaves during the vegetative phase, but later undergoes transformations, originating in the inflorescence, and thereafter retakes its vegetative activity, producing the crown of the fruit (Augusto and da Cunha, 2005). The transition from the vegetative phase to flowering is very important for pineapple plants, since flowering is the first step of the sexual reproduction (Bernier et al., 1993), resulting in fruit production, which is the main objective in rendering plants profitable.
Flowering is a unique and integrated process of very complex nature and multifactorial control (Bernier et al., 1993). In general, two phases are observed during flowering: floral initiation and floral development. In the first one, it is necessary that some events take place in the stem apex and lateral buds, in an irreversible way, with the initiation of flowering, the first step for the formation of flowers, denominated "evocation" (Kinet et al., 1981). Flower initiation takes place at the terminal axis of the stem. Stages of development after its appearance are called "half-inch open heart" and "one-inch open heart". At these stages, the center is open by approximately 1.25 and 2.5 cm, respectively, and the red inflorescence is clearly visible below the opening (Fig. 2, a, b). The inflorescence then becomes cone-shaped (Fig. 3, a, b, c). Three to four weeks after the one-inch open heart stage, blue flower petals can be seen appearing at the bottom (Fig. 4a), then at the middle (Fig. 4b) and finally at the

tip (Fig. 4c) of the cone- shaped inflorescence. Low levels of IAA, GA3 and zeatin and high levels of ethylene, ABA and 2iP facilitated inflorescence initiation. Nonetheless, high levels of zeatin, IAA and GA3 and low levels of ethylene and ABA facilitated inflorescence development (Sheng-hui et al., 2011). Before all flowers open, the earliest petals will have begun to dry. Opened pineapple flowers could serve as entry points to pathogens causing diseases. Ethylene application in the form of ethrel prevents pineapple flowers from opening (Obrero and Schnitzler, 1986). This practice, however, is costly and laborious. After all petals have dried, the inflorescence is said to be at the "dry petal" stage (Fig. 4, d). Its surface is dull, individual fruitlets ("eyes") are pointed, and a crown begins to develop (https://www.ctahr.hawaii.edu/oc/freepubs/pdf/f_n-7.pdf).

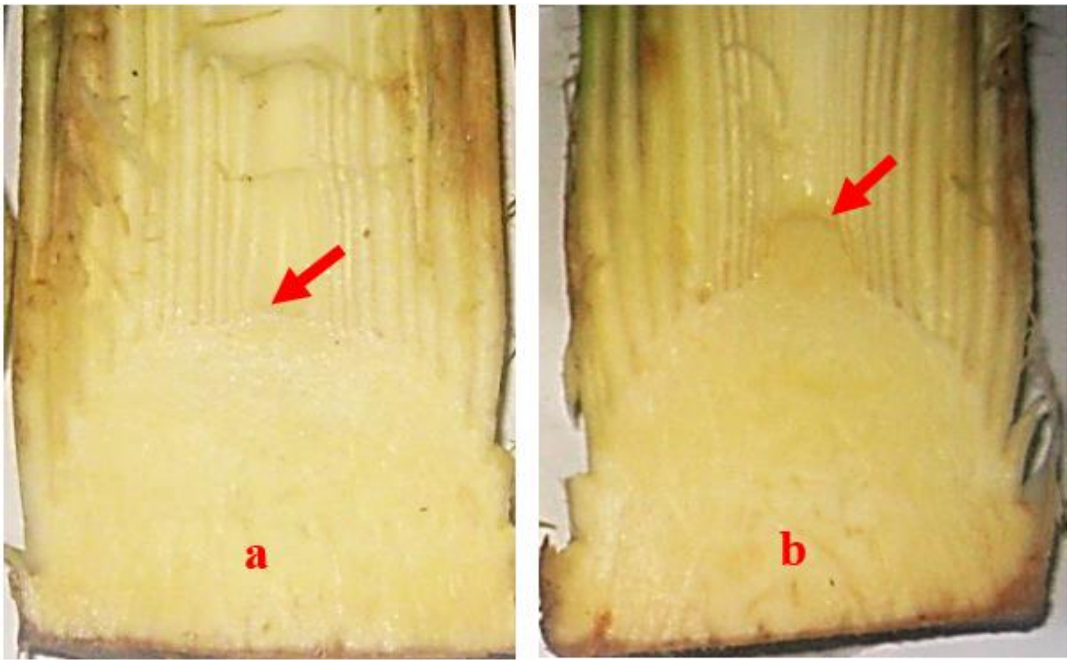


Fig. 1. Non-differentiated (a) and differentiated (b) pineapple plants. In a non-differentiated pineapple, the growing point remains rounded; in a differentiated pineapple, the growing point becomes pointed

