Characteristics of Color Development and Relationship between Anthocyanin Synthesis and Phenylalanine Ammonia-lyase Activity in 'Starking Delicious', 'Fuji' and 'Mutsu' Apple Fruits¹

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Summary

To elucidate the characteristics of color development in apple cvs 'Starking Delicious' ('SD'), 'Fuji' and 'Mutsu', the effects of light on anthocyanin synthesis and L-phenylalanine ammonia-lyase (PAL) activity were investigated. Irradiation with white light and simultaneous irradiation with white and ultraviolet light with an emission peak at 312 nm (white+UV 312) were used. When the whole fruit skin was examined, 'SD' produced higher anthocyanin levels than 'Fuji' especially under white light. In skin disks, however, anthocyanin synthesis was increased in 'Fuji' to the same level as in 'SD'. The yellow-colored cultivar, 'Mutsu', did not respond to white light, and only produced a small amount of anthocyanin under white+UV 312 light. In whole fruit skin at least, there was a close correlation between anthocyanin synthesis and PAL activity. In skin disks, PAL activity was far higher than in whole fruit skin, however, anthocyanin synthesis did not show a similar increase in PAL activity. In 'Mutsu', white light did not induce anthocyanin synthesis although it stimulated PAL activity to the same extent as the white+UV 312 light.

Introduction

Under field conditions, color development of apple fruit differs between cultivars. Red-colored cultivars such as 'Starking Delicious' ('SD') and 'Jonathan' develop a deep red color indicating higher anthocyanin levels, while yellow-colored cultivars such as 'Golden Delicious' and 'Mutsu' produce only a small amount of anthocyanin. 'Fuji', the leading cultivar in Japan, is intermediate and does not often develop adequate red color, especially within the tree canopy.

Anthocyanin synthesis in apple fruit is also influenced by many other factors such as light(2, 8), temperature(5, 9), fruit maturity (6) and wounding(4). We showed previously in 'Jonathan' that simultaneous irradiation with red and UV-B (ultraviolet light of 280–320 nm) light had a synergistically stimulative effect on anthocyanin synthesis and suggested that this synergism might play an im-

portant role in developing the desirable red color under field light conditions(2). This synergism may also be involved in the differing color development depending on cultivar and position of fruit within the tree, because of poor penetration of UV-B into the tree canopy.

Anthocyanin synthesis in several plant tissues is associated with increased L-phenylalanine ammonia-lyase (PAL) (EC. 4.3.1.5) activity(7, 1). In apple skin, Faragher and Chalmers studied the effects of UV light and wounding on anthocyanin synthesis and PAL activity and suggested that PAL controlled the rate of anthocyanin synthesis in whole fruit skin(4). However, the UV light which they used had a emission peak at 254 nm and was not UV-B.

In this report, we investigated anthocyanin synthesis and PAL activity in response to white light and white+UV 312 (light source used for UV-B) light, and discussed the relationship between anthocyanin synthesis and

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PAL activity, in the three apple cvs 'SD', 'Fuji' and 'Mutsu'.

Materials and methods

Apple fruits of cvs 'SD', 'Fuji' and 'Mutsu' were harvested one month before ripening from the experimental orchard of the Faculty of Agriculture, Tohoku University in Sendai. Intact fruits and skin disks were compared. The skin disks, 11 mm in diameter with 1~2 mm cortical tissue attached, were cut from several fruits (30~50 disks/fruit), washed briefly in 0.3 M sucrose and rinsed with distilled water. They were then placed on filter paper saturated with distilled water, covered with a layer of thin polyvinyl chloride film and irradiated.

Irradiation

White light was obtained from white fluorescent lamps (FL 20 S.W, Toshiba, Tokyo) with filters which eliminated radiation of wavelengths below 390 nm. White+UV 312 light was provided by a combination of the white fluorescent lamps and UV fluorescent lamps (FL 20 S.E, Toshiba, Tokyo) with filters which eliminated radiation of wavelengths below 290 nm. Details of technical data have been shown previously(2). All irradiations and incubations in the dark was carried out at 15°C.

Determination of anthocyanin and PAL activity

In whole fruit experiments, skin disks were cut from the reddest area of each fruit just after irradiation. Anthocyanin was determined using five skin disks (taken from each fruit in the whole fruit experiments) as described previously(2). PAL activity was determined using 21 skin disks (7 from each of three fruit in the case of the whole fruit experiments) as described by Faragher and Chalmers(4).

Anthocyanin determination was replicated 4 times, and the PAL assay was replicated 3 times.

