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Short communication

Expression of expansin genes in the pulp and the dehiscence zone of ripening durian (*Durio zibethinus*) fruit

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ABSTRACT

Durian (*Durio zibethinus*) fruit was harvested at the commercially mature stage and stored at 25 °C. Durian fruit have 3–5 longitudinal dehiscence zones (DZs) in the peel, which are up to 40 cm long and 2 cm thick in large fruit. Dehiscence started a week after harvest, was hastened by exogenous ethylene, and delayed by 1-methylcyclopropene (1-MCP), showing that it is regulated by endogenous ethylene. Three genes encoding α-expansins (*DzEXP1-3*) were isolated. In the expression of these genes increased, prior to dehiscence. Pulp firmness decreased during storage. The decrease was hastened by ethylene and delayed by 1-methylcyclopropene (1-MCP). Exogenous ethylene promoted gene expression of *DzEXP1* both in the DZs and in the pulp. It had a smaller effect on *DzEXP2* in the zones and pulp, but did not affect *DzEXP3* expression. 1-MCP inhibited the expression of *DzEXP1* and, somewhat less, of *DzEXP2*, but did not affect *DzEXP3* expression, both in DZs and pulp. It is concluded that the close relationship between expression of *DzEXP1* and *DzEXP2* and both dehiscence and fruit softening suggests that these genes are involved in both processes.

Introduction

Durian (*Durio zibethinus*) fruit have 3–5 longitudinal sutures in the peel (pericarp; husk) covering the entire fruit, i.e. from its apical to basal end. These sutures open in overripe fruit, a process called dehiscence. Durian dehiscence zones (DZs) can be up to about 40 cm long in large fruit, and due to the thick peel, up to 2 cm thick. As there can be five DZs per fruit, the total cell separation area is as much as about 400 cm^2 per fruit. This means that durian fruit might have among the largest cell separation surfaces in the plant kingdom.

Dehiscence of durian fruit was hastened by exogenous ethylene (Sriyook et al., 1994; Khurnpoon et al., 2008), but the effect of an ethylene inhibitor apparently has not been reported. It therefore is not yet clear if the process is regulated by endogenous ethylene. Dehiscence has been studied in many systems (Addicott, 1982), recently mostly in anthers (Wilson et al., 2011) and in *Arabidopsis* seed pods. In *Arabidopsis*, mutants with non-functional ethylene receptors showed normal dehiscence, and treatment with inhibitors of ethylene action had no effect. The authors nonetheless suggested that a low threshold level of ethylene might be needed for cell separation (Roberts et al., 2002). In some other systems dehiscence was clearly promoted by ethylene, e.g. in the fruit peel of *Juglans* and *Carya* species (Addicott, 1982) and in tobacco anthers (Wilson et al., 2011). Dehiscence of the anthers in tobacco flowers was inhibited by 1-MCP, suggesting that is regulated by endogenous ethylene (Wilson et al., 2011).

At least the early stages of dehiscence are due to cell separation. This separation is the same as in abscission (Roberts et al., 2002). Durian fruit dehiscence is preceded by an increase in soluble pectin

Abbreviation: DZ, dehiscence zone.

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levels, concomitant with an increase in smaller pectin molecules, which indicates pectin degradation. Hemicellulose is also broken down. Increased activity has been reported of polygalacturonase, β-1,4-endoglucanase, pectin (methyl) esterase, and β-galactosidase (Khurnpoon et al., 2008). Khurnpoon (2007) sprayed durian fruit with gibberellic acid, which delayed dehiscence. The treatment reduced the activities of polygalacturonase, β-1,4-endoglucanase, pectin (methyl) esterase and β-galactosidase in the DZ, indicating that these enzymes are involved in the separation process.

The flesh (pulp) of durian softens quite rapidly after harvest. Exogenous ethylene induces earlier pulp softening (Sriyook et al., 1994; Maninang et al., 2011; Siriphanich et al., 2011) while 1-MCP can delay it (Maninang et al., 2011). Pulp softening is thus regulated by endogenous ethylene.

Expansins are among several enzymes involved in fruit flesh softening, at least in tomato (Hiwasa et al., 2003; Sane et al., 2005; Kunyamee et al., 2008; Yu et al., 2012). The expression of expansin genes, and fruit softening, was hastened by ethylene in e.g. tomato (Rose et al., 1997) and mango (Sane et al., 2005) fruit. In persimmons, fruit softening and the expression of expansin genes was delayed by treatment with gibberellic acid, which also delayed ethylene production (Zhang et al., 2012). In tomato, suppression of an expansin gene inhibited fruit softening, while over expression of this gene hastened softening (Brummell et al., 1999a,b). Expansins apparently disrupt the non-covalent bond between matrix glycans and cellulose microfibrils in cell walls (Rose et al., 1997; Dal Santo et al., 2013), but details of its action have not yet been elucidated (Dal Santo et al., 2013).