(a) 1 cm red inflorescence (b) 2 cm red inflorescence

Fig. 2. Red inflorescence development of pineapple observed at 46-55 days after forcing

(a) Early cone (b) Middle cone (c) Late cone

Fig. 3. Pineapple fruit, forming a conical shape at 55-62 days after forcing; peduncle (p) holding the fruit is visible during these stages

(a) Early whorl (~65 DAF) (b) Middle whorl (~70 DAF)

(c) Last whorl (~75 DAF) (d) Dry petal stage (79-90 DAF)

Fig. 4. Pineapple inflorescence showing the different stages in petal development

The floral differentiation of pineapple plants has another uniqueness: it can be triggered artificially, by chemical substances, which are also related to the natural flowering. The sensitivity of the pineapple plant to natural or artificial flowering is related to the age or size of the plant. The first one is related to induction by environmental factors, and the second by means of the chemical products, generally by phytohormones or plant growth regulators. In both cases, there is the involvement of hormones synthesized by the plant, such as indoleacetic acid (IAA) and ethylene, the latter being the real inducing factor (Burg and Burg, 1966; Augusto and da Cunha, 2005).

The natural flowering of pineapple is a very complex process which makes several challenges. Pineapple plants tend to flower from late-fall to early-winter, although it may also happen in other seasons, depending on the region. Natural flowering can result in serious losses to growers all over the world, because it affects crop management, fruit harvest and sale, thereby reducing crop revenues. These losses become worse if flowering is precocious, when plants are not enough developed to produce fruits with commercial value. Besides, the differences in the susceptibility of plants to flowering have been observed. In general, the largest plants are more susceptible. Despite this, it has also been observed that small plants can be susceptible to natural and artificial inductive flowering signals (Augusto and da Cunha, 2005).

Pineapple fruit development

The pineapple is technically called a ‘sorosis’- a fusing of many fruits together to form one unit. Each “eye” (fruitlet) is a complete fruit. Flowering starts at the bottom of the sorosis and continues up as a spiral to the last eye. When the formation of the fruitlets stops, the growing points revert to a vegetative state and the top (crown) is formed (Fig. 5). With good management practices, they will all fill out to give a well-shaped, high-yielding fruit (https://www.daf.qld.gov.au/__data/assets/pdf_file/0007/66247/Ch1-The- Pineapple.pdf).

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. Applications of auxin, giberrellin, or cytokinin could can enhance the fruit size, weight and qualities in pineapple. Apart from delaying or hastening pineapple flowering, the mixture of naphthalene and indole compounds such as α-naphthaleneacetic (2000 ppm), β-naphthoxyacetic (1000 ppm), indole acetic (200 ppm) and indolebutyric (100 ppm) acids, and amides (50 ppm) and alkali metal salts (30 ppm) strengthened pineapple peduncle, improved shell firmness, increased pineapple size and boosted weight by around 0.5 kg per fruit (Mehrlich, 1948).

