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Communication

Sunlight-stimulated phenylalanine ammonia-lyase (PAL) activity and anthocyanin accumulation in exocarp of 'Mahajanaka' mango

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Abstract: The activity of phenylalanine ammonia-lyase (PAL) required for anthocyanin synthesis was stimulated by sunlight exposure resulting in the development of red colour in 'Mahajanaka' mango exocarp, which occurred only on the sunlight-exposed side of the fruit. The accumulation of anthocyanin was concurrent with the increase in PAL activity in the mature stage of the fruit. The exposed side of the fruit had higher PAL activity, endogenous sugar content, and anthocyanin accumulation than the unexposed side. It is concluded that sunlight increases red colour development of the mango exocarp by inducing PAL activity. Exposure to sunlight also enhances endogenous sugar accumulation in mango fruit.

Keywords: 'Mahajanaka' mango, PAL activity, anthocyanin in fruit exocarp, sunlight-induced anthocyanin synthesis, endogenous sugars

INTRODUCTION

'Mahajanaka' mango (*Mangifera indica* Linn. cv. Mahajanaka) is a hybrid cultivar between 'Nang Klang Wan' and 'Sunset' cultivars in Chiang Mai province, Thailand. The fruit is elongated and similar to 'Nang Klang Wan', with a thick exocarp and long edible portion of the thick yellowish-orange mesocarp. It is soft and has a good flavour and a thin pyrene, which makes it suitable for consuming and processing. The red colour of the exocarp is similar in intensity to

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'Sunset' mango and is due to anthocyanins [1-3]. However, 'Mahajanaka' mango develops non-uniform red exocarp colouration during fruit development. This reduces the value and quality of the fruit as it is unacceptable for foreign importers and consumers in Thailand.

Light induces anthocyanin synthesis and red colour formation in plants. Sunlight increases the activity of the key enzymes for anthocyanin synthesis, especially phenylalanine ammonia-lyase (PAL), dihydroflavonol reductase (DFR), anthocyanin synthase (ANS) and UDP-glucose flavonoid 3-o-glucosyltransferase (UFGT) [4-6]. Light also induces flavonoid synthesis involving several photoreceptors such as phytochromes and the photosynthetic system [6-7]. In an experiment performed by Singh et al. [6], etiolated maize seedlings grown for six days in dark conditions were exposed to sunlight for 4 hours and transferred back to darkness. Anthocyanins were found to form in all vegetative organs with a slow increase during 4-16 hours after exposure to sunlight and a rapid increase after 16-24 hours. PAL was also found to be stimulated with two distinct peaks of activity: the first peak during 4-12 hours and the second peak during 12-24 hours after exposure. Sunlight intensity in different conditions during fruit development seems to affect anthocyanin accumulation. As reported for apple (*Malus domestica*) in China [8], the cultivars 'Starkrimson' (with red exocarp) and 'Golden Delicious' (with yellow exocarp) in a highly irradiated area had higher anthocyanin accumulation than in the same cultivars grown in a less irradiated area. In both areas, PAL activity in the red apple exocarp was also higher than in the yellow one.

Sunlight regulates anthocyanin formation in vegetative tissues and organs (root, mesocotyl, leaf and coleoptile). More anthocyanin accumulates in sun-exposed exocarp than in shaded one [7-9]. In 'Elstar', 'Jonagold', 'Elshof' and 'Red Elstar' apples, the levels of cyanidin 3-galactoside (anthocyanin) and quercetin 3-glycoside (flavonol) in the sun-exposed exocarp of individual fruit were much higher than in the shaded side [10]. Anthocyanin levels were highest in fruits borne on the top of the tree and the outer tree parts, whereas the lowest levels of anthocyanin were found in fruits from shaded parts of the tree [7]. 'Delicious' apple fruits were covered with one-, two-, or three-layered paper bags to inhibit anthocyanin accumulation in the exocarp. After the bags were opened and exposed to light for three days, the fruit treated with the three-layered bag had the highest anthocyanin accumulation [9]. In 'Early Black' cranberry fruits, natural light conditions increased total anthocyanin levels by 75.3 % and 87.2 % after 24 and 48 hours respectively of water immersion [11].