Ethylene-induced abscission of *Sambucus nigra* leaves was accompanied by increased expression of two expansin genes, suggesting that these genes might be involved in cell separation (Belfield et al., 2005). A possible role of expansins in dehiscence systems, including that in durian fruit, has apparently not yet been described. Accordingly, we isolated three expansin genes from the DZs and the fruit flesh (pulp) of durian and studied gene expression in these tissues. Treatments with ethylene and by 1-MCP were used to investigate the temporal relationship between gene expression and both dehiscence and fruit softening. We hypothesized that one or more expansin genes play a role in both dehiscence and softening of durian fruit. Changes in gene expression would support this notion.

Materials and methods

Plant material

Durian (*Durio zibethinus* Murr.) fruit cv. Monthong was obtained directly from an orchard in Chantaburi Province, Thailand. Harvest occurred 106 days after anthesis. Fruit were dipped in 500 $\mu L\,L^{-1}$ imazalil at the orchard, directly after picking. Fruit were air-dried for 2 h and transported to the laboratory in a temperature-controlled truck (20 °C) where they arrived within 12 h of harvest. Fruit were selected for uniformity of maturity and size.

Ethylene and 1-MCP treatments

In one group (72 fruit) about 0.5 mL of 480 $mL\,L^{-1}$ ethephon was brushed at the cut surface of the stalk. This is referred to as the ethylene treatment. A second group was placed in a sealed container (71 L) and treated with 1-methylcyclopropene (1-MCP) for 12 h at 25 °C (1-MCP treatment). 1-MCP was generated by adding water to 1-MCP (EthylBloc® from Floralife, Walterboro, SC, USA) powder, in a glass vial. This resulted in a final concentration of 1000 $nL\,L^{-1}$ of 1-MCP in the air. Fans were used in the chambers to maintain air circulation. Control untreated fruit were immediately stored at 25 °C and 85–90% RH, while fruit in the two treatments were stored at these conditions after the treatments.

Ethylene production

Ten fruit from each treatment were individually weighed and fruit were placed in 18.5 L plastic containers, 1 fruit per container, which were held at 25 °C. Air in the containers was flushed before closure. RH in the closed containers was 85–90%. After 30 min, a 1 mL gas sample was taken from the headspace, using a syringe. Ethylene was measured using a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan), equipped with a flame ionization detector and a 2.1 m × 2.4 mm stainless steel column filled with activated alumina. The column, injector and detector temperatures were 80, 150 and 150 °C, respectively.

Dehiscence

At regular intervals nine fruit per replication were visually scored for dehiscence, following Khurnpoon et al. (2008). These scores were: 0 = no dehiscence, 1 = dehiscence up to 1/4 of suture length, 2 = dehiscence up to 1/2 of suture length, 3 = dehiscence up to 3/4 of suture length, and 4 = dehiscence along entire suture. The dehiscence index of a fruit was calculated as follows:

$$\text{Dehiscence index} = \frac{\text{Dehiscence classification level} \times \text{Number of sutures at that level}}{(\text{Number of sutures})}$$

Firmness

At intervals 9 fruit per treatment were taken at random and assessed for pulp firmness. Firmness was measured using a FT-011 penetrometer (Effegi, Alfonsine, Italy) equipped with a cylindrical plunger, 0.5 cm in diameter. The plunger was inserted in the middle of the pulp mass, to a depth of 0.5 cm. The force was expressed as N per square cm.

Isolation of expansin genes

After measurement of firmness, pulp tissue was cut into 0.5 cm × 0.5 cm × 0.5 cm pieces, immediately frozen in liquid nitrogen and stored at −70 °C until further use. Material from the DZ was collected by excising a 0.5 cm band of tissue along the sutures. The material was cut into 0.5 cm × 1.0 cm × 1.0 cm pieces, immediately frozen in liquid nitrogen and stored at −70 °C until further use. Three batches of fruit were used at each measurement point, each batch containing 9 fruit.

Approximately 10 g of frozen samples were ground in a mixing mill (MM 301, Retsch, Haan, Germany). Total RNA was isolated from 2 g of pulp and dehiscence tissues, following the method of Chang et al. (1993). RNA was measured spectrophotometrically at 240, 260, and 280 nm. The RNA quality was confirmed by gel electrophoresis on a 0.8% agarose gel. All RNA samples were treated with DNase (DNA-free, Ambion, Thermo Fisher, Waltham MA, USA) to eliminate DNA contamination. cDNA was synthesized from 4 μg of DNA-free RNA following the protocol of SuperscriptIII kit (Invitrogen, Thermo Fisher, Waltham, MA, USA). Fifty-fold diluted cDNA was used for quantitative real-time RT-PCR.

Expansin genes were isolated using degenerate primers and 3′ race (Supplementary Table 1) designed from conserved regions, following the protocol of GeneRacer (Invitrogen). All primers were designed with Vector NTI 11.0 (Invitrogen). The size of the qPCR products was between 100 and 150 bp.