Forced flowering of pineapple (*Ananas comosus* cv. Tainon 16) in response to ethephon with or without calcium carbonate

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**Abstract:** As a gaseous plant hormone, ethylene is responsible for inducing the initiation of reproductive development in pineapple. Reproductive development can be forced in pineapple (*Ananas comosus*) throughout the year with ethylene. Ethephon is the most common agent applied in pineapple forcing with high uniformity and convenience. We studied the effect of additive added to the ethephon solution on the plant and fruit of pineapple. Shoot apices of pineapple treated twice to evaluate the effect. Results showed that 800mg/L ethephon could induce flowering successfully; addition of calcium carbonate could reduce the dose of ethephon significantly from 800 to 50. Furthermore, the addition of calcium carbonate not only caused the prolongation of the peduncle and increasing of slip number, but also increased fruitlet numbers and fruit weight with a highly significant difference. 100mg/L ethephon with 0.04% calcium carbonate was the optimum forcing treatment for Tainon 16 cultivar.

1. Introduction

Pineapple (*Ananas comosus*) is a kind of typical tropical fruits in China. One plant only bear one fruit, so it is very important for pineapple to get synchronized flowering[1], especially for those cultivars applied for fresh consumption. Natural flowering can cause serious scheduling problems for orchardists. Reproductive development in pineapple can be induced by short day-length and cool night temperatures under natural climate conditions in winter[2]; however, other stresses can also induce flowering[3]. In Zhanjiang (latitude20°-21°35’N), natural induction begins in December as a result of successive drops in temperature and little rainfall from October[4].

Natural flowering induction in pineapple in Zhanjiang area is assumed to be principally regulated by ethylene burst in the shoot apical meristem because of lower night temperatures in winter. At the same time, recent research showed that natural flowering of ‘Tainon 17’ pineapple can be prevented with aviglycine, which is an inhibitor of ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity inhibiting ethylene biosynthesis[5-6]. Any chemical or physical agents which can promote ethylene release or stimulate ethylene production by the plant can force pineapple flowering to get high uniformity of flowering and harvest in commercial production, such as Ethephon, calcium carbide, NAA, and so on. Ethephon is the most common agent applied in pineapple forcing with high uniformity and convenience which degrades to produce ethylene and may also stimulate ethylene production by the plant, was identified in the 1960s[7]. It was reported that adjusting the acidity of the ethephon solution to a pH 8 or 10 can accelerate the release of ethylene increasing, then reduce the concentration of ehephon to 20-40mg/L from 400-600mg/L with additive of 0.04% calcium carbonate to the solutiong of ethephon in Smooth Cayenne[8]. However, no proofs showed that the difference of
the influence of the ethephone with or without calcium carbonate on the plant growth and fruit quality. The cultivar ‘Tainon 16’, which was bred by Taiwan Chiayi agricultural experimental institute in 1995. As an excellent cultivar for fresh market with high sugar content and low acidity, it became more and more popular since introduced to mainland of China \[9-10\]. This cultivar is difficult to force in off-season production and the forcing efficiency and fruit quality are greatly influenced by both the kind and concentration of forcing agent. Ethephon with the concentration of 800mg/L were applied successfully to force plants at Hainan province \[9\]. However, with the development of social economy and improvement of people’s living standards, the less the chemical agents applied, the more popular the fruits would be and more friendly to the environment. Hence, the present study was conducted in order to obtain the optimum Ethephon concentration, and the difference of Ethephon with or without calcium carbonate on the fruit and plants after forcing were also analyzed.

2. Materials and methods

2.1. Materials and reagents
The experiment was conducted in November on Guangqian farm, Suixi County, Guangdong province, China. One-year-old cultivar ‘Tainon 16’ was used in all the experiments. Uniformed pineapple plants were selected and marked and given no nitrogen fertilizer a month before forcing. The Ethephon was 40% obtained by Pengpu chemical factory of shanghai, china and calcium carbonate was of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Plants were treated by different levels Ethephon solution (50 mg/L, 100 mg/L, 200 mg/L) with 0.04% calcium carbonate by pouring to the center of the rosette of the plant. Another treatment was conducted with only Ethephon solution with the concentration of 200mg/L and 800 mg/L. Control plants were treated with water. 60mL solution were applied for each plant. And a week later the above-mentioned treatment was conducted again to ensure satisfactory results. The experiment was repeated at least three times with three replicates in each test.

2.2 Investigation of pineapple flowering and appearance quality
50 days later, the forcing result including the flowering date and flowering rate was investigated and counted. 180 days later, the fruits of experimental plants were harvested and the fruit quality including appearance such as the fruit shape index (longitudinal diameter / horizontal diameter) In addition the fruitlet number and fruit weight were also be counted.

2.3 Chemical analyses of fruit internal quality
The total titratable acidity was assessed as outlined by AOAC \[11\]. Vitamin C was determined by the Dichloroindophenol method AOAC \[12\]. Soluble solids were determined using an Abbe refractometer \[13\]. Total carbohydrates were estimated by the Anthrone Method \[14\].

2.4 Data analysis
DPS 9.5 (Statistical software, Zhejiang university, China) was used to analyze the data. Multiple Range Test (LSD) was used to assess differences among different treatments at 95% confidence level.

