

Full Length Research Paper

Changes in endogenous hormone concentrations during inflorescence induction and development in pineapple (*Ananas comosus* cv. Smooth Cayenne) by ethephon

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This study investigated the changes of five endogenous hormones in the shoot apex and the white bases of D-leaf during the inflorescence induction and development of 'Smooth Cayenne' pineapple, using 14-month-old pot-grown plants as material and ethephon as flower forcing agent. Results showed that application of ethephon increased the level of endogenous ethylene (C₂H₄), abscisic acid (ABA) and 2-isopentyl adenine (2-iP) while it decreased the concentration of indole-3-acetic acid (IAA), gibberellic acid (GA₃) and zeatin (ZT), and led to the transition of vegetative growth to inflorescence initiation. After inflorescence initiation, the contents of C₂H₄, ABA and 2-iP declined but the contents of IAA, ABA and ZT increased. These results indicated that low levels of IAA, GA₃ and ZT and high levels of C₂H₄, ABA and 2-iP facilitated inflorescence initiation, while high levels of ZT, IAA and GA₃ and low levels of C₂H₄ and ABA facilitated inflorescence development.

Key words: Pineapple, inflorescence initiation, inflorescence development, endogenous hormone.

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merrill) is native to southern Brazil and Paraguay. Its pulp and products are very popular and widely consumed by all the people over the world. As a kind of herbaceous perennial plant, the spike-like inflorescence exerts from the leaf bunches on the top then develops to a multiple accessory fruit (Bartholomew et al., 2003).

The flowering initiation of pineapple plants is affected by the plant weight, varieties and environment conditions (Cunha, 2005). If the plants are too small they cannot be induced to flower successfully even if treated with ethephon (Van de Poel et al., 2009). When the plant

reaches the ripe-to-flower stage, low temperature or cold treatment can stimulate the D-leaf to release ethylene, which then leads to flowering (Min and Bartholomew, 1997). Usually, intervarietal hybrids are more likely to flower naturally (Bartholomew et al., 2003). However, natural flowering out of season can cause precocious fruiting, decreased yield or disrupt the forcing plan of growers (Scott, 1997).

'Smooth Cayenne' is a wonderful processing cultivar because it is high yielding, adaptable and has good canning characters (Bartholomew et al., 2003). However, in China, this cultivar is highly sensitive to natural flowering during winter months. The natural flowering rate is beyond 95% for large plants and about 50% in mid-sized plants (having 30 to 35 leaves that are longer than 30 cm). To understand the flowering mechanism of pineapple, 'Smooth Cayenne' was selected to investigate endogenous hormone changes during inflorescence induction and development induced by ethephon.

Plant hormones are chemicals occurring naturally within plant tissues, which are generally active at very low concentrations. They control the shape of the plant and

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Abbreviations: ABA, Abscisic acid; ZT, zeatin; IAA, indole-3-acetic acid; GA₃, gibberellic acid; C₂H₄, ethylene; 2iP, 2-isopentyl adenine; AVG, aminoethoxyvinylglycine; HPLC, high-performance liquid chromatography; FID, flame ionization detector; GC, gas chromatograph.

mediate growth response on external factors such as light and temperature. Up till now, five groups of plant hormone were found namely; auxins, cytokinins, gibberellins, ethylene and abscisic acid (George et al., 2008). Indole-3-acetic acid (IAA) is the major auxin involved in many of the physiological processes in plants (Arteca, 1996). Gibberellic acid (GA_3), is the first gibberellin to be structurally characterized and it is not easily degraded to inactive conjugates by plant metabolism. Of the naturally occurring cytokinins, only zeatin and 2-isopentyl adenine (2iP) have some use in plant tissue culture showing that both of them are active. Therefore, the content of IAA, GA_3 , abscisic acid (ABA) zeatin (ZT), 2-isopentyl adenine (2-iP) and ethylene (C_2H_4) were measured in this study.

