Abscisic Acid Metabolism during Fruit Development and Maturation of Mangosteens

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ABSTRACT. Endogenous abscisic acid (ABA), its 2-trans isomer (trans-ABA), phasic acid (PA), and dihydrophaseic acid (DPA) concentrations were quantified in the peel, aril, and seed of mangosteen (Garcinia mangostana L.). Changes in carbon dioxide (CO₂) and ethylene (C₂H₄) production and 1-aminocyclopropane-1-carboxylic acid (ACC) concentration in the peel and aril were also examined. ACC concentration and CO₂ and C₂H₄ production were high at the beginning of fruit development and gradually decreased toward harvest, which confirms that mangosteen is a nonclimacteric fruit. In the peel and aril, the increase in ABA concentration preceded the decrease in peel firmness and coloring of the peel. This suggests that ABA may induce the maturation of mangosteens. The state of ABA metabolism varied with the part of fruit. In the peel, PA and DPA were not considered to be predominant metabolites of ABA because their concentrations were low compared to ABA throughout fruit development. In contrast, in the aril and seed, it is possible that the PA-DPA pathway may be a main pathway of ABA metabolism because the concentrations of DPA in the aril and of PA in the seed directly coincided with the concentrations of ABA. The differences in the ABA metabolites between aril and seed may be caused by the rate of ABA metabolism. The concentrations of ABA and its metabolite in the seed decreased toward harvest.

Mangosteen (Garcinia mangostana L.) is cultivated in the humid tropics and is known as the queen of fruit because of its excellent taste, flavor, and shape. Although the changes in sugar and acid concentrations in fruit have been investigated (Ratanamarno et al., 1999), no study on physiologically active substances significant to development and maturation of the fruit has been published. Ethylene (C₂H₄) in climacteric fruit (Abeles et al., 1992) and abscisic acid (ABA) in nonclimacteric fruit (Kondo and Gemma, 1993; Kondo and Kawai, 1998) have been particularly associated with maturation and ripening. Mangosteens have been classified as nonclimacteric based on the respiratory pattern after harvest (Nakasone and Paull, 1998). However, the roles of C₂H₄ and ABA during fruit development are yet unknown.

In general, most tropical fruit store poorly, e.g., rambutans (Nephelium lappaceum L.) stored at temperatures higher than 10 °C become unmarketable within 4 d (O’Hare, 1995). In contrast, mangosteens can be stored for 2 to 3 weeks at room temperature, and for =1 month at 9 to 12 °C (Salunkhe and Desai, 1984). Mangosteen peel is =10 mm thick covering the white edible aril. The changes and concentrations of ABA in the peel and pulp tissues of rambutan differed throughout fruit development (Kondo et al., 2001). Therefore, in order to understand the development and maturation of mangosteen fruit, the peel and aril should both be investigated. The formation of C₂H₄ proceeds through l-methionine, S-adenosylmethionine (SAM), and 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979). The changes of ACC concentration coincide with those of C₂H₄ production in many cases (Hyodo et al., 1985). The previous reports showed that ABA concentrations increased rapidly from maturation toward harvest in climacteric apples, but those in nonclimacteric sweet cherries increased before maturation and then decreased toward harvest (Kondo and Tomiyama, 1998; Kondo et al., 1999). The changes in ABA, which differ with each fruit, suggest that the role of ABA may vary among fruit. Plants generally metabolize ABA through the unstable intermediate 8-OH-ABA to form phaseic acid (PA) through the action of a monoxygenase, and then form dihydrophaseic acid (DPA) through the action of a reductase (Zeevaart et al., 1991). If ABA is associated with the development and maturation of mangosteens, the examination of the changes in its metabolites may help to clarify the effect of ABA on fruit development.

In this study, ACC in the peel and aril of mangosteen was analyzed during fruit development and maturation. At the same time, ABA, its 2-trans isomer (trans-ABA), PA and DPA in the peel, aril, and seed were also quantified. The role of these substances in fruit development and maturation is discussed.

Materials and Methods

PLANT MATERIAL. Three randomly selected 5- to 6-year-old mangosteen trees of a local variety from a commercial orchard in Rayong province, Eastern Cape (lat. 13°N), Thailand were used for this experiment in 2000 and 2001. Fruit were collected from 14 d after full bloom (DAFB) until harvest (84 DAFB) at 7 to 14 d intervals for analyses of CO₂, C₂H₄, ACC, ABA, and ABA metabolites. Since the seed begins developing from the inner carpel wall just before 70 DAFB, the seed was collected on and after 70 DAFB. The peel without the outermost woody tissue, aril, and seed samples were immediately frozen in liquid N₂ and lyophilized. The peel color of two positions on the longitudinal part of the fruit was measured by a color-difference meter (CR-200; Minolta, Tokyo). Hue angle (0° = red-purple, 90° = yellow, 180° = blue-green, 270° = blue) was...
calculated according to the method reported by McGuire (1992). After peeling the outermost woody peel, the firmness of the fruit peel was determined with a Texture Analyzer (TA-XT2; Charpa Techcenter, Bangkok, Thailand) equipped with a needle of 2 mm in diameter. Nine fruit (three fruit per tree) were used for these measurements.