3. Results and discussion

3.1 Efficiency of different flowering induction agents
At between 30 and 40 days after treatment, visible changes could be observed. A round coin-sized inflorescence emerged in the heart of the plant, and crown leaves are visible, the leaves (bracts) in the centre of the leaf whorl have turned red. Then at between 60 and 70 days, the floret can be seen. Higher concentration of Ethephon significantly enhanced the flowering percentage, and if applied with calcium carbonate, Ethephon concentration will decrease at large scale at only 50mg/L (Table 1). Both the control plants and plants with 200mg/L treatment did not show any flowering. Plants with
800mg/L treatment caused a perfect homogeneity percentage of 100%. The addition of calcium carbonate decreased the acidity of the inducer solution to a pH 8.5 increases the efficiency of ethephon, making possible the use of reduced amounts of the product, once the release of ethylene is favored in alkaline solutions [8]. Compared with treatments which added calcium carbonate, plants treated with only Ethephon burst into blossom and matured a week earlier than three other treatments (table 1).

Pineapple is a kind of multiple fruit with many fruitlets. Fruitlet numbers decide the fruit size to some extent. Forcing treatment not only affected the flowering rate and flowering date but also affected the length of peduncle, slip number, fruitlet number and fruit weight (table 2). Plants treated by Ethephon with calcium carbonate bear fruits with much longer peduncle, more slips, more fruitlet number than by only Ethephon. All the treatments from 50 to 200mg/L Ethephon with calcium carbonate caused highly significant difference from only Ethephon treatment in length of peduncle, slip number, fruitlet number. As for fruit weight, 100-200mg/L Ethephon with 0.04% calcium carbonate was highly significant higher than 800 mg/L Ethephon treatment and 50mg/L Ethephon with 0.04% calcium carbonate treatment.

### Table 1. the flowering rate and date by different forcing treatments

| Treatment                      | flowering rate (%) | flowering dates from treatment | Maturing days from treatment |
|--------------------------------|--------------------|--------------------------------|-----------------------------|
| CK                             | 0                  | /                              | /                           |
| 200 mg/L ethephon              | 0                  | /                              | /                           |
| 800mg/L ethephon               | 100                | 60                            | 185                         |
| 50mg/L ethephon with CaCO3     | 100                | 67                            | 192                         |
| 100mg/L ethephon with CaCO3    | 100                | 67                            | 192                         |
| 200mg/L ethephon with CaCO3    | 100                | 67                            | 192                         |

* Different lowercase/capital letters in the same column indicate significance of difference at $P<0.05$ / $P<0.01$ levels. The same below.

### Table 2. Slip number and peduncle length of plants by different forcing treatments

| Treatment                      | Length of peduncle(cm) | Slip number | Fruitlet number | Fruit weight(kg) |
|--------------------------------|------------------------|-------------|-----------------|-----------------|
| 800mg/L ethephon               | 9.54bB                 | 1.16bB      | 100bB           | 1.21bA          |
| 50mg/L ethephon with CaCO3     | 20.17aA                | 4.00aA      | 127aA           | 1.29abA         |
| 100mg/L ethephon with CaCO3    | 20.96aA                | 4.13aA      | 136aA           | 1.47aA          |
| 200mg/L ethephon with CaCO3    | 19.24aA                | 3.53aA      | 129aA           | 1.35aA          |

3.2 The interior quality of pineapple fruits by different forcing treatments

The content of vitamin C, sugars and acids in mature fruits from different forcing treatment were measured in this experiment. Results showed that there were no significant different in the content of vitamin C, total acids and total soluble solids. However, the total soluble sugar content in both 50mg/L and 100mg/L Ethephon 0.04% CaCO3 treatment was highly significantly higher than by 800 mg/L Ethephon treatment and 200 mg/L Ethephon with 0.04% CaCO3 treatment. Total soluble sugar is assumed to be influenced by the length of peduncle because the longer peduncle was, the much more sunshine would be obtained.

### Table 3. Interior quality of pineapple fruits by different forcing treatments

| Treatment                      | Vitamin C(mg.100g^{-1} FW) | Total acid (%) | Total soluble sugar | Total soluble solids |
|--------------------------------|-----------------------------|----------------|---------------------|---------------------|
| 800mg/L ethephon               | 9.61a                       | 0.41a          | 7.57bB              | 17.08ab             |
| 50mg/L ethephon with 0.04% CaCO3| 9.81a                       | 0.37ab         | 9.47aAB             | 17.78ab             |
| 100mg/L ethephon with 0.04% CaCO3| 9.75a                       | 0.37ab         | 9.68aA              | 18.64a              |
The technique of Tainon 16 pineapple was studied in the research. Results showed that 800mg/L Ethephon could induce flowering successfully, addition of calcium carbonate could reduce the dose of Ethephon significantly from 800 to 50. Furthermore, the addition of calcium carbonate not only caused the prolongation of the peducle and increasing of slip number, but also increased fruilet weight with a highly significant difference. 100mg/L Ethephon with 0.04% calcium carbonate was the optimum forcing treatment for Tainon 16 cultivar.

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