MATERIALS AND METHODS

Pineapple (*A. comosus* cv. Smooth Cayenne) plants were potted and grown in a greenhouse in South Subtropical Crops Research Institute of China Academy of Tropical Agriculture Science. The size of pot was 40 cm in diameter and 50 cm in height, containing 10 kg growth medium which was a mixture of brick-red soil and cow manure (9:1). Plants were irrigated every 7 days and fertilized every month.

14-month-old plants were selected for flower forcing. To get 100% flowering rate and orderly development, no nitrogen was supplied in a month, and there was no irrigation in two weeks before forcing. The forcing solution containing 400 mg/l ethephon and 2% urea was applied into the shoot apex at 50 ml for each plant (Liu, 2009). At the mean time, only 2% urea solution was applied to the control plants. The experiment was repeated three times in different blocks.

Ten shoot apices (about 1.5 cm^3) and 10 white bases of D-leaf (defined as the youngest physiologically mature and fourth visible leaf from the shoot apex (Devadas, 2005)) were sampled from each treatment every four days. Five apices were immediately put into formalin acetic alcohol (FAA) solutions for anatomy analysis; and the remaining five apices and 10 white bases of D-leaf were immediately put into an ice bag and taken to the laboratory. Fresh tissues were used for C_2H_4 determination while other endogenous hormone analysis used tissues stored at -80°C .

Determination of C_2H_4 concentration

C_2H_4 concentration was determined following the method described by Min and Bartholomew (1997) using a gas chromatograph (Agilent 6890 GC; Agilent Technologies, Inc., USA). C_2H_4 was measured with a HP-Plot Q fused silica capillary column and a FID (flame ionization detector) detector. The oven temperature was 60°C and the FID detector temperature was 250°C . Nitrogen (99.99%) was the carrier gas and the column flow rate was 6 ml/min. A known concentration (100 ppm) of ethylene gas (Shanghai Weichuang Standard Gas Limited Company, Shanghai, China) was used as the standard.

Determination of IAA, ZT, ABA and GA_3 concentration

IAA, ZT, ABA and GA_3 were extracted and analyzed using HPLC (high-performance liquid chromatography) following the method described by Zeng et al. (2008) with a few modifications. All the operations were performed under weak illumination. For ABA, IAA

and GA_3 , the eluting agent was a mixture of methanol, acetonitrile (CH_3CN) and phosphorous buffer (pH3.5) (20:60:20, vvv), and the detecting wavelengths were 210, 210 and 240 nm, respectively. For ZT and 2-iP, the eluting agent was a mixture of methanol, acetonitrile (CH_3CN) and phosphorous buffer (pH 3.5) (15:15:70, vvv), and the detecting wavelength was 254 nm. Standards of ABA, IAA, GA_3 , ZT and 2-iP (Sigma Corporation, USA) were used to calculate their concentrations.

Determination of inflorescence developmental stage

Paraffin serial sectioning method was used to determine the stage of inflorescence development. The method was as described by Zhang et al. (1983). The fixed materials were stained with hematoxylin before embedding and sectioning. Samples were observed and photographed under an Olympus BH2 microscope.

RESULTS

Inflorescence development in pineapple plants forced by ethephon

The paraffin section results showed that all the plants without ethephon treatment remained in the state of vegetative growth, as the shoot apex was flat and the phyllome wrapped around shoot apex very closely (Figure 1A). However, plants that were treated with ethephon showed sign of inflorescence development eight days after the applications. The apex widened, the phyllome stopped developing and the inflorescence primordium formed (Figure 1B). 12 days later, sepal, petal, pistil and stamen differentiated successively in the floret of the first-layer (Figure 1C). 32 days later, the shoot apex stopped floret differentiation and resumed to the vegetative state (Figure 1D).