**PREPARATION OF PHASEIC ACID AND DIHYDROPHASEIC ACID.** β-Hydroxy-β-methylglutaric ester of 8′-hydroxycabiac acid isolated from immature seeds of black locust (*Robinia pseudacacia* L.) (Hirai et al., 1978) was used to prepare PA. The ester (43 mg) was dissolved in a mixture of 1 mL of methanol and 2 mL of 2 M NaOH aqueous solution, and left at room temperature for 5 h. The solution was diluted with 20 mL of water, and partitioned with 10 mL of ethyl acetate for four times at pH 2. The organic layer was washed with water, dried over sodium sulfate, and filtered. The filtrate was concentrated and subjected to silica gel (13 g) chromatography, using mixtures of toluene and ethyl acetate as the eluent. The materials eluted with 50% and 60% ethyl acetate were combined, and recrystallized from a mixture of n-hexane and ethyl acetate to give PA (17 mg). PA (8 mg) was dissolved in 1 mL of methanol, and methylated with 3 M of diazomethane ether solution to give PA methyl ester. PA methyl ester (8 mg) was dissolved in a mixture of 1 mL of methanol and 1 mL of water, and to that solution sodium borohydride (10 mg) was added. The reaction mixture was stirred at 0 °C for 1 h, diluted with 15 mL of water, and partitioned with 10 mL of ethyl acetate for four times. The organic layer was washed with water, dried over sodium sulfate, and filtered. The filtrate was concentrated and subjected to silica gel (6 g) chromatography, using a mixture of 2 toluene:3 ethyl acetate as the eluent, to give epi-DPA methyl ester (3 mg) and DPA methyl ester (3 mg). DPA methyl ester (3 mg) was dissolved in a mixture of 0.2 M of methanol and 1.5 M of 0.1 m NaOH aqueous solution, and left at room temperature for 3 h. The solution was diluted with 10 mL of water, and partitioned with 5 mL of ethyl acetate for four times at pH 2. The organic layer was washed with water, dried over sodium sulfate, and filtered. The filtrate was concentrated to give DPA (2 mg). Identification of PA and DPA was done by their 'H NMR spectra and mass spectra of their methyl esters (Milborrow, 1975).

**CO₂, C₆H₁₂, AND ACC ANALYSIS.** For CO₂ and C₆H₁₂ analyses, three fruit selected randomly were sealed in 650-mL glass vials for 3 h at 25 °C immediately after harvest. One milliliter headspace gas containing CO₂ and C₆H₁₂ was analyzed by gas liquid chromatography (GLC) (GC-380; GL Sciences, Tokyo) equipped with porous polymers column (Porapak Q, Waters, Milford, Conn.; 2.2 mm i.d. ×2.0 m). ACC concentrations in the peel and aril (0.5 g DW) were determined with a Texture Analyzer (TA-XT2; Charpa Techcenter, Bangkok, Thailand) equipped with a needle of 2 mm in diameter. Nine fruit (three fruit per tree) were used for these measurements.

**ABA, trans-ABA, PA, and DPA ANALYSIS.** ABA, trans-ABA, PA, and DPA in the peel, aril, and seed (1 g DW) were extracted using 0.2 μg (±)[3′,5′,7,7′,7″-H] ABA (d₆-ABA) as the internal standard in 80% methanol. The extract was first filtered through glass fiber filters (GFP; Kiriyama Instrument, Tokyo), and then reduced to the aqueous phase in vacuo. The pH was changed to 2.5 using 0.1 M phosphoric acid. ABA, trans-ABA, PA, and DPA were extracted using dichloromethane. The solvent was removed in vacuo, and the residue was redissolved in 1 mL of 4.8 M acetonitrile and introduced into a high-performance liquid chromatography (HPLC) (Japan Spectroscopic, Tokyo) equipped with an ODS column (Mightysil RP-18, Kanto Chemical, Tokyo; 4.6 mm i.d. ×25 cm). The detector was set at 254 nm, and the flow rate of eluent was 1.5 mL·min⁻¹ following a gradient program of acetonitrile containing 20 mm acetic acid (4.8 to 9.6 m acetonitrile over 30 min and then held at 9.6 m for 5 min). The fractions which contained ABA, trans-ABA, PA, and DPA standards were collected, dried in vacuo and then methylated with diazomethane. This method yielded a recovery rate of 68% to 71% for ABA, trans-ABA, PA, and DPA. The ABA-, trans-ABA-, PA-, and DPA-methyl esters were redisolved in methanol and measured by gas chromatography–mass spectrometry (GC–MS) (QP 5000; Shimadzu Scientific Instruments, Kyoto, Japan: column was CP-Sil 5CB (Chrompack, Middelburg, Netherlands; 0.25 mm i.d. × 25 m, 0.25 mm film thickness); linear He flow was set at 50.2 cm·s⁻¹, column temperature was a step gradient of 60 °C for 2 min, 60 to 270 °C at 10 °C·min⁻¹ and 270 °C for 35 min; and electron potential of 70 eV). Selected ion monitoring mode (SIM) was used to perform quantitative analyses. ABA-, trans-ABA-, PA-, and DPA-methyl esters had the following retention times: d₆-ABAs, 19.44 min; trans-ABA, 20.56 min; PA, 20.18 min; DPA, 20.85 min. Ions were measured for d₆-ABA and d₆-trans-ABA/d₆-ABA as m/z 260, 194 and 190, respectively. Ions were measured at m/z 276 and 294 for PA, and m/z 278 and 296 for DPA.