Light indirectly increases anthocyanin accumulation by increasing endogenous sugars, which induce the gene expression of key enzymes in anthocyanin biosynthetic pathways, especially PAL enzyme. In 'Comet' red radish hypocotyls during cultivation in light (~50 $\mu E/m^2s$), total sugars (glucose, fructose and sucrose) increased in 21 days after sowing, and the sugar accumulation enhanced anthocyanin production in the hypocotyl [12]. In particular, sucrose-induced anthocyanin production occurred via increased gene expression of chalcone synthase (*CHS*) and anthocyanidin synthase (*ANS*) in 'Comet' red radish hypocotyls with three times higher ratio of *CHS:ANS* expression from planting until six days after [13]. In the 'Incicle' white radish, the two anthocyanin-specific genes (*PAL* and *CHS*) responded to sucrose, and other genes such as chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), *DFR*, and *ANS* were weakly expressed, causing less anthocyanin accumulation [13]. When Lisianthus (*Eustoma grandiflorum* cv. Royal Purple), a potted

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plant, was cultured in high-intensity light (15,000 lux), the petals had a higher accumulation of soluble sugars than that in a low light intensity (1,000 lux), and the high light intensity condition also induced the expression of *CHS*, *CHI* and *DFR* in anthocyanin synthesis [14]. In grape berries (*Vitis vinifera* cv. Shiraz) grown in sunlight, the total soluble solids of the fresh tissue increased during 8-16 weeks after flowering, along with an increase in anthocyanin accumulation in the berry exocarp after 10 weeks. Expression of seven anthocyanin biosynthesis genes (*PAL*, *CHS*, *CHI*, *F3H*, *DFR*, leucoanthocyanindin dioxygenase (*LDOX*) and *UFGT*) were enhanced during fruit development, especially *UFGT*, which was expressed only 10-16 weeks after flowering and coincided with anthocyanin accumulation in the berry exocarp [15]. Sunlight also promoted the synthesis of endogenous sugars, which induced the gene expression for increasing anthocyanin synthesis in *V. vinifera* cell suspension [16] and 'Comet' radish hypocotyl [12].

The two important factors affecting anthocyanin synthesis and accumulation are light and sugars. Fructose and sucrose were found to promote red colour development in the exocarp of 'Mahajanaka' mango [2], while the involvement of sunlight in red colour development has not been investigated. The objective of this study is to investigate the effects of sunlight on PAL enzyme activity, sugar accumulation, and anthocyanin accumulation during the development of 'Mahajanaka' mango exocarp.

MATERIALS AND METHODS

Plant Stock

Forty 7-year-old 'Mahajanaka' mango trees from an orchard in Chiang Mai province were used in this study. The experiment started in January and continued until the end of May 2010. Inflorescences were tagged to record the age of fruits from the day after full bloom (DAFB). After that, fruits at 70 DAFB (approximately 2 weeks prior to the beginning of red colour development in the fruit's exocarp as reported by Boonkan [1]) which hung on the outer part of the canopy were sampled at 7-day intervals until fruit maturity at 126 DAFB. The exocarp of each fruit was divided into 2 sides: the sunlight-facing side and the shaded side; the former was exposed to sunlight in the morning until midday. A total of 360 fruits were used in this experiment. The freshly harvested fruits were transported within 1 hour to Chiang Mai University laboratory for analysis. The experiment followed a completely randomised design, with each treatment being applied to 40 randomly selected trees.

Methods

Extraction and analysis of PAL activity from the exocarp (1-mm thick) was performed by a method modified of those of Faragher and Chalmer [17] and Arakawa et al. [18]. The absorbance was compared with that of standard cinnamic acid and presented as nmole/mg protein·hour. Protein quantity was measured according to the method of Lowry et al. [19] and expressed as mg/g fresh weight.

Extraction and analysis of reducing sugars and total sugar content were determined by the modified Somogyi and Nelson method [20-21] The absorbance was measured at 540 nm and

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compared with standard D-glucose. Determination of total anthocyanin content was conducted according to Ranganna [22]. The absorbance of the extract was measured at 535 nm.

The exocarp colours were measured with a colourimeter. The chromaticity was measured as a* value, which measures the greenness and redness on a scale of -60 to +60. A minus a* value means a green colour and a positive value, a red colour.

The statistical packages for the social science (SPSS) software for Windows was used in this experiment for analysis of variance (ANOVA) and least-significant difference (LSD) at 95% confidence level of each variable value under a completely randomised design (CRD).

RESULTS

PAL activity on the exposed side of mango fruit exocarp increased during two periods in fruit development at 91 and 119 DAFB, and was significantly ($p = 0.05$) higher between 84-126 DAFB than the unexposed side (Figure 1). Similar results were found on the unexposed side, where PAL activity also showed two peaks, though not coinciding with those from the exposed side, at 77 and 126 DAFB, and was lower than that on the exposed side (Figure 1).

