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The crown plays an important role in maintaining quality of harvested pineapple

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ABSTRACT

Pineapple is a unique fruit partly because it has a beautiful crown and consumers around the world regard the crown as an integral part of pineapple fruit. However, farmers in some countries detach the crown at harvest and use it for propagation. It is not clear whether the detachment of crown affects quality of harvested pineapple. This study shows that decrowning aggravated internal browning by 55.2% and reduced SSC/TA ratio by 2.2, following 9-d storage, suggesting that decrowning deteriorated quality of the flesh and shortened shelf-life of fruit. Furthermore, decrowning increased reactive oxygen species (ROS), malondialdehyde (MDA), and phenolics levels, and upregulated polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) gene expression and activity, suggesting keeping crown intact prevented ROS generation and lipid peroxidation, and inhibiting phenolics biosynthesis and oxidation. Moreover, decrowning increased endogenous GAs(GA_1 and GA_4) and decreased endogenous ABA in pineapple tissues, suggesting crown is important for keeping balance between GAs and ABA. Exogenous application of ABA inhibited IB in pineapple with intact crown more effectively than in decrowned one, ABA application to crown only controlled IB as effectively as to both crown and fruit, and decrowning following ABA application to crown significantly compromised the efficacy. These suggest that the crown is the main source of endogenous ABA and that IB control depends on continuous supply of ABA from the crown. This study provides possibility for effectively controlling IB.

1. Introduction

Pineapple (*Ananas comosus* L. [Merr.]) is a perennial herb native to the American tropics (Evans et al., 2002). It contributes to over 20% of the world production of tropical fruits. Nearly 70% of the pineapple is consumed as fresh fruit in producing countries (Tassew, 2014). Pineapple is a unique fruit, not only because of its special flavor and attractive pulp color, but also because of its top, or crown. However, the beautiful crown may sometimes be a "burden" to businessmen, for its big size, normally 20%–80% of that of fruit in volume. Although it is clear that the crown would increase logistical expenses during storage and transport, pineapple industry in different countries deals with it in different ways. In Australia, the crown is detached at harvest and used for propagation. In some of the other pineapple growing countries, such as India (Mhatre, 2007) and Nigeria (Agogbua and Osuji, 2011; Salami, 2013), crowns are the preferred propagation material. In China, however, the crown is regarded as an essential part of pineapple product, and the wholesalers and retailers all keep the crown intact throughout the marketing chain. Apart from logistical and aesthetical consideration, the detachment of crown, leaving a considerable wound, may cause postharvest disorders and affect quality of pineapple, but which, to the authors' knowledge, no research has so far addressed.

Among postharvest disorders of pineapple, internal browning attracts most attention from researchers around the world, as most pineapple varieties are susceptible (Hassan et al., 2010), and IB often causes severe economical losses in producing countries (Rohrbach and Paull, 1982; Smith, 1983; Ko et al., 2006; Hong et al., 2013). In some countries like Australia, over the past decade, farmers have switched other varieties to such as 'Gold' or 'MD-2', which are less sensitive to IB. In China, however, the susceptible variety 'Comte de Paris' still makes up 90% of pineapple production.

Abbreviations: ABA, abscisic acid; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; IB, internal browning; MDA, malondialdehyde; TS, tungstate; ROS, reactive oxygen species; AsA, ascorbic acid; SSC, soluble solid concentrations; TA, titratable acids; TPC, total phenolic compounds; FW, fresh weight; $O_2^{\bullet-}$, superoxide radicals; H_2O_2, hydrogen peroxide; OD, optical density.

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The aim of this study is to investigate whether and how the crown affects IB and quality of pineapple, hoping to develop techniques that help maintain quality and reduce postharvest losses.