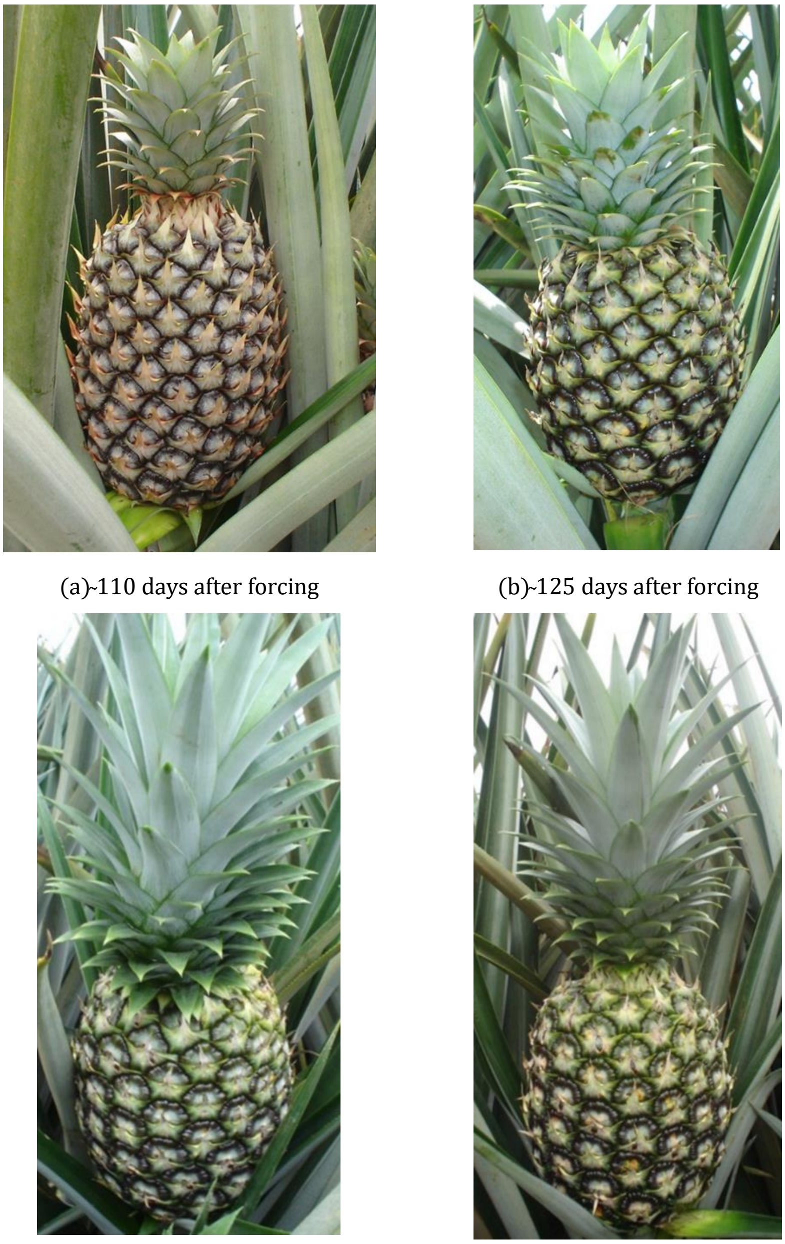
In a study conducted by Suwandi et al. (2016), gibberellin alone (100 or 200 ppm) or in combination with cytokinin (24 or 48 ppm) increased the fruit weight, harvest index and fruit crown length and delayed fruit maturity (5 days) of ‘Smooth Cayenne’ pineapple. Treatment with 100 ppm of gibberellin, when combined with 24 ppm of cytokinin, was the best treatment for improvement of the pineapple harvest index and fruit quality. In another study, exogenous application of GA3 at 50 ppm increased fruit weight of ‘Comte de Paris’ pineapple by 20.3% versus the untreated group. Percentage of S phase (DNA replication) cells in treated fruits did not differ significantly from the control. GA_3 in pineapple fruits affect cell-surface enlargement more than they assist in proliferating cell count. Furthermore, GA3 promotes fruit development in the transverse direction than in the longitudinal direction of pineapple fruits, and increases vitamin C content (Li et al., 2011).

Conclusion

Phytohormones are naturally-occurring substances that are produced by plants. They promote vegetative and reproductive development and hasten senescence. Similar to other crops, phytohormones affect the flowering and fruit development of pineapple.

In commercial pineapple production, synchronized flowering can be achieved through the application of products containing ethylene or auxin. These two hormones are involved in inducing flower formation in pineapple plants. During flower initiation and flowering in pineapple, phytohormones act in combination, thereby interacting with each other. Low levels of IAA, GA3 and zeatin and high levels of ethylene, ABA and 2iP facilitated inflorescence initiation (Sheng-hui et al., 2011), whereas high levels of zeatin, IAA and GA3 and low levels of ethylene and ABA facilitated inflorescence development (Sheng-hui et al., 2011).

In pineapple fruit development, the presence of auxin, gibberellin and cytokinin can improve fruit size and quality.



(a)-110 days after forcing (b)-125 days after forcing

(c)-155 days after forcing (d)-175 days after forcing

Fig. 5. Latter stages of pineapple fruit development. Note the changes in peel color, fruit, and crown as the fruit matures

Application of the mixture of naphthalene and indole compounds such as α-naphthaleneacetic (2000 ppm), β-naphthoxyacetic (1000 ppm), indole acetic (200 ppm) and indolebutyric (100 ppm) acids, and amides (50 ppm) and alkali metal salts (30 ppm) strengthened pineapple peduncle, improved the firmness of shell and increased pineapple size and weight by around 0.5 kg per fruit (Mehrlich, 1948). Likewise, application of gibberellin alone (100 or 200 ppm) or in combination with cytokinin (24 or 48 ppm) increased the fruit weight, harvest index and fruit crown length, while delaying fruit maturity (5 days) of 'Smooth Cayenne' pineapple (Suwandi et al., 2016). Treatment with 100 ppm of gibberellin combined with 24 ppm of cytokinin was the best treatment for improvement of the pineapple harvest index and fruit quality (Suwandi et al., 2016). Li et al. (2011) reported that exogenous application of GA3 at 50 ppm increased fruit weight of 'Comte de Paris' pineapple by 20.3% versus the untreated group. Percentage of S phase (DNA replication) cells in GA_3-treated fruits did not vary greatly from the untreated. GA3 affected cell-surface enlargement rather than the increase in cell number of pineapple fruit. Furthermore, GA_3 increased dimensions in the transverse direction than in the longitudinal direction of pineapple fruits, and increased vitamin C content (Li et al., 2011).