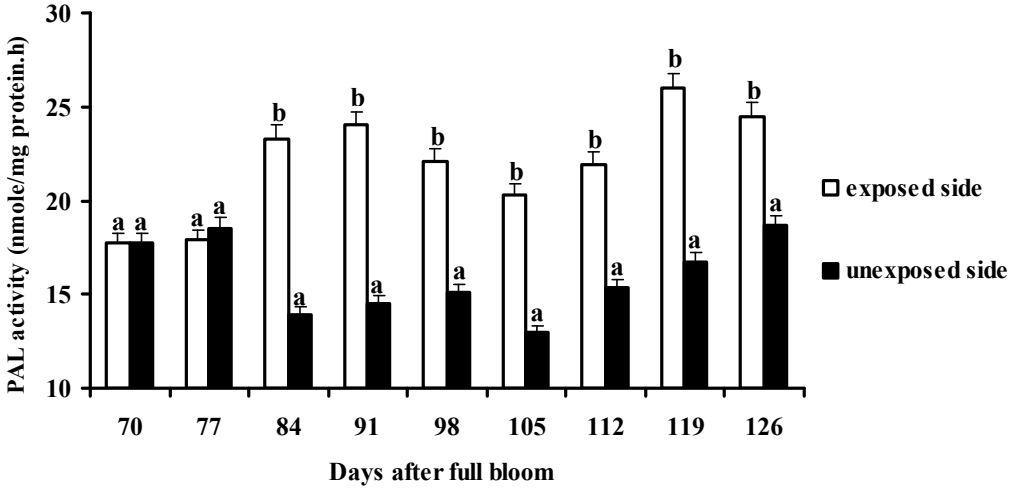

Figure 1. Effect of sunlight on PAL activity of 'Mahajanaka' mango fruit exocarp between 70-126 DAFB. Bars with different letters are significantly different ($p \leq 0.05$). Vertical bars indicate ±SE.

The reducing sugar content of the exocarp increased to the highest level at 91 DAFB and then decreased slightly and increased again at 126 DAFB (Figure 2). The exposed side had a higher reducing sugar content compared with the unexposed side throughout fruit development (significant at $p = 0.05$).

The total sugar content was relatively constant during 77-91 DAFB; after that it tended to increase. The highest content was detected at 112 DAFB for the exposed side. Total sugar content of the unexposed side had the same trend throughout fruit development (Figure 3) but was found to be lower than that of the exposed side (significant at $p = 0.05$).

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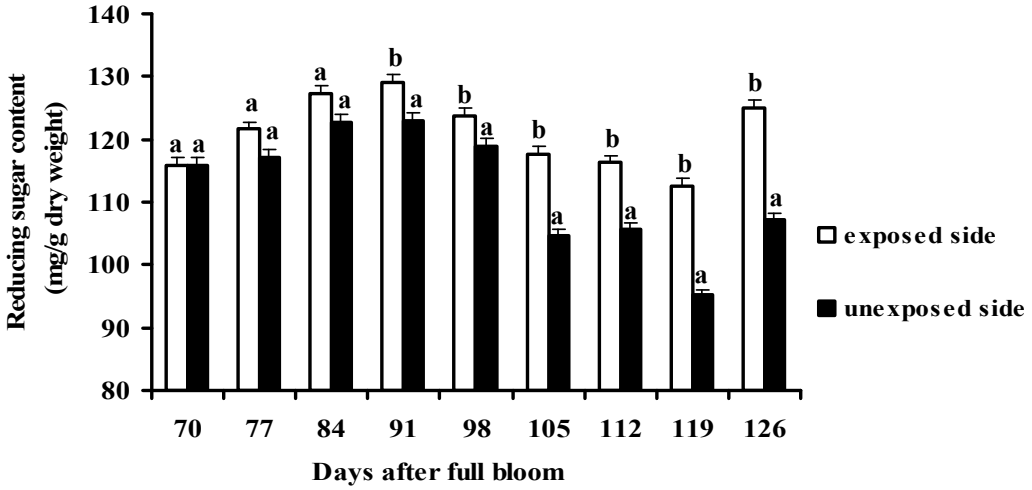

Figure 2. Effect of sunlight on reducing sugar content of 'Mahajanaka' mango fruit exocarp between 70-126 DAFB. Bars with different letters are significantly different ($p \leq 0.05$). Vertical bars indicate ±SE.