Changes in contents of endogenous hormones during inflorescence initiation and development

Immediately after ethephon application, the liberations of ethylene in both shoot apex and D-leaf increased very significantly (Figure 2A). In the 8th day, it reached the climax ($F = 1354.47$, $p < 0.01$). At the mean time, the structure of the shoot apex also changed, such as, the shoot apex widened, the inflorescence primordium was formed and the leaf primordium grew very slowly (Figure 1B). However, liberation of ethylene decreased to the control level at 12 days (Figure 2A), which indicates that ethephon treatment did not affect the release of endogenous ethylene once inflorescence initiation was completed.

ABA contents in both D-leaf and shoot apex reached climax four days after ethephon application (Figure 2B). After that, the ABA content in the shoot apex decreased continuously. The ABA content in D-leaf also decreased, but not continuously. In the mid and late stage of the inflorescence development, ABA content in the D-leaf

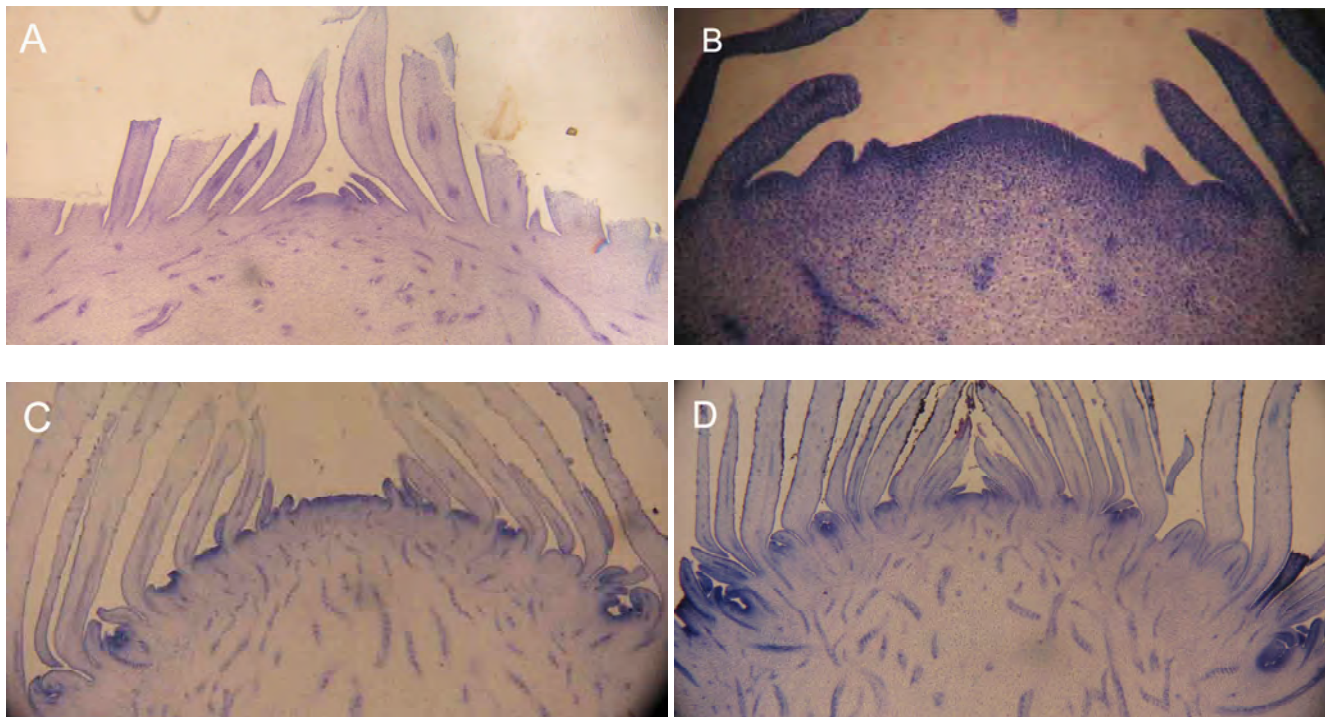


Figure 1. A to D, paraffin sections of shoot apex of pineapple (*Ananas comosus* cv. Smooth Cayenne) at different developmental stages. A, the vegetative shoot apex of pineapple plants, $\times 40$; B, the shoot apex of pineapple plant 8 days after ethephon application, showing the inflorescence initiation, $\times 40$; C, the shoot apex of pineapple plant 12 days after ethephon application, showing the development of floret, $\times 40$; D, the shoot apex of pineapple plant 32 days after ethephon application, showing the returning to vegetative state of the shoot apex, $\times 40$.