Based on the findings of different cases of research, various phytohormones are involved in pineapple inflorescence initiation and inflorescence development. These include auxin, gibberellin, cytokinin, ethylene, and abscisic acid (ABA). Auxin, gibberellin and cytokinin are the phytohormones that improve the fruit size and quality of pineapple. With the increasing prices of synthetic fertilizers nowadays, the application of phytohormones may be considered as an alternative that can be used by commercial pineapple growers to produce marketable fruits at a lower input cost.

Acknowledgments

The author is grateful to Central Mindanao University and Department of Science and Technology for the scholarship grant.

Conflict of Interest

The author declares no conflict of interest in this work.

References

Augusto G, da Cunha P. 2005. Applied aspects of pineapple flowering. Bragantia Revista de Ciencias Agronomicas 64(4), 499-516.

Augusto G, da Cunha P, Reinhardt, DH. 2004. Inhibiting natural flowering on pineapple. ISHS Newsletter of the Pineapple Working Group 11, 17.

Bartholomew DP, Malezieux E, Sanewski GM, Sinclair E. 2003. “Inflorescence and fruit development and yield,” in The Pineapple: Botany, Production and Uses, Ed. D. P. Bartholomew, R. E. Paull, K. G. Rohrbach. New York, USA: CABI Publishing, 167-202.

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. 1993. Physiological signals that induce flowering. The Plant Cell 5, 1147-1155.

Bhowmick N, Suresh CP, Paul PK, Ranjit K, Pal K, Deb P, Gurung S. 2010. Effect of ethrel on fruiting characteristics of pineapple in Cooch Behar District, West Bengal, India. ISHS Newsletter of the Pineapple Working Group 17, 19-20.

Bose T, Aich KK, Mitra SK, Sen SK. 1983. Regulation of flowering in pineapple the year round. Indian Agricultural Journal 27, 331-336.

Burg SP, Burg EA. 1966. Auxin-induced ethylene formation and its relation to flowering in the pineapple. Science 152(3726), 1269.

Cameron JS, Dennis FG. 1986. The carbohydrate-nitrogen relationship and flowering/fruiting: Kraus and Kraybill revisited. HortScience 21(5), 1099-1102.

Dass HC, Randhawa GS, Singh HP, Ganapathy KM. 1976. Effect of pH and urea on the efficacy of ethephon for induction of flowering in pineapple. Scientia Horticulturae 5, 265-268.

Dayondon RR, Valleser VC. 2018. Effects of urea and calcium-boron applied at flower bud stage on ‘MD2’ pineapple fruit. International Journal of Scientific and Research Publications 8(6), 322-328. DOI: 10.29322/IJSRP.8.6.2018.p7842

de Poyle V, de Croylaan W. 2009a. A novel flowering induction agent for pineapple. ISHS Newsletter of the Pineapple Working Group 16, 6.

de Poyle V, de Croylaan W. 2009b. MD-2 pineapple plant maturity and flowering. ISHS Newsletter of the Pineapple Working Group 16, 10.

de Wilde RC. 1971. Practical applications of (2-chloroethyl) phosphonic acid in agricultural production. HortScience 6(4), 70.

Fournier BP, Soler A, Alphonsine PAM. 2007. Growth characteristics of the pineapple cultivars ‘MD2’ and ‘Floran 41’ compared with ‘Smooth Cayenne’. ISHS Newsletter of the Pineapple Working Group 14, 18.

Haque MA, Sakimin SZ, Jaafar NM, Ding P, Sarker BC, Islam MS, Yusop MK. 2022. Transient application of foliar urea with N-(n-Butyl) thiophosphoric triamide on N metabolism of pineapple under controlled condition. Scientia Horticulturae 295, 110822. https://doi.org/10.1016/j.scienta.2021.110822

Hussain MJ, Ulla MA, Salim MMR, Rahman MM, Sarker MMR. 2008. Effect of different application time of ethrel on off-season pineapple production at Srimongal, Sylhet. International Journal of Sustainable Crop Production 3(6), 68-71.

Kinet JM, Sachs RM, Bernier G. 1981. The physiology of flowering. The development of flowers. Boca Raton: CRC Press.