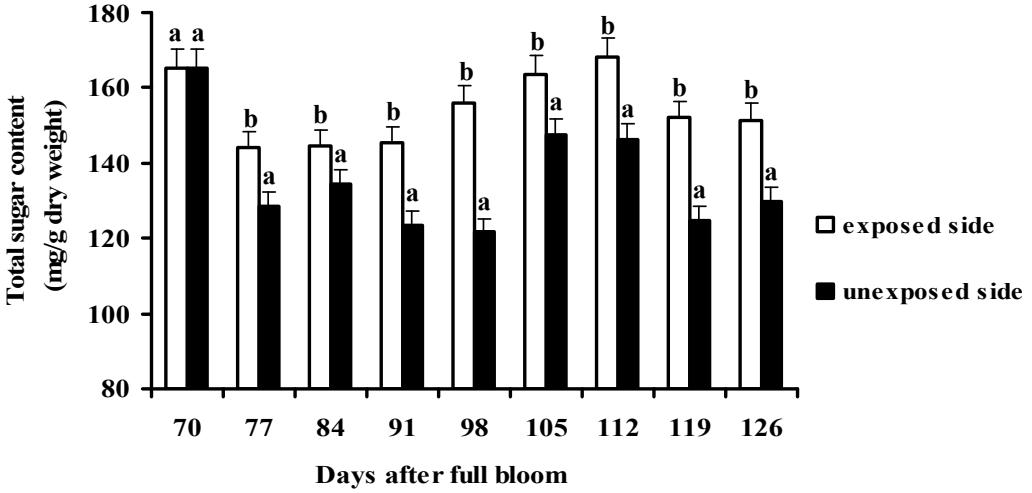

Figure 3. Effect of sunlight on total sugar content of 'Mahajanaka' mango fruit exocarp between 70-126 DAFB. Bars with different letters are significantly different ($p \leq 0.05$). Vertical bars indicate ±SE.

The total anthocyanin content of the exocarp significantly increased on the exposed side as compared with the unexposed side (Figure 4). Anthocyanin content of the exposed side started to increase at 84 DAFB and rose to its highest level at 112 DAFB, which was significantly higher than that of the unexposed side. The total anthocyanin content of the unexposed side remained relatively constant between 70-91 DAFB and then gradually decreased.

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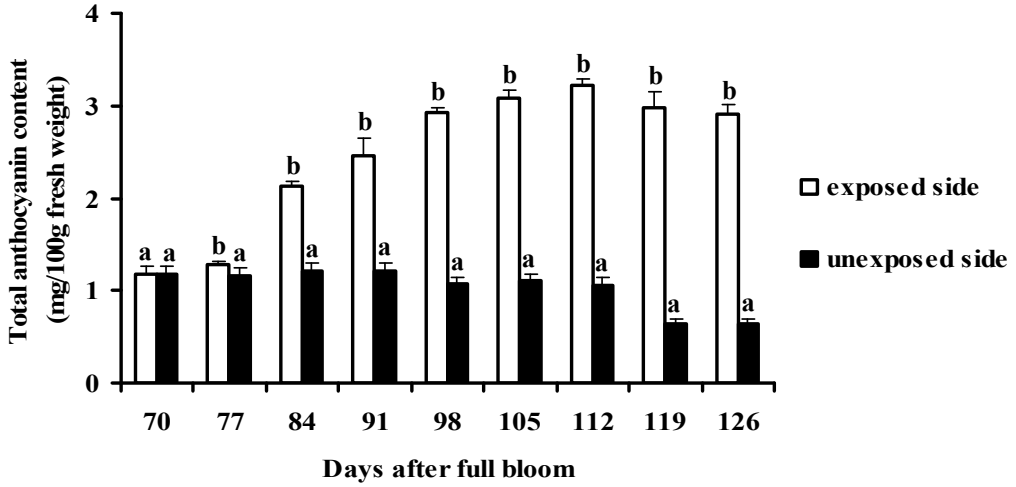

Figure 4. Effect of sunlight on total anthocyanin content of 'Mahajanaka' mango fruit exocarp between 70-126 DAFB. Bars with different letters are significantly different ($p \leq 0.05$). Vertical bars indicate ±SE.

The change of a* value of the exocarp was investigated during fruit development (Figure 5). Red colouration of the exposed side of the fruit was first observed at 84 DAFB and the highest a* value was observed at 112 DAFB, indicating maximum red colour development. The unexposed side had a minus a* value indicating a green exocarp. The a* values of all treatments were significantly different throughout the fruit development period.