Results and discussion

Effect of fluence rate of white and white+UV 312 light on anthocyanin synthesis

1. Whole fruit skin

Fig. 1. Effect of fluence rate of white (a) and white+UV 312 (b) light on anthocyanin synthesis in the skin of 'Starking Delicious' and 'Fuji' whole fruit. Anthocyanin was assayed after 96 h irradiation. Vertical bars indicate standard deviations.

Under white light, anthocyanin synthesis in 'SD' increased linearly with fluence rate and reached $4.8 \mu\text{g cm}^{-2}$ at 17 W m^{-2} . Anthocyanin synthesis in 'Fuji' was lower and was saturated at 13 W m^{-2} with a value of $1.2 \mu\text{g cm}^{-2}$ (Fig. 1-a). 'Mutsu' produced no anthocyanin under the white light.

White+UV 312 light was much more effective in anthocyanin synthesis than white light, despite the lower intensity used (Fig. 1-b). Anthocyanin synthesis in 'SD' was saturated at 4 W m^{-2} with a value of $9 \mu\text{g cm}^{-2}$. While anthocyanin synthesis in 'Fuji' was lower than that in 'SD', it was not saturated until 5.3 W m^{-2} , at a value of $4 \mu\text{g cm}^{-2}$.

Fig. 2. Effect of fluence rate of white+UV 312 light on anthocyanin synthesis in whole fruit and skin disks of 'Mutsu'. Anthocyanin was assayed after 96 h irradiation. Vertical bars indicate standard deviations.

cm⁻². 'Mutsu' produced anthocyanin only under white+UV 312 light, with a maximum value as low as 0.9 μg cm⁻² at 5 W m⁻² (Fig. 2). These results show that the ability of whole fruit skin to produce anthocyanin differs with cultivars and light quality. 'SD' has a high ability to produce anthocyanin in response to both the white and white+UV 312 light, but that of 'Mutsu' is very low and the addition of UV 312 to the white light is essential for the development of red color, although strong coloring can not be expected. 'Fuji' is an intermediate cultivar and produces only a little anthocyanin under white light but much more anthocyanin under white+UV 312 light. This characteristic of 'Fuji' may be a reason why fruit of this cultivar shows poor red color within the tree canopy, where penetration of UV-B is greatly limited.

2. Skin disks

Anthocyanin synthesis in skin disks was much higher than in whole fruit skin (Fig. 3). In 'Fuji' especially, it was about 7 times as high as in whole fruit skin at 17 W m⁻² of white light. This means that the latent potential of this cultivar to produce anthocyanin under white light can be fully expressed by wounding. 'Mutsu', however, did not produce anthocyanin under white light

Fig. 3. Effect of fluence rate of white (a) and white+UV 312 (b) light on anthocyanin synthesis in skin disks of 'Starking Delicious' and 'Fuji'. Anthocyanin was assayed after 96 h irradiation. Vertical bars indicate standard deviations.

even in skin disks, although, under white+UV 312 light, skin disks produced about three times as much anthocyanin as the whole fruit skin (Fig. 2). Wounding is known to stimulate anthocyanin synthesis(4). However, the results above show that the wounding effect on anthocyanin synthesis differs with cultivars. Thus, it must be noted that the results of anthocyanin synthesis obtained with skin disks are not necessarily applicable to whole fruit skin.

Relationship between anthocyanin synthesis and PAL activity

1. Whole fruit skin

As in the previous experiments, using

Fig. 4. Anthocyanin accumulation (a) and PAL activity (b) in skin of whole apple fruit of 'Starking Delicious' irradiated with white (16.9 Wm^{-2}) and white+UV 312 (5.3 Wm^{-2} in total) light. Vertical bars indicate standard deviations.

whole fruit skin, 'SD' produced a greater amount of anthocyanin than 'Fuji', with white+UV 312 light being more effective than white light alone in both cultivars as in the previous experiments (Figs. 4 and 5 vs. Figs. 1 and 3). PAL activity reached a maximum after 24 h of irradiation, and was much higher under white+UV 312 light than white light, irrespective of cultivars. In whole fruit skin of 'Mutsu', a small amount of anthocyanin ($0.9 \mu\text{g cm}^{-2}$) was produced under white+UV 312 light only, but no measurable PAL activity was found.

2. Skin disks

In skin disks, PAL activity was stimulated

Fig. 5. Anthocyanin accumulation (a) and PAL activity (b) in skin of whole apple fruit of 'Fuji' irradiated with white (16.9 Wm^{-2}) and white+UV 312 (5.3 Wm^{-2} in total) light. Vertical bars indicate standard deviations.

more than in whole fruit skin. Anthocyanin synthesis, however, did not increase to the same extent as PAL activity (Figs. 6 and 7). Anthocyanin synthesis in the three cultivars proceeded at a constant rate for 72 h of the irradiation, while PAL activity reached a maximum after 48 h of irradiation and then declined regardless of light quality. White+UV 312 light stimulated PAL activity more than white light alone, with maximum activity differing little between 'SD' and 'Fuji'. When skin disks were kept in the dark, PAL activity increased slowly for at least 72 h. Although this level was much lower than when disks were irradiated, it was higher than when the whole fruit was irradiated.