Fig. 1. Changes in peel, aril, and seed weight per fruit, peel firmness, and peel hue angle in mangosteens. Hue angle = [(ATAN (b/a)/2π) × 360°, 0° = red-purple, 90° = yellow, 180° = blue-green, 270° = blue]. Data are the means of nine fruit.
ABA and trans-ABA concentrations were calculated from the ratio of peak areas for m/z 190 (d_0)/194 (d_6). PA calculations came from the ratio of peak areas for m/z 276 (d_6)-PA/194 (d_6)-ABA, and DPA calculations came from those for m/z 278 (d_6)-DPA/194 (d_6)-ABA. Each concentration was calculated using a linear regression equation based on each standard chemical of different concentrations and (d_6)-ABA. Fragmentation patterns were compared with those of the chemical standards in total ion monitoring mode (TIM) to identify ABA-, trans-ABA-, PA-, and DPA-methyl esters in the samples.

Statistical analysis. Data were subjected to analyses of variance and LSD procedures were used for mean separation (SAS Institute, Cary, N.C.).

Results and Discussion

The weight of peel and aril of mangosteens increased until harvest (84 DAFB) (Fig. 1). In contrast, the seed weight increased slightly until 70 DAFB, and thereafter no increase in weight was observed. Peel firmness fluctuated between 6 and 7 N·mm⁻² until 77 DAFB, then decreased rapidly at 84 DAFB. Hue angle of the peel also decreased dramatically after 77 DAFB. These shifts indicate that 77 DAFB may be a transition from development to maturation. Production of CO₂ and C₂H₄ was highest at 14 DAFB and decreased gradually toward harvest (Fig. 2). In both the peel and aril, ACC concentrations were high at the beginning of fruit development and decreased with DAFB. Compared to climacteric fruit such as winter squash (Hyodo et al., 1985) and apples (Kondo et al., 1991), the ACC concentration and C₂H₄ production in mangosteens were very low. It has been reported that in apples and tomatoes, the stimulating effect of ABA on C₂H₄ production caused an increase in ACC synthesis (Lara and Vendrell, 2000; Riov et al., 1990). However, in our study, this association was never observed as C₂H₄ production from fruit and ACC concentrations of peel and aril decreased toward harvest but ABA increased. The results confirm that mangosteen is a nonclimacteric fruit.

The changes in ABA and its metabolites differed between the peel and aril. In the peel, ABA fluctuated around 20 mmol·kg⁻¹ DW until 70 DAFB, but there was an increase from this time until 84 DAFB when it reached a maximum of 25 mmol·kg⁻¹ DW (Fig. 3). The concentration of trans-ABA was twice of that of ABA in stored apples (Bangerth, 1982). Although this suggests that trans-ABA may increase with fruit senescence, a relationship between maturation of mangosteen peel and trans-ABA was not observed. ABA is metabolized to its β-D-glucose ester through estification and to PA and DPA by hydroxylation and reduction, respectively (Walton, 1980). If PA and DPA are primary metabolites of ABA in mangosteen peel, the level of fluctuations of PA or DPA should correlate with that of ABA. However in our study, PA and DPA concentrations were very low compared to ABA concentrations. Therefore, it is possible that the PA-DPA pathway may not be the main pathway that ABA is metabolized in mangosteen peel. When ABA was applied exogenously to sweet cherries, it was metabolized to the β-D-glucose ester (Kondo and Tomiyama, 1998). Our results suggest that β-D-glucose ester, formed by esterification, may also be the predominant metabolite of ABA in mangosteen peel. Although β-D-glucose ester does not have physiological activity, ABA is released under mild alkaline conditions. Thus, β-D-glucose ester is suspected to be the source of ABA. If β-D-glucose ester is converted to ABA in mangosteen peel, it may have a role in the senescence of peel tissue. However, research has shown that the ABA base-labile conjugate formed in tomato plants fed with [⁴C]-(R, S)-ABA could not be metabolized to ABA (Walton, 1980).