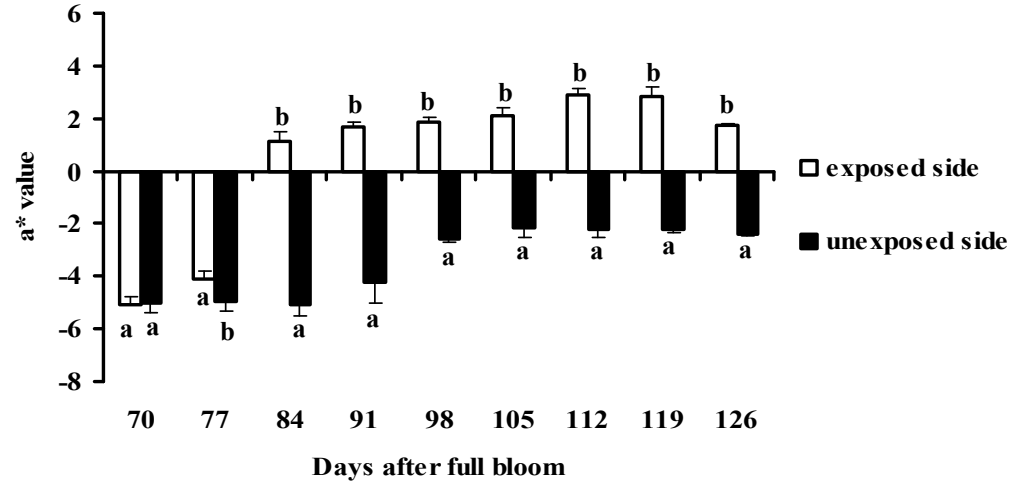

Figure 5. Effect of sunlight on a* values of 'Mahajanaka' mango fruit exocarp between 70-126 DAFB. Bars with different letters are significantly different ($p \leq 0.05$). Vertical bars indicate ±SE.

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DISCUSSION

An accumulation of anthocyanin in the sunlight-exposed side of 'Mahajanaka' mango fruit exocarp was concurrent with an increase in PAL activity in the mature stage at 105-126 DAFB but not at 70-98 DAFB (Figures 1 and 4). It is possible that the first peak of PAL activity in the immature fruits is necessary for phenolic metabolism that is directed to the synthesis of simple phenols and flavonoids during fruit development. However, the relationship between PAL activity and anthocyanin accumulation varies according to the stage of fruit development, which is similar to what occurs in apple exocarp [5, 8].

Similar to our results, Awad et al. [7] found that increasing anthocyanin synthesis and development of red colour in apple exocarp depend on sunlight. This is true in apples receiving sunlight from the top of the tree, which is exposed to higher light intensity. These fruits tend to have more anthocyanin synthesis than those at other positions. Visible light (400-700 nm) and also UV-B (312 nm) have the most important role in promoting red exocarp colour development in apples [23]. A highly exposed place increases the efficiency of anthocyanin synthesis and stimulates higher anthocyanin accumulation compared to a shaded area [8]. Other studies have shown that the photosynthesis pathway stimulates the synthesis of precursors for anthocyanin production [23-24].

In our experiment, sunlight also stimulates an increase of reducing sugar and total sugar content largely on the exposed side of the exocarp, where the change in total sugar content closely follows the change in anthocyanin level except at 70-77 DAFB. Total sugar content also parallels PAL activity but only in mature fruits. Transport of sugars, especially sucrose from the source (leaf) to the sink (fruit), is stimulated by sunlight. Sucrose is translocated to fruit via phloem causing an increase in endogenous sugars [25]. This is consistent with the report of Guan and Janes [26], who found that the amount of sugar in tomato fruit (*Lycopersicon esculentum*) accumulated in light is higher than in the dark. Light stimulates uptake, metabolism and storage of sugars, especially sucrose, which is a major sugar translocated into the tomato fruit. Light-grown fruits have higher carbohydrate content than dark-grown fruits, as well as higher ADP glucose pyrophosphorylase activity, which is correlated with a high starch level in tomato fruit [27].

Endogenous sugars induce gene expression of PAL enzyme, the key enzyme in the anthocyanin synthesis pathway. According to Vitrac et al. [16], the result of *Vitis vinifera* cell suspension cultures showed that sucrose promotes anthocyanin production and recognises the general signal transduction such as Ca^{+}, calmodulin and protein kinase/phosphatases for inducing anthocyanin biosynthesis. In red radish hypocotyls treated with exogenous sucrose, the expression levels of *PAL*, *CHS*, *CHI*, *F3H*, *DFR* and *ANS* for anthocyanin biosynthesis were enhanced [13]. In corn leaves (cv. Suweon 19), sucrose, which is important for controlling *CHS*, *CHI*, *F3H*, *DFR* and *ANS*, was shown to produce the greatest stimulation of anthocyanin formation [28].

CONCLUSIONS

Sunlight induces red colour development in mango fruit exocarp by promoting anthocyanin synthesis via increasing PAL activity. Sunlight also increases endogenous sugars (reducing sugars and total sugars) during mango fruit development.

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