Fig. 6. Anthocyanin accumulation (a) and PAL activity (b) in skin disks of 'Starking Delicious' irradiated with white (16.9Wm^{-2}) and white+UV 312 (5.3Wm^{-2} in total) light. Vertical bars indicate standard deviations.

In 'Mutsu', PAL activity was also stimulated by both light treatments, with the maximum activity differing little between the two light treatments, and anthocyanin synthesis occurring only under white+UV 312 light (Fig. 8).

In whole fruit at least, there was a close correlation between anthocyanin and PAL activity. Thus, it appears that PAL acts as the rate limiting enzyme for anthocyanin synthesis. White+UV 312 light induced higher PAL activity than white light even at a lower fluence rate, a fact which indicates that UV 312 light is more effective than white light in increasing PAL activity.

Fig. 7. Anthocyanin accumulation (a) and PAL activity (b) in skin disks of 'Fuji' irradiated with white (16.9Wm^{-2}) and white+UV 312 (5.3Wm^{-2} in total) light. Vertical bars indicate standard deviations.

Such an effect agrees with the results obtained with *Haplopappus gracilis* and parsley, where UV light of 320~350 nm (11) and lower than 345 nm (3) increased the PAL activity.

In skin disks, however, PAL activity increased to a much greater extent than anthocyanin synthesis. This difference has also been recognized by other workers (4, 10) and it has been suggested that anthocyanin synthesis is saturated at a much lower level of PAL activity than that found in skin disks. Moreover, in skin disks, a large number of other phenolic compounds besides anthocyanin are known to be produced, perhaps through surplus PAL activity. In addition,

Fig. 8. Anthocyanin accumulation (a) and PAL activity (b) in skin disks of 'Mutsu' irradiated with white (16.9 Wm^{-2}) and white+UV 312 (5.3 Wm^{-2} in total) light. Vertical bars indicate standard deviations.

Tan showed that wounding (by preparing skin disks) increased PAL activity by destroying the ability of the tissue to synthesize PAL inactivating systems (PAL-IS) (10). In our results, the increase in PAL activity following wounding was accentuated by white light as well as by UV 312 light, but it is not known whether or not the increase in PAL activity is due to the suppression of PAL-IS.

The results obtained with skin disks indicate that PAL is not the only critical enzyme regulating anthocyanin synthesis in apple skin, although its activity may control the rate of anthocyanin synthesis in whole fruit skin. Other rate limiting enzyme(s) must

be found in the pathway resulting in ultimate synthesis of anthocyanin. Further, our results suggest that the light requirement of the two enzymes studied differs with cultivars; thus white light is effective in activating both enzymes in 'SD' and 'Fuji' but not in 'Mutsu', but UV 312 is effective, though to different degrees in the three cultivars. A comparative study of such varietal differences might be very useful in elucidating the regulatory mechanism of anthocyanin synthesis in apple skin.

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リンゴ3品種, ‘スターキング・デリシャス’, ‘ふじ’ 及び ‘陸奥’ の着色特性
及びアントシアニン生成と L-フェニルアラニンアンモニアリアーゼ
活性との関係

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摘 要

リンゴ3品種, ‘スターキング・デリシャス’ (‘SD’), ‘ふじ’ 及び ‘陸奥’ の着色特性を知るために, 光強度とアントシアニン生成量との関係及びアントシアニン生成と L-フェニルアラニンアンモニアリアーゼ (PAL) 活性との関係について調査した. 白色光 (white) 及び白色光と紫外線の混合光 (white+UV 312) を用いた. 果実照射では ‘SD’ はアントシアニン生成能が最も高く, ‘ふじ’ は ‘SD’ に比べて特に白色光での生成能が劣っていた. 果皮片照射ではアントシアニン生成が促進され,

特に ‘ふじ’ の白色光での生成能が著しく促進された. 黄色品種である ‘陸奥’ は, 白色光では全くアントシアニンを生成せず混合光で少量のアントシアニンを生成した. 果実照射においてはアントシアニン生成と PAL 活性の間には密接な関係が認められた. 果皮片照射ではアントシアニン生成の促進以上に PAL 活性が著しく促進された. 特に ‘陸奥’ では白色光照射による PAL 活性の上昇は混合光照射のそれと大差なかったが, アントシアニンは全く生成されなかった.