A previous report showed that ABA applied exogenously promoted anthocyanin biosynthesis in sweet cherry peel (Kondo and Gemma, 1993). In our study, the increase in endogenous ABA which occurs before the decrease in peel firmness or coloring after 77 DAFB suggests that ABA may induce these changes in the peel. ABA concentrations in the aril were relatively high at 28 DAFB and decreased until 56 DAFB but then increased again toward 84 DAFB (Fig. 4). The concentrations of trans-ABA fluctuated around 0.2 µmol·kg⁻¹ DW throughout development. PA changed similarly with ABA except at 84 DAFB. PA has been found to gradually increase as ABA decreases during the storage of peaches (Tsuchida et al., 1990). However, a similar phenomenon was not observed during the development of mangosteen in our study, and the concentrations of PA were 6% to 10% of ABA concentrations. However, this result may imply that ABA is metabolized to PA, except in the peel, as explained in the following. If PA is further metabolized immediately, low PA concentrations are expected. In practice, concentration of DPA, which is the primary metabolite of PA, corresponded to ABA concentrations throughout development. DPA concentrations increased rapidly from 2 µmol·kg⁻¹ DW at 28 DAFB to 17 µmol·kg⁻¹ DW at 42 DAFB. This increase is more than five times that of ABA (3 µmol·kg⁻¹ DW) which increased from 28 to 42 DAFB. The variation in both should coincide if DPA is the final metabolite of ABA. However, the downstream metabolite of DPA is unknown. There are no known reports on the rate of metabolism.

![Fig. 2. ACC concentrations and CO₂ and C₂H₄ productions in mangosteens. Data are the means of three replications.](image-url)
of ABA to DPA in fruit. If the ABA concentrations before 28 DAFB are higher than the level at 28 DAFB, then DPA may increase between 28 and 42 DAFB. There may be a small lag time between the decrease of ABA and increase of DPA. This conclusion is supported by the level of ABA decreasing 3 \( \mu \text{mol·kg}^{-1} \text{DW} \) at 42 to 56 DAFB and coinciding with the increase of a similar level of DPA at 56 to 77 DAFB. Our results suggest that PA and DPA may be the predominant metabolite of ABA in the aril of mangosteen. The physiological activity is 5% for PA and almost none for DPA compared to that of ABA (Hirai, 2001). Thus, their influences on fruit development may not be important, even if PA and DPA increased during the development stage. However, the significant increases of ABA after 56 DAFB in the aril and after 70 DAFB in the peel suggest that ABA may play a role in inducing fruit maturation in mangosteens. ABA, which increases toward harvest as observed in the peel and aril of mangosteen, differed from that of rambutans and sweet cherries, which are also nonclimacteric. In rambutans and sweet cherries, which have inferior storability, ABA increases earlier during development and reaches a peak before maturation (Kondo and Tomiyama, 1998; Kondo et al., 2001). In nonclimacteric fruit, the pattern of ABA increasing and peaking at harvest may indicate superior storability.

Tropical fruit seeds are typically recalcitrant, and lose their ability to germinate under low temperature and desiccating conditions (Nakamura, 1994). The continued presence of ABA may induce and maintain dormancy (Black, 1991). The low endogenous ABA concentrations in maturing seeds (Fig. 5) may relate to the lack of dormancy in the mangosteen seed. ABA metabolism in the seed differed from both the peel and aril in that the concentration of PA was high, and the concentration of DPA was very low in seed. The high PA concentration suggests that the primary metabolic pathway of ABA in the seed may be the PA-DPA pathway. However, the high concentration of PA and the low concentration of DPA imply a lower state of ABA metabolism in the seed than in the aril. These hypotheses on ABA metabolism in mangosteens, however, require further examination using radio-labeled ABA.

In summary, the changes in ACC concentrations in the peel and aril, and changes in \( \text{C}_2\text{H}_4 \) and \( \text{CO}_2 \) production by fruit confirmed that mangosteens are nonclimacteric. The increase in ABA in the peel and aril preceded coloring and the decrease in peel firmness. Therefore, ABA may trigger fruit maturation in mangosteens. In the aril and seed, PA and DPA were considered to be the predominant metabolites of ABA. In contrast, the concentrations of PA and DPA in the peel were very low.

**Literature Cited**