Li Y-H, Wu Y-J, Wu B, Zou M-H, Zhang Z, Sun, G-M. 2011. Exogenous gibberellic acid increases the fruit weight of ‘Comte de Paris’ pineapple by enlarging flesh cells without negative effects on fruit quality. Acta Physiologiae Plantarum 33, 1715-1722. DOI 10.1007/s11738-010-0708-2.

Liu SH, Dou MA, Zang XP, Sun GM. 2009. Flower forcing technique by ethephon in pineapple. ISHS Newsletter Pineapple Working Group 17, 22-23.

Malip M. 2010. New formulation for the flowering of pineapple var. Maspine. ISHS Newsletter Pineapple Working Group 17, 18.

Maruthasalam S, Shiu LY, Loganathan M, Lien WC, Liu, Y L,…. Lin CH. 2009. Forced flowering of pineapple (*Ananas comosus* cv. Tainon 17) in response to cold stress, ethephon and calcium carbide with or without activated charcoal. Plant Growth Regulator. DOI 10.1007/s10725-009-9421-9.

Mehrlich FP. 1948. Treatment of pineapple plants with plant hormones. US Patent No. 2,441,163. United States Patent Office.

Min XJ, Bartholomew DP. 1996. Effect of plant growth regulators on ethylene production, 1-aminocyclopropane-1- carboxylic acid oxidase activity, and initation of inflorescence development of pineapple. Journal of Plant Growth Regulator 15(3), 121-128.

NOSB Materials Database. 2007. Ethylene. Crops. Retrieved January 28, 2022, from https://www.ams.usda.gov/sites/default/files/media/Ethylene%201%20TR%202007.pdf.

Obrero FP, Schnitzler WH. 1986. Method for treating pineapples to inhibit opening of the pineapple flowers. European Patent No. 0 275 708. European Patent Office.

Pineapple News. 1995. Borax or solubor for ethrel induction? ISHS Pineapple Working Group Newsletter 1(2), 5.

Py C, Lacoeuilhe J-J, Teisson C. 1987. The pineapple: Cultivation and uses. Paris, Maisonneuve and Larose.

Py C, Lossois P. 1962. Prevision de recolte en culture d'ananas. Etudes de correlations. Deuxieme partie. Fruits 17, 75-87.

Reinhardt DH, da Cunha GAP. 1987. Determinação do período critic de competição de ervas daninhas na cultura do abacaxi 'Pérola'. Pesquisa Agropecuária Brasileira (Brasília) 19(4), 461-467.

Sheng-hui L, Xiao-ping Z, Guang-ming S. 2011. Changes in endogenous hormone concentrations during inflorescence induction and development in pineapple (*Ananas comosus* cv. Smooth Cayenne) by ethephon. African Journal of Biotechnology 10(53), 10892-10899.

Soler A. 2008. Considerations on growth characteristics of different pineapple varieties in Côte d'Ivoire, La Reunion and Caribbean Islands. 6th International Pineapple Symposium. Joa Pessao, Brazil.

Suresh CP, Pall R, Deb P, Bhutia KD, Roy A, Hasan MA. 2010. Effect of NAA and Ethrel on yield and quality of pineapple (*Ananas comosus* L. (Merr)). ISHS Newsletter Pineapple Working Group 17, 20.

Suwandi T, Dewi K, Cahyono P. 2016. Pineapple harvest index and fruit quality improvement by application of gibberellin and cytokinin. Fruits 71(4), 209-214.

Trejo D, Bañuelos J, Gavito ME, Sangabriel-Conde, W. 2020. High phosphorus fertilization reduces mycorrhizal colonization and plant biomass of three cultivars of pineapple. Terra LatinoAmericana 38(4), 853-858. DOI: https://doi.org/10.28940/terra.v38i4.701

Uddin MN, Rahman MM, Quasem A. 1998. Studies on year round production of pineapple using ethrel and calcium carbide. Bangladesh Journal of Agricultural Research 23, 617-624.

Valleser VC. 2018. Plant age and rate of flower inducer affects flower initiation of 'MD2' pineapple. International Journal of Research and Review 5(4), 27-32.

Valleser VC. 2019. Phosphorus nutrition provoked improvement on the growth and yield of 'MD-2' pineapple. Pertanika Journal of Tropical Agricicultural Science 42(2), 467- 478.

Wang R-H, Hsu Y-M, Bartholomew DP, Maruthasalam S, Lin C-H. 2007. Delaying natural flowering in pineapple through foliar application of aviglycine, an inhibitor of ethylene biosynthesis. HortScience 42(5), 1188-1191.