2. Materials and methods

2.1. Plant materials and treatment

Pineapple (*Ananas comosus* L. cv 'Comte de Paris',) fruit of 'Queen' group of at 70% maturity (commercial maturity in China, with fruit being green with a trace of yellow, a stage when the fruit has reached full size), 120 d after flowering, were collected from a commercial plantation in Xuwen County, Guangdong Province and transported 300 km to Guangdong Province Key Laboratory of Postharvest Physiology and Technology of Fruits and Vegetables, College of Horticulture, South China Agricultural University, Guangzhou, Guangdong Province. Experiments were conducted with 'spring pineapple', which is harvested in period between March and June, and 'autumn/winter pineapple', harvested between September to February. No experiment was conducted in summer, because farmers don't produce pineapple during that period, as the pineapple is not profitable in the presence of many other fancy fruits produced in summer. All experiments were repeated at least two times with pineapple from different seasons, as it has long been noticed that the 'spring pineapple' is less susceptible to IB than 'autumn/winter pineapple'(Zhang et al., 2015). Fruit uniform in size (average weight about 500 g) and free of physical injury or disease symptoms were selected. Regarding the crown, there are two treatments: fruit was either kept with intact crown (Crowned) or with the crown removed by cutting off (De-crowned). Solution of $GA_{4/7}$ (580 μM) or ABA (380 μM), containing 0.01% Tween 80, was sprayed as fine mist to the crown or fruit or both the crown and fruit until runoff and air-dried. Each treatment was applied to three replications, each containing 20 fruit. Following treatment, pineapples were wrapped with perforated (2 perforations per bag, each 5 mm in diameter) polyethylene film 0.04 mm thick, and kept at 20 °C in the dark at 95% RH. Samples of pulp tissues were collected at 6 h, 12 h, 24 h, 3 d, 6 d, 9 d and 12 d, frozen in liquid nitrogen and stored at −80 °C.

2.2. Internal browning severity assessment

IB incidence was assessed as we described previously (Zhang et al., 2015).

2.3. Evaluation of fruit skin yellowing and weight loss

The skin yellowing was determined using a 0–5 scale, where 0 = fruit skin is all green; 1 = fruit skin with yellow area <25%; 2 = 25–50% yellow area; 3 = 50–75%; 4 = yellow area >75%; 5 = all yellow. The yellowing index (RI) was calculated from these scores: $RI = \sum (N_X \times X)/\sum N_X$, where X represents rate of yellowing (0–5), and N_X represents the number of fruit at the corresponding rate.

Weight loss rate was assessed by weighing pineapple fruit for each treatment before and after storage. Three replicates, each with 10 fruit, were performed for each treatment.

2.4. Determination of soluble solids, titratable acids and ascorbic acid

For this, pulp 0.5 cm from the core and 1 cm from the fruit surface in the middle section of fruit was used. Pineapple pulp (100 g) was cut into small pieces, homogenized and filtered. The filtrate was used for measuring soluble solids concentrations (SSC), titratable acid (TA) (in g citric acid per 100 g) and ascorbic acid (AsA) contents according to methods described by Lu et al. (2010).

2.5. Determination of $O_2^{\bullet -}$, H_2O_2 and MDA

The contents of superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were determined according the method described by Zhang et al. (2015).

2.6. Assay of total phenolic compounds (TPC)

The contents of TPC were detected using the method we described previously (Zhang et al., 2015). Results were calculated and expressed as milli-grams of gallic acid equivalent (GAE) per kilogram of fresh samples.

2.7. Assay of enzyme activity

Polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were extracted and activity assayed according to the methods we described previously (Zhang et al., 2015). Here one unit of PPO was defined as an increase in A_{398} of 10 min^{-1} and one unit of PAL was defined as an increase in A_{290} of 10 h^{-1}.

2.8. RNA extraction, cloning and sequencing of PPO and PAL

The pineapple pulp samples from 3 fruit were cut into small pieces, well mixed, frozen in liquid nitrogen, and then ground to a fine powder in the presence of liquid nitrogen. Total RNA was extracted using the hot borate method of Wan and Wilkins (1994). First strand cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-rad) according to the manufacturer's protocol.

2.9. Gene expression analysis by semi-quantitative RT-PCR

Semi-quantitative RT-PCR was used for the analysis of PAL and PPO transcript accumulation in pineapple pulp following the method of we described previously (Zhang et al., 2010). The primers (Table 1) were designed according to known sequences of PAL (accession number AY098511.1) and PPO (accession number AF261957.1). The actin was PCR amplified as an internal control.

The PCR reaction mixture (20 μl) contained 2.5 μl, 10 μl PCR buffer, 2 μl dNTPs (2 Mm), 1 μl (10 Mm) of each primers and 0.25 μl Taq (5 U, Fermentas). The cycling conditions of POD and PPO were 94 °C 2 min for initial denaturation and a total of 27

Table 1
Specific sequences of primers for semi-quantitative RT-PCR.

Gene	Primer sequence (5′-3′)	Expected size (bp)	Genbank Accession NO
PPO	F: AGTGCCTGGTTTAGGTGTATT R: TGATGGTGGATTGGTATGG	410	AF261957.1
PAL	F: *GCTCCTCCCTAACGAACCTGTA* R: CTGAAGTCCGCCGTCAAGAAC	429	AY098511.1