increased slowly. Senescence of D-leaf during inflorescence development may cause the rise in ABA content. ABA contents of both D-leaf and shoot apex in the control plants did not show any dramatic changes (Figure 2B). These results indicate that the application of ethephon could cause increase in the contents of endogenous ABA, so high contents of endogenous ABA facilitated inflorescence initiation, but was not necessary for floret development.

GA_3 contents in the D-leaf and shoot apex in the ethephon treated plants were lower than that in the control plants both in the process of inflorescence initiation and development (Figure 2C). GA_3 contents in the D-leaf and shoot apex dropped quickly four days after ethephon application. GA_3 content in the D-leaf kept dropping until 20 days after ethephon application, and then increased slowly. GA_3 content in the shoot apex kept dropping until 16 days after ethephon application, and then increased slowly.

IAA and ZT contents in both D-leaf and shoot apex in ethephon-treated plants were lower than that in the control plants at the early stage of inflorescence formation, but was higher than that in the control plants at later stage of inflorescence formation. Shortly after ethephon application, they decreased and reached the lowest point at four days after ethephon treatment. After

that, they increased continuously until 28 days after ethephon treatment. The changing curve of IAA content was similar to that of ZT, except that the increasing extent of IAA content was larger than that of ZT and the rise of IAA content was later than that of ZT.

2-iP content in the shoot apex of ethephon treated plant was much higher than that of the control and the content in the shoot apex was much higher than that in the D-leaf. The content of 2-iP in both shoot apex and D-leaf increased rapidly after the ethephon treatment and reached the climax at 8 days, which was remarkably higher than that of any other stage, then declined sharply. From 12 days, the content began to increase in a small scale until the end of differentiation (Figure 2D and E).

Changes in ratios between endogenous hormones during inflorescence initiation and development

This study analyzed changes in ratios between endogenous hormones during inflorescence initiation and development. The results show that in plants treated with ethephon, the ratio of C_2H_4/GA_3 , C_2H_4/ZT and C_2H_4/ABA all reached the climax at 8 days after ethephon application, and then dropped dramatically to the control level at 12 days after ethephon application (Figure 3A).

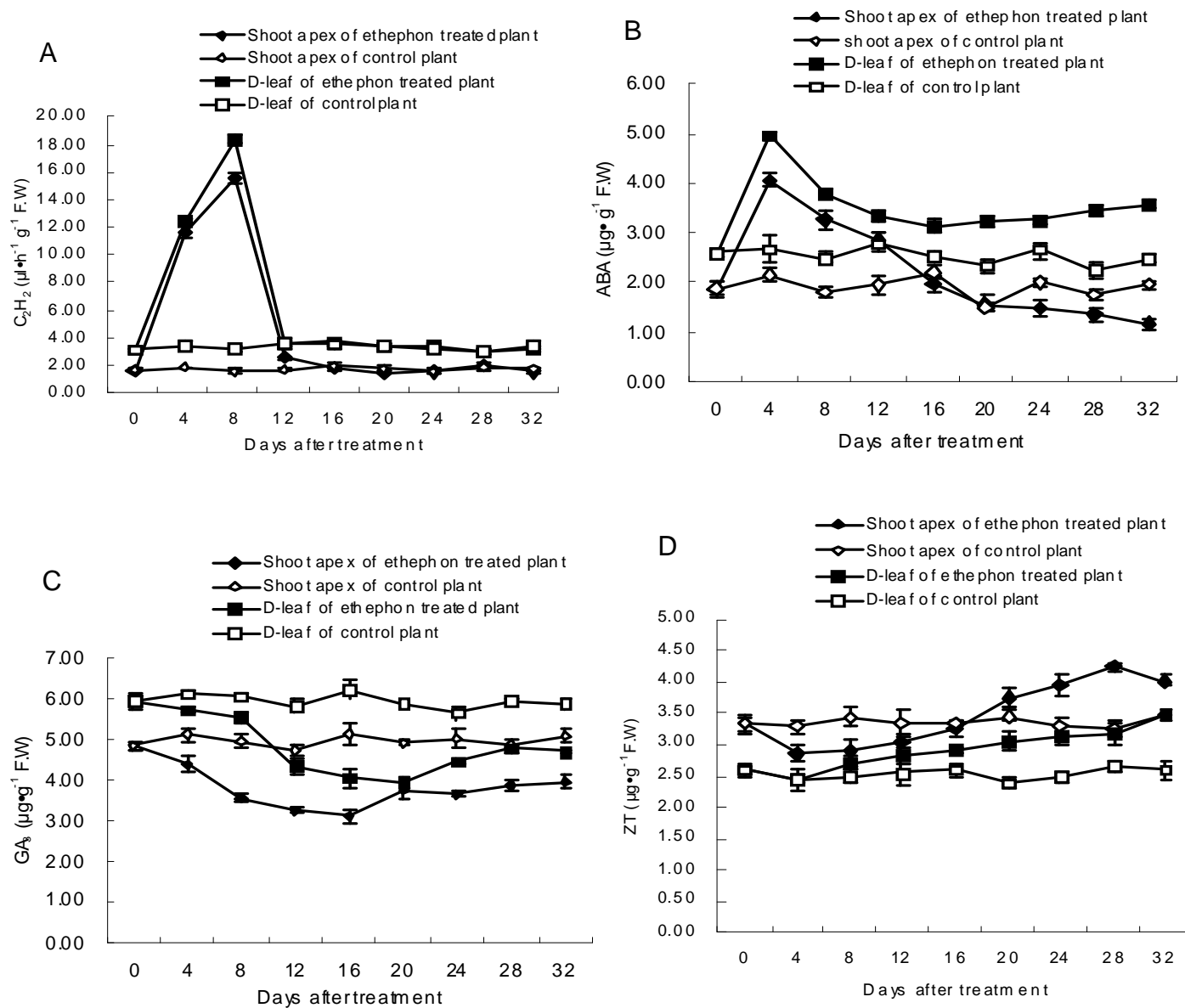


Figure 2. A to F, changes in concentrations of the six hormones in shoot apex and white base of D-leaf of pineapple plants treated with 50 ml of ethephon and control plants. Each data point represents the mean SE of three replicate (n=3). A, C₂H₄; B, ABA (Abs); C, GA₃; D, IAA; E, ZT; F, 2-iP.

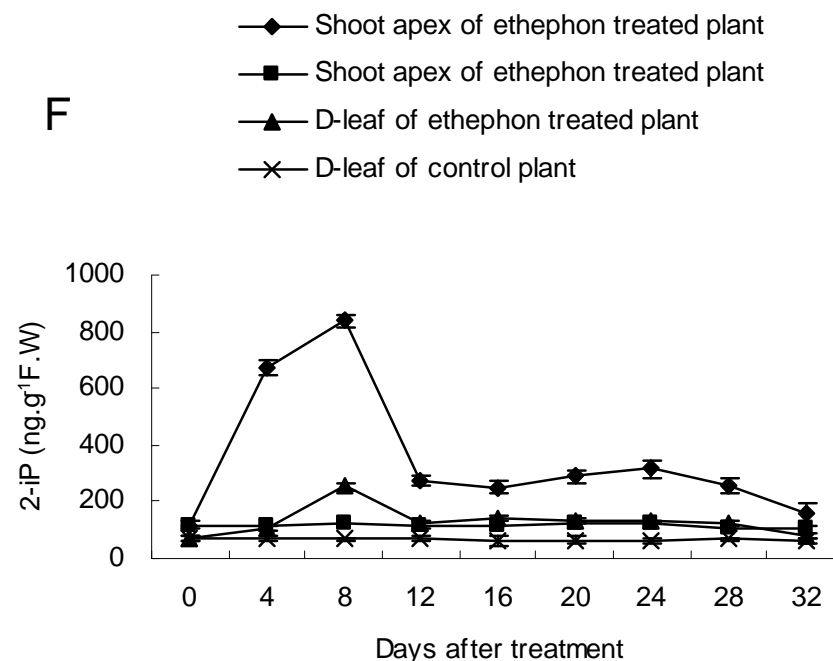
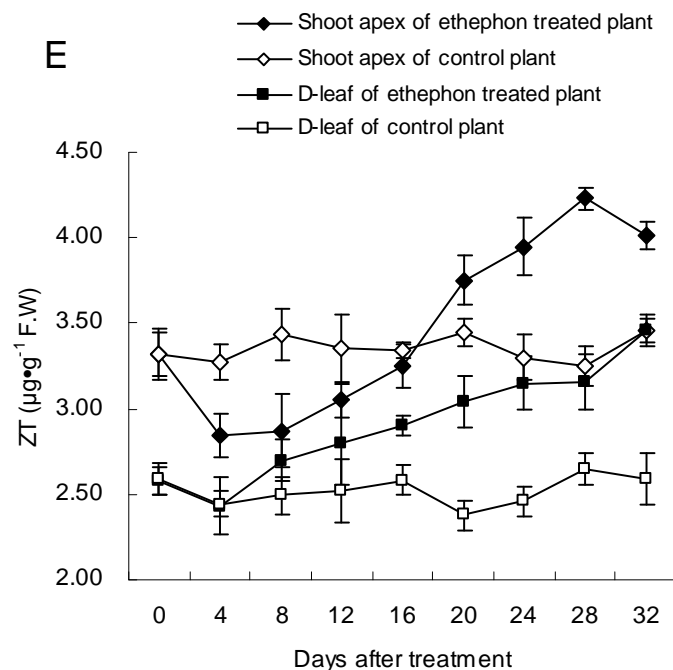


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Among them, the ratio of C_2H_4/GA_3 was much higher than that of C_2H_4/ZT and C_2H_4/ABA . The ratio of ABA/ZT , ABA/IAA and ABA/GA_3 reached the climax at 4 days after ethephon application, and then dropped continuously to the control level at 24 days after ethephon application (Figure 3B). Obviously, C_2H_4 played a more important role in pineapple's flowering induction than ABA; while GA_3 played a more important role in flowering inhibition than ZT and IAA. The ratio of $(C_2H_4 + ABA)/(GA_3 + ZT + IAA)$ increased immediately after ethephon treatment and reached the peak at 8 days after treatment, and then decreased to the control level at 12 days after treatment (Figure 3C).

DISCUSSION

The relationship between ethylene and pineapple flower induction

Many studies confirmed that ethylene (C_2H_4) plays an important role in flowering induction of fruit trees. In mango trees, fruiting shoots contain higher ethylene content than vegetative shoots, and shoots in on-year trees also contain higher ethylene content than the ones in off-year trees, indicating that endogenous ethylene is an important flowering induction factor (Saidha et al, 1983). In apple trees, ethylene level in bearing shoots is thrice that of vegetative buds (Klein

and Faust, 1978). In twisted shoots apple, IAA and cytokinins decreases while ethylene accumulates rapidly, and if the shoot is treated with Aminoethoxyvinylglycine (AVG, a C_2H_4 synthesis inhibitor), the concentrations of both IAA and cytokinins increase, resulting in inhibition of inflorescence formation (Sanyal and Bangerth, 1998). In this study, application of 400 mg/l ethephon induced pineapple plants to release large amounts of C_2H_4 , which reached the climax when the inflorescence initiated. Both the changing curve of C_2H_4 and that of the ratios between C_2H_4 and other endogenous hormones indicate that C_2H_4 was a predominant factor in pineapple flowering induction.

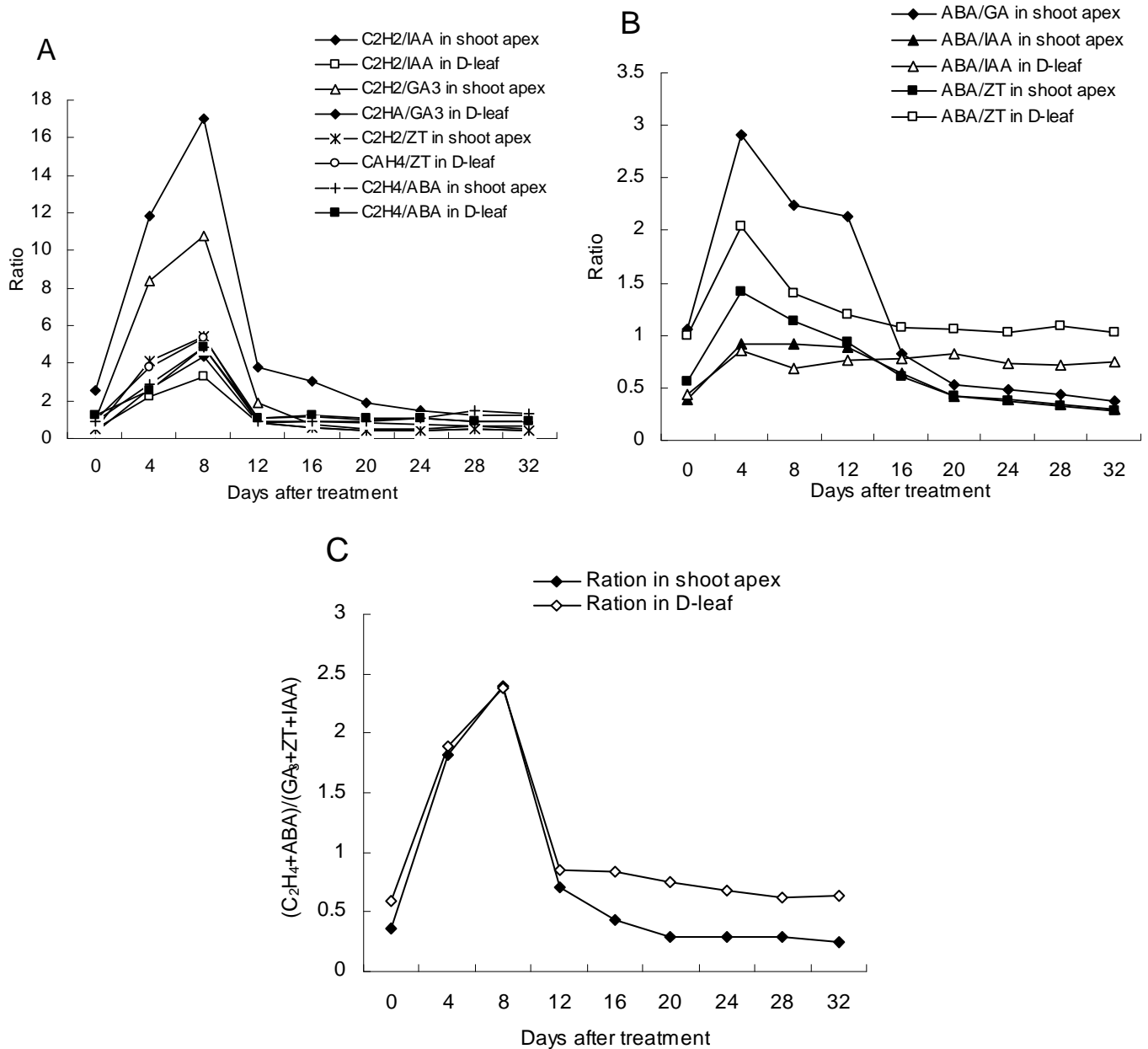


Figure 3. A to C, changes of the ratios between different hormones in shoot apex and white base of D-leaf of pineapple plants treated with 50 ml of ethephon. A, ratios between ethylene (C₂H₄) and other hormones; B, ratios between ABA (Abscisic acid) and other hormones; C, ratio between (C₂H₄+ ABA) and (GA₃+ZT+IAA).

The relationship between ABA and pineapple flower induction

ABA distributes ubiquitously in all organs and tissues of plants, and is abundant in the will-be falling organs and tissues, especially in stress conditions (Wu, 2008). Endogenous ABA plays a role in flower induction of mango and litchi trees (Chacko, 1986; Hou, 1987). Mei and Tan, (2002) proposed that in flower induction, ABA may modulate the ratio or balance between hormones.

In this study, ABA content increased shortly after ethephon application, then decreased during floret formation, indicating that ABA played a cooperative role in pineapple flowering induction.

The relationship between IAA, GA₃ and pineapple flower differentiation

There are different results about the effects of IAA and

GA₃ on flower differentiation. Bangerth (1998) argued that the reduction of IAA is a necessary condition for apple flowering. While for Shorttube Lycoris, relatively high IAA contents benefit flowering induction, and lower IAA is good for flower formation (Wang, 2004). As for GA₃, it stimulates flowering of coffee (Ursulak et al., 1990), but inhibits flowering of poplars (Wang and Xu, 2001). In this study, IAA decreased quickly after forced flowering by ethephon and increased during the floret development, indicating that lower IAA level was good for pineapple's flowering initiation and higher IAA level was good for floret development. GA₃ might have the same effect as IAA on pineapple's flowering induction and floret development, indicated by their similar changing curve after ethephon application.

The relationship between cytokinins and flower differentiation

Cytokinins could promote cell division and flower initiation in many fruits; synthesized in roots then transported to the shoot with the help of leaf transpiration. So, the leaf play more important role than nutrient supply (Luckwill, 1970). Li et al., (1985) reported that after the critical period of flower bud differentiation, the cytokinins level in the shoot of on-year Litchi trees increased gradually and reached the climax when floral organ began to differentiate, however, it kept much lower status in the vegetative buds of off-year trees. Shi (2005) also reported that cytokinins played a key role in flower differentiation of ornamental pineapple. In this study, ZT increased continuously and slowly during the floret development, indicating that ZT had no effect on flowering induction but was needed at the stage of floret development. Because ZT level indicates the active extend of cell division and metabolism of plant (Zhang et al., 1990), higher level of ZT in floret development observed in this study might be a signal of the higher quality of floral organ differentiated. As another kind of active cytokinins, 2-iP content was much lower than ZT, and the two substances demonstrated different changing rule after the treatment; 2-iP increased quickly but ZT decreased, which was similar to *Aechmea fasciata* cv variegata (Shi, 2005). After the flower initiation, ZT exhibited a violent increment until the end of differentiation, but 2-iP just increased a little bit. This study indicate that ZT had no effect on inflorescence initiation, but had a role in floret development of pineapple, and 2-iP might have positive effect on the inflorescence initiation, but little influence on floret development.

The relationship between hormone balance and flower differentiation

There are many kinds of hormones that affect and control flower bud differentiation of fruit trees. Luckwill (1970) first

put forward that it is the balance of endogenous hormones that affect and control plant's flowering. An et al. (2009) suggested that the balance of GA₃/cytokinins or GA₃ affect apple's flower differentiation, while GA₃ is the inhibitor and cytokinins is the promoter. In this study, among the six hormones tested, C₂H₄ contents changed dramatically, and the ratios of C₂H₄ between the other four hormones changed in a similar way. These results indicate that C₂H₄ played a predominant role in pineapple's flowering induction, while ABA and 2-iP was a co-operative factor, and the other three hormones inhibited flowering